

## Subterranean Fishes of North America: Amblyopsidae

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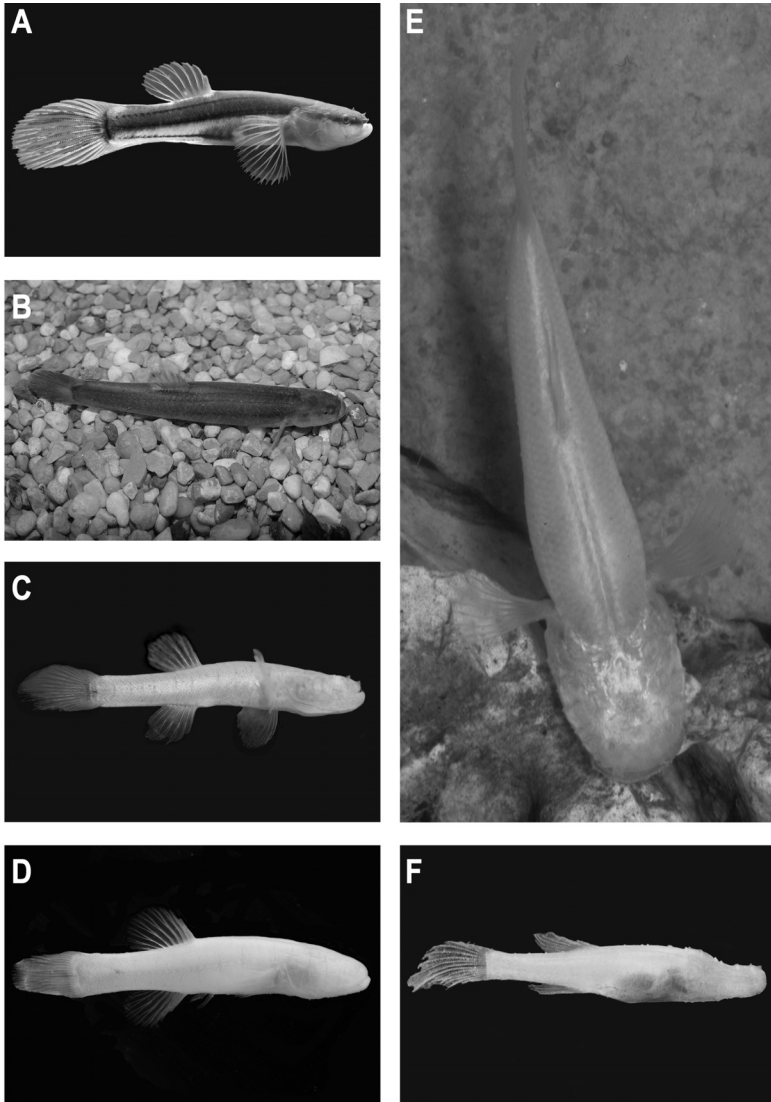
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### INTRODUCTION

The Amblyopsid cavefishes, family Amblyopsidae, have been viewed as a model system for studying the ecological and evolutionary processes of cave adaptation because the four cave-restricted species in the family represent a range of troglomorphy that reflect variable durations of isolation in caves (Poulson 1963, Poulson and White 1969). This group has both intrigued and excited biologists since the discovery and description of *Amblyopsis spelaea*, the first troglobitic fish ever described, in the early 1840s. Other than the Mexican cavefish (*Astyanax fasciatus*), cave Amblyopsids are the most comprehensively studied troglobitic fishes (Poulson, this volume).

The Amblyopsidae (Fig. 1) includes species with some unique features for all cavefish. *Typhlichthys subterraneus* is the most widely distributed of any cavefish species. Its distribution spans more than 5° of latitude and 1 million km<sup>2</sup> (Proudlove 2006). *Amblyopsis spelaea* is the only cavefish known to incubate eggs in its gill chamber. In fact, this species is the only one of the approximately 1100 species in North America with this behavior. The Amblyopsidae is the most speciose family of subterranean fishes in the United States containing four of the eight species recognized. Two other



**Fig. 1** Members of the Amblyopsidae. The family includes (A) the surface-dwelling swampfish (*Chologaster cornuta*), (B) the troglophile spring cavefish (*Forbesichthys agassizii*), and four troglobites: (C) the southern cavefish (*Typhlichthys subterraneus*), (D) the northern cavefish (*Amblyopsis spelaea*), (E) the Ozark cavefish (*A. rosae*), and (F) the Alabama cavefish (*Speoplatyrhinus poulsoni*). Photos courtesy of Uland Thomas (A), Dante Fenolio (E), and Richard Mayden (F).

*Color image of this figure appears in the color plate section at the end of the book.*

families have species adapted to subterranean habitats, the Ictaluridae and Cottidae. Two species of ictalurids are endemic to the Edwards Aquifer of Texas, the widemouth blindcat (*Satan eurystomus*) and the toothless blindcat (*Trogloglanis pattersoni*); and troglomorphic populations of sculpins (*Cottus* sp.) are known from Missouri and Pennsylvania.

Our primary aims of this chapter are to provide an up-to-date, comprehensive review of the pertinent literature and unpublished research (Niemiller on phylogenetics and phylogeography and Poulson on pigment, foraging patterns, metabolic efficiencies, and metabolic acclimation to temperature) regarding Amblyopsid cavefishes, including their surface and troglomorphic relatives in the family. We begin with a synopsis of the history of Amblyopsid research from the early 1840s to the present day. Here we discuss the significant studies and researchers that have shaped the knowledge base. Next, we review the systematic relationships and taxonomy of the family. The Amblyopsidae have only recently been the subject of phylogenetic examination using molecular markers and we include preliminary data here. We then discuss the biogeography, habitat, and distribution of the family. In the next section, we review morphology of the family in general and morphology of cave adaptation in particular. We include some new data on pigment systems. Subsequently, we summarize aspects of ecology, life history, and behavior including topics such as reproduction, diet, parasites and disease, longevity, metabolism, and demography. Here we also include some new data. Finally, we end with a discussion of the conservation status and threats to members of the family. In each section, we suggest avenues of future research that are needed.

Our review is intended to be as detailed and comprehensive as possible and to include the majority of relevant references. With this in mind, we hope that this chapter will serve as both a stimulus for future research and an exhaustive bibliographic reference regarding Amblyopsid biology.

## I. HISTORY OF AMBLYOPSID RESEARCH

In this section, we provide a brief history of Amblyopsid research beginning with the discovery of the first member of the family in the early 1840s. By no means is this review meant to encompass all papers on Amblyopsids. Instead, we attempt to outline the major studies and prominent players in Amblyopsid research, particularly during the early years from 1840 to 1910. For more complete reviews on the history of hypogean fish research including Amblyopsids, readers should peruse the works of Romero (2001) and Proudlove (2006).

The description of the northern cavefish, *Amblyopsis spelaea* (= *A. spelaeus*), from the River Styx in Mammoth Cave, Kentucky, by DeKay (1842) represents the first scientifically acknowledged report of a troglomorphic fish species (Romero 2001, Proudlove 2006). However, probable reports of *A. spelaea* occurred earlier. James Flint in 1820 recounted a conversation with a local in Indiana who stated that a neighbor found 'blind fishes' when digging a well (Flint 1820, Romero 2001, Proudlove 2006). The first published account of *A. spelaea* from the Mammoth Cave area appeared in the book *An Excursion to the Mammoth Cave, and the Barrens of Kentucky* by Robert Davidson (Davidson 1840). His observations represent the first biological observations of the species, and of a troglolithic fish (Proudlove 2006).

The original description of *A. spelaea* by DeKay was not very detailed but incited the interest of other researchers. Wyman (1843ab, 1851, 1854ab, 1872) conducted first detailed examination of the internal anatomy and brain. Tellkamp (1844) provided detailed descriptions of *A. spelaea* from Mammoth Cave. Amblyopsids were of particular interest to Jean Louis Agassiz who published the first insight on the potential importance of troglomorphic fishes to biological research in 1847 (Romero 2001). Agassiz (1847) described a research plan involving *A. spelaea* to investigate the embryology, anatomy, and effects of light on the species. Although he never followed through with his plans, several of his students investigated aspects of Amblyopsid biology (see below). Agassiz (1853) did, however, describe the epigeal swampfish, *Chologaster cornuta* (= *C. cornutus*).

Between 1850 and 1900, troglomorphic fishes, and Amblyopsids in particular, were prominent in the debate on evolution. Several prominent biologists including Frederick Ward Putnam (a former student of Agassiz), Alpheus Packard, Edward Lankester, Alpheus Hyatt, Jeffries Wyman, and Carl H. Eigenmann discussed the evolution of troglomorphy in cave fauna, and of cavefish especially. Their influence on and contributions to cave biology are eloquently detailed by Romero (2001). Also during this time period, four Amblyopsid species were described (three are currently recognized). The southern cavefish, *Typhlichthys subterraneus*, was described by Girard (1859) from a well near Bowling Green, Kentucky. Girard thought this new species was a transitional form between *A. spelaea* and the entirely epigeal species, *C. cornuta*. In 1872, Frederick Ward Putnam described the spring cavefish from a well near Lebanon, Tennessee (Putnam 1872). Ten years later, Forbes (1882) described *C. papilliferus*.

At the end of nineteenth and into the twentieth century, Carl H. Eigenmann was the prominent figure in cave research. Between 1887 and 1909, much of his work focused on understanding the loss of visual structures in cave vertebrates. Amblyopsids were included in many of

his studies. In 1898, Eigenmann described the Ozark cavefish, *Amblyopsis rosae* (= *Typhlichthys rosae*). In 1905, Eigenmann (1905) described both *T. osborni* and *T. wyandotte*. Both species would later be synonymized under *T. subterraneus*. Between 1898 and 1905 alone, he published at least 39 papers and abstracts on cave vertebrates focusing on the morphology and development of loss of vision (Romero 2001). His work culminated in his 1909 book titled *Cave Vertebrates of North America: A Study in Degenerative Evolution* (Eigenmann 1909) in which he detailed the structure, development, and degeneration of the eye in several cave-dwelling vertebrates known to science at the time. Amblyopsids were a major focus of his investigations in this volume. The volume also includes an important chapter by Fernandus Payne on laboratory observations and experiments on the feeding and sensory capabilities of *A. spelaea*. Between the writings of Eigenmann and the early 1960s, few studies centered on Amblyopsids.

However, the family received renewed interest from biologists beginning with the systematic investigation by Woods and Inger (1957). In 1955, Thomas L. Poulson began a comparative study of the Amblyopsidae. His Ph.D. dissertation included aspects of morphology, physiology, life history, ecology, and behavior. Using Amblyopsids as a 'natural evolutionary experiment', Poulson (1960, 1963) inferred evolutionary and ecological patterns of cave adaptation and attempted to outline a step-by-step process of increasing subterranean (troglomorphic) specialization from preadapted surface to obligate cave-dwelling species. Poulson has continued to study evolutionary and ecological aspects of cave adaptation to the present day. His multiple hypotheses and tests of these hypotheses have influenced many other researchers. In fact, the most recent Amblyopsid to be described, the Alabama cavefish (*Speoplatyrhinus poulsoni*), was named in his honor (Cooper and Kuehne 1974), partly because of his predictions of what the next stage of troglomorphy would look like. Much of our knowledge about the biology of Amblyopsid cavefishes has been obtained by the numerous studies he has conducted, many of which are referenced in more detail in this chapter. He continues to study these fishes and several new workups of old unpublished data are included in this chapter.

Amblyopsid cavefishes continue to intrigue and excite biologists attempting to discern the ecological and evolutionary facets of cave adaptation. Although many studies in the last twenty years have focused on the demography, distribution, conservation status, and threats of the cave-dwelling species, other aspects of biology including the phylogenetic relationships, biogeography, and life history continue to evoke interest. We have attempted to summarize what we feel are the most relevant

references for each of these areas in subsequent sections of this chapter. We apologize in advance to authors of any extensive or insightful studies we may have missed and hope the authors will work with us to redress any such omissions.

## II. TAXONOMY AND SYSTEMATICS

The Amblyopsidae have been known to science since the 1840s. The family-group name (Amblyopsidae-Amblyopsini) was first used by Bonaparte (1846). Previously, the family names Heteropygii and Hypsaeidae were used by Tellkamp (1844, 1845) and Storer (1846) respectively, but these names are considered unavailable because they were not formed from the stem of an available genus-group name (Poly and Proudlove 2004). Aspects of taxonomy and systematics of the family have been examined by Cox (1905), Eigenmann (1909), Woods and Inger (1957), Swofford *et al.* (1980), Swofford (1982), Bergstrom *et al.* (1995), Bergstrom (1997), and Niemiller and Fitzpatrick (2008), Near *et al.* (unpublished data). The latter four studies were molecular in nature. More recently, Poly and Proudlove (2004) reviewed taxonomy and systematic relationships of the Amblyopsidae.

Presently five genera and six species are recognized within the Amblyopsidae (Fig. 1). These include the epigeal swampfish (*Chologaster cornuta* Agassiz), a troglophile, the spring cavefish (*Forbesichthys agassizii* Putnam), and four troglobitic species in order of presumed increasing time of isolation in caves: southern cavefish (*Typhlichthys subterraneus* Girard), northern cavefish (*Amblyopsis spelaea* DeKay), Ozark cavefish (*A. rosae* Eigenmann), and the Alabama cavefish (*Speoplatyrhinus poulsoni* Cooper and Kuehne). We will briefly discuss the higher-level relationships of the Amblyopsidae before discussing nomenclature and interspecific relationships within the family.

### A. Higher-level Relationships

The Amblyopsidae have been considered the sister group to the pirate perches, family Aphredoderidae (Rosen 1962, Patterson 1981, Patterson and Rosen 1989) but also share a close affinity with the trout perches, family Percopsidae (Rosen 1962). Together, these three families have been included in the order Percopsiformes (Greenwood *et al.* 1966, Nelson 1984, 2006). However, some have questioned the monophyly of this order. Murray and Wilson (1999) suggested Amblyopsids might be more closely related to the Anacanthines and proposed recognition of Amblyopsids as a distinct order, the Amblyopsiformes. Poly in Poly and Proudlove

(2004) suggested that Amblyopsids might be related to Gobioids because of the distribution of Amblyopsids in relation to the former Mississippi Embayment (see below), similarities in patterns of neuromasts on the body, and similarities in morphology, particularly when compared with troglobitic Gobioids. McAllister (1968) also suggested a possible relationship between Gobioids and Amblyopsids. Wiley *et al.* (2000) resolved a sister relationship between trout perches and pirate perches based on a combined analysis of morphological and molecular characters but did not include any Amblyopsid species. More recently, a molecular study using both mitochondrial and nuclear markers and including members of all three families supports the inclusion of the Amblyopsidae in the order Percopsiformes with a sister relationship between cavefishes and pirate perches (Smith and Wheeler 2006). Immunological evidence also supports the inclusion of the Amblyopsidae in the order Percopsiformes (Kalayil and Clay 1976).

## B. Taxonomy

Agassiz (1853) described the swampfish (Fig. 1A) from ditches in rice fields in South Carolina. Since the description, taxonomy has remained stable. Only two synonyms exist: *Chologaster cornutus* (Agassiz 1853) and *C. avitus* (Jordan and Jenkins in Jordan 1889). The swampfish generally is considered the most basal Amblyopsid. Like the other Amblyopsids, little molecular work has been conducted on the species. The most substantial is the allozyme study by Swofford (1982) who found considerable differentiation among *C. cornuta* populations both among and within drainages. Preliminary evidence using mitochondrial and nuclear DNA markers also indicate significant genetic differentiation across the species range (Niemiller and Fitzpatrick 2008, Near *et al.*, unpublished data). Poulson (1960, 1963) also found considerable variation in adult body size and meristic characters among drainages.

The spring cavefish (Fig. 1B) was originally described as *C. agassizi* by Putnam (1872) from a well near Lebanon, Wilson Co., Tennessee. Later, Forbes (1882) described *C. papilliferus* from a spring in western Union Co., Illinois, on the basis of coloration differences between the Tennessee and Illinois populations. Jordan and Evermann (1927) erected a new genus, *Forbesella*, citing that the subterranean nature of spring cavefish warrants separate recognition. Jordan (1929) later replaced *Forbesella* with *Forbesichthys*, as the former was preoccupied in tunicates. This genus is still considered a junior synonym of *Chologaster* by some authors, however. Woods and Inger (1957) noted that populations of spring cavefish from southern Illinois, central Kentucky, and central Tennessee all differed slightly but did not warrant specific or subspecific designation. Therefore,

*C. papilliferus* was synonymized under *C. agassizi* and their revision has been followed by most subsequent authors with the exception of Clay (1975) who maintained that *C. agassizi* and *C. papilliferus* are specifically distinct. Allozyme analyses by Swofford (1982) revealed considerable differentiation between populations that justified resurrection of the genus *Forbesichthys*, which was elevated later by Page and Burr (1991). Spring cavefish have not been the subject of phylogenetic studies since Swofford (1982); however, recent evidence suggests that populations from Illinois (formerly *papilliferus*) and Tennessee may be phylogenetically distinct at both mitochondrial and nuclear markers (Near *et al.*, unpublished data). Kentucky populations have yet to be examined.

The southern cavefish (Fig. 1C) was described by Girard (1859) from a well near Bowling Green, Warren Co., Kentucky. Later, Eigenmann (1905) described both *T. osborni* and *T. wyandotte* based on differences in head width and eye diameter. *Typhlichthys osborni* was described from Horse Cave, Kentucky. *Typhlichthys wyandotte* was described from a well near Corydon, Indiana, that was later destroyed. Recently, a well-like entrance into a cave on the property of a car dealership in Corydon was discovered and is believed to represent the type locality (Black in Lewis 2002). Regardless, this species is generally considered invalid and was not listed as a locality in Woods and Inger (1957). Recent surveys in the vicinity of Corydon have failed to document *T. subterraneus*, finding only *A. spelaea* (Lewis 1998, Lewis and Sollman 1999). *Typhlichthys eigenmanni* (*nomen nudum*) was described as a fourth species in the genus from Camden Co., Missouri (likely River Cave). Recently, Parenti (2006) proposed that *T. eigenmanni* Charlton (1933) is a subjective synonym of *T. subterraneus*. Woods and Inger (1957) synonymized all species under *T. subterraneus* on the basis of lack of any clear geographic pattern in morphological variation. A population from Sloans Valley Cave, Pulaski Co., Kentucky, differs in several ways from populations to the southwest in Tennessee and may represent an undescribed species (Cooper and Beiter 1972, Burry and Warren 1986). However, further details regarding this population have not been published.

Because of the extensive distribution of *T. subterraneus* and the results of molecular studies of other troglobites (especially Culver *et al.* 1995), some authors have speculated that *T. subterraneus* actually represents several independent invasions and, therefore, distinct lineages (Swofford 1982, Barr and Holsinger 1985, Holsinger 2000, Poulson, this volume). Indeed, electrophoretic allozyme analyses by Swofford (1982) showed considerable differentiation among morphologically similar populations of *Typhlichthys*, even those that are geographically close, suggestive of multiple, independent invasions and limited gene flow. In fact, six of the thirteen



populations Swofford sampled were monomorphic and for different allozyme alleles. However, owing to small sample size, Swofford's study was limited in its ability to distinguish modular or hierarchical subdivision from a continuous relationship between genetic and geographic distance. More recently, Bergstrom *et al.* (1995) and Bergstrom (1997) investigated phylogenetic relationships of populations west of the Mississippi River using mitochondrial DNA. Although limited, these studies revealed considerable variation among populations. Likewise, Niemiller and Fitzpatrick (2008) examined genetic variation among eastern populations of *Typhlichthys* in Alabama, Georgia, and Tennessee. Again, significant genetic divergence was observed and both mitochondrial and nuclear DNA variation was structured among hydrological drainages. Molecular and morphological evidence also indicate that Arkansas populations warrant recognition of a distinct species (Graening *et al.*, unpublished data). These studies support the hypothesis that morphological similarity is the result of parallel evolution rather than significant dispersal and gene flow across major drainage and river divides (Cooper and Iles 1971, Barr and Holsinger 1985).

The northern cavefish (Fig. 1D) was the first troglomorphic fish ever described in the scientific literature. The formal description is based on fish from Mammoth Cave, Edmonson Co., Kentucky by DeKay (1842). Originally spelled *Amblyopsis spelaeus*, Woods and Inger (1957) corrected the name to *A. spelaea* and provided the most important definition of the species (Proudlove 2006). Swofford (1982) found that populations north and south of the Ohio River are monomorphic for the same allozyme alleles with no heterozygosity. The data do not contradict Poulson (1960) who observed a distinct split in morphological variation across the Ohio River. Rather than indicating pre-Pleistocene dispersal as Poulson (1960) proposed, Swofford concluded that populations north and south of the river likely are of more recent origin during the Pleistocene and insufficient time has elapsed for significant genetic differentiation. Clearly more work is needed to elucidate the biogeographic history of the species. No studies to date have examined mitochondrial or nuclear DNA differentiation within the species.

The Ozark cavefish (Fig. 1E) was first reported from specimens collected from caves near Sarcoxie, Jasper Co., Missouri, by Garman (1889) and identified as *T. subterraneus*. The name *Typhlichthys rosae* was applied to the species in several of Eigenmann's papers in 1898 (for a review see Poly and Proudlove 2004). The Ozark cavefish was reclassified as *Troglichthys rosae* by Eigenmann (1898, 1899a) and this name stood until Woods and Inger (1957) placed the species in the genus *Amblyopsis*, on the basis of morphological similarity with *A. spelaea*. Phylogenetic studies that

included samples of *A. rosae* have revealed considerable divergence across the range. Although Swofford (1982) sampled just two populations, one from the Neosho River drainage in Jasper Co., Missouri, and the other from the Illinois River drainage in Benton Co., Arkansas, these populations were well differentiated and with very low heterozygosities for allozyme loci. The phylogenetic studies of Bergstrom *et al.* (1995) and Bergstrom (1997) also revealed distinct genetic structure associated with each major drainage. Bergstrom (1997) proposed that mitochondrial genetic divergences were so pronounced that at least subspecific designation was warranted. At least four genetically differentiated groups associated with distinct drainage basins were identified: 1) Illinois River drainage in northwestern Arkansas, 2) White River drainage in southwestern Missouri, 3) Neosho River drainage in southwestern Missouri, and 4) Neosho River drainage in northeastern Oklahoma. Genetic variation within drainages also suggested that many localities are genetically isolated and, therefore, constitute distinct endemic populations (Noltie and Wicks 2001).

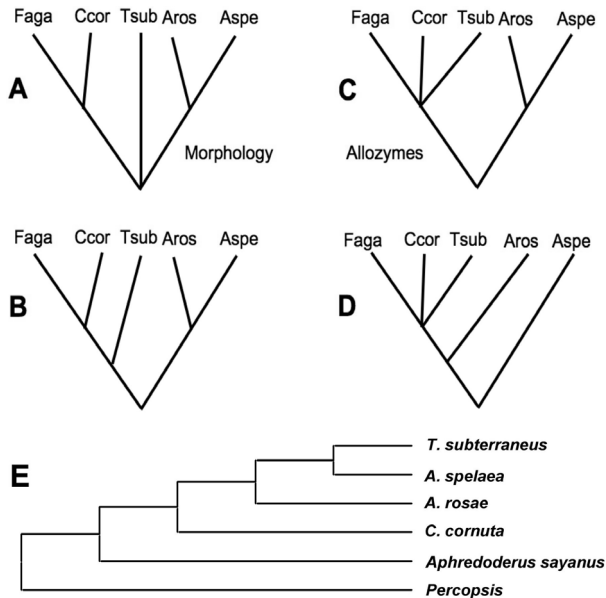
Cooper and Kuehne (1974) described the Alabama cavefish (Fig. 1F), *Speoplatyrhinus poulsoni*, from Key Cave, Lauderdale Co., Alabama, in honor of Thomas L. Poulson. Little is known about how this species is related to other Amblyopsids. Because *S. poulsoni* is the most troglomorphic of all the subterranean Amblyopsids and thought to represent a relict population, the single cave endemic probably is not closely related to any of the other species (Proudlove 2006). It has at least three features that are completely unlike other Amblyopsids: arrangement and relative sizes of otoliths, size and extent of caudal papillae (Cooper and Kuehne 1974), and absence of any tactile receptors on the head (Poulson, personal observation). In addition, there are two extreme neotenic features: the absence of branched fin rays in adults and the absence of a bilateral supraopercular papilla opening to the head subdermal lateral line system (Cooper and Kuehne 1974).

### C. Interspecific Relationships

Although Amblyopsids have received considerable attention from evolutionary biologists and ichthyologists, comparatively little work has been conducted on the systematics of the family, particularly at the molecular level. Prior to the study of Woods and Inger (1957), nine species in five genera were recognized. As mentioned above, the authors synonymized all species of *Typhlichthys* under *T. subterraneus* and moved *Troglichthys rosae* into the genus *Amblyopsis* and *Forbesichthys* into *Chologaster*. Likewise, *C. papilliferus* was synonymized with *C. agassizi*. Woods and Inger (1957) suggested two phylogenies representing the

interspecific relationships of the family (Fig. 2A–B) relying primarily on the presence or absence of two characters: (i) scleral cartilages in the eye and (ii) postcleithrum in the pectoral girdle. However, a reanalysis by Swofford (1982) using the same characters as Woods and Inger (1957) does not support either proposed phylogeny. Instead, Swofford (1982) proposed the phylogenies presented in Fig. 2C–D. Accordingly, the resurrection of the genus *Forbesichthys* was recommended because *C. agassizii* and *C. cornuta* are considerably divergent morphologically according to meristic characters (Woods and Inger 1957) and genetically (Swofford 1982). This treatment was preferred over the more drastic alternative, which involved synonymizing *Typhlichthys* under *Chologaster*.

Uyeno (pers. comm.) examined karyotypes of three Amblyopsid species. Both *F. agassizii* from Rich Pond, Kentucky, and from Wolf Lake, Illinois, and *A. spelaea* had  $2n = 24$  chromosomes noting that both of these species had very similar karyotypes. Uyeno also examined the karyotype



**Fig. 2** Phylogenetic hypotheses of the interspecific relationships of the Amblyopidae and according to the (A and B) morphological examinations of Woods and Inger (1957), (C and D) allozyme studies of Swofford (1982), and (E) mitochondrial DNA (Niemiller and Fitzpatrick 2008, Near *et al.*, unpublished data). Faga = *F. agassizii*, Ccor = *C. cornuta*, Tsub = *T. subterraneus*, Aros = *A. rosae*, and Aspe = *A. spelaea*. *Forbesichthys agassizii* and *S. poulsoni* are not represented in the mtDNA phylogeny.

of two populations of *T. subterraneus*, Cave City, Kentucky, and Thomason Cave, Alabama. In *T. subterraneus*, the karyotype was  $2n = 46$  including one pair of rather large metacentric chromosomes. Karyotypes have not been examined for *C. cornuta*, *A. rosae*, and *S. poulsoni*.

Bergstrom *et al.* (1995) and Bergstrom (1997) were the first to examine interspecific relationships of Amblyopsids using DNA sequence data. Using a portion of the mitochondrial NADH-dehydrogenase subunit-2 (NAD2) gene, their analyses resolved a sister relationship between *A. rosae* and all other Amblyopsids (*S. poulsoni* was not included), but low levels of differentiation were found between *C. cornuta*, *F. agassizii*, *A. spelaea*, and some *T. subterraneus* populations. Their ND2 phylogeny clearly differs from the phylogenetic hypotheses of Woods and Inger (1957) and Swofford (1982). Unfortunately, DNA samples from *C. cornuta*, *F. agassizii*, and *A. spelaea* were extracted from formalin-preserved tissues and were contaminated. Therefore, the relationships resolved likely do not reflect the true history of the ND2 gene. However, the intraspecific relationships reported within *A. rosae* and western populations of *T. subterraneus* are believed to be accurate given that extractions were made from freshly collected tissue.

Recent phylogenetic work utilizing both mtDNA and nuclear DNA markers has revealed a different relationship (Fig. 2E) among the Amblyopsids (Niemiller and Fitzpatrick 2008, Near *et al.*, unpublished data). MtDNA supports the basal position of *C. cornuta* within the Amblyopsidae. Monophyly of *Amblyopsis* is not supported with the mtDNA dataset. However, other nuclear markers need to be examined before taxonomic revisions are made.

The Alabama cavefish has not been included in any systematic treatment of the family. Boschung and Mayden (2004) state that *Typhlichthys* and *Speoplatyrhinus* form a monophyletic group that is sister to a clade comprising *Forbesichthys* and *Chologaster*. This statement likely is a typo and *Amblyopsis* should be replaced for *Speoplatyrhinus*. Proudlove (2006) speculated that *S. poulsoni* is probably not closely related to other species in the family. On several counts it is the most neotenic, most troglomorphic, and most distinct species in the family (Cooper and Kuehne 1974, Poulson 1985; and Taxonomy). The exact placement of *Speoplatyrhinus* within the family remains unknown and will remain so until a fresh tissue sample can be obtained.

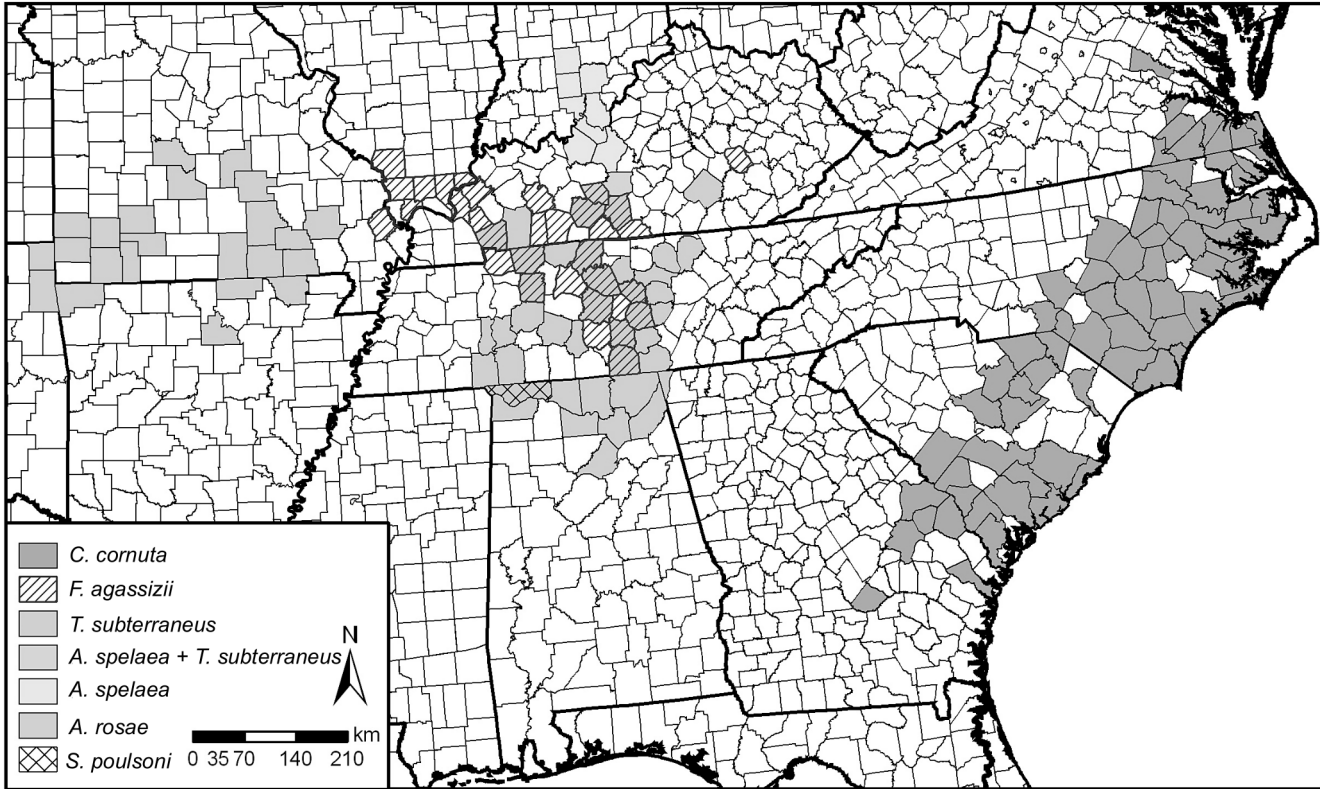
### III. DISTRIBUTION, BIOGEOGRAPHY AND HABITAT

The present-day distributions of the Amblyopsid species have been influenced by climatic, geological, and ecological factors throughout their

evolution. All Amblyopsids occur in eastern North America (Fig. 3). All but one species, the epigean *C. cornuta*, occur in the Interior Low Plateau or Ozark Plateau where the ancient former position of the Cretaceous Mississippi Embayment bounds their collective distributions to the south and the Pleistocene glacial advance bands the distribution to the north. In this section we discuss the distribution, phylogeographic history, and habitat preferences of the each species within the Amblyopsidae beginning with epigean swampfish and advancing our discussion toward the troglobitic forms. In the process, we discuss the several hypotheses that have been posited explaining the biogeographic patterns observed.

The swampfish is the only entirely epigean Amblyopsid and the only species of the family found in the Coastal Plain of the southeastern United States (Fig. 4). It occurs from southeastern Virginia to east-central Georgia (Cooper and Rohde 1980, Rohde *et al.* 1994). It typically occurs in heavily vegetated and shaded lowland swamps, swampy creeks, and backwater habitats that are tannin-stained and acidic (Cooper and Rohde 1980, Rohde *et al.* 1994, Ross and Rohde 2003). Water temperatures in these habitats rarely exceed 25°C (Poulson 1963) and pH ranges 5.7–6.8 (M.D. Norman in Jenkins and Burkhead 1994). Swampfish also have been collected from sites with salinities up to 5‰ in North Carolina but are not found in brackish water. Woods and Inger (1957) hypothesized that the ancestor to *C. cornuta* migrated from the Interior Low Plateau southward and around the southern Appalachians via continuous swamp habitat that fringed the location of the border of the ancient Mississippi Embayment. However, recent molecular evidence suggests an opposite route, as *C. cornuta* is the most basal member of the Amblyopsidae (Niemiller and Fitzpatrick 2008, Near *et al.*, unpublished data). A more plausible scenario would be that a *C. cornuta*-like ancestor inhabited swamps and wetlands adjacent to the karst regions of the Interior Low and Ozark Plateaus and likely the Coastal Plain during the Miocene. As the climate became more arid during this period, some populations became isolated in springs and caves eventually giving rise to the troglobitic forms, whereas other populations remained living in a swamp-like habitat.

However, the absence of *C. cornuta* from the Gulf Coastal Plain has puzzled past researchers. Woods and Inger (1957) felt that *C. cornuta* formerly inhabited this area in the past because several community associates, such as *Aphredoderus sayanus* and *Umbra pygmaea*, have distributions that range into the Gulf Coastal Plain. They speculated that *C. cornuta* might have been extirpated from the Gulf Coastal Plain when their swamp-like habitat dried up because of prolonged drought and was not able to recolonize because of poor dispersal ability. According to the scenario outlined above, we also believe *C. cornuta* or a *cornuta*-like ancestor inhabited portions of the Gulf Coastal Plain and was extirpated. Meristic data (Poulson 1960)



**Fig. 3** Distribution by county of the Amblyopsidae in the eastern United States. Only the swampfish, *C. cornuta*, is found outside the Interior Low Plateau or Ozark Plateau.

*Color image of this figure appears in the color plate section at the end of the book.*

and both allozyme data (Swofford 1982) and DNA sequence data (Near and Niemiller, unpublished data) indicate that dispersal in *C. cornuta* is limited. Swofford (1982) observed significant differentiation even among populations in the same hydrological drainage and concluded that *C. cornuta* is highly specific in its habitat requirements, and, consequently, is unable to disperse over long distances unless corridors of suitable habitat are available. Because *C. cornuta* cannot tolerate brackish water (Woods and Inger 1957), dispersal may only be achieved by headwater exchange (Swofford 1982). However, fine-scale genetic analyses are needed to test this hypothesis.

The spring cavefish occurs from south-central Tennessee northward into central and western Kentucky, then westward following the Shawnee Hills of southern Illinois and the Benton Hills west of the Mississippi River in southeastern Missouri (Fig. 3). Spring cavefish occur at the interface of epigeal and subterranean habitats bridging the threshold toward a troglobitic life. Although it occurs in caves throughout most of its range, *F. agassizii* is most abundant in springs, spring runs, and spring seeps (Smith and Welch 1978). Both habitat types are utilized, however, as fish often emerge from subterranean haunts at dusk to feed and then later retreat back underground before dawn. Fish also can be found underneath rocks in springs and spring runs during the day. In Tennessee, *F. agassizii* is common in dense vegetation associated with springs and spring-fed streams (Etnier and Starnes 1993) and have been collected in very low numbers in caves. Illinois populations in the LaRue-Pine Hills Ecological Area have been taken at springs in the swamp during winter (Smith and Welch 1978), but it is unknown whether fish actively use or successfully disperse through swampy, lentic habitats.

In Kentucky and Tennessee, spring cavefish occur in springs and caves, including the Mammoth Cave system, from the Highland Rim physiographic province of the Tennessee River drainage, middle and lower Cumberland River drainage, and the upper Barren-Green River system of Kentucky (Etnier and Starnes 1993). Spring cavefish have not been collected in Alabama, but the close proximity of a population in the Elk River drainage in Franklin Co., Tennessee (Armstrong and Williams 1971), led Boschung and Mayden (2004) to speculate that the species likely occurred in springs associated with the Elk River system in Alabama in the past. *Forbesichthys agassizii* has been collected from springs issuing from the base of limestone bluffs of the Shawnee Hills and Benton Hills at the edge of the Mississippi River alluvial plain in southwestern Illinois (Weise 1957) and southeastern Missouri (McDonald and Pflieger 1979) respectively. Until recently, the Shawnee Hills and Benton Hills populations likely were continuous and were isolated around 2,000 years ago when the Mississippi River was diverted through the narrow gap between these regions (McDonald and Pflieger 1979).



*Forbesichthys* shows a little less allozyme differentiation than *C. cornuta* with an average heterozygosity of 0.028 compared to 0.040 (Swofford 1982). This suggests that *F. agassizii* populations in Kentucky and portions of the Highland Rim in Tennessee utilize surface corridors for dispersal (Swofford 1982), particularly when compared with more differentiation in troglobitic *T. subterraneus* populations over a similar geographic area. However, molecular evidence indicates that populations of *Forbesichthys* from Illinois and Tennessee are distinct and likely isolated and suggestive that long distance dispersal is rare. Although cave populations of *F. agassizii* exist, subterranean dispersal seems unlikely, given low abundance in caves, low tolerance to starvation, relatively poor food finding ability, and short life span (see below). But surface dispersal along rivers between springs may be possible since Woods and Inger (1957) report several specimens collected from surface streams.

Of the four troglobitic Amblyopsids, three occur in the Interior Low Plateau and the other occurs in the Ozark Plateau (Fig. 3). Collectively, their distributions lie north of the boundary of the ancient Mississippi Embayment and south of the Pleistocene glacial boundary. Woods and Inger (1957) proposed that a surface ancestor entered caves sometime during the Tertiary Period and gradually dispersed into the areas where they occur today.

However, it is unlikely that subterranean dispersal alone can account for the widespread collective distribution of the troglobitic Amblyopsids, and of *T. subterraneus* in particular. Dispersal through surface watercourses is unlikely (Poulson 1960, Woods and Inger 1957) given the separation of some populations by over 1000 surface river miles (Woods and Inger 1957). Fish are very rarely observed in surface streams just downstream of springs after flood events. Although most fish likely perish, some might find their way back underground further downstream. It is much more likely that fish could move through cavernous river bottoms or solution channels (Poulson 1960), however. Although little direct evidence supports this avenue as a significant form of dispersal, indirect evidence indicates that surface rivers do not significantly impede dispersal of subterranean species. First, the thick horizontal Ordovician and Mississippi limestone formations of the Interior Low Plateau and Ozark Plateau and the groundwater systems contained therein pass underneath major surface rivers, including the Cumberland, Tennessee, Ohio, and Mississippi rivers. Populations of the troglobitic species occur on either side of these big rivers (e.g., *A. spelaea* north and south of the Ohio River; *T. subterraneus* north and south of the Tennessee River in Alabama) and, therefore, rivers do not appear to pose significant barriers to dispersal (Woods and Inger 1957). Rotenoned Southern cavefish apparently floated to the river surface



from conduits in a limestone riverbed during a fish survey at a prospective dam site (Mohr and Poulson 1966). Second, populations on opposite sides of major rivers often show little genetic variation. Populations of *T. subterraneus* in the same hydrological drainage but on opposite sides of a river are poorly genetically differentiated (Niemiller and Fitzpatrick 2008) indicating that either these populations have been recently isolated or that some migration is occurring between them.

Contemporary surface drainage divides also may not impede subterranean dispersal. Woods and Inger (1957) reported the presence of *T. subterraneus* from a spring well near the crest of the Arkansas/White River watershed divide. The spring well lies at an elevation of 1360 ft at the top of the Springfield Plateau and fish potentially can move from one watershed to the other. This is supported by the observation of very genetically different southern cavefish within the same population in the Salem Plateau (Bergstrom 1997). Although this is consistent with movement between watersheds, there are other explanations for considerable genetic diversity within a population such as lineage sorting. The recharge basins in this area are large and contiguous with few dry or faulted barriers between basins. Therefore, some individuals can potentially move between recharge basins (Noltie and Wicks 2001). However, other molecular evidence indicates that many eastern populations of *T. subterraneus* are confined to distinct hydrological drainage basins (Niemiller and Fitzpatrick 2008), as genetic structure is highly correlated with hydrological structure. Poulson (1960) argued that populations of *T. subterraneus* along the Eastern Highland Rim and western escarpment of the Cumberland Plateau were more similar than elsewhere in the range. He hypothesized that the observed homogeneity was because of occasional dispersal by cave headwater stream capture. However, both the electrophoretic data of Swofford (1982) and genetic analyses of (Niemiller and Fitzpatrick 2008, Niemiller *et al.*, unpublished data) indicate that morphological similarity of these populations is not due to homogenizing gene flow.

The southern cavefish is the most widely distributed troglobitic fish in North America. Its range is discontinuous and divided into two main components east and west of the Mississippi River: an eastern component that extends along the Cumberland Plateau and through Interior Low Plateau from central Kentucky (Mammoth Cave region) southward into central Tennessee, northern Alabama, and northwestern Georgia, and a western component that includes the Ozark Plateau of central and southeastern Missouri and northeastern Arkansas (Fig. 3). The distribution of the species was once thought to include southern Indiana and northeastern Oklahoma. These records are now thought to be erroneous. Mayden and Cross (1983) showed that all specimens of southern cavefish from northeastern Oklahoma are in fact *A. rosae*.

Similarly, records of *T. subterraneus* from Green Co., Missouri, are incorrect and actually represent *A. rosae* (Jones and Taber 1985). Therefore, *A. rosae* and *T. subterraneus* should no longer be considered sympatric (Proudlove 2006). Eigenmann (1905) described *T. wyandotte* from a well near Corydon, Indiana, in the heart of the range of *A. spelaea* and this was almost certainly a misidentification.

There do not appear to be clear differences among the habitats of Amblyopsid cavefish. Most cavefish habitats are at or near the water table (Poulson 1963) and the types of organic and inorganic substrates vary enormously within and among caves for all four troglobitic Amblyopsids (e.g., Fig. 3 in Poulson 1963). Also, water velocity among seasons and among years varies tremendously for most cavefish habitats. Nevertheless, some intriguing regional differences in geology and related size and integration of caves in the Ozarks exist for *T. subterraneus* and *A. rosae*. These differences may relate to food supply and degree of isolation (see below and Poulson, this volume). In addition, the geology of the karstic strata inhabited by *T. subterraneus*, at least in the Salem Plateau, suggests that southern cavefish reside at appreciable depth below the surface (Noltie and Wicks 2001). As mentioned above, evidence suggests that this species can move between recharge basins; however, dispersal between basins appears to be low (Swofford 1982, Niemiller and Fitzpatrick 2008, Niemiller *et al.*, unpublished data). In contrast, *A. rosae* lives in cave systems that are smaller, shallower, and much less likely to be interconnected (Noltie and Wicks 2001).

The extensive but highly disjunct geographic range of *T. subterraneus* may be the result of multiple, independent invasions and subsequent dispersal (Swofford 1982, Barr and Holsinger 1985, Holsinger 2000, Poulson, this volume). Woods and Inger (1957) hypothesized a single invasion of subterranean waters by *Typhlichthys* and a separate invasion for *Amblyopsis*. However, others have proposed a multiple-invasion scenario for *Typhlichthys* as well as the other troglobitic species. Swofford (1982) offered two possible scenarios to explain the distribution of *T. subterraneus*. First, the ancestor of *T. subterraneus* was a widely distributed epigeal species preadapted to cave life and possibly similar to *F. agassizii* today. During the Pleistocene, it originally occupied a more northern distribution but was forced southward during glacial periods then underground during the warmer and drier interglacial periods. Alternatively, the surface ancestor may have resembled *F. agassizii*, having occupied a variety of habitats from streams to springs to caves. As climatic conditions changed, surface populations were extirpated leaving only cave populations scattered over a broad area but genetically isolated from one another. In both of these scenarios, some range expansions through subterranean dispersal may occur accounting for some of the current distribution patterns that are difficult to interpret otherwise (Swofford 1982).

Swofford's alternative scenarios also can be applied to both *Amblyopsis* species. Willis and Brown (1985) hypothesized a similar vicariant scenario in which the surface ancestor of cave *Amblyopsis* inhabited swamps during the wetter periods of the Pleistocene becoming restricted to swamp-like springs and caves during drier periods. Rather than just one or two invasions, possibly each current cavefish population represents a separate invasion event. The actual number of independent subterranean invasions likely falls somewhere in between the two extremes. Recent molecular evidence also indicates that the major lineages within the *Amblyopsidae* originated well before the Pleistocene (Bergstrom 1997, Near *et al.*, unpublished data), however the glacial cycles likely had a profound effect on the current distributions and intraspecific relationships within each species. These studies also support a multi-invasion scenario and support the hypothesis that morphological similarity is the result of parallel evolution rather than significant dispersal and gene flow across major drainage and river divides.

If current taxonomy is indicative of past evolutionary history, then a plausible biogeographic hypothesis explaining the distributions of both *Amblyopsis* species is difficult to frame. Woods and Inger (1957) proposed two hypotheses to explain the distribution of *Amblyopsis*. First, the disjunct ranges of the two species could have resulted by dispersal from a more northern former distribution above the limit of the glacial ice sheets. The ancestor was subsequently split and diverged as a direct result of Pleistocene glaciations. Since no evidence has ever indicated that any *Amblyopsis* existed north of the karst regions of the Interior Low Plateau, they favored a second hypothesis that postulated an ancestral *Amblyopsis* that ranged widely through the limestone plateaus and were isolated into two groups by range expansion of the more competitive *Typhlichthys*. Bergstrom (1997) places the timing of the split between *Typhlichthys* and *Amblyopsis* around 8 mya and provides a biogeographic scenario consistent with this and subsequent splits within the respective lineages (Noltie and Wicks 2001).

Recent molecular is inconsistent with the above scenarios since *Amblyopsis*, as currently recognized, does not form a monophyletic group. "with *A. rosae* diverging much earlier." Obviously, more research is needed to elucidate the large-scale biogeographic patterns of the family. At a finer scale, considerable work has been conducted on the distribution and habitat of *A. spelaea* and *A. rosae*.

The northern cavefish follows a narrow arc of karst, from near the city of Bedford in Lawrence Co., south-central Indiana, southward to the Mammoth Cave area in central Kentucky. It has a distribution through portions of ten counties (Fig. 3). Karst landscapes start with the Mitchell

Plain in Indiana and continue as the Pennyroyal Plain in Kentucky. In the most recent assessment of the species distribution, Pearson and Boston (1995) reported 76 localities from five counties in Indiana and 38 localities from five counties in Kentucky; however, some localities no longer exist because of quarry operations and highway construction. Northern cavefish have the largest populations in caves with deep pools and moderately deep shoals. The best habitats have ledges, overhangs, and backwater areas that serve as refugia during floods. Substrate type and particle size are quite variable (Fig. 3 in Poulson 1960). The cave types flood regularly and have high food supplies of plankton and isopods (see below, Food Supplies in Caves).

The distribution of the northern cavefish is bounded by the East Fork White River to the north. Harsh periglacial conditions and alteration and impounding of major rivers north of this area may have limited the northern range of *A. spelaea* (Keith 1988). The southern extent of the species' range is the Mammoth Cave system. It is not clear why its distribution does not extend further to the south or immediately east to the sinkhole plain where the range of *T. subterraneus* starts. In Mammoth Cave itself, *T. subterraneus* and *A. spelaea* occur in different but contiguous habitats.

Mammoth Cave is one of only a few sites with syntopic species of cavefish. Typically only a single species of troglobitic fish is found within a cave system (Weber 2000) and this is the case within the Amblyopsidae. However, two instances of syntopy have been observed in the family. First, *A. spelaea*, *T. subterraneus*, and the troglophile *F. agassizii* co-occur within the Mammoth Cave system in Kentucky. In the Mammoth Cave system, *T. subterraneus* and *A. spelaea* are syntopic only where habitat is sufficiently diverse to allow habitat segregation (Poulson and White 1969, Poulson 1992, Poulson, this volume).

In the Mammoth Cave system, segregation of the three species of Amblyopsids has been hypothesized as follows. Spring cavefish are washed into the cave from sinking streams during flood events but only survive as long as a year when the subterranean food supply is well above average. Although the species can subsist on the increase in food availability, reproduction is unlikely as not enough food can be consumed to support its energetic demands and higher reproductive output compared with the troglobitic species. With regards to the two troglobitic species that inhabit the Mammoth Cave system, their distributions can possibly be explained by competitive interactions (Woods and Inger 1957, Poulson 1992). Southern cavefish are found in the master drains of vertical shafts, whereas *A. spelaea* inhabit deeper water with decreasing food availability further downstream. It remains unclear whether *A. spelaea* is precluded from upstream sections because of its larger size or by aggressive

dominance of *T. subterraneus* (Poulson 1992). Alternatively, *T. subterraneus* may be excluded from downstream habitats by decreased food supply or by competitive dominance by *A. spelaea*. The other observed syntopic interaction between two Amblyopsids involves *T. subterraneus* and *S. poulsoni* and is discussed below.

The distribution of *A. rosae* is limited to caves in the Springfield Plateau of the Ozark Highlands Province of northwestern Arkansas, southwestern Missouri, and northeastern Oklahoma. (Fig. 3) The Springfield Plateau encompasses ca. 21,000 km<sup>2</sup> that is drained by the White River to the south and east, the Neosho River to the west, and the Osage River to the north. Historically, *A. rosae* occurred in 24 caves in nine counties with unconfirmed reports increasing occurrence to 52 caves in 14 counties (Brown and Todd 1987). Willis and Brown (1985) confirmed *A. rosae* in just four caves in Arkansas, five in Missouri, and three in Oklahoma. Brown and Todd (1987) reported three additional localities in Benton Co., Arkansas. Currently, *A. rosae* is known from 47 localities (G.O. Graening, unpublished data): 11 caves in one county in Arkansas, 24 in seven counties in Missouri, and 12 in two counties Oklahoma. These localities occur in the main section of the plateau; however, no localities are known from the section of the plateau that extends southeast into Arkansas and from the central area in and around McDonald Co., Missouri (Noltie and Wicks 2001).

Ozark cavefish are confined to the karstic layer at or immediately adjacent to the surface based on the stratigraphy of the Springfield Plateau limestones and underlying shale layer (Noltie and Wicks 2001). The majority of cavefish localities are caves developed in the shallow and thin Boone and Burlington limestones and intersect the water table close to the surface (Noltie and Wicks 2001). Fish are generally observed in two types of subterranean habitats: 1) laterally-oriented cave streams that occupy conduits accessible by humans that intersect the surface near the base of Springfield Plateau escarpment either along the bluff line or of river valley walls, and 2) karst windows and wells that typically intersect conduits that are too small for humans to penetrate (Poulson 1960, USFWS 1989, Noltie and Wicks 2001). Cave streams inhabited by *A. rosae* typically are small. Karst window and well localities often occur toward the interior of the plateau and are not associated with the plateau escarpment. These are windows to human-inaccessible habitat that may be phreatic. These areas may serve as refugia, but also may provide avenues for dispersal and gene flow. Poulson (this volume and below) believes that these areas contain a very small portion of the overall cavefish population. Genetic studies are consistent with both the hypothesis of small numbers and limited dispersal since there is pronounced variation among populations (Swofford 1982, Bergstrom *et al.* 1995, Bergstrom 1997).

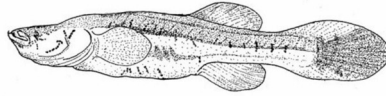
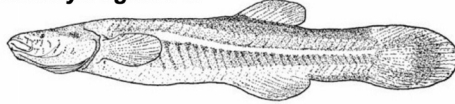
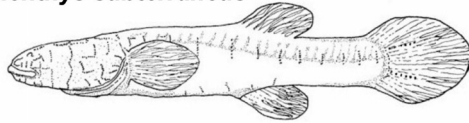
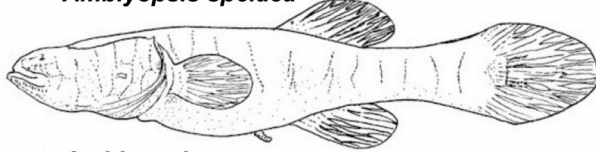
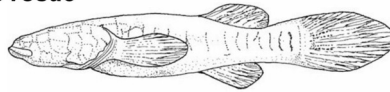
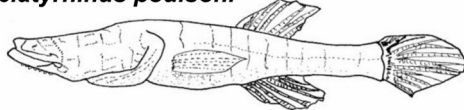
Optimum Ozark cavefish habitat occurs in caves with large colonies of gray bats, *Myotis grisescens* (Brown and Todd 1987) or comparatively large sources of allochthonous matter (USFWS 1989). Five of the 14 caves with *A. rosae* populations reported by Willis and Brown (1985) contained bat colonies and another six caves contained guano piles indicating past use. These caves had the highest food supplies based on isopod counts in leaf pack traps and copepods and cladocerans in plankton samples. Ozark cavefish occur in small cave streams where they reside in quiet pools from a few centimeters to 4 m in depth with chert rubble or silt-sand substrates (Poulson 1963).

The Alabama cavefish, *Speoplatyrhinus poulsoni*, is the most troglomorphic Amblyopsid and its single occurrence (Fig. 3) in Key Cave, Lauderdale Co., Alabama, is thought to be a relict population of a once more widely distributed species. It is known from just five pools within Key Cave, Lauderdale Co., Alabama (Kuhajda and Mayden 2001). The maze-like cave system is developed in the Mississippian-aged Tuscumba Limestone that approximately lies along the ancient Cretaceous shoreline of the Mississippi Embayment (Cooper and Kuehne 1974). The pools inhabited by *S. poulsoni* lie in a zone of seasonal oscillation of the water table where pools that form during high water become isolated during drier conditions (Trajano 2001). Many of the pools are extremely deep with depths up to 5 m (Kuhajda and Mayden 2001) and some are situated adjacent to bat roosts where guano occasionally slides into and likely enriches the aquatic environment (Cooper and Kuehne 1974). Recently, Kuhajda and Mayden (2001) reported the capture in 1995 of a single *T. subterraneus* from Key Cave. Until this observation, the two species were thought to be allopatric. Although the effect of *T. subterraneus* on *S. poulsoni* is unknown, the absence of *S. poulsoni* from adjacent caves where *T. subterraneus* have been observed suggests that competitive interactions have and might currently be influencing the distribution of *S. poulsoni*. This hypothesis has not been examined but Poulson (this volume) argues that it is unlikely. Rather he proposes demographic swamping as an alternative hypothesis.

## IV. MORPHOLOGY

### A. Family Characteristics

Members of the Amblyopsidae (Fig. 4; Table 1) are characterized by possessing (i) a large, flat head and a tubular, non-streamlined body, (ii) an oblique mouth with the lower jaw protruding beyond the upper jaw, (iii) a segmented premaxilla, (iv) jugular position of the anus and urogenital

***Chologaster cornuta******Forbesichthys agassizii******Typhlichthys subterraneus******Amblyopsis spelaea******Amblyopsis rosae******Speoplatyrhinus poulsoni***

**Fig. 4** The Amblyopsid cavefishes include an epigeal, troglomorphic, and four troglobitic species that exhibit a range of troglomorphy. The fish illustrated are drawn to scale of average-sized adults. Modified from drawing by John Ellis in Romero (2004).

pore, (v) reduced head lateral line canals and absence of the trunk lateral line canal but the presence of superficial papillae (neuromasts) arranged in distinct rows on the head and body, and papillae of unknown function in 2–4 rows on the caudal fin, (vi) small, embedded cycloid scales except on the head, (vii) six branchiostegal rays, (viii) presence of a swim bladder, (ix) tubular anterior nostrils, (x) absence of pelvic fins (except rudimentary in *A. spelaea*). Troglobitic species also can be characterized by (i) lack of externally visible eyes, (ii) reduced pigmentation, (iii) hypertrophy of the superficial lateral line system that includes an extensive system of elevated neuromasts arranged in distinct ridges, (iv) hypertrophy of the semicircular canals and otoliths, and (v) presence of highly-developed

**Table 1** Meristic characters for species in the Amblyopsidae.

<i>Species</i>	<i>Dorsal fin rays</i>	<i>Anal fin rays</i>	<i>Pectoral fin rays</i>	<i>Caudal fin rays</i>	<i>Caudal fins</i>	<i>Pelvic fin rays</i>	<i>Vertebrae</i>	<i>Post-cleithrum</i>	<i>Penultimate vertebrae bend</i>
<i>Chologaster cornuta</i>	11 (9–12)	9 (9–10)	10 (10–11)	10 (9–11)	branched	absent	27–29	present	straight
<i>Forbesichthys agassizii</i>	10 (9–11)	10 (9–11)	9–10 (9–11)	14–15 (11–17)	branched	absent	33–35	present	upwards slightly
<i>Typhlichthys subterraneus</i>	8–9 (7–10)	8–9 (7–10)	10–11 (9–12)	12–13 (10–15)	branched	absent	28–29	present	upwards slightly
<i>Amblyopsis spelaea</i>	10 (9–11)	9–10 (8–11)	10 (9–11)	12–13 (11–13)	branched	4 (0–6) <sup>1</sup>	29–30	absent	upwards strongly
<i>Amblyopsis rosae</i>	7–8 (7–9)	8 (8–9)	10 (10–12)	10 (9–11)	branched	absent	28	absent	straight
<i>Speoplatyrhinus poulsoni</i>	9 (9–10)	8 (8–9)	9 (9–11)	22 (21–22)	not branched	absent	27–28	absent	

<sup>1</sup>Frequency distribution of pelvic fin rays in *A. spelaea*:

0 rays – 1, 1 ray – 1, 2 rays – 2, 3 rays – 18, 4 rays – 58, 5 rays – 7, 6 rays – 1



caudal sensory papillae. Amblyopsids are also characterized by having dorsal and anal fins that are similar in shape with the dorsal fin origin anterior to the anal fin origin. All fins lack spines (although the first ray in the dorsal fin of *T. subterraneus* has been reported as spinuous). Dorsal fin rays range 7–12, anal fins 7–11 rays, and pectoral fins 9–12 rays. Pelvic fins are absent except in *A. spelaea* that have 0–6 rays. The caudal fin may be elliptical, lanceolate, or rounded with 9–22 branched rays. No fin rays are branched in *S. poulsoni*. The urogenital pore is positioned just anterior to the anal fin at hatching and migrates anteriorly until it occupies a jugular position in adults (Woods and Inger 1957).

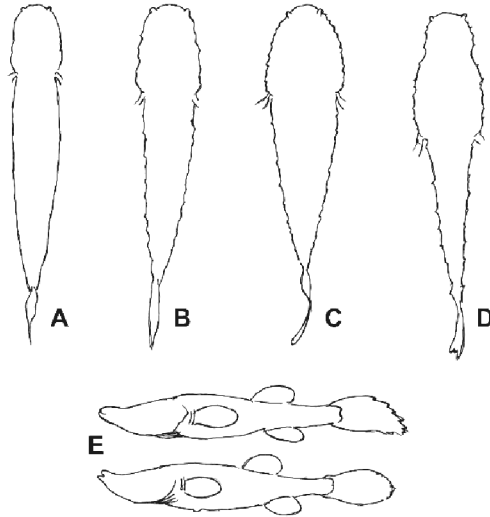
## B. Sexual Dimorphism

Most Amblyopsids cannot be sexed using external morphology. Spring cavefish cannot be sexed using external morphology, although Weise (1957) noted that in the spring almost all adult individuals could be sexed by observing the gonads through the translucent body wall. This is rarely the case for cave Amblyopsids that seem to have low clutch sizes (see Fecundity).

Male swampfish can be distinguished from females by the presence of a Y-shaped appendage that develops on the snout. This appendage is believed to be a contact-stimulatory organ implicated in courtship behavior (Poulson 1960) and pheromone chemoreception (Jenkins and Burkhead 1994), although its function is unknown.

## C. Neoteny

Poulson believes that there is good evidence for neoteny in the Amblyopsidae. He predicted the characteristics of his namesake based on increasing neoteny from *Typhlichthys* to *Amblyopsis*. Absence of bifurcate fin rays in adult *S. poulsoni* is the most extreme neotenic trait for troglobitic Amblyopsids (Cooper and Kuehne 1974, Weber 2000) as this condition is also found in 15–17 mm SL *T. subterraneus*. Moreover, adult *S. poulsoni* and 15–20 mm *T. subterraneus* and *A. spelaea* are similar in head shape and body proportions (Fig. 5 and Poulson in Culver 1982). Some of the specialized morphological and behavioral traits in troglobitic Amblyopsids, such as longer fins, larger heads, and increased exposure of neuromast organs, may be explained by neoteny which is one kind of heterochrony (Cooper and Kuehne 1974, Trajano 2001).



**Fig. 5** Scale drawings (Poulson and Cooper) of the body shapes and relative head sizes of Amblyopsid fishes. With increasing cave adaptation heads become relatively larger and bodies smaller. Dorsal views from left to right are *F. agassizii*, *T. subterraneus*, *A. spelaea*, and *S. poulsoni*. That these are neotenic trends is suggested by the side views of a 48 mm SL *Speoplatyrhinus* and a 13 mm SL *Amblyopsis*. Can you tell which is which?

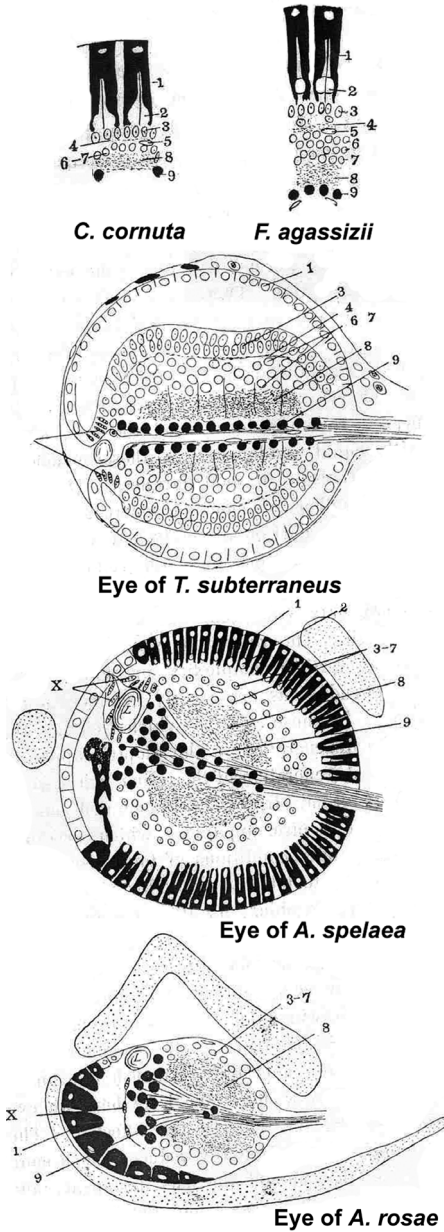
## D. Eyes

Carl Eigenmann's classic study (1897) of *The Eyes of the Amblyopsidae* remains the best ever. It is a paradigm for the comparative approach to understanding a process; degeneration of eyes both phyletically, among the five species known at the time, and ontogenetically, across almost the known body lengths of each species. Eigenmann's discussions of mechanisms also are a paradigm for scientific study. He used evidence to falsify some but not reject others of six alternative hypotheses (see Poulson's this volume).

Eigenmann's conclusions (1897, p. 587 and Summary Points 1–23), based on the best-developed eyes for each species, are that phyletically there is increasing degeneration and simplification of eyes from *Chologaster* to *Typhlichthys* to *Amblyopsis spelaea* to *Troglichthys* (= *Amblyopsis*) *rosae*. Of the parts of fish eyes missing or indistinguishable histologically (Table 2) the numbers are *C. cornuta* 0, *F. agassizii* 1, *Typhlichthys* (Kentucky) 7, *A. spelaea* (Indiana) 11, and *A. rosae* (Missouri) 12. The maximum eye sizes in micra are 1100 for *C. cornuta*, 760–930 for *F. agassizii*, 180 for *T. subterraneus*, 200 for *A. spelaea*, and 85 for *A. rosae*. Figure 6 shows these differences in parts and in eye size.

**Table 2** Summary of parts of the eye of members of the Amblyopsidae. The eyes of cave Amblyopsids are reduced in different ways but overall the number of parts absent, reduced/vestigial/undefined/merged has increased from *T. subterraneus* to *A. spelaea* to *A. rosae*. This pattern is consistent with the hypothesis that rudimentation of eyes is an index of evolutionary time isolated in caves. From Eigenmann 1897. The eye of *S. poulsoni* has not been histologically examined.

Species	<i>C. cornuta</i>	<i>F. agassizii</i>	<i>T. subterraneus</i>	<i>A. spelaea</i>	<i>A. rosae</i>
Habitat	Swamp	Spring	Cave	Cave	Cave
Retina					
1. Pigment epithelium (PE)	Defined	Defined	Defined	Defined	Imperfect
Pigment in PE	Present	Present	Absent	Present	Reduced
2. Rods and cones	Defined	Defined	Undefined/Absent	Undefined	Cones absent
3. Outer nuclear layer	1 layer of cells	3 layers of cells	Undefined	Merged	Undefined/Absent
4. Outer plexiform layer	Defined	Defined	Defined	Undefined	Absent
5. Horizontal cells	Defined	Defined	Undefined	Undefined	Undefined
6. Inner nuclear layer	1 layer	3 layers	Defined	Merged	Undefined
8. Inner plexiform layer	Defined	Defined	Defined	Defined	Defined
9. Ganglion layer	Defined	Defined	Defined	Funnel-shaped	Reduced
Optic nerve connection to brain	Yes	Yes	Yes No	No	No
Focusing					
Lens	Present	Present	Vestigial/Absent	Vestigial/Absent	Vestigial/Absent
Ciliary muscles	Present	Absent	Absent	Absent	Absent
Eye muscles	Normal	Normal	Absent	Reduced	Reduced/Absent
Nutritive					
Vitreous body	Present	Present	Vestigial	Vestigial	Vestigial
Hyaloid membrane	Present	Present	Present	Absent	Absent
Scleral cartilages	Absent	Absent	Absent	Present	Present
Pupil	Open	Open	Open	Closed	Closed
Maximum eye diameter (mm)	1.10	0.76 – 0.93	0.18	0.2	0.09
Eye parts absent	0	1	3	3	7
Eye parts vestigial	0	0	4	8	5
Variability within and between individuals	none	none	some	great	great



**Fig. 6** Scale drawings (from Eigenmann 1897) of the vestigial eyes of cave Amblyopsids with the retinas of *Chologaster* to the same scale (thus complete eyes would be ~10x as large). The numbers of the retinal layers are identified on [Table 2](#) of eye parts. "X" is the degenerate lens or its parts. Not shown are the vestigial eye muscles of *Amblyopsis spelaea*.

Eigenmann (1897) stressed the fact that eyes of each species “degenerate along different lines”. For example, *Typhlichthys* eyes are the least degenerate overall for a cave Amblyopsid but lack eye muscles, scleral cartilages, and pigment epithelium. On the other hand, the overall more degenerate eyes of *A. spelaea* have some eye muscles, clear scleral cartilages, and well developed pigment epithelium. Nonetheless, in general, Eigenmann stated that “the eye of *Amblyopsis spelaea* represents one of the stages through which the eye of *Troglichthys* passed (phyletically)”.

Ontogenetically each species’ eyes develop to a maximum state of tissue differentiation and then become more simplified and degenerate with advancing age (Eigenmann 1897). In addition, older fish of the same size have more variable eye development and there is more right to left side variation of eyes in the same individual. In *A. spelaea*, for which the largest range of sizes was studied (6 to 130 mm), Eigenmann found that the degeneration started in the earliest stages of eye development and that the eyes had degenerated completely in the largest fish with one eye even being absent in a 130 mm individual. These patterns are consistent with that for cave *Astyanax fasciatus* with increased variability in the largest individuals among individuals and in right to left side asymmetry within individuals.

Poulson (1963, Table 2 in 1985) reported on the sizes and allometric growth constants ( $b$ ) of eyes and brain optic lobes for five species and optic lobe volume in all six species including *S. poulsoni*. The values reported here are for the populations of each species with the greatest available size range since accuracy of allometric constant depends on having a full size range; the size values are for 45 mm SL fish on the fitted line of part size vs. body length. The size ranges in mm SL and localities are as follows: *C. cornuta*, Roquist Creek, North Carolina, 7–50; *F. agassizii* Rich Pond, Kentucky, 15–55; *T. subterraneus*, Shelta Cave, Alabama, 17–65; *A. spelaea*, Letch Cave, Kentucky, 8–90; and *A. rosae*, Cave Springs Cave, Arkansas, 18–55. There are differences among populations related to both locality and growth rate that are discussed elsewhere (Poulson 1960 and this volume).

As for eyes and optic lobe length, there is a decline in optic lobe volume in cubic mm from 2.12 in *C. cornuta* to 1.53 in *F. agassizii* to similar values for the first three troglobites (1.01, 1.37, and 0.96) to the minimum value of 0.38 for *Speoplatyrhinus*. The values for allometric growth constant of optic lobe length are ~0.80 and ~0.60 for the nontroglobitic species to ~0.40 for *Typhlichthys* to ~0.60 for *A. spelaea* to 0.20 for *A. rosae*. There was too small a range in body size of *S. poulsoni* specimens to estimate an allometric constant but it appears to be the lowest of all.

There is a trend of increasing and then decreasing variance of eye and optic lobe size from epigeal to troglomorphic to the most troglolitic Amblyopsids (Fig. 13 in Poulson 1960, Table XIV in Poulson 1963). Along with this pattern there is a decrease in size and allometric constant for eyes and optic lobe length. In 1960, Poulson interpreted these trends in the context of a balance between natural selection and mutations of rudimentation. Selection for maintenance of vision or against mutations of rudimentation is seen in both non-troglolitic species since size and allometric constants are high but with distinctly greater variability in *F. agassizii*. *Typhlichthys* eyes are about 80 percent smaller with much less positive allometry and still greater variance. Compared to *F. agassizii*, its optic lobe is about 30 percent smaller, variance is the same and allometry is a little less positive. In *A. spelaea*, eyes and optic lobes and their allometric constants are slightly larger than for *Typhlichthys* despite a more degenerate eye and an increased variability of eye size in adults. Poulson's interpretation for *Typhlichthys* and *A. spelaea* was, and still is, of mutations of rudimentation predominately influencing the patterns since eyes had already been reduced so much. In *A. rosae*, eyes and optic lobes and their allometric constants are all lower than for *A. spelaea* but variance for both is as low as for *C. cornuta*! This decreased variability is clearly related to a longer evolutionary time in caves but its mechanistic explanation is elusive. One hypothesis for decrease in visual system variability is that the visual system is already about as reduced as possible (Fig. 2 in Poulson 1985). But *Speoplatyrhinus* has even more reduced optic lobes!

It may be relevant to the trends in visual system variability that the average heterozygosities for 14 allozyme loci also decline with increasing troglomorphy (Swofford 1982). Poulson (1985) hypothesized that "selection for some alleles and chance fixation of the most common alleles gave rise to increasing uniformity." Swofford (pers. comm.) believes that we cannot differentiate between stochastic effects and selection with his data.

## E. Pigmentation

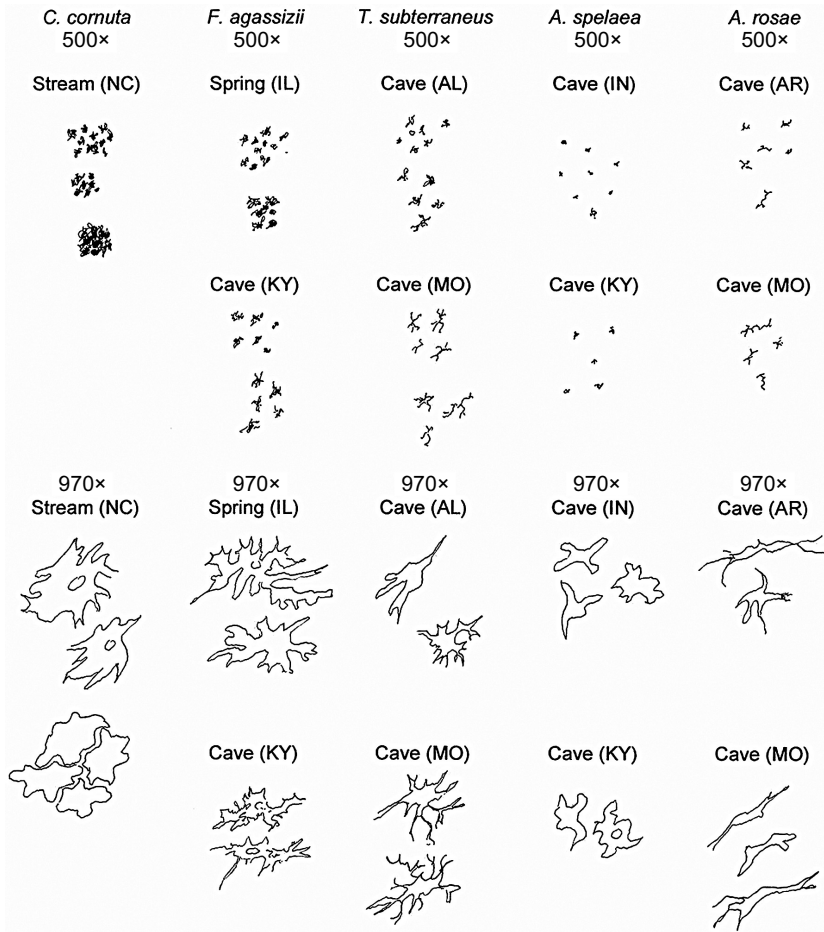
Other than the rudimentation of eyes, reduction of pigment cells and pigment are the most commonly observed reductive troglomorphies in vertebrate troglolites (Langecker 2000). Reduction of melanin can occur at many steps with many controlled by single genes (Jeffery, this volume). Size and number of pigmented melanophores is controlled by polygenic systems in an additive manner (Wilkens 1988). In many cases, the genetic bases for reduction are not the same in different subterranean fish species. In the Mexican cavefish, albinism in different populations is caused by independent mutations in a single gene, *Oca2* (Protas *et al.* 2006). Mutations

that affect the polygenic system result in continuous variation towards complete depigmentation at slower rates than mutations that result in the loss of the ability to synthesize melanin (Trajano 2007). Accordingly, it has been argued that the loss of pigmentation as a proxy for relative age of subterranean inhabitation should be restricted to related species that have retained melanin where the extent of depigmentation is caused by mutations in the polygenic system (Trajano 2007).

Aspects of the developmental, physiological, and genetic mechanisms of pigment rudimentation have not been investigated in the Amblyopsidae. Only Poulson (Table 15 in 1960, 1963, Table 2 in 1985, and new data herein) has quantified the melanophore sizes and numbers and dispersion of melanin granules within melanophores in the Amblyopsidae. As with eyes, there is a decline for all of these measures in numbers (densities), allometry for number, and size through the epigeal, troglomorphic, and troglobitic species. All of the data are consistent with increasing durations of evolutionary time and increases in elaborated traits from *Typhlichthys* to *A. spelaea* to *A. rosae* to *S. poulsoni*.

The original observations (Poulson 1960, 1985) were made with a dissecting microscope and the new data are on excised skin wet mounts examined with a compound microscope at 500 $\times$  and 970 $\times$  (oil immersion). At 500 $\times$ , 5–8 melanophores were scored for shape and melanin particle dispersion in each individual. Dispersion scores are as used in zebra fish from 1 punctate (maximum aggregation of melanosomes) to 2 amoeboid to 3 stellate to 4 stellate-reticulate to 5 reticulate (maximum dispersion of melanosomes). At 970 $\times$ , for each individual 2–3 melanophores were scored for intensity of melanin deposition in melanosomes, the integrity of limiting membranes, presence of a nucleus, and details of the protoplasmic projections (dendrites or filaments).

With the exception of several individuals in one population of *A. rosae* (Roy Pierson Cave, Missouri) and one population of *Typhlichthys* (River Cave, Missouri) with no pigmented melanophores visible with a dissecting microscope (Fig. 7; Table 3), the melanophores in all species appear to have fully developed melanin granules (melanosomes) with dense deposition of melanin (electron microscope examination may reveal differences in the ontogeny of melanophores and melanosomes among the cave species). There is a trend, with increasing troglomorphy among the species, for the troglobites to have an increasing proportion of melanophores in the superficial muscles, body cavity mesenteries, and brain meninges but this has not been quantified. The other trend common to all three troglobites is for expanded melanophores (with dispersed melanin granules) to be less symmetric/stellate and more irregularly elongate. They have more projections having open ends, and they have less distinct limiting membranes overall (Fig. 7).



**Fig. 7** Drawings of pigmented melanophores. For each of 1–2 localities for each species of Amblyopsid, extremes of melanophore size (microns) and shape are shown for three fish examined at 500×. Also, the range of branching patterns are shown from examination at 970× (oil immersion). See text for methods and interpretations.

*Chologaster cornuta* possesses the most melanophores that are also the largest. *Chologaster cornuta* is an attractive fish with a striking color pattern due partly to three kinds of chromatophores (Figs. 1A and 7). Despite being nocturnal, the pattern is a combination of disruptive and counter-shading camouflage with the swampfish's habit of resting during the day among dense vegetation with dappled sunlight. In live fish (but not in preserved fish) there appear to be three kinds of chromatophores: xanthophores and,



**Table 3** Pigmented melanophores (45mm SL fish). See Fig. 7 for appearance of melanophores at 500× and 970×. See text for methods and detailed results. The decline in density and size of melanophores is consistent with the hypothesis that the amount of rudimentation is an index of evolutionary time isolated in caves.

Trait	<i>C. cornuta</i>	<i>F. agassizii (spring)</i>	<i>F. agassizii (cave)</i>	<i>T. subterraneus</i>	<i>A. spelaea</i>	<i>A. rosae</i>	<i>S. poulsoni</i>
Number on cheek	<=60	35	25	0	0	0	0
<i>b</i>	0.71	0.42	-0.40	-	-	-	-
Number on body	>103	> 100	~ 50	12	6	0.7	0.6
<i>b</i>	+?	+?	+?	1.5	-1.1	-2.4	-?
Micra <sup>2</sup>	100	83	50	21	16	15	10?
Micron length							
Mean	32	65	55	42	31	35	20?
Range	15-50	20-150	15-100	33-200	21-45	8-200	na
Dispersion							
Score (1-5)	5	3-5	4-5	1-5	2-3	1-5	4-5

perhaps, two types of melanophores. Swampfish are chocolate brown dorsally with a sharp border grading to orange-yellowish ventrally and is reddish on the opercles (xanthophores). Two to three stripes are present laterally with one at the brown to orange-yellowish border. Only the melanophores are easily quantified in this species. With full dispersion of melanin (score of 5), the cheek melanophores average 0.10 mm in diameter with up to  $\geq 60$  in a reference area of  $0.175 \text{ mm}^2$ . The allometric constant of increase in numbers with standard length is 0.71 for a population that grows up to 50 mm SL. Melanophores on the body are too numerous to count and determine dispersion pattern accurately. Subjectively, however, these melanophores all have fully dispersed melanin with a score of 5.

Swampfish body melanophores are dense, dark, and either packed like paving stones or overlapping, especially along the three black stripes. They range in estimated maximum dimension from 15 to 65 microns with most 40–50. It remains to be seen how dispersion would change under different light and background conditions, but the disruptive color pattern and counter-shading suggests that adapting to light levels and backgrounds may not be necessary.

*Forbesichthys agassizii* is dull brown dorsally to slightly lighter ventrally (Fig. 1B) with only one kind of chromatophore-melanophores. Fish have poorly defined stripes, one mid-dorsal and two lateral, and slightly more melanophores along the edges of the myomeres than elsewhere. The range in size of melanophores in micra among individuals and populations is 20–150. All melanophores have many projections (10–21 per cell with most blunt-ended and few thinning at the ends) with melanin granule dispersion of  $3 < 4 < 5$ . The melanophores on the cheek of the Pine Bluffs fish (spring-cave N = 9) increase from 35 to 45 per  $.175 \text{ mm}^2$  with growth and an allometric constant of 0.42 while for Mammoth Cave spring cavefish (in cave N = 6), cell numbers decline from 25 to 15 per  $.175 \text{ mm}^2$  with an allometric constant of  $-0.60$ .

These data suggest that light intensity will affect number, density and perhaps range of dispersion within a fish ontogenetically. In the spring-cave populations, melanophores have a score of 3–5 for range of dispersion from reticulate to fully dispersed melanin granules. Poulson does not have comparable data for the Mammoth Cave fish but the pale color suggests that the range would include punctate (score of 1). To extend this argument, Poulson expects to see that spring-cave populations will increase melanophore melanin dispersion and density of melanophores if kept in the light.

Live *T. subterraneus* from all localities appear white to pearly opalescent (some guanine?) and in high-resolution photographs outlines of the tiny embedded scales are visible as slightly grayer color (Fig. 1C). Kentucky

populations have visible blobs of fat around the vestigial eyes. Compared to *Chologaster*, all *Typhlichthys* populations (three from east of the Mississippi River and two from west of the Mississippi River sample sizes 4–13) have comparable to somewhat higher densities of melanophores but much smaller surface areas of each pigmented melanophore. Unlike in *Chologaster*, it is rare to see melanophores contiguous and there is only one individual where some melanophores overlapped. In preserved specimens, the visible melanophores are concentrated along the dorsal myomere borders and scattered elsewhere on the body. Melanophores are not visible on the cheek and the number in the reference  $0.175 \text{ mm}^2$  pectoral area increase from 2 to 12 with growth with an allometric constant of 1.50. Melanin dispersion in these melanophores ranges from mostly stellate (score of 3) to reticulate (score of  $4 < 5$ ). Their longest axis (6–130 micra) is much greater than the narrowest (3–20 micra). There are some pigmented melanophores on the surface of muscle under the dermis and relatively more in the internal connective tissues.

For the populations with the greatest size range and sample sizes, Shelta Cave, Alabama (N = 13, 13–60 mm SL) and The Gulf, Missouri (N = 13, 21–30 mm SL), there are interesting differences (Fig. 7), which Poulson hypothesizes are related to the open, partially sunlit, and very deep sinkhole of The Gulf (the same differences are seen with the Shelta fish 21–32 mm SL as a control for size). Of the Gulf fish melanophores, the dispersion scores are one (0), two (0), three (7), four (19) and five (16); whereas for Shelta fish, the scores are one (20), two (9), three (9), four (4), and five (5). The Shelta fish are not only more variable between individuals (e.g., one with no pigmented melanophores, one with all score one and one with all score 5) but also within individuals (e.g. one with all 1, one 1-3, and one 3-5). Poulson suggests that the greater uniformity and darker melanophores in The Gulf are due to the low to medium light levels. This is consistent with substantial increases in pigmentation seen by several workers in *Typhlichthys* kept in the light in the laboratory (e.g., Woods and Inger 1957).

Compared to *Typhlichthys*, *A. spelaea* melanophores are much reduced in numbers, sizes, and ranges of melanin granule dispersion. The data are mean melanophore area in  $\text{mm}^2 \times 10^{-3}$  from *T. subterraneus* to *A. spelaea* (0.21 to 0.06), range of longest dimension in micra of melanophores (8–135 with a median of 75 to 23–65 with a median of 36), number per  $0.175 \text{ mm}^2$  reference area (90 to 6), and range of dispersion (1–5 with a mean of 4.4 to 2–3 with a mean of 2.4). In addition, for the same dispersion scores of 3, *A. spelaea* melanophores have about half as many projections (4.0) as *T. subterraneus* (7.0). And unlike for *Typhlichthys*, neither number nor darkness of melanophores increases in the light (Eigenmann 1897,

Poulson, personal observations). In fact, the allometric constant for number of melanophores is negative (-1.10) and some larger fish have no melanophores (vs. 1.50 for *Typhlichthys*). All of these data are consistent with increasing accumulation of rudimentation mutations at the *A. spelaea* grade of evolutionary troglomorphy.

Compared to *A. spelaea*, *A. rosae* has a somewhat different pattern of pigment loss but overall, in contrast to a much greater rudimentation of eyes, shows only somewhat more rudimentation of pigmented melanophores. Mean melanophore area in  $\text{mm}^2 \times 10^{-3}$  is not as reduced in *A. rosae* compared to *A. spelaea* (0.15 to 0.06) the range of longest dimension in micra of melanophores is greater (12–200 with a median of 42 to 23–65 with a median of 36), and the range of melanin granule dispersion is greater (1–5 with a mean 4.1 to 2–3 with a mean of 3.4). However, melanophore numbers per  $0.175 \text{ mm}^2$  reference area is less in *A. rosae* ( $0.7 < 6.0$ ). There was no difference in number of melanophore projections for the same dispersion ( $5.3 \sim 4.0$ ). As for *A. spelaea*, *A. rosae* does not become darker in the light and the negative allometric constant is still more negative (-2.37) with some large individuals with no melanophores.

## F. Brain Pattern as a Clue to Sensory Systems

In teleost fish, many brain sensory input areas are visible as separate parts so sensory abilities can be inferred from brain pattern (Evans 1940). All the following comparisons are for 45 mm SL Amblyopsid fish from double log plots of size of part versus standard length.

### ***Vision and Reactions to Light***

Even the surface-living, nocturnal Amblyopsid with the largest eyes, *C. cornuta*, has very small eyes and optic lobes compared to the diurnal *Fundulus notatus* topminnow (Figure 11 in Poulson 1960). Both estimated eye and optic lobe volumes for *F. notatus* are  $\sim 12\times$  that for *C. cornuta*.

Both *C. cornuta* (Poulson, personal observations) and *F. agassizii* (Weise 1957, Poulson, personal observations) hide in vegetation or under objects in even low levels of ambient light. Even blinded *F. agassizii* show no change in swimming patterns or food-finding ability (Payne in Eigenmann 1909). Spring cavefish certainly uses their eyes to detect light intensity since the species exits caves into springs after dark and retreats back underground as dawn approaches (Weise 1957, Hill 1966; Poulson, personal observation). *Amblyopsis spelaea* show very weakly developed negative photokinesis even when vestigial eyes of 15–25 mm TL fish are removed (Payne 1907).

Comparative data on Amblyopsid visual systems can be found in Table 2 and Fig. 6). Maximum eye diameters in mm are greatest in the non-troglobitic species and decrease in the troglobitic species. No data are available for *S. poulsoni* but much smaller eyes are expected based on very small optic lobe length. Optic lobe volumes in mm<sup>3</sup> also are greatest in the nontroglobitic species and smallest in the most troglomorphic species, *S. poulsoni*. If we could measure optic tectum volume, Poulson expects that lamellar organization of visual fish will be absent in all the troglobites even if some optic tracts have been taken over by other sensory systems. Even further optic tectum rudimentation is predicted with increasing evolutionary time in caves. See the details of eye structure rudimentation in the earlier section on eyes.

Response to light has been investigated for several Amblyopsid species. Poulson (1963) noted both *C. cornuta* and *F. agassizii* are negatively phototactic, i.e., directed movement away from light. Among the other Amblyopsid species, *A. spelaea* is reported to be negatively photokinetic, i.e., lower activity in the dark resulting in more time spent in the dark end of a gradient (Payne 1907, Eigenmann 1909, Green and Romero 1997), whereas *T. subterraneus* is indifferent to light (Eigenmann 1909, Verrier 1929, Green and Romero 1997). Photokinetic response may be linked to functioning of the pineal organ in Amblyopsids (Green and Romero 1997). The pineal organ is poorly developed and functionally ineffective in *T. subterraneus* (McNulty 1978b), which is indifferent to light. Although the pineal organ of *A. spelaea* has not been examined, Green and Romero (1997) predict that the organ is photosensitively functional, although role of extraocular, extrapineal photoreception cannot be readily dismissed. Photokinetic responses have not been investigated for *S. poulsoni*. Likewise, variation among populations within species has not been examined.

Poulson believes that the weak and varying photokinetic responses to low light intensities among troglobitic Amblyopsids (e.g., Payne 1907, Poulson, unpublished data) have no ecological or evolutionary significance. Part of his reasoning is that *A. rosae* and *T. subterraneus* change from negatively to positively or neutrally photokinetic with increase in size. This could be because of decrease in transparency of the head tissues above the brain. Nerves and the brain of many organisms respond electrically to bright light. Payne removed eyes of small *A. spelaea* and found no change in their weak negative photokinesis. Furthermore, it is extremely rare that any cave Amblyopsid is exposed to a light gradient where water flows into or out of a cave. In one such case (the caves of Spring Mill State Park where Eigenmann and Payne worked) *A. spelaea* avoids the silty areas in a 150 m stretch before the stream exits a cave but can be seen swimming in the twilight zone of rocky areas at the entrance to the next cave 50 m

downstream across a karst window (Payne 1907, Poulson, unpublished data). In another case (The Gulf in Missouri), *T. subterraneus* preferentially forages in the light areas along the sides of a steep-sided and deep karst window, probably because of higher food densities.

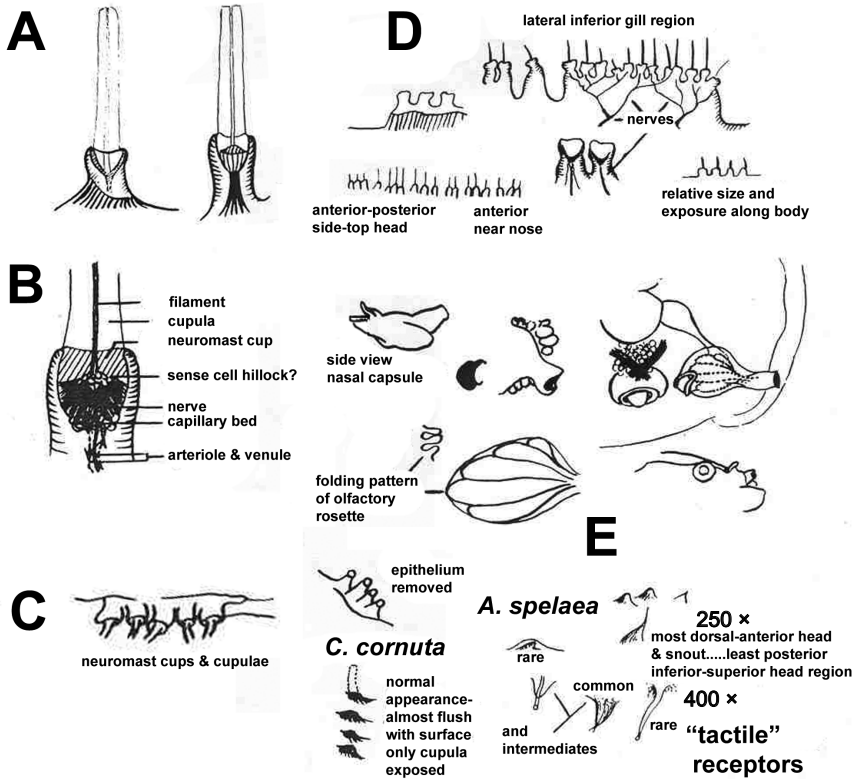
### **Taste**

The sense of taste (contact chemoreception) does not seem to be important for Amblyopsids. Anatomically the sense organs on the head do not look like taste buds and neither the skin brain centers (a median 'facial lobe' of the medulla) nor mouth brain center (paired 'vagal lobes' of the medulla) can even be identified visually. Smith and Welch (1978) noted that spring cavefish rely on gustation rather than visual or olfactory cues to determine prey edibility. *Forbesichthys* that ingest and then spit out inedible items that researchers wiggle near them may be using some mouth taste, but there is sometimes inedible organic matter or bat guano as a small component of gut contents in troglobitic Amblyopsids. This suggests that they are not as good as human babies that can selectively sort and spit out peas from a mush of potatoes or oatmeal (Poulson, personal observations).

### **Olfaction**

The sense of smell (distance chemoreception) is inferred to be better developed in all Amblyopsids than in *Fundulus notatus* based both on external olfactory rosette area and internal brain olfactory lobe length (Table 4, Fig. 8, Fig. 11 in Poulson 1960, Fig. 4 in Poulson 1963). All Amblyopsids have a tubular intake and pore output for water that somehow is drawn across the olfactory rosette. Since every rosette surface cell has a cilium (Claude Baker, personal communication) Poulson presumes that the surface area of olfactory rosettes is a good index of olfactory capacity. Among Amblyopsid species, there is no discernable change with increased troglomorphy in olfactory lobe size, no pattern for number of lobes of the olfactory rosette, and only modest increase in estimated olfactory rosette area (Table 4, Fig. 8, Fig. 11 in Poulson 1960, Fig. 4 in Poulson 1963, Table 2 in Poulson 1985). This lack of a trend suggests to Poulson that cave Amblyopsids do not exhibit much enhanced chemoreceptive abilities.

Fernandus Payne (in Eigenmann 1909), Poulson (1960), and Hill (1966) have done simple behavioral feeding experiments and neither *F. agassizii* nor *A. spelaea* react positively to smells of live or dead prey that are the main food items in their guts and in their environments. However, both the troglophile and troglobite react to and even seize moving cotton balls, sticks or wires. This is not surprising with the overwhelming importance of lateral line neuromast and tactile senses (see below).



**Fig. 8** Drawings by Poulson of sensory system anatomy of Amblyopsid fishes. Shown are “tactile” receptors of *Amblyopsis spelaea*, the olfactory apparatus of *Forbesichthys agassizii*, and the difference in exposure of neuromasts in *Forbesichthys agassizii* and *Chologaster cornuta*.

If not used in feeding, what is the function of olfaction in Amblyopsids? At present this is an unsolved mystery. Experiments on neurophysiological responses to serial dilutions of amino acids have not been conducted but lack of behavioral response to live but immobile prey only millimeters away suggest that olfactory detection of prey is not important. Aumiller and Noltie (2003) provided evidence that *T. subterraneus* detects and is attracted to exudates of both conspecifics and prey (amphipods) in a laboratory setting but the study did not differentiate between olfaction and gustation, nor is there any behavioral responses to an injured fish that suggests the presence of alarm odors (i.e., shreckstoff). Likewise, sex-related variation in responses has not been addressed. However, Aumiller and Noltie suggest that cavefish may rely on chemoreception when locating conspecifics during the reproductive season or detecting

**Table 4** Sensory systems of Amblyopsid fishes listed in order of increased (+++) to no (0) evolutionary elaboration with life in caves. Based on Poulson (1960, 1963, 1985, unpublished data) and Cooper (unpublished data). Standardized to 45 mm SL. Where there is a trend, species are given rank from least elaborated (1) to most elaborated (6). Cave species are listed in order of increasing time of isolation in caves (1–4) based on eye and pigment rudimentation.

<i>Species</i>	<i>C. cornuta</i>	<i>F. agassizii</i>	<i>T. subterraneus</i>	<i>A. spelaea</i>	<i>A. rosae</i>	<i>S. poulsoni</i>
<b>Habitat</b>	Swamp	Spring	Cave1	Cave2	Cave3	Cave4
<b>H<sub>2</sub>O Movement (Lateral Line)</b>						
Lateral line neuromast num. in rows 2–4	21.6 (1)	28.5 (2)	45.6 (6)	37 (4)	32.5 (3)	41.9 (5)
Head surface area (mm <sup>2</sup> )	443 (2)	329 (1)	666 (3)	615 (3)	683 (3)	666 (3)
Height of cupula + neuromast (mm)	0.3 (1)	0.66 (2)	0.84 (3)	1.14 (6)	0.8 (3)	0.96 (5)
Forebrain 'computer' volume (mm <sup>3</sup> )	0.5 (1)	0.9 (2)	1.15 (3)	1.3 (5)	1.25 (5)	1.15 (3)
<b>Equilibrium (Otoliths &amp; Semicircular Canals)</b>						
Sagittus length (mm)	0.69 (1)	0.96 (2)	2.1 (4)	2.05 (4)	2.07 (4)	1.3 (3)
SS Canal complex (mm)	1.65 (1)	2.15 (2)	4.1 (5)	4 (5)	3.48 (4)	3.15 (3)
Cerebellum 'computer' volume (mm <sup>3</sup> )	0.67 (1)	0.7 (1)	1.04 (4)	1.48 (5)	1.49 (5)	0.9 (2)
<b>Caudal Papillae (Unknown Function)</b>						
Total number	24? (1)	36? (2)	89 (3)	96 (5)	111 (6)	88 (3)
Relative size	1	1	1.5	1.5	1.7	3.2
<b>Tactile Receptors</b>						
Number on head (0.175 mm <sup>2</sup> )	8 (1)	14 (2)	21 (4)	16 (2)	18 (4)	0 (0)
Relative size	1	1.5	1.5	2.5	2	0
Number of types	1	1	1	2–3	1–2	0
<b>Smell (Distance Chemoreception)</b>						
Number of lobes in olfactory rosette	4	5	6	8	5	5
Olfactory rosette surface area (mm <sup>2</sup> )	0.045	0.05	0.062	0.07	0.071	0.066
Olfactory 'computer' lobe length (mm)	0.53	0.55	0.47	0.60	0.62	0.45



potential food sources via exudates dispersed in groundwater flows over long distances. These hypotheses remain to be critically tested.

Poulson (this volume) argues that critical testing of the importance of olfaction must involve realistic scales of volume, mixing of currents, and extreme dilution effects expected in nature. Even in a moderate-sized artificial stream, Bechler (personal communication) saw only occasional agonistic responses of ripe males and females of four Amblyopsid species tested intraspecifically. In addition, Poulson (in Mohr and Poulson 1966) once spent 24 hours watching six presumed pairs of large *A. spelaea* in Upper Twin Cave, Indiana. Even though they were close to each other, he could detect no change in behavior when they closely approached each other, seemingly by chance. Poulson has watched newly hatched *A. spelaea* freeze when approached by an adult in the field. One hatchling that moved was eaten immediately and cannibalism is known to occur when population densities are high (Poulson 1969; Hill 1966). Bechler found that a 'freeze' reaction is a submissive act seen in all Amblyopsid species that show agonistic behavior (Bechler 1983). All of these observations are consistent with the importance of lateral line sensing and not olfaction.

### **Mechanosensory Lateral Line System**

Eigenmann (1909) thought that the large number of lines of pores or ridges on the head and body in all Amblyopsids were tactile organs. In fact, each ridge or line of pores is one part of a lateral line system that is most sensitive to water movement. The lateral line system has sense organs called neuromasts that are part of two systems. The head canal system has only three pores opening to the surface visible with a dissecting microscope. With histological sections there appear to be neuromasts under each of a number of tiny openings all along the subdermal head canals (Claude Baker, personal communication). The much more extensive superficial system has large neuromasts that are arranged in lines on the body surface. In many teleosts, superficial neuromasts are scattered, much smaller, and much more numerous than in the Amblyopsids (Montgomery *et al.* 2001 for *Astyanax fasciatus*; Claude Baker, personal communication, for *Aphredoderus*). In all cases, each neuromast consists of several sensory cells with cilia embedded in a gelatinous cupula (Fig. 8A and B; Fig. 4 in Poulson 1963).

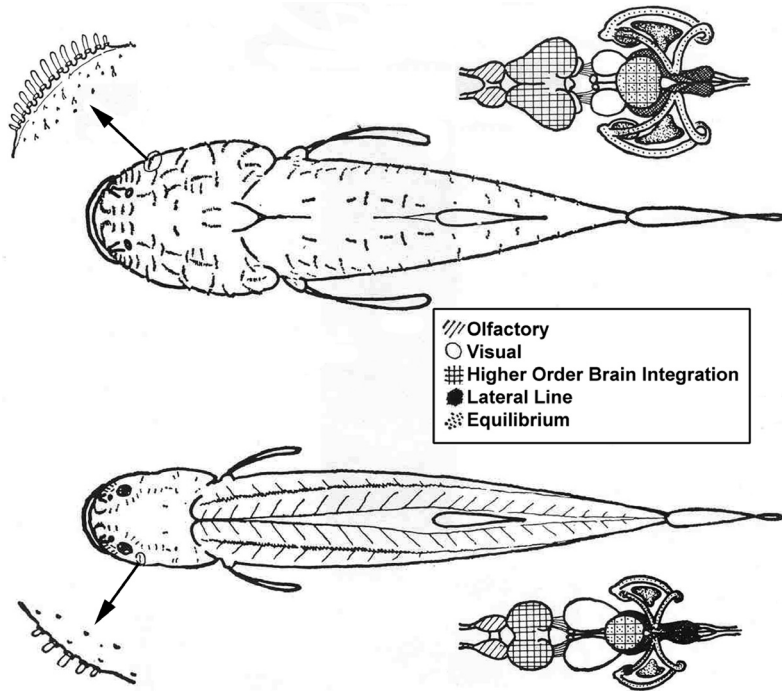
Montgomery *et al.* (2001) provide a short and lucid summary of the mechanosensory lateral line system anatomy and how it works in teleost fish. The authors go on to summarize a variety of neuroanatomical, behavioral, selective pharmacological inhibition, and neurophysiological evidence including some on *Astyanax fasciatus* cavefish that suggest different functions for subdermal canal neuromasts, mostly on the

head, and superficial neuromasts, all over the body. The canal system neuromasts filter out low frequency noise and are most sensitive to high frequency water movements generated by localized moving prey. In contrast, the superficial neuromasts are most sensitive to low frequency water movement generated by changes in water flow velocity around the fish ('hydrodynamic imaging') as it approaches an obstacle or swims upstream in a current (rheotaxis). Nonetheless, superficial neuromasts in some species react to water displacement rather than to velocity and this seems to be the case in Amblyopsids.

Poulson's (1960, 1963) data are consistent with the head neuromast ridges detecting live prey. In fact, the cupulae of anesthetized Amblyopsids can be seen visibly to move under dark field of a dissecting microscope to tethered moving prey. For large water fleas (*Daphnia magna*), *F. agassizii* cupulae move at 10 mm distance while for *T. subterraneus* and *A. spelaea* the distance is 20–40 mm. For small amphipods (*Hyalella azteca*), *F. agassizii* cupulae move at 20–30 mm, *A. spelaea* cupulae at 30–45 mm, and *T. subterraneus* cupulae at 40–50 mm. These are a little less than the distances at which live fish orient toward and then capture the same prey items. The longer distance for reaction of the troglobites is related to more elevated neuromast ridges and longer cupulae in *T. subterraneus* and *A. rosae* compared to *F. agassizii*. In *C. cornuta*, the neuromasts are in lines but they are not elevated and the cupulae are very short (Fig. 8 and Fig. 4 in Poulson 1963).

The system of superficial ridges of neuromasts, each with 10–20 neuromasts, is especially hypertrophied in all the troglobites (Figs. 9 and 10) and so Poulson (1960, 1963, 1985) asserted that it was their primary sensory system. This is consistent with relative enlargement of the brain primary sensory nerve projections of lateral line to the cristae cerebelli on the medulla and eminentia granularis on the sides of the cerebellum. Higher sensory processing and integration of the lateral line inputs with other sensory systems is in the forebrain (telencephalon), which is also hypertrophied in all the troglobites (Fig. 9; Fig. 3 in Poulson 1963; Table 2 in Poulson 1985). The many neuromast ridges on the head, with vertical and horizontal orientations of adjacent ridges (Fig. 10) must give rich detail of water movement information for the forebrain to interpret.

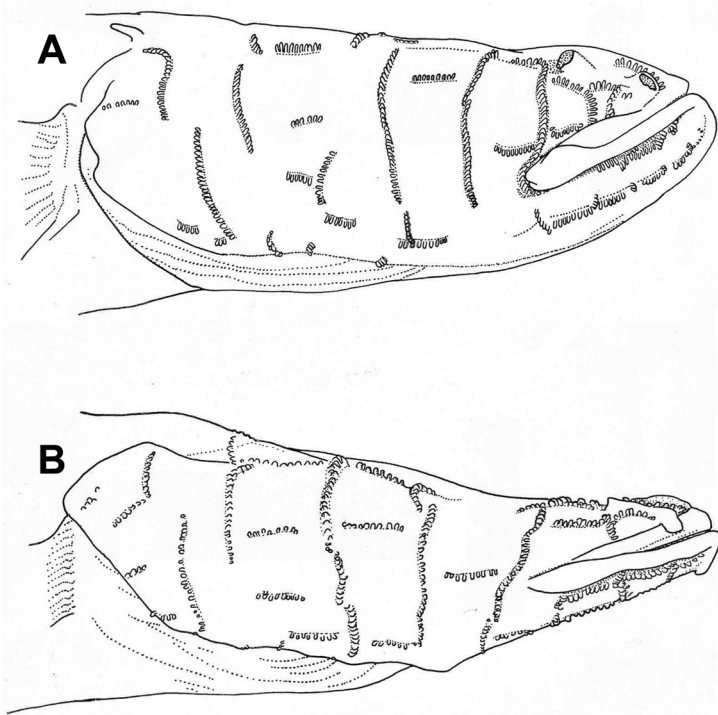
In addition to the advantage of a relatively large head having more elaborate superficial neuromasts, there is a side benefit of better 'hydrodynamic imaging'. A larger head allows slower swimming to detect and avoid obstacles. Slower swimming generates less noise for the neuromasts to detect moving prey. Montgomery *et al.* (2001) write, "Although hydrodynamic imaging may not be the sole prerogative of blind cavefish, these fish appear to have evolved rather sophisticated mechanisms for processing and using the images."



**Fig. 9** Scale drawings (from Poulson and White 1969) contrasting the troglolithic *Amblyopsis spelaea* with its trogliphilic relative *Forbesichthys agassizii*. Compared to *Amblyopsis*, each of the lateral line rows in *Forbesichthys* has fewer neuromasts, they are less elevated above the skin, and their brain ‘computer’ centers are less hypertrophied, but its eyes and optic lobes are larger. Also, the presumed ‘tactile’ receptors (shown by the enlarged lateral line rows), are smaller and fewer in kind than for *Amblyopsis*. Note also the difference in semicircular canals and otoliths. However, there is no difference in olfactory lobes of the brain.

### ***Rheotaxis Depends on the Lateral Line System***

Except perhaps for *C. cornuta*, all Amblyopsids show positive rheotaxis (e.g., Tables 13 and 14 in Poulson 1960). *Chologaster cornuta* rarely is found in flowing streams. The difference among species in rheotaxis is of degree. Allochthonous food renewal is almost always associated with late winter/spring increases in water input to caves. *Amblyopsis spelaea* will swim in the strong currents and lives in cave streams that almost always have appreciable current. As floods begin and the current becomes too strong (up to  $7 \text{ m}^3 \text{ sec}^{-1}$ ) they move to the bottom of deep pools and into backwaters. *Typhlichthys* in L&N Cave, Kentucky, are exquisitely sensitive



**Fig. 10** Scale drawings (courtesy of John E. Cooper) of the heads of *Typhlichthys subterraneus* and *Speoplatyrhinus poulsoni*. Note the relatively longer head and shovel-nosed shape of *S. poulsoni* head, which are neotenic trends. Both species show the convergent similarity in lateral line ridges or “stitches”. Each stitch has four to as many as 39 neuromasts cups.

to slight increases in current; they seek shelter hours before we can detect an oncoming flood (Pearson, personal communication). *Forbesichthys* rely on swimming upstream to return to caves every day (Weise 1957) or at the end of the growing season (Hill 1966).

### ***Tactile Receptors and Thigmotaxis***

In fish, the brain primary input for tactile information, the somato-sensory lobe, is hard to distinguish from that for lateral line input since they go to the same medulla area and share cranial nerves V and VII. A further complication is that the short neuromasts could function as tactile receptors when Amblyopsids strike at moving prey or are touched by moving prey (think about seeing light when you are hit on the eye in the dark). Also, the small size of what are presumed to be tactile receptors on the head

and around the mouth of both *Chologaster* and *Forbesichthys* makes it very difficult to quantify numbers using a dissecting microscope, especially with the heavily pigmented epithelium.

Despite the above problems, Poulson has attempted to quantify putative tactile receptors in all the Amblyopsids. All have what are presumed to be tactile sense organs concentrated on the heads with relative densities on the body << side of head < top of head above the mouth << under the mouth and lips. The incomplete data for number of tactile receptors per 0.175 mm<sup>2</sup> are as follows: for the side of the head, *T. subterraneus* 3–8 = *A. spelaea* 4–16 = *A. rosae* 4–7; for the top of the snout, *A. spelaea* 1–4 << *A. rosae* 11–15; and for under the mouth and lips, *A. spelaea* 16–20 < *A. rosae* 25–35. On the side of the head, both *F. agassizii* and all three troglobites have similar densities of these presumed tactile receptors but they are larger in the troglobites and especially larger and more diverse in shape in *A. spelaea* (Fig. 8, Fig. 4 in Poulson 1963). *S. poulsoni* has none of these receptors anywhere on its head or body.

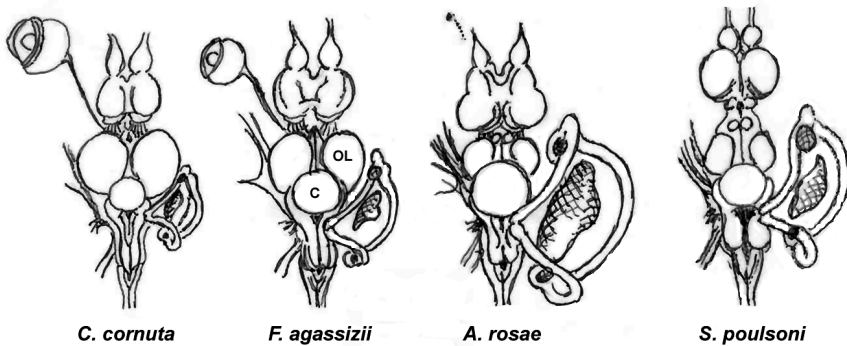
Despite relatively low numbers of tactile receptors and superficial neuromasts, both *C. cornuta* and *F. agassizii* behaviorally show strong thigmotaxis and *Typhlichthys* shows stronger thigmotaxis than either species of *Amblyopsis*. In spring cavefish, Weise (1957) found that strong thigmotaxis overshadows negative phototaxis. Spring cavefish exhibit strong preferences for cover objects, even if those objects are well illuminated. When resting, *F. agassizii* and especially *C. cornuta* are often in groups touching each other. In aquaria and in the field, *C. cornuta* rests amongst dense vegetation during the day and *F. agassizii* rests under sticks and rocks during the day. In an artificial stream *F. agassizii* spends > 90% of its time under rocks and *Typhlichthys* spends much more of its time under or next to objects resting than either species of *Amblyopsis* (Bechler, personal communication).

During foraging and prey capture, lateral line and touch receptors probably act in a complementary way. Poulson's (1960) and others' observations (Eigenmann 1909, Weise 1957) show that Amblyopsids capture the same live prey by a combination of lunge and grab. They do not appear to use gape and suck feeding. Troglobitic cavefish initiate capture at the same distances that cupulae can be seen to move but *F. agassizii* appears to contact the prey before attack. *Chologaster cornuta* will not capture prey unless it contacts the head or lips; whereas *F. agassizii* will turn and grab prey touching any parts of its body especially if it has previously captured one or more prey. Weise (1957) notes for *F. agassizii* that "an amphipod is taken by a vicious sidewise jerking of the head and is immediately swallowed." Spring cavefish will take three to four 10–15 mm amphipods in a few seconds. *Amblyopsis spelaea* also 'jerks and grabs' as it captures a large prey item.

### Static and Dynamic Equilibrium Reception

As with all vertebrates, teleost fish and Amblyopsids have semicircular canals and otoliths. The sensory organs associated with each are modified neuromasts. The semicircular canals detect rates and directions of movement while the otoliths detect body position even at rest.

The three semicircular canals are all filled with a viscous fluid and are oriented at right angles to each other; the neuromasts in the ampullae at the end of each canal detect inertial movement of fluid in the three canals in each of three dimensions as a fish swims or stops. The three calcium carbonate otoliths detect position in three planes even while a fish is not moving. The otoliths rest on a group of neuromasts such that the null or usual body orientation results in no nerve impulses. In the null position the otolith presses evenly on all the sensory hairs but any change in position results in an uneven loading of the otolith on the hairs and increasing frequency of nerve impulses with increasing departure from the null position. The saggitta is the largest otolith and it detects deviation from a horizontal position. The much smaller astericus and lapillus give information when the fish is oriented in other planes.



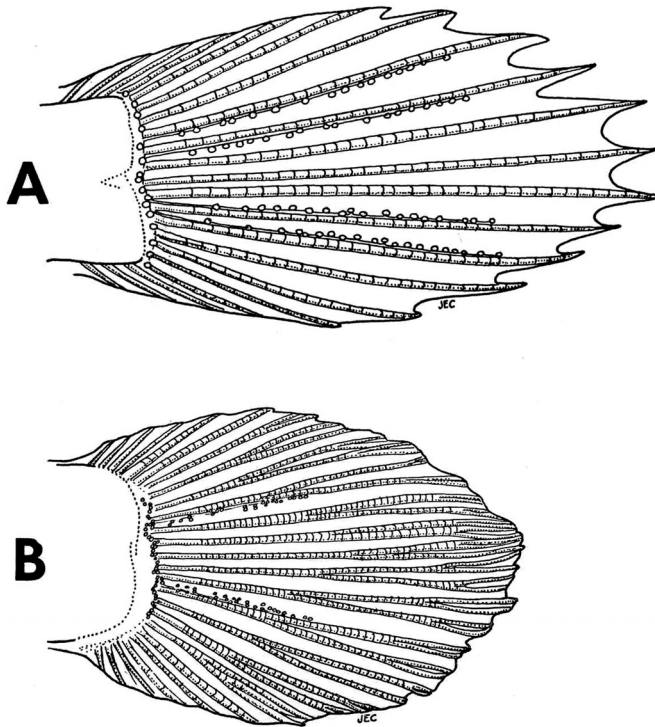
**Fig. 11** Scale drawings (Poulson) of eyes and optic nerve (where present), brains, and semicircular canals (SSC) with otoliths of Amblyopsid fishes. *Chologaster cornuta* is the small-eyed swampfish with largest eyes and optic lobes (ol) and smallest forebrain, cerebellum, SSCs and otoliths. *Forbesichthys agassizii* has intermediate brain proportions. *Amblyopsis rosae* shows the convergent proportions also seen in *Typhlichthys subterraneus* and *Amblyopsis spelaea* but has the smallest eyes and optic lobe and the largest forebrain, cerebellum, SSCs and otoliths. *Speoplatyrhinus poulsoni* shows still smaller optic lobes but its cerebellum and SSCs are not as hypertrophied. Note that it has a different relative size of the otoliths than for the other Amblyopsids. Also note that there is no trend in relative development of olfactory lobes at the front of the brain. See Fig. 9 for the relation of brain proportions to body shape and relative development of the lateral line system.

Ordinarily, the static equilibrium system, the otoliths, and dynamic equilibrium system, the semicircular canals, operate in concert with vision. So it is perhaps not surprising among the Amblyopsids that the reduced eye *Forbesichthys*, that cannot see images (Eigenmann 1909), and blind cavefish have hypertrophied semicircular canals, otoliths, and the cerebellum brain integrative area for these two systems (Figs. 8 and 11, Fig. 3 in Poulson 1963). As with the lateral line system and its brain input and integrative centers, there is little further increase in relative size of these systems with increased evolutionary time in caves from *F. agassizii* to *T. subterraneus* to *A. spelaea* to *A. rosae* to *S. poulsoni* (Table 4, Table 2 in Poulson 1985).

It is of interest to discuss why the increase in semicircular canals and otoliths seen in essentially blind and nocturnal *Forbesichthys* is not sufficient to compensate for lack of vision. The answer appears to be that the relatively larger head in all the troglobites has been selected in part to increase the lateral line sensory system (above). The larger head in turn makes it necessary to have further hypertrophy of the semicircular canals and otoliths. Fine *et al.*'s (1987) analysis of the largest known semicircular canals and otoliths in any teleost is instructive. The fish in question is a deep-sea Ophidiid with an even larger head relative to body size than in *S. poulsoni*. It carries to extremes the theoretical need for large canals in fish generally, because fish have no neck and swim in a viscous medium (Jones and Spells 1963). With increased relative head size the radius of curvature and canal diameters must be larger to have the same neurophysiological gain in sensitivity. In addition, a larger head results in less inertial change when turning and so requires larger canals to detect. The deep sea Ophidiid has a very large head, approximately 10 × the volume of the body. The relative head to body volumes in Amblyopsids range from about equal in *S. poulsoni* to about 10% in *C. cornuta*. Even *C. cornuta* has a relatively large head for teleosts.

### **A Mystery Sensory System: The Caudal Papillae**

All Amblyopsids have a vertical row of papillae at the base of the caudal plus 2–5 horizontal rows extending out along the fin. These are larger and more rounded than the largest presumed tactile sense organs elsewhere on the body. Based on drawings in Woods and Inger (1957), papillae are slightly larger on *T. subterraneus* and both *Amblyopsis* species than on either *C. cornuta* or *F. agassizii*. In addition, total numbers of these papillae are *T. subterraneus* 27 <= *A. spelaea* 30 < *A. rosae* 36. Based on very accurate drawings by John Cooper (Fig. 12), the densities, placement, and sizes of the papillae are strikingly different in *T. subterraneus* (Alabama) and *S. poulsoni*. *Typhlichthys* papillae are much smaller and more crowded than



**Fig. 12** Scale drawing (courtesy of John E. Cooper) of the caudal fins of *Typhlichthys subterraneus* and *Speoplatyrhinus poulsoni*. The unbranched fin rays of *S. poulsoni* is a neotenic trait. The especially large caudal papillae (function unknown) of *S. poulsoni* are much larger than those of *T. subterraneus* and all other cave Amblyopsids.

in *S. poulsoni*; with shorter total row length (8.5 mm *T. subterraneus* << 24.8 mm *S. poulsoni*) and fewer rows (3 vs. 5) over less fin area. *Typhlichthys* has 70 papillae with 12–17 per mm and *S. poulsoni* has 88 papillae with 3–5 per mm. Obviously, we need equally careful measurements and counts on all the other species to see if there are differences among other species.

## V. PREY DETECTION AND AVAILABILITY

All of the available field and gut content evidence is consistent with the laboratory feeding observations (reviewed above) that show that the superficial lateral line system is necessary and probably sufficient to explain the diet of Amblyopsid fish. All items in the gut are certain to have been alive and moving prey, and predominant prey in the gut match the distribution and abundance of prey in the field. This is true in terms



of frequency of occurrence, abundance, and volume. If prey are similar in these regards, then the species that is most active, and thus most easily detected with the lateral line system, is more frequent and abundant in fish guts. Thus, the fish are not showing a preference for particular food items because they take whatever they can detect.

### **A. Data for *Chologaster* and *Forbesichthys* Gut Contents**

The data are excellent, in terms of sample sizes (hundreds to thousands of fish) and time of year of sampling for *C. cornuta* (Ross and Rohde 2003) and *F. agassizii* (Weise 1957; Hill 1966). The match of prey in the gut and prey in the field was remarkably similar in *C. cornuta* from Black Swamp Creek, North Carolina, and *F. agassizii* from Pine Hills, Illinois, cave-spring runs. Both sites have dense vegetation and hiding places so visual predators of the Amblyopsids are probably unimportant. In both sites Gammarid amphipods were the most frequent, most numerous, and largest food items in the Amblyopsid guts and in the field. Of the items eaten the amphipods are also the most active and so most easily detectable using the superficial lateral line system. The Rich Pond spring runs did not have amphipods but did have abundant Chironomid midge larvae and small oligochaetes with a high diversity of other potential prey. The *Forbesichthys* guts had more midges than worms. Hill (1966) attributed this to the greater activity levels of the midges, which made them more detectable by the fish.

At Rich Pond, Kentucky, there is a huge difference in food supply and feeding outside the cave and inside (Hill 1966). All surface-collected fish had very high condition factors and full guts. The cave source of the Rich Pond spring run had almost no live prey compared to the super-abundance of prey outside. Guts of fish 35–40 mm SL spending their first winter in the cave were 99.9% empty and fish had low condition factors. Guts of fish spending their second winter in the cave were only 18% empty with the rest having fish (cannibalism) and only slightly poorer condition factors than when they were outside the spring before.

### **B. Cave Amblyopsid Gut Contents as Samplers of Prey Density**

For Amblyopsid troglobites the fish are a better sampler than we are of prey availability in the caves with their very low food supplies (Poulson and Lavoie 2000, Poulson 2005). Poulson (1960, unpublished data) has some gut content data for several localities with sample sizes in the 50–100 range and where we can both easily census fish and crayfish and can

occasionally census amphipods and isopods by examining loose rocks. Harpacticoid and Cyclopoid copepods are virtually impossible to census accurately and they are the most frequent and numerous prey in guts of cavefish.

Here we present some representative data for *T. subterraneus* from Shelta Cave, Alabama, *A. spelaea* from Donaldson Cave, Indiana, and *A. rosae* from Cave Springs Cave, Arkansas (a gray bat maternity cave). For four fish each of 37–43 mm SL the gut contents were as follows with total numbers of each prey and % frequency (25, 50, 75, or 100%). All the data were taken at low water times well after the most recent flood. Four *T. subterraneus* had 167 copepods (100% frequency), 13 tiny ostracods (25%), 1 amphipod (25%), and 1 tiny shrimp (25%). Four *A. spelaea* had 65 copepods (100% frequency), 7 isopods (75%), and 1 amphipod (25%). Larger *A. spelaea* had more isopods, some amphipods, and one fish even had a tiny crayfish. Four *A. rosae* had 65 copepods (100% frequency), 8 cladocerans (25%), 4 isopods (50%), and one 10 mm crayfish (25%).

Extensive checks of guts in museum collections for other caves with lower censused numbers of each Amblyopsid species show that troglobites NEVER had an empty rectum or intestine and rarely had an empty stomach. In contrast, six of ten *Forbesichthys* from Mammoth Cave had completely empty guts in the year when Poulson censused fifteen fish that had survived after being washed in from far upstream sinking creeks. For those four individuals with any food in their guts, the maximum was 6 copepods!

### **C. Is *Forbesichthys* Truly Troglomorphic?**

Hill's (1966) data on *Forbesichthys* at Rich Pond reviewed above is consistent with our current view that spring cavefish are not ordinarily able to complete their life history in caves because of inadequate food supplies. Rich Pond has many thousands of *Forbesichthys* that eat frequently and grow quickly in the spring run. However, 6–30 mm SL fish in the cave had empty guts and had poor condition factors. Even 40–60 mm individuals had only cannibalized fish in their guts. In caves with the same very low food densities, of copepods >> isopods > amphipods > tiny crayfish or salamanders, all troglomorphic Amblyopsids have food in their guts.

### **D. Gut Contents in Relation to Prey Densities**

An early review of relative live food occurrence in relation to gut contents for all the Amblyopsids (Poulson 1960) is consistent with our present generalization that fish eat the most available and fastest moving prey.

The habitats of *C. cornuta* and *F. agassizii* have abundant prey with 2–4 orders of magnitude greater numbers and volume than any cave.

Here are some data for surface habitats for *C. cornuta* and *F. agassizii*. A 0.25 ft<sup>2</sup> Ekman dredge sample, in Rockfish Creek, North Carolina, in April had three odonate nymphs, 15 small amphipods, 121 copepods, 52 ostracods, and many rotifers. In the guts of the 16 *C. cornuta* collected at the same time there were 17 taxa but they were predominately amphipods (60% frequency, 41% numbers, and 80% volume). In a Pine Bluffs, Illinois, spring run with a cave-spring population of around 150 *Forbesichthys*, large (10–15 mm) *Gammarus troglophilus* have astounding densities year around of 10–25 per 0.25 ft<sup>2</sup>. Isopods and flatworms make up the rest of the macroscopic fauna but neither is found in *F. agassizii* stomachs. As Weise (1957) states the fish are monophagous on amphipods.

The best comparative data on food scarcity in caves is from Hill (1966) since he made weekly censuses of *Forbesichthys* abundance, sizes, and gut contents along with Ekman dredge samples in both the cave and surface spring run at Rich Pond, Kentucky. During the spring months as the water table rises and the cave waters flow out into the huge spring run, surface prey became abundant but inside the cave there was almost no food. The extremes of prey in Ekman dredge samples outside in the spring run vs. inside the cave were as follows: chironomid larvae 300–1200 versus 0–6, oligochaetes 600–1200 versus 0–2, nematodes 0–500 versus 0–1, copepods 1–250 versus 0–20, and ostracods 1–120 vs. none. Outside fish guts were completely full with 70–80% chironomids and 10–20% oligochaetes by volume; inside the cave fish guts were empty.

Only some caves with Amblyopsid troglobites studied by Poulson (1960) have more live prey than in the Rich Pond cave. Even the best *A. spelaea* cave (Upper Twin Cave in Indiana) in terms of abundance with 86–130 cavefish (Fig. 4 in Poulson 1960) and 45–80 cave crayfish had 1–5 isopods and 0–1 amphipods in ten Ekman samples. However, plankton samples of 800 liters ranged from 0–300 ml ETOH displacement (Scott 1907). During a spring flood, the 300 ml consisted of 70% silt and 15% leaf fragments by volume. The remaining 45 ml had an incredible number of plankters including 1998 *Bosmina* cladocerans, 410 adult copepods + 319 nauplii, and 717 rotifers. This cave is fed in part by a sinking stream and both the plankton and the benthos are more than an order of magnitude more than in the most food rich caves Poulson sampled with other species of Amblyopsids. We do not have data on food supply for the two *Amblyopsis spelaea* caves with the highest densities of cavefish known: Under the Road Cave had an estimated 4199 fish per hectare in a 300 m stream segment with virtually no visible organic matter (Table 5, Poulson 1969) and Penitentiary Cave had an estimated 2643 fish per hectare in a

1200 m series of rimstone pools with 10-30 cm of leaf, twig, and acorn fragments (Pearson, personal communication). Seven caves with the highest visually censused populations of *Typhlichthys* had peak numbers of 4, 6, 11, 16, 20, 23, and 64 fish and for *A. rosae*, three caves had 7, 11, and 72 fish. The two most abundant *Typhlichthys* caves had only 2–4 copepods, 0–3 ostracods, and 0–2 rotifers in one 800 liter plankton sample and no fauna in ten Ekman dredge samples. However, 40–50 cave crayfish were sampled. The most abundant *A. rosae* cave had 40 copepods, 6 ostracods, and 1 isopod in an 800-liter plankton sample and the benthos could not be sampled among the large rock rubble; however fish gut samples show that isopods, amphipods, and small crayfish are present.

## VI. ECOLOGY, LIFE HISTORY AND BEHAVIOR

In this section, we discuss aspects of Amblyopsid ecology, life history, and behavior, including such topics such as reproduction, metabolism, demography, and agonistic behavior. Much of the information assembled to date is from the works of Poulson, Bechler, and colleagues. However, many questions remain unanswered.

### A. Life History Patterns

#### *Hypotheses and Theory*

On the basis of scale marks, size-frequency distributions, egg size and number, and metabolic rates Poulson (1960, 1963, 1985) inferred that there is a trend of increasing longevity, decreasing growth rates, and decreasing metabolic rates from the epigeal *C. cornuta*, to the troglophile *F. agassizii*, to the three troglobitic cavefish known at the time. For the troglobites, he hypothesized that increasing life span, lowered metabolic rates, and large eggs resulting in large young are adaptations to low food supply in caves. On the basis of gaps in the size-frequency distribution with no small fish and some data on gill surface area, he later inferred that his namesake *S. poulsoni* has an even longer and more energy efficient life history and metabolic pattern.

In the context of life history theory (Poulson 2001b and this volume) the Amblyopsids represent the extremes of very high  $r$  to very low  $r$  (instantaneous population growth rate in the logistic equation  $dN/dt = rN(K-N/K)$ ). At the *C. cornuta* extreme of high  $r$ , there is a 1–2 year lifespan and one-time reproduction (semelparity) at an age of  $\leq 1$  year. The tradeoffs are of population booms when times are good and busts when times are bad. Associated with this pattern are spreading of risk

of mortality among numerous small eggs that result in small young. *Amblyopsis spelaea* represents the other extreme of low  $r$  with a 20–30 year lifespan (see below) and an age at first reproduction no earlier than 6 years. The tradeoffs are of population ‘fizz’ when times are good and population ‘fizzle’ when times are bad. Associated with this pattern are spreading of risk of reproductive failure among many attempts (iteroparity) with few large eggs and large resulting young at each reproductive attempt.

Iteroparity likely evolved in cave Amblyopsids, and in many other cave species, because of extreme selective pressures on energy economy at all life history intervals and because adults are top predators and essentially immune from predation (Poulson 2001b). In the surface species, *C. cornuta* and *F. agassizii*, death likely occurs after a single reproductive event shortly after reaching sexual maturity. For both these species, almost all adult females breed in a given year (Poulson 1963, Ross and Rohde 2003, Hill 1966). Older individuals in their second or third years probably represent those that failed to reproduce at an earlier age.

For cave species food limitation is the likely selective force limiting reproduction and reproductive output. In wet years when food availability is higher, a larger proportion of females likely reproduce. Contrastingly, in poor years when food availability is severely limited, perhaps all females fail to reproduce. Iteroparity and increased longevity reduce the risk of population extirpation when food supplies are too low for an extended period of time to allow females to reproduce. However, mark-recapture measures of growth rate (below) show that cave Amblyopsids are much older than Poulson (1963, 1985) originally suggested based on scale marks and so the reproductive potential of each species may be considerably less. This means that conservation of various populations may be more critical than we currently think (D. Bechler, personal communication).

### **Tests of Hypotheses: Life History Patterns**

In cave Amblyopsids, only a fraction of all sexually mature females reproduce in a given year. In the three species for which reproductive data existed, Poulson (1963) estimated that 50% of *T. subterraneus*, 20% of *A. rosae*, and only 10% of *A. spelaea* females reproduce in a given year. Based on better data and inferred longer longevities, his revised estimates (Poulson 1985) were 30% *T. subterraneus*, 5% *A. spelaea*, and 25% *A. rosae*. Based on a thorough review of even more data and the knowledge from growth rates of even greater longevities, he now estimates the percent of mature females reproducing in a year averaged over a decade for all Amblyopsids as 100% *C. cornuta*, 70% *F. agassizii*, 20% *A. rosae*, 10% *Typhlichthys*, and 5% *A. spelaea*. These numbers may be too high for the troglobites if preliminary data hold up showing sex ratios of all Amblyopsids as almost 2 to 1 for

adults. The revised percents of females reproducing over a decade then become 13% *A. rosae* > 7% *T. subterraneus* > 3% *A. spelaea*).

The Ross and Rohde (2003) life history study of *C. cornuta* is a paradigm for rigor and completeness. It redresses the odd situation that this most common and accessible (epigeic) species of Amblyopsid was the least studied and least understood. The life history of this Amblyopsid was previously only inferred from size-frequency distributions and ovarian egg counts from small samples from many localities each on usually only one date (Fig. 4 in Poulson 1960; Ross and Rohde 2003). From collections at one site on 25 dates with samples of 24–41 fish over one year, Ross and Rohde could follow the growth rates of young of the year over 65 days in spring from 8–10 mm SL to 13–18 mm SL for males (~4 mm growth per month) and to 23–30 mm for females (~8 mm growth per month). By 14 June, the few surviving adults were gone and presumably dead. The first year fish growth rate slowed in summer and increased in fall. By early January, males were 19–25 mm and females 29–37 mm SL. We now think that troglobitic Amblyopsids of these sizes are as old as 3–6 years even with energetic adaptations to orders of magnitude lower food supplies.

From all of their data, Ross and Rohde (2003) agree with Poulson's earlier inference that *C. cornuta* has a one year life cycle and dies after reproduction (semelparity). Their data show a sharp 2-month decline of mean gonosomatic index from 11% to 1% and from 1% to 0% in the next month (GSI = weight of ovaries as % total body weight less ovary weight). The initiation of this short spawning season coincided with a sharp February to March rise in stream temperature from 5 to 15°C by mid May when 8–10 mm young of the year were sampled. There were only two of this size so the smallest young were undoubtedly missed with relatively coarse meshed seines.

Even Ross and Rohde's extensive data on ovarian egg number and diameter are difficult to interpret and compare to other Amblyopsids. They wisely used only ovaries that were "tight and full of eggs" to estimate fecundity in 158 females 20–47 mm SL. There was huge variation in egg diameter from 1.14–1.78 mm (mean 1.48) and in number of eggs from 6–98 (mean 24.7). Together this indicates great variation in both clutch size and size of smallest free-swimming hatchlings. These data are difficult to compare to other Amblyopsids that grow much larger (females assayed for fecundity are 45–65 mm SL). Females from the population Ross and Rohde studied mostly grew to a maximum of 39 mm with 3 of 156 larger (45 mm = 33 eggs, 41 mm = 71 eggs, and 47 mm = 98 eggs). From a sample size of only three females from two other populations where average maximum SL is in the mid 40s, the fecundities are much higher (42 mm = 339 and 45 mm = 426 eggs in one population and 44 mm = 252 eggs in

another population) than the fecundities Poulson used for comparison to other Amblyopsids (Table 6 in Poulson 1960). Also the egg diameters are higher than the 0.9–1.2 mm Poulson (1960) reported and used to estimate reproductive risk (Poulson 1985).

Loren G. Hill's (1966) Ph.D. dissertation on *F. agassizii* is in many ways the most complete study ever done on one population of Amblyopsid. In his Rich Pond, Kentucky, study site there is a population size of many thousands of fish and he collected samples of up to 100 on each date throughout the year. He followed growth, feeding, gut contents, condition factor, fecundity, and ontogeny of vent migration and scale development weekly for the growing season. He studied fish both in the cave and in the spring-run into which the fish moved in late winter, as water started to flow from the cave. Fish returned to the cave in summer, as the surface waters dried up, and spawned in the cave the following winter.

Rich Pond has similarities and differences to the Pine Bluffs, Illinois, spring-cave populations (Weise 1957). Pine Bluff populations are similar in fecundity, growth rates, and 3–4 year classes (size cohorts and scale annuli). However, it is different in the much smaller habitat area and much smaller populations (Weise 1957, Smith and Welch 1978). In eight Pine Bluffs springs studied by Petersen mark-recapture, the population estimates were 1–302 with a conservative total of 862 (Smith and Welch 1978). The food available was abundant under rocks and in watercress in epigeal spring runs with 291–902 individuals per square foot of five taxa. In every monthly sample over a year a large *Gammarus* species (10–15 mm) comprised 50–80% of numbers and 90–95 % of volume in the field and > 98% by volume in adult fish guts (Weise 1957). Both Weise and Smith and Welch agree that most fish exit the underground into the spring a half hour after dark and return about one hour before dawn (a few remain under rocks outside during the day). Thus, they are extremely negatively phototactic and positively thigmotactic. They also agree that spawning occurs underground since gravid fish disappear from the surface in January and February and no gravid fish are found on the surface after that.

### **Tests of Hypotheses: Growth Rates and Age**

Since Poulson's (1985) latest summary of data and hypotheses based on scale marks and presumed cohorts, various workers have done mark-recapture studies to obtain data on growth rates of *F. agassizii* (Smith and Welch 1978), *A. spelaea* (N. Welch, personal communication), *T. subterraneus* and *A. spelaea* (W. Pearson, personal communication), and *A. rosae* (Brown and Johnson 2001). All of these studies show that the ages and growth rates match those inferred from scale marks and distinct size classes in

*Forbesichthys* (2–3 years) but are underestimates of age by  $2\text{--}4 \times$  for all the trogllobites. Thus, potential lifespans are 16–24 years for *Typhlichthys*, 30–45 years for *A. spelaea*, 8–12 years for *A. rosae*, and 16–24 years for *Speoplatyrhinus*. A large range in age for the same size fish within each species is due in part, Poulson now believes, to the great variation in growth rate in fish eating just copepods, fish also eating a few isopods and amphipods, and fish that show huge growth rate spurts when they are lucky enough to capture and eat a large prey item (Poulson 2001b).

### **Tests of Hypotheses: Fecundity, Egg Size and Cost of Reproduction**

Cave Amblyopsids tend to have not only fewer, but also larger and potentially more nutrient-rich eggs than their surface counterparts. As in other cave organisms, larger eggs also contain more yolk and produce larger larvae. By investing more yolk volume per egg, larvae hatch at larger size and are larger in size when all yolk is absorbed. Therefore, individuals have greater starvation tolerance, greater swimming ability when foraging but also when avoiding predation, and can accommodate a wider range of prey sizes when all yolk reserves are depleted. All of these are adaptations that increase survival in a food-poor environment.

How well does a more careful evaluation fit Poulson's (1985) hypotheses about the advantages of increasing egg size with decreasing clutch size with cave adaptation? Data on size of fry when their yolk sac are absorbed is a better measure of sizes of eggs laid than is the sizes of eggs in ovaries of gravid females. The reason is that *Forbesichthys* ovarian eggs at Rich Pond range from 1.5 to 2.0 mm but the sizes of hatchling fry after yolk sac absorption are all 6.0 mm (Hill 1966) suggesting that laid eggs are 2.0 mm. The only other species where we are certain about size of post yolk absorption fry is in *A. spelaea* where we can occasionally find females brooding 2.3 mm eggs in their branchial cavities and find free-swimming 8.5 mm hatchlings (e.g., Eigenmann 1909, Poulson 1960, Pearson, this volume). Thus, an increase in egg diameter from 2.0 to 2.3 translates to an increase in post yolk absorption fry size from 6.0 to 8.5 mm SL. What does this size difference mean to fry?

From data on fish (Blaxter 1983, Hempel 1965, Hunter 1972) an increase in egg size of 2.5 mm translates to the following advantages for fry. There is an estimated 2.5-fold increase in maximum size of prey and increase in range of food item sizes that can be eaten (mouth size), a 3-fold increase in volume of water searched per time (swimming speed), a better ability to escape predators (swimming speed), and a 3-fold decrease in density of prey needed to maintain weight (resistance to starvation). This is about the advantage of increased egg size in trogllobitic Amblyopsids (from



1.8 mm eggs in *C. cornuta* to 2.0 mm eggs in *F. agassizii* to 2.2 mm eggs in *A. rosae* to 2.3 mm eggs in *T. subterraneus* and *A. spelaea*) that Poulson (1985) calculated. It does not seem like a big difference but it still seems evolutionarily significant with the extremely low food supplies in caves.

For an egg-laying female, the evolutionary reduction in estimated clutch volume from *C. cornuta* to *A. rosae* is an important aspect of overall increase in energy economy. Poulson has seen photos or read about live females with egg-swollen bodies and easily visible eggs but only for *C. cornuta* and *F. agassizii* (Ross and Rohde 2003, Weise 1957). Poulson now believes that the estimates of clutch size based on ovarian eggs (i.e., Table 2 in Poulson 1985) are too high for cave species but we have no way of knowing by how much.

Poulson has recalculated aspects of reproductive cost that he presented in 1985. The new data (Table 5) are based on average clutch sizes, maximum ova diameter, and average weight of reproducing females. For a *C. cornuta* of 42 mm SL and a weight of 1.0 g with a clutch of 152 eggs of 1.8 mm diameter, the total egg volume is 466 mm<sup>3</sup> with 466 mm<sup>3</sup> per gram of female. For a *F. agassizii* of 60 mm SL and a weight of 3.0 g with a clutch of 154 eggs of 2.0 mm diameter, the total egg volume is 645 mm<sup>3</sup> with 215 mm<sup>3</sup> per gram of female. For a *Typhlichthys* of 50 mm SL and a weight of 1.6 g with a clutch of 58 eggs of 2.3 mm diameter, the total egg volume is 370 mm<sup>3</sup> with 231 mm<sup>3</sup> per gram of female. For an *A. spelaea* of 65 mm SL and a weight of 5.2 g with a clutch of 65 eggs of 2.3 mm diameter, the total egg volume is 414 mm<sup>3</sup> with 80 mm<sup>3</sup> per gram of female. Finally, for an *A. rosae* of 45 mm SL and a weight of 2.7 g with a clutch of 34 eggs of 2.2 mm diameter, the total egg volume is 190 mm<sup>3</sup> with 70 mm<sup>3</sup> per gram of female. In summary, the cost of eggs measured as mm<sup>3</sup> per gram of female declines with increasing cave adaptation with *C. cornuta* > *F. agassizii* <= *T. subterraneus* >> *A. spelaea* >= *A. rosae*.

We get a different perspective if we compare these volumes converted to calories per day over four months (the time observed for eggs to mature *in vivo* in nature) with routine metabolic rate converted to calories per day (Table 5). The data are calories per day routine metabolic rate + calories per day to grow eggs (% due to egg growth): *C. cornuta* 18.4 + 2.9 (16%) << *F. agassizii* 45.0 + 4.0 (9%) >> *A. spelaea* 18.7 + 2.6 (14%) > *T. subterraneus* 13.4 + 2.3 (17%) > *A. rosae* 9.8 + 1.2 (12%). Thus, from this perspective Poulson's (1969) statement would seem to be wrong: "The high cost of reproduction is a crucial problem to be solved in adapting to the cave environment". The low absolute values for *A. rosae* only helps explain why it has the highest estimated percent of females reproducing each year (20% compared to 10% in *T. subterraneus* and 5% in *A. spelaea*). But comparing this to *C. cornuta* (100%) and *F. agassizii* (70%) it does appear

**Table 5** Calculation of reproductive costs and comparison to percent of females reproducing and maximum population density. See text for methods. The advantage of large egg size for fry size is not offset by a high cost of egg production and total mm<sup>3</sup> of eggs per g of female declines. In addition, the restrictions of low food supply in cave (*F. agassizii* spring vs. cave) are offset among the cave species by increasing efficiencies that is reflected by increasing population densities and percent females breeding.

<i>Species</i>	<i>C. cornuta</i>	<i>F. agassizii</i>	<i>F. agassizii</i>	<i>T. subterraneus</i>	<i>A. spelaea</i>	<i>A. rosae</i>	<i>S. poulsoni</i>
Habitat	Surface	Spring	Cave	Cave	Cave	Cave	Cave
Free-swimming fry (mm SL)	4.5 <sup>a</sup>	6.0	na	8.5 <sup>a</sup>	8.5	8.0 <sup>a</sup>	
Maximum egg diameter (mm)	1.8	2.0	na	2.3	2.3	2.2	
Egg volume (mm <sup>3</sup> )	3.05	4.19	4.19	6.38	6.38	5.58	
Clutch size	152	154	0	< 58?	65	< 34?	
Female size (mm SL)	42	60	40	50	65	45	
Egg volume per g female mass	466	254	na	231	80	70	
Cal eggs	2.9	4.0	na	2.3	2.6	1.2	
RMR Cal	18.4	45.0	na	13.4	18.7	9.8	
Estimated % females breeding/yr	100	70	0	10	5	20	
Estimated reproductions/lifetime	1	1	0	2–3	3–5	2–3	
Lifespan	1–2	2–4	2–4	16–24	30–45	16–24	16–24
Max density hectare <sup>-1</sup>	42,000	80,000	0–56	864	4199	2134	

<sup>a</sup>estimated

that the statement is correct. The reason is that caves have such low food supplies compared to swamp and spring-run habitats.

With the low volume of eggs per gram of female in troglobites, we would not expect to notice large swollen bodies in females ready to spawn. Also, there would not seem to be a high energetic risk given the great energetic economy in all the species. Poulson now predicts a flexibility of clutch size in all the troglobites based on differences among years in food supply (e.g., Pearson this volume for a rare year with ~20% of adult female *A. spelaea* with branchial eggs and hatchlings versus usual maximum of 5%) and his lucky large meal hypothesis (Poulson 2001b).

## B. Branchial Brooding and Reproductive Behavior

There is a morphological reason to expect all Amblyopsids to branchially brood eggs but it has only been seen in *A. spelaea*. The jugular position of the genital papilla in adults and the attachment of gill membranes to the isthmus in all Amblyopsids suggest that all species brood eggs in their branchial cavities (Woods and Inger 1957, Poulson 1963). Some have thought *A. rosae* brood eggs in the branchial cavity (Breder and Rosen 1966, Balon 1975). It has been suggested that non-incubated eggs are more susceptible to predation in the cave environment (Noltie and Wicks 2001); however, direct evidence is lacking. Conversely, evidence against branchial incubation in the other Amblyopsids exists. At least two researchers have suggested that total egg volume in ripe Amblyopsids exceeds branchial volume in Amblyopsids other than *A. spelaea*. Here we do some calculations to determine whether we agree.

We start by calculating branchial volume of a 65 mm SL incubating *A. spelaea* with 60 eggs each 2.3 mm diameter:  $6.38 \text{ mm}^3 \times 60 = 383 \text{ mm}^3$ . This is about  $4 \times$  the branchial volume Poulson (2001a) estimated from linear estimates for a 45 mm fish. If we proportionally increase the 45 mm estimate to 65 mm/45 mm the estimate is still only  $121 \text{ mm}^3$  and about a third of the observed volume of eggs in the females branchial cavity. We suggest that this continued discrepancy is due to the likelihood that  $121 \text{ mm}^3$  is a large underestimate because brooding females have obviously puffed up cheeks with a completely distended branchial cavity. If we apply a proportional correction to Poulson's estimate of *A. rosae* branchial volume for a 45 mm SL female we get  $260 \text{ mm}^3$  for a branchial cavity that is completely distended ( $383 \text{ mm}^3 / 121 \text{ mm}^3 \times 82 \text{ mm}^3$  linear estimate for *A. rosae*). If this 45 mm fish had a clutch size of 34 eggs each 2.2 mm in diameter (Table 2 in Poulson 1985) the clutch volume would be  $190 \text{ mm}^3$  or about 73 percent of the estimated distended branchial volume. On this basis, we disagree with Ginny Adams (personal communication)

that total ovarian egg volume of *A. rosae* exceeds its distended branchial volume. In fact, we believe that *Amblyopsis* clutch volumes are even less (see reasoning in the previous section). On the other hand, we agree with Jenkins and Burkhead (1994) that 339 and 426 ova would not fit in the branchial cavities of female *C. cornuta* of 42 and 46 mm SL. Our estimate of distended branchial volume of 42–46 mm SL *C. cornuta* is about 110–130 mm<sup>3</sup> and using a 1.8 mm egg of 3.05 mm<sup>3</sup> volume (since we believe that females only lay eggs at or near maximum size) the clutch volumes are 600 mm<sup>3</sup> and 754 mm<sup>3</sup>. Unlike the case for either *Amblyopsis* species, these clutch volumes hugely exceed our newly estimated distended branchial volume (~1220 mm<sup>3</sup> >>>> 130 mm<sup>3</sup>). Ross and Rohde's (2003) observations are consistent with our conclusion that *C. cornuta* cannot carry its large clutches of eggs in its branchial cavity. Ross and Rohde were very careful to hold many newly captured large gravid females in jars but never saw eggs or fry in or released from a branchial cavity in 13 years of field study. They speculate that swampfish likely deposit eggs somewhere in the stream. Swampfish have been observed building a nest-like depression under a rock in an aquarium (J.S. Smith, personal communication in Ross and Rohde 2003).

Poulson thinks that the especially low reproductive effort of eggs per gram for *A. spelaea* (~80) may be related to the constraint of branchial brooding that has been verified many times for this species (Eigenmann 1898, Poulson 1960, Pearson, this volume). Clearly there could be no more eggs than observed in fish with extremely distended branchial cavities. In this context, it is of interest that Eigenmann reported a few cases of decrease of eggs in the branchial chamber in three cases where he kept careful track (57 → 34, 61 → 43, and 65 → 51). This could be due to failure of development and/or swallowing some eggs. It seems clear that the + tradeoff of branchial incubation is protection of eggs and new hatched fry but the potential – tradeoff of not being able to feed while incubating may not be important. The metabolic rate of *A. spelaea* is low and fish survive more than a year without feeding. Whatever the negatives, it is clear that females could not hold any more eggs in their fully distended branchial cavity than the reported numbers of 61–70 for 60–75 mm SL females.

The mode of egg-laying and question of parental care both remain mysteries to be solved in the future. Dave Bechler has, in our opinion, the most 'green thumb' for maintaining troglobitic *Amblyopsids* for long periods with high food supplies. But, despite many females developing some visible eggs, Bechler (personal communication) has never seen evidence of reproductive behavior much less egg-laying.

### C. Population Size and Population Density

Adaptation to low food supply in caves can perhaps be best seen by comparing the population density per hectare for the best habitats for all the species with the maximum numbers estimated from 4–12 censuses over 1–12 years. For estimates we used a multiplier factor of 1.5–2.5  $\times$  from mark-recapture population size calculations in habitats of different complexities and numbers of hiding places. The *C. cornuta* population studied by Ross and Rohde (2003) was in an area of only 50 m<sup>2</sup> and had an estimated 42,000 fish per hectare (2.5  $\times$  maximum number of 84 censused)! The *F. agassizii* population studied by Weise (1967) was in only 25 m<sup>2</sup> and had an estimated 80,000 fish per hectare (2.5  $\times$  maximum number of 104 censused) while the Mammoth Cave population studied by Poulson was in a 5400 m<sup>2</sup> habitat that had 0 fish per hectare (11 of 12 years) and an estimated 56 fish per hectare one year (2.0  $\times$  maximum number of 15 censused). Given the highest caloric cost for metabolic rate and eggs for all species (45 and 4.0 cal per day) it is not surprising that *Forbesichthys* rarely if ever reproduces in caves.

Compared to the spring-cave *Forbesichthys*, all the troglobites had higher densities in caves due to their lower metabolic rate and greater foraging efficiencies. *Typhlichthys* in Shelta Cave, Alabama, were in a 7,800 m<sup>2</sup> of prime habitat and had an estimated 229 fish per hectare (2.5  $\times$  maximum of 64 censused) plus as many as 116 crayfish and 30 shrimp per hectare. *Typhlichthys* in Blowing Springs Cave, Tennessee were in an 1100 m<sup>2</sup> area and had an estimated 524 fish per hectare (2.5  $\times$  maximum of 23 censused). With much lower metabolic rates both species of *Amblyopsis* had much higher estimated population sizes and much higher densities. *Amblyopsis spelaea* in Upper Twin Cave, Indiana, were in a 3600 m<sup>2</sup> habitat and had an estimated 417 fish per hectare (2.5  $\times$  maximum of 130 censused) and in Under the Road Cave, Kentucky in only 300 m<sup>2</sup> of habitat there were an incredible estimated 4199 per hectare (1.5  $\times$  maximum of 84 censused). And Pearson and Boston (1995) estimated 2643 *A. spelaea* per hectare in 3702 m<sup>2</sup> of Penitentiary Cave (1.9  $\times$  519 censused). *Amblyopsis rosae* in Logan Cave, Arkansas, in 1100 m<sup>2</sup> of prime habitat had an estimated 1160 fish per hectare (2.5  $\times$  maximum 51 censused) and in Cave Springs Cave, Arkansas, in 1300 m<sup>2</sup> of prime habitat had an estimated 2134 fish per hectare (2.5  $\times$  maximum of 111 censused). In summary, the estimated maximum density of fish in the 1 - 2 best habitats for each species is as follows. In food-rich surface habitats there were 42,000 *C. cornuta* per hectare and 80,000 *F. agassizii* per hectare. *Forbesichthys* in a cave had only 0–56 fish per hectare <<< 524 and 864 for *T. subterraneus* << 2643 and 4199 for *A. spelaea* >= 932 and 2134 for *A. rosae*.

Despite striking energy economies that have allowed high population densities, there appears to be density-dependent population regulation for the populations that have been most accurately censused over a period of years (Poulson 1969). The first evidence is the irregular appearance of small size classes in all troglobites (Fig. 4 in Poulson 1960). Next, there are slow growth rates when eating predominately copepods. Finally, the density-dependent population regulation inferred for *A. spelaea*, including cannibalism of 8–10 mm fry, suggests energetic limitation on reproduction despite low metabolic rates, efficient foraging, and absence of empty guts at any time.

The data that led to Poulson's (1969) inference of population regulation was based on a nine censuses from 1957–1964 of all sizes of *A. spelaea* in Upper Twin Cave, Indiana. The numbers fluctuated from 84–130 (1.55 ×) but the total mass fluctuated less from 363–465 grams (1.28 ×) and the total metabolic demand (based on routine metabolic rates) fluctuated still less from 153–180 ml O<sub>2</sub> per day (1.17 ×). In addition, the number of incubating females ranged from 0–3 per census and two instances of new hatched young. At the time, Poulson suggested that in years with highest metabolic demand there might be density-dependent cannibalism. An inadvertent manipulative experiment confirmed this hypothesis. About 30 fish of 50–70 mm SL were collected from Under the Road Cave (population 84 fish at the time) for histological and behavioral studies and guts of five fish had small fish ~ 1–2 years of age that had been cannibalized. Two years later, Poulson censused 67 fish including nine fry 9–11 mm SL and there were no cannibalized fish in the guts of the eight large fish collected for anatomical studies.

#### **D. Timing of Reproduction**

Troglobitic species in many systems are subject to pronounced seasonality in food availability and water levels (flooding). Accordingly, many species have reproductive cycles synchronized with seasonality. In Amblyopsids, peaks in reproduction occur just after spring floods (Poulson and Smith 1969). Synchronization during this time is adaptive because offspring survival is maximized. Young are produced shortly after spring floods when food availability is still high, yet mortality due to extreme flows is reduced (Poulson and Smith 1969). However, timing of these cues is unpredictable and may occur from late fall into spring. Rises in water level and alkalinity coupled with subtle drops in water temperature may be triggers to reproduction and synchronization of circannian rhythms of reproductive readiness (Poulson 1963, Jegla and Poulson 1970). Synchronization of circannian rhythms of reproductive cycles would allow

cave Amblyopsids to maximize reproductive readiness when chances for reproductive success also are at a maximum (Poulson and White 1969).

The reproductive cycle in the epigeal Amblyopsid is clear and is probably cued by photoperiod and water temperature. In a detailed year long study at one site Ross and Rohde (2003) showed that *C. cornuta* exhibits a fairly short, spring spawning season that coincides with a fast increase in water temperature from 5 to 15°C. Ovaries begin to enlarge in late fall, show peak development in winter, and all females are spent in April. Throughout the range, reproduction occurs from early March to late-April (Poulson 1963, de Rageot 1992, Jenkins and Burkhead 1994; Ross and Rohde 2003) although a few individuals may spawn in late February. Gravid females with large ova have been collected from 29 January–20 February in the Tar River drainage (Jenkins and Burkhead 1994), and free-living young as small as 9 mm have been collected in mid-May (Rohde and Ross in Jenkins and Burkhead 1994).

The seasonality of reproduction in *F. agassizii* is also clear. In the Illinois populations studied by Weise (1957) and Smith and Welch (1978), most adults presumably spawn underground in late winter based on near disappearance of adults from springs. Ova begin to enlarge in the fall reaching mature size in January when adults move underground (Weise 1957, Poulson 1963). Subterranean spawning is believed to occur from January through April and peaks in February when water levels typically are at their maximum. Fry appear and adults return to the surface by early May. The evidence for seasonality of reproduction in Rich Pond, Kentucky populations is even more clear because Hill sampled biweekly year-round in both the surface and cave habitats. In the cave in winter, both males and females have developed full gonads. Spent males and immatures leave the cave as water levels increase and start to spill out to form the seasonal surface spring run. But spent females and newly hatched young do not exit the cave until 1.5 months later. From this observation, Hill reasonably inferred some parental care though fecundities and egg volumes are much too high for branchial brooding to be possible.

Data on the yearly reproductive cycle for the cave Amblyopsids are comparatively less detailed with *A. spelaea* being the best-documented species. Poulson and Smith (1969) inferred seasonal reproductive cycles for *A. spelaea* based on times of year when gravid females, incubating females with different stages of egg development, and newly hatched and very small young are observed (unpublished data; Eigenmann 1899a). Thus in *A. spelaea*, breeding appears to occur during high water levels from February through April. Females brood eggs in their gill cavities until hatching and hold young until yolk reserves are used up. Eigenmann (1909) showed that this takes 4–5 months. Fry begin to appear in late summer and early fall

(e.g., Fig. 4 in Poulson 1960 for Under the Road Cave). The reproductive cycle of *A. rosae* is thought to closely parallel that of *A. spelaea* but the evidence is scant. Gravid females of *A. rosae* have been found from late August through December (Poulson 1963, Adams and Johnson 2001). On one occasion three 10 mm SL fish were observed in mid-July (Adams and Johnson 2001). Except for one other case of an 11 mm SL fish, the smallest fish ever observed are in the mid 20 mm range. The reproductive cycle of *T. subterraneus* appears to be similar but even less evidence is available. Breeding likely occurs in the spring when water levels are at their highest. However, no newly hatched fish have ever been seen or collected and the smallest fish ever observed documented are 15–20 mm (Fig. 4 in Poulson 1960 for Shelta Cave, Alabama) that are possibly nearly a year old. No data on the reproductive cycle of *S. poulsoni* exist.

## E. Growth and Development

Generally, cave organisms exhibit reduced growth rates and delayed development and maturity compared to related surface species. Reduced growth rates represent an adaptive response to low food supplies in cave environments because less energy over a given amount of time is needed (Hüppop 2000). Within the Amblyopsidae, growth and developmental rates decrease with increasing cave adaptation (Poulson 1963).

The fastest growth rates occur in the epigeal species. First-year swampfish from North Carolina grow 9.0 mm SL on average from June to January and reach a mean size of 32.6 mm SL by 22 months of age (Ross and Rohde 2003). Slow to no growth occurs during summer, presumably because of lower feeding rates during this time period. An explanation for slow or no growth in the summer might be elevated metabolic rates because of elevated habitat temperature combined with reduced food supplies.

First year spring cavefish grow 10–20 mm per year on average and also exhibit variable growth rates from season to season (Smith and Welch 1978, Hill 1966). Hill (1971) studied squamation and pigmentation development in *F. agassizii*. Scale primordia first appear on the caudal peduncle at around six weeks. By 12 weeks, both squamation and pigmentation pattern are well developed.

Growth rates for troglotic Amblyopsids are substantially slower with estimates of 1.0–1.25 mm month<sup>-1</sup> for *T. subterraneus*, 1.0 mm month<sup>-1</sup> for *A. spelaea*, and 0.9 mm month<sup>-1</sup> for *A. rosae* based on putative annual scale marks (Poulson 1963 estimated the number of annuli on the scales photographs shown in Figure 8 of Woods and Inger (1957) as 4+ *T. subterraneus*, 3+ to 4 *A. rosae*, and 4+ to 6 *A. spelaea*). Using mark-



recapture, Brown (1996) observed growth rates in *A. rosae* at Logan Cave lower than those inferred by Poulson from scale marks (1963). Some fish species do not always show annulus formation because limited feeding precludes annulus development. Thus, it may be reasonable to assume that some cave Amblyopsid populations fail to form annuli in a given year because of low food availability (D. Bechler, personal communication). Therefore, age and growth estimates based on annuli counts are surely underestimates.

Excluding 20 individuals that decreased in size, Brown and Johnson (2001) observed growth rates of 48 *A. rosae* that were recaptured at least once. Growth rates declined with increasing size. Individuals 30–39 mm TL grew, on average, 0.7 mm month<sup>-1</sup>; 40–49 mm TL, 0.3 mm month<sup>-1</sup>, and 50+ mm TL, only 0.06 mm month<sup>-1</sup>. However, some individuals experienced much higher growth rates >1 mm month<sup>-1</sup>. Observed spurts in growth were not always correlated with increasing food availability, and are influenced by other factors including age, sex, reproduction, and other exogenous factors (Brown 1996, Trajano 2001). One explanation for observed growth spurts is the rare capture of large prey, such as salamander larvae or crayfish (Poulson 2001b). With a highly efficient metabolism, these rare feeding events could lead to significant spurts of growth much higher than average.

Sexual maturity is also delayed in cave Amblyopsids. Both *C. cornuta* and *F. agassizii* reach sexual maturity around 12 months of age. The development of the Y-shaped snout appendage in male *C. cornuta* parallels sexual maturity. Ross and Rohde (2003) documented the development of this appendage noting that it is not discernable by 16 mm SL, but can be readily observed by 23–25 mm SL with full development by 25 mm SL at 13–14 months. On the other hand based on scale mark estimates of growth rate, *T. subterraneus*, *A. rosae*, and *A. spelaea* may take a minimum of 2, 3, and 4 years respectively, to reach sexual maturity. Estimates for the cave species are based on Poulson's (1963) original examination of growth annuli, and, therefore, are very conservative. More reasonable estimates are 4, 6, and 10 years for age at first reproduction of *T. subterraneus*, *A. rosae*, and *A. spelaea*.

Vent migration is characteristic of the Amblyopsidae and Aphredoderidae (Poulson 1963). Like other developmental stages, time from hatching to completion of vent migration is longest in the troglobitic species (Poulson 1963). Ross and Rohde (2003) have traced migration of the vent in swampfish. Anterior migration of the vent with increasing body size is most rapid from 10–17 mm SL and decreases significantly with very little change  $\geq$  19 mm SL. Hill (1966) also found that vent migration to the jugular position was complete in 16–18 mm SL *Forbesichthys*. And,

Eigenmann (1909) found that the process took longer for *A. spelaea*. As in all species the vent is located posterior to the pelvic fins in newly hatched young. But in *A. spelaea* 25 mm in length, the vent has only migrated forward in front of the pelvic fins and by 35 mm, it is still positioned just posterior to the pectoral fins.

## F. Longevity

Increased longevity of cave organisms compared to their surface relatives is one of several life history adaptations toward a low-r strategy by which cave organisms cope with limited food resources. Prolonged life spans, coupled with a trend from semelparity to iteroparity, increases the chance of population persistence over time, as a population is less likely to be extirpated during times of extremely low food supplies that result in little to no recruitment (Hüppop 2000).

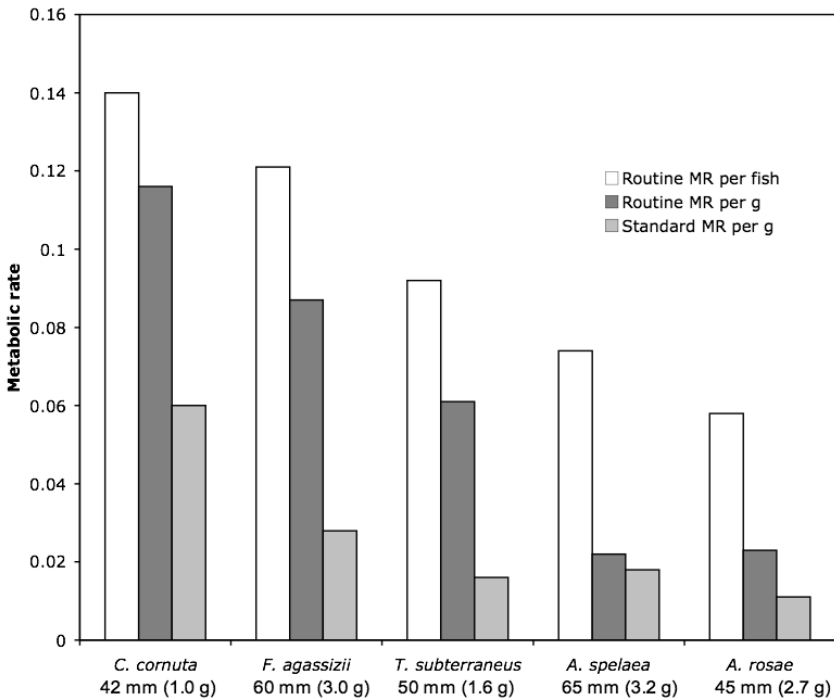
This pattern is evident in the Amblyopsidae as longevity inferred from scale marks increases with increased adaptation to cave environments (Poulson 1963). The shortest life spans are observed in the epigeic *C. cornuta* and troglophile *F. agassizii*. Previously, maximum longevity of *C. cornuta* was estimated at 14–15 months (Poulson 1963) with a few two-year-old fish reported (Rhode and Ross 1986). However, a recent study indicates that several swampfish lived up to 22 months with one living 26 months (Ross and Rohde 2003). Spring cavefish are known to live up to three years (Hill 1966, Smith and Welch 1978). It is likely that, as in many short-lived species, death occurs after a single reproductive attempt (semelparity). Therefore, older individuals are those that simply did not acquire enough resources to reproduce at a younger age.

Troglobitic species live considerably longer. Conservatively based on scale marks, southern cavefish were estimated to live 3–4 years (Poulson 1963); however, individuals have been maintained in captivity for over a decade and likely live considerably longer than initial estimates in nature (Noltie and Wicks 2001). Poulson (1963) originally estimated the average longevity of the more troglomorphic species, *A. spelaea* and *A. rosae*, at about 7 and 5 years, respectively. However, Poulson (2001) later questioned his original longevity estimates of the troglobitic species stating they may be off by a factor of 3–4, partly because of the difficulty in determining scale annuli in larger individuals but primarily because of observed growth rates of marked individuals in nature (see above Growth Rates). Accordingly, maximum life spans for *A. spelaea* and *A. rosae* may actually be as long as 24–28 and 15–19 years, respectively. The maximum life span of *S. poulsoni* is unknown, although the largest specimen collected, a 58.3 mm SL female, was estimated to be as old as 8 years (Cooper and Kuehne 1974, USFWS

1982). This estimate was based on the analysis by Poulson of scale marks in a 42 mm SL individual based on the same the criteria used to estimate age in other Amblyopsids. One clearly larger *S. poulsoni* have been observed but not measured in Key Cave (J. Cooper, personal communication) and unquestionably represents an older individual.

## G. Metabolic Rates

A trend toward reduction in metabolic rate with increasing cave adaptation exists within the Amblyopsidae (Poulson 1963, 1985). Poulson (1963) found that standard, routine, and active metabolic rates decrease with increasing cave adaptation (Fig. 13). The cause of decreased standard metabolic rate is a combination of a decrease in gill surface area and reduction in the volume and rate of ventilation (Poulson and White 1969, Poulson 2001a). Poulson (2001a) found that the strongest correlates of metabolic rate reduction in Amblyopsids were reduction in ventilation frequency and volume > reduction in brain metabolic rate > reduction



**Fig. 13** Standard and routine metabolic rates (MR) in Amblyopsid cavefishes in  $\text{ml O}_2 \text{g}^{-1} \text{h}^{-1}$ . Routine metabolic rate for sizes of fish specified are in  $\text{ml O}_2 \text{hr}^{-1}$ .

in gill surface area. No single physiological or morphological trait was in the same rank order as the overall decline in metabolic rate. However, collectively the reductions were in the same order as metabolic rates. To Poulson this is consistent with a general pattern of different pathways to convergent evolution of complex traits. Among Amblyopsid species there were no differences with cave adaptation in histological indices of thyroid activity in adults, although subtle increases in thyroid follicle cell height and colloid vacuolization from the non-growing season (fall/winter) to the growing season (spring/summer) were detected. This was most noticeable within *A. rosae*.

Adams and Johnson (2001) documented a statistically significant effect of body mass on metabolic rate that differed among seasons for a population of *A. rosae* at Logan Cave in Arkansas. A positive relationship was observed during summer and autumn followed by a negative relationship during winter and spring. The authors speculated that seasonal relationships between body mass and metabolic rate may reflect alterations in environmental conditions, such as food availability and ambient dissolved oxygen, however other factors, such as biased sex ratios or seasonal and size susceptibility to handling stress may influence metabolism. But Poulson wonders whether the effects are biologically significant given the small sample sizes and no plausible explanations.

In a study on acclimation of metabolic rate to temperature (next section) the routine metabolic rate of many individuals of each species was measured at 15°C. These data are more extensive than Poulson's (1960) data and include fish from other localities so it is of interest to compare the results. The data are for *C. cornuta* (4 of 23–32 mm SL from Cashie Creek, South Carolina), *F. agassizii* (10 of 27–32 mm from Pine Bluffs, Illinois and 14 of 32–68 mm from Rich Pond, Kentucky), *T. subterraneus* (13 of 29–41 mm from Cave City, Kentucky), *A. spelaea* (13 of 35–70 mm from Sig Chatlet Cave, Kentucky), and *A. rosae* (7 of 36–46 mm from Cave Springs Cave, Arkansas).

Here are minimum and maximum routine metabolic rates ml O<sub>2</sub> g<sup>-1</sup> hr<sup>-1</sup> at 15°C with the average and the range among individuals (standardized to a 1 gram fish):

<i>C. cornuta</i>	maximum 0.190 (0.142–0.298) and minimum 0.116 (0.095–0.132)
<i>F. agassizii</i>	maximum 0.137 (0.039–0.396) and minimum 0.087 (0.029–0.092)
<i>T. subterraneus</i>	maximum 0.082 (0.026–0.181) and minimum 0.061 (0.015–0.136)
<i>A. spelaea</i>	maximum 0.037 (0.015–0.093) and minimum 0.023 (0.010–0.023)

*A. rosae* maximum 0.031 (0.012–0.070) and minimum 0.026 (0.008–0.070)

This decline in routine metabolic rate *C. cornuta* > *F. agassizii* >> *T. subterraneus* > *A. spelaea* = *A. rosae* is really close to that reported by Poulson in (1985).

## H. Tolerance of Temperatures from 5 to 25°C

In these studies of acclimation, data were taken in a torus respirometer. An oxygen electrode was used to measure metabolic rate and an infrared detector was used to measure spontaneous swimming activity.

The results are that metabolic rate and spontaneous activity of all Amblyopsid species acclimate to a wide range of temperatures. This is yet another bit of evidence that belies the constant cave paradigm. In retrospect, it is not surprising that even troglobitic Amblyopsids acclimate since they must deal with floods that bring in prey or allochthonous organic matter that their troglobitic prey need. At the end of winter, rain and snow melt result in cold water entering the cave. In more rare summer floods, the water entering the cave is warm. The Pine Bluffs populations of *F. agassizii* are in springs that only vary from 11–15°C but they acclimate to temperatures of 5, 10, 15, 20, and 25°C the same way as fish from Rich Pond, Kentucky that experience water temperatures of 7–26°C.

The data (Poulson, previously unpublished) show that there is no difference in degree of acclimation among the species and each species shows acclimated metabolic rates and spontaneous activity that are essentially the same from 5 to 25°C. Thus, each species shows almost perfect compensation for temperature. Put another way, metabolic rates and activity are the same in fish acclimated to 5, 10, 15, 20, and 25°C (no significant differences between rates at any temperature for any species). The process of acclimation takes 1–3 weeks depending on the temperature difference. Initially, fish taken from 15 to 5°C show a reduced metabolic rate and activity and fish taken from 15 to 25°C show an elevated metabolic rate and activity.

## I. Starvation Resistance

Poulson (1961, 1963) crudely compared starvation resistance in Amblyopsids and found increasing ability to cope with food scarcity from the epigeic *C. cornuta* to the most troglobitic species, *A. rosae*. *Chologaster cornuta* showed the greatest loss in body mass and lowest starvation resistance, expiring after only 45 days (Table 6). On the other hand, *A. rosae* lost only 9% body mass after 20 days and survived over 13 months

**Table 6** Effects of starvation on Amblyopsid cavefishes under aphotic conditions (from Poulson 1960). Decreasing standard metabolic rate (SMR) reflects decreased rate of weight loss during starvation and decreased routine metabolic rate (RMR) reflects increased time until spontaneous activity stops during starvation. Data standardized to 45 mm SL fish. Metabolic rates in ml O<sub>2</sub> g<sup>-1</sup> hr<sup>-1</sup>.

Species	Habitat	Body mass <sup>1</sup>	SMR	Activity <sup>2</sup>	Minimum – Maximum RMR
<i>C. cornuta</i>	Swamp	34	0.065	46	0.085–0.298
<i>F. agassizii</i>	Spring	24	0.028	116	0.029–0.396
<i>T. subterraneus</i>	Cave	15	0.016	230	0.015–0.181
<i>A. spelaea</i>	Cave	13	0.018	280	0.006–0.093
<i>A. rosae</i>	Cave	9	0.011	400	0.006–0.070

<sup>1</sup> % loss of body mass after 20 days

<sup>2</sup> End of spontaneous activity in days

without food. *Amblyopsis spelaea* can survive up to 20 months without food (Sloan in Eigenmann 1909).

Starvation resistance in cavefish can be achieved by four primary means, acting alone or in concert (Hüppop 2000): 1) consumption of large amounts or a single large prey item at a single feeding event, 2) reduction of activity and metabolic rate, 3) storage of large amounts of fat, and 4) reduction of the digestive absorptive epithelium or reduction of gut length. The first three mechanisms are evident in Amblyopsids. Large prey items occasionally have been observed in the stomach contents of collected fish. Second, metabolic rate decreases within increasing cave adaptation (see Metabolic Rates above and Poulson 1960, 1985, 2001). However, in Poulson's studies, activity of some of the trogllobites actually increased over the first six weeks before decreasing later. The observed initial increase in activity has been attributed to searching for food. Lastly, although fat was not directly measured, Poulson (1960) noted that the trogllobitic species possessed larger fat stores compared to *C. cornuta* and *F. agassizii*. Thus, fat reserves (though much lower than in *Prietella* and *Astyanax* cavefish), coupled with low metabolic rates explain the increased survival time of trogllobitic Amblyopsids under starvation (Hüppop 2000).

However, more detailed studies, like those on other cavefishes (especially by Hüppop 2000), are needed to assess the ability to recover weight after varying amounts of weight loss and to store large amounts of fat quickly when food is available. In addition, it is important to compare the mechanisms of resistance to starvation seen in epigeal fish to determine the degree of preadaptation. This has been done for *Astyanax fasciatus* (Hüppop 2000) but not for Amblyopsids. For example, all vertebrates, including humans, when deprived of food first use glycogen, then fat, and as a last resort protein resulting in muscle wasting. Associated with these changes is a reduction in activity and basal metabolic rate.

## J. Circadian and Circannian Rhythms

Little information exists about endogenous cycles in cave organisms (Langecker 2000) but some evidence is available for cave Amblyopsids. Some troglotic species have retained circadian oxygen consumption but have lost circadian activity, as their rhythms cannot be entrained to light-dark cycles (Poulson and Jegla 1969, Poulson and White 1969). This has been viewed as evidence that entrainment of activity cycles to the environment is not maintained by selection in subterranean habitats (Poulson and White 1969) as has been observed in *F. agassizii* (Poulson and Jegla 1969). With regard to endogenous annual (circannian) rhythms, both *A. spelaea* and *A. rosae* exhibit a well-defined yearly reproductive cycle (see above 'Timing of Reproduction'). Because caves do not have reliable seasonal cues (Poulson and Smith 1969), circannian rhythms are adaptive to maximize reproductive readiness when chances for reproductive success also are at a maximum at unpredictable times of the year (Poulson and White 1969).

## K. Agonistic Behavior

Agonistic behavior of Amblyopsids has been investigated in detail by Bechler (1980, 1981, 1983). He examined intraspecific dyadic interactions (1 resident and 1 intruder) in 80 liter or 160 liter aquaria with a rock hiding place in each. Only *C. cornuta* showed no agonistic behavior and Poulson suggests that this is because no resources are defendable in its epigeal plant and debris-choked habitat with extremely high food densities.

Among the four other species Bechler documented two submissive acts, "freeze" and "escape", in all species. This is the only study to document "freezing" in hypogean fishes (Parzefall 2000). Poulson has observed freezing by recently hatched *A. spelaea* under threat of cannibalism. Large *Forbesichthys* also cannibalize smaller fish (Hill 1966) and freezing should be an excellent defense since Amblyopsids use only lateral line to detect other fish and prey. Thus it is not surprising that fish that perceived that they are losing in an agonistic encounter 'froze' more often than escaping by fleeing. This allowed them to avoid the most intense kinds of acts.

In order of increasing intensity and decreasing frequency Bechler documented tail beat > chase > attack = head butt > bite and jaw lock (Table 7). The only metric that was in the order of increasing time isolated in caves was total number of agonistic bouts with *F. agassizii* 158 >> *T. subterraneus* 59 = *A. spelaea* 61 >> *A. rosae* 21. Mean duration of bouts had *T. subterraneus* and *A. spelaea* reversed in rank order with *F. agassizii* 53 sec = *A. spelaea* 57 sec >> *T. subterraneus* 26 sec > *A. rosae* 18 sec. Of the six

**Table 7** Agonistic behavior in Amblyopsid fishes. From Bechler (1983). In paired interaction tests (1 resident and 1 intruder) *C. cornuta* showed no agonistic behavior. Among the one troglophile and three troglobites, three species showed five to six of six kinds of acts (*F. agassizii* = *A. spelaea* <= *T. subterraneus*) but the most cave-adapted species, *A. rosae*, showed only one of six. See text for interpretation.

Species	<i>C. cornuta</i>	<i>F. agassizii</i>	<i>T. subterraneus</i>	<i>A. spelaea</i>	<i>A. rosae</i>
Habitat	Surface	Spring/Cave	Cave	Cave	Cave
Aggressive acts					
Tail-beat	-	+++	+++	++	++
Head-butt	-	+	+	++	-
Attack	-	+++	+	+	-
Bite	-	+	+	++	-
Chase	-	+	++	++	-
Jaw-lock	-	+(2)	-	+(1)	-
Submissive acts					
Freeze	-	+++	+++	+++	+++
Escape	-	++	++	++	++
Total no. acts	0	158	59	61	21
Mean duration of acts (sec)	0	53	26	57	18

- not observed; + rare; ++ regular; +++ frequent; ++++ always

possible aggressive acts there was less of a trend among species with *F. agassizii*, *T. subterraneus*, and *A. spelaea* showing 5–6 acts and *A. rosae* with showing only tail-beating (Table 7).

One clear difference among species is that *F. agassizii* and *T. subterraneus* always initiated agonistic acts from under or next to rock shelter whereas *A. spelaea* and *A. rosae* patrolled the whole tank and did not set up “stations”. This is consonant with the high importance of thigmotaxis to *F. agassizii* and *T. subterraneus* (see Thigmotaxis above). Bechler (personal communication) noted the same prevalence of thigmotaxis with long periods of rest in *F. agassizii* and *T. subterraneus* in a 6.3 m long 4.1 m<sup>2</sup> artificial stream with many rocks. And he noted that both *A. spelaea* and *A. rosae* were active almost all the time and did not set up “stations”. To make these observations, Bechler recorded the behavior of four individuals of each species periodically by videotape for 30 days. The four individuals of each species “encountered each other” (within 15 cm) much more infrequently and showed many fewer agonistic interactions per encounter than did the two individuals in aquaria of orders of magnitude smaller volumes. In fact, in aquaria pairs of individuals always were agonistic when encountering each other whereas in the stream the percentages of encounters with any agonistic behavior were 55% *T. subterraneus* > 27% *A. spelaea* = 26% *A. rosae* >= 21% *F. agassizii*. In addition, the bout lengths were



shorter and the agonistic acts less intense than in aquaria. The density of four fish in 4.1 m<sup>2</sup> of artificial stream translates to 9,662 per hectare, which is twice the highest we have ever estimated in nature for any cave Amblyopsid. So, it seems to us that in caves encounters would be even less frequent. In fact, neither we or Bechler (personal communication) have ever seen agonistic interactions in the field with hundreds of observations of fish within 15 cm of one another.

Bechler (1983) viewed degenerative evolution as the most likely mechanism for reduction of agonistic behavior in Amblyopsids suggesting conservation of energy due to other adaptations to subterranean life. Thus reduction in metabolic rate and fecundity with increased swimming efficiency reduced selective pressures caused by limited food availability and allowed for reduction in agonistic behavior. He suggested that retention of some levels of agonistic behavior in *A. spelaea* might reflect lower levels of subterranean adaptation or the possibility of defense of prime foraging areas in riffle and pool cave streams. Agonistic behavior has not been observed in *A. spelaea* in nature and Poulson thinks that it is unlikely.

Poulson (this volume) argues that there seems to be no defensible resource in caves and so we should not expect territoriality and associated agonistic behavior to be important. This suggestion is consistent with the lack of any agonistic behavior in *C. cornuta* where there is clearly no defensible resource or food in the dense vegetation in which it lives.

## L. Territoriality and Social Groups

Although epigeal species are often territorial or form social groups, troglitic species generally are found in low population densities and usually solitary with a large home range (Langecker 2000). In general, populations of *A. spelaea* and *T. subterraneus* are low in density (but see Poulson 1969 and above TESTS OF HYPOTHESES: FECUNDITY AND EGG SIZE) and individuals are irregularly distributed over suitable habitat (Poulson 1963). Individual fish have large home ranges, cover long distances in search of food, and never defend areas (Poulson 1963, Mohr and Poulson 1966). However, during aggression trials by Bechler (1983), *F. agassizii* and *T. subterraneus* established distinct territories in aquaria with rocks ("stations"), whereas the less sedentary species, *A. spelaea* and *A. rosae*, patrolled the entire tank. Cavefish do not display schooling behavior, although individuals are sometimes observed in close proximity typically around food sources (e.g., underneath a bat roost). Even in caves with the largest populations, 70-80% of fish are found in perhaps only 10-20% of the accessible cave stream (Brown and Johnson 2001, Poulson, unpublished data, Pearson, personal communication).

## M. Food Habits

The reported diet of Amblyopsids has been well characterized and was treated in the context of prey detection earlier in this chapter (V. Prey Detection and Availability). All Amblyopsids eat live, moving prey with invertebrates comprising most of the diet. However, cannibalism has been documented in the family and small amounts of nonliving food such as bat guano and detritus have been observed in the stomach contents of some species. These are probably ingested along with live prey but would provide very much lower nutritional benefit per volume than live prey.

Swampfish are likely crepuscular or night feeders (Poulson 1963) principally feeding on amphipods, chironomids, and cladocerans with amphipods comprising the majority of diet (G.B. Pardue and M.T. Huish in Jenkins and Burkhead 1994, Ross and Rohde 2003). Other potential prey items include nematodes, ostracods, ephemeropterans, odonates, plecopterans, megalopterans, coleopterans, trichopterans, and other dipterans. Fifty-three percent of 289 stomachs of day-collected fish examined by Ross and Rohde (2003) were empty from one North Carolina site; however, several fish had noticeable food in the intestines. Prey diversity and percent occurrence and abundance of each organism in the diet is greatest from January–April then substantially decreases in summer and fall (Ross and Rohde 2003). Cannibalism has not been reported in *C. cornuta*.

The diet of *F. agassizii* varies geographically and between cave and surface populations. Illinois populations feed almost exclusively on *Gammarus* amphipods (Forbes and Richardson 1908, Layne and Thompson 1952, Gunning and Lewis 1955, Weise 1957), although amphipods (Weise 1957), insect remnants (Gunning and Lewis 1955), and detritus (Gunning and Lewis 1955) also have been found in stomachs. In Kentucky, surface fish feed principally on chironomids but also copepods, oligochaetes, nematodes, and ostracods (Hill 1969a). On the contrary, individuals of the same population are strongly cannibalistic on younger individuals when in the cave part of the habitat. In caves cannibalism may represent an alternative feeding strategy in response to competition for more typical but extremely rare invertebrate food sources. In rare years when *F. agassizii* occurs in the Mammoth Cave food supply is much greater than usual (Poulson, unpublished data).

The diets of the troglobitic Amblyopsids consists largely of copepods and isopods but rare, larger meals, such as crayfish, salamander larvae, or conspecifics, may result in high growth efficiency and a burst in growth rate (Poulson 2001). An assortment of prey have been reported in stomach contents of *T. subterraneus* including copepods, amphipods,

isopods, trichopteran and tenebrionid larvae, cladocerans, isopods, and crayfish (Poulson 1960, 1963, Cooper and Beiter 1972); however, copepods are the primary food source accounting for 60–90% of the diet by volume (Poulson 1963). The diet of *A. spelaea* includes copepods, amphipods, isopods, and salamander larvae (Clay 1975). *Amblyopsis spelaea* smaller than 45 mm total length chiefly eat copepods, whereas those in excess of 45 mm may consume amphipods, isopods, and crayfish (Poulson 1963). Larger fish also occasionally cannibalize smaller fish. Few studies have investigated the food habits of *A. rosae*, but gut contents show that the diet of Ozark cavefish comprises the majority of available food sources in the caves they inhabit. Copepods constitute up to 90% of the diet by volume, but isopods, amphipods, cladocerans, ostracods, small crayfish, coleopteran larvae, plecopterans, crickets, and small salamanders also are taken (Poulson 1960, 1963). Bat guano has also been found in stomach contents. It has been suggested that younger individuals are cannibalized. However, Adams and Johnson (2001) observed a presumed parental-young aggregation of *A. rosae* for more than six weeks with no evidence of cannibalism. It remains unknown how long such parent-kin interactions occur and whether kin recognition exists. Based on stomach contents, extent of fat deposits, and annulus formation, *A. rosae* fed less in the fall than other times of the year (Poulson 1960). The diet of *S. poulsoni* has not been examined but likely consists of amphipods, isopods, and small crayfish (Boschung and Mayden 2004).

## N. Predators

Troglobitic Amblyopsids are at the top of the food chain in most cave systems that they inhabit, and, therefore, have few natural predators. Epigeal fishes may potentially prey on cave Amblyopsids (Brown 1996, Poly and Boucher 1996), as well as crayfish (Romero 1998a), and troglobitic salamanders. Young cavefish are susceptible to cannibalism by larger adults (Poulson 1963), as has been documented in *A. spelaea* and one cave population of *F. agassizii*. Cannibalism may serve as one means to regulate population densities in a food-limited environment (Poulson 1969). Epigeal populations of *F. agassizii* likely are occasional prey for other fish, snakes, birds, and mammals (Smith and Welch 1978). At Rich Pond in Kentucky, natural predation is seasonally heavy (J.E. Cooper in Smith and Welch 1978). Epigeal predators, such as raccoons, fish, and water snakes, also may be potential threats to cave-inhabiting Amblyopsids; however, no cases of predation on cave-dwelling Amblyopsids by epigeal predators have been documented even in cave entrance areas.

## O. Parasites and Diseases

Like many cave vertebrates, few parasites have been observed afflicting Amblyopsid hosts. Proteocephalan cestodes have been collected from the pyloric caeca of *A. spelaea* and *F. agassizii*. Three *Proteocephalus poulsoni* were collected from two northern cavefish from Under the Road Cave in Breckinridge Co., Kentucky (Whittaker and Zober 1978). Whittaker and Hill (1968) described *P. chologasteri* from spring cavefish. In southern Illinois, 71 percent of fish examined were parasitized by cestodes and other internal parasites (G. Garoian in Smith and Welch 1978). The acanthocephalan *Neoechinorhynchus cylindratus* has been reported from the intestines of *A. spelaea* (Nickol and Whittaker 1978) but neither frequency nor incidence was reported. In cursory examination of many Amblyopsid guts for food item analyses, Poulson has never seen an obvious parasite.

Reports of external parasites are even fewer. A species of copepod, *Cauloxenus stygius*, is an obligate ectoparasite found on the upper lip of northern cavefish. Little is known about the species. Its distribution is believed to be that of its host but the parasite has rarely been observed and few records exist (Cope 1872, Blatchley 1897, Giovannoli 1933, Lewis 2002ab, Poulson, unpublished data). In other Amblyopsids, small, unidentified leeches also have been reported for *F. agassizii* in Illinois (Smith and Welch 1978). Additional studies using a comparative framework are needed to not only document additional parasitic taxa, but to also compare frequency and density of occurrence between the epigeal, troglolithic, and troglobitic Amblyopsids.

Few diseased Amblyopsid cavefishes have been reported in nature. Fournie and Overstreet (1985) reported on an adult *F. agassizii* from Union Co., Illinois, with a retinoblastoma on the right side of the head. This condition may be related to chromosomal abnormalities. At least one other individual collected at the same spring had a similar tumor in appearance and eventually died after the tumor involved the entire head. However, this specimen was not available for histological examination (Bechler, pers. comm. in Fournie and Overstreet 1985).

In troglobitic Amblyopsids, Pearson and Boston (1995) documented a bacterial infection of the fins of *A. spelaea* at Donaldson Cave, Lawrence Co., Indiana. All individuals observed had extremely shortened pectoral and caudal fins with ragged margins. Many fish also had small, red spots scattered over the body. During a recent trip to Donaldson Cave, this condition was not observed (Niemiller, unpublished data). Broken-back syndrome was noted for only three years in perhaps 10% of fish from this site. The likely cause was exposure to pesticide and other chemical contamination since the surface watershed was in row crops and pasture

(Keith and Gray 1979, Keith and Poulson 1981). Gas bubble disease has been documented in recently collected *T. subterraneus* at a spring site in Missouri (Schubert *et al.* 1993). Southern cavefish from Missouri may be particularly susceptible to this disease because of the depths at which individuals reside (Schubert *et al.* 1993, Noltie and Wicks 2001).

There is some logic to believe that caves may be a refuge from many kinds of diseases or parasites so Poulson (this volume) has suggested that we consider this hypothesis carefully and at least keep good records. A corollary is that absence of parasitism and low incidence of disease may have led to evolutionary rudimentation of immune defenses. However, the recovery of many individual *A. spelaea* from fin rot in one cave (W. Pearson, personal communication) may be evidence of perfectly adequate immune responses. It will be worthwhile to compare the Amblyopsids for incidence of and recovery from common aquarium fish diseases.

## P. Abundance and Population Sizes

Few studies have attempted to quantify population sizes and relative abundance of Amblyopsids, including *C. cornuta* and *F. agassizii*. The few studies that have attempted to quantify population sizes via techniques such as mark-recapture or survey removal have focused on caves that are known to contain relatively large populations. Other studies for which the most reliable estimates of abundance have been obtained have focused on the species of conservation concern. Additional demographic studies, including long-term censuses, are needed for both epigeal and subterranean populations.

In general, the majority of cave Amblyopsids localities yield few fish sightings during single surveys. Although this may be a real reflection of actual abundance in some instances, it is important to realize that the distribution and abundance of these troglobitic species, perhaps with the exception of *S. poulsoni*, likely is greater than currently realized. Localities for which cavefish have been reported represent but a fraction of total available habitat accessible to fish. This fact was clearly illustrated during the Maramec fertilizer pipeline break that resulted in the death of nearly 1,000 southern cavefish and likely many more (see discussion in Noltie and Wicks 2001) from a drainage basin with few records documented previously. The problem with inferring population densities from such fish kill is that we do not know the volume or extent of habitat impacted.

Most observations of cavefish are restricted to caves near the surface and there is some controversy as to whether even the best cavefish caves are sources or sinks (see Poulson, this volume). Poulson believes that populations that we can sample make up the majority of the total number

of fish in a cave watershed. Part of his logic is that cavefish will come to be found in the highest available food areas and these are likely to be in shallow caves with allochthonous inputs and a combination of deep pools and shoals. Further we usually find most fish in the highest food supply areas of these caves (see above 'Population Size and Population Density'). He also argues that only few fish will be in deep phreatic habitats with little food input or the most upstream parts of caves inaccessible to humans. He bases this second inference on both Cave Springs Cave, Arkansas, with about 100 *A. rosae* seen on each visit, and Upper Twin Cave, Indiana, with 84–130 *A. spelaea* seen on each visit. As one goes far upstream in both these caves the number of cavefish drops to none as the water gets shallower and faster flowing with no refuges during floods.

Consequently, habitats where we see no fish or only a few at each visit may be population sinks and not sources. Wells and short stream segments encountered in an otherwise dry cave may not be representative of the habitat that most troglobitic Amblyopsids inhabit. To be sure cavefish can disperse through and occupy submerged passages inaccessible to humans but these habitats are probably neither usual for the fish nor optimal. This does not mean, however, that these fish are doomed. They may at least be potential dispersers. They could move long distances given their long lives, low metabolic rates, and foraging efficiencies. In those caves where we always see 6–10 fish they may even be slowly reproducing. If so, these sinks could become a source for re-colonization if some disaster befalls the fish in the best caves or habitats.

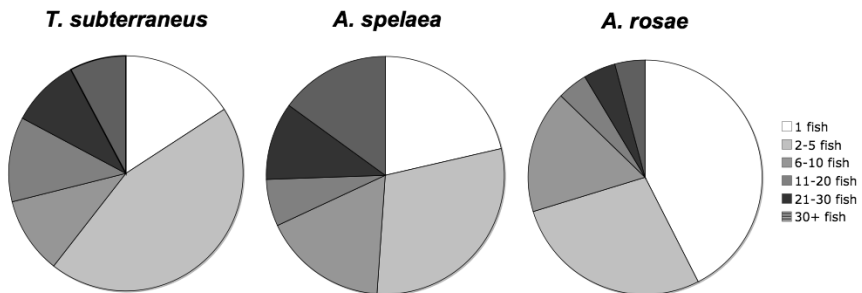
Swampfish are reported as generally rare or uncommon throughout their range (Poulson 1963, Cooper and Rohde 1980, Shute *et al.* 1981, Jenkins and Burkhead 1994). However, its reported rarity may be more of a sampling artifact than a reflection of true abundance (Ross and Rohde 2003). Abundance estimates may be biased as most sampling occurs during daylight (Jenkins and Burkhead 1994), whereas *C. cornuta* are most active at night and occur in habitats difficult to seine or electro-fish. In Virginia, the largest series was taken in the Blackwater River drainage via a nonselective ichthyocide (Jenkins and Burkhead 1994). Poulson found that repeated kick-seining at the edges of dense weed beds routinely led to capture of 1–3 swampfish at each attempt for up to six repeats at the same spot. Ross and Rohde's made many collections over a year in a short section of stream and the maximum density estimated from their data is 42,000 per hectare ( $84 \times 2.5$  in  $50 \text{ m}^2$ ).

Historically, *F. agassizii* has been considered rare to uncommon throughout much of its range. Smith and Welch (1978) estimated less than a thousand individuals from eight springs in Union Co., Illinois, and around 40 individuals at Cave Springs Cave in Union Co. However, many

hundreds of specimens have been accessioned from a large spring run at Rich Pond in Warren Co., Kentucky. In Tennessee, *F. agassizii* can be locally abundant in ideal habitats (Etnier and Starnes 1993), and, like *C. cornuta*, is easily overlooked because of its nocturnal and reclusive habits (Smith and Welch 1978). Apparent abundance of *F. agassizii* is dependent upon light levels, groundwater velocity, and season (N.M. Welch in Smith 1979). Pine Hills IL spring runs have as many as 80,000 fish per hectare whereas in Mammoth Cave most often had 0 fish per hectare and 50 per hectare only in a year with high food input (Poulson 1969).

We suggest that most localities for cave Amblyopids are population sinks and a very small percentage are population sources. Figure 14 shows that there were <10 fish per survey in 68% of *A. spelaea* caves, 72% of *Typhlichthys* caves, and 87% of *A. rosae* caves. Conversely, there were 30+ fish per survey in only 5% of *A. rosae* caves, 8% of *Typhlichthys* caves, and 14% of *A. spelaea* caves. This pattern is even more accentuated if we consider the 2–3 caves that have 46–87% of all individuals observed for each species.

For *Typhlichthys* the top three caves account for 46% of the 354 fish censused in 39 caves by Niemiller. One population in Putnam County, Tennessee, had 121 fish in a 400 m stream section (Niemiller, personal observation) with an estimated 864 per hectare. In Shelta Cave, Alabama, on one trip there were 64 fish in an area under a gray bat colony (Poulson 1960) with an estimated 229 per hectare ( $64 \times 2.5$  in 7000 m<sup>2</sup>). In several caves, we routinely see > 40 fish per visit and in two caves these moderate numbers are consistent over a period of years. From most to least numbers per trip, Herring Cave in Rutherford Co, Tennessee had 47, 39, 37, and 32 fish. Blowing Springs Cave in Coffee Co., Tennessee had 52, 37, 31, and 26



**Fig. 14** Portion of cave Amblyopsid localities with the maximum number of observed fish during a single survey correspond to the following categories: 1 fish, 2–5 fish, 6–10 fish, 11–20 fish, 21–30 fish, and 30+ fish.

fish including recent surveys by Niemiller and surveys from the 1960s by Poulson.

For *A. spelaea*, the top three caves by number of fish observations account for 87% of the 1591 fish censused in 47 caves, and these caves have had high populations at every survey over the past 50 years (Poulson 1969, Pearson and Boston 1995). During their surveys in the early 1990s, Pearson and Boston (1995) conducted mark-recapture and census studies in several *A. spelaea* caves in Indiana and Kentucky. During a single trip in March 1994, they counted 515 fish at Penitentiary Cave in Breckinridge Co., Kentucky. Over 100 fish were observed during single surveys of two other caves in Kentucky and the Donaldson-Twin Cave complex in Lawrence Co., Indiana. Blue Springs Caverns is also reported to contain a large *A. spelaea* population in excess of 150 individuals (Welch in Keith 1988). Poulson (1969 and VI C this chapter) estimated maximum population densities of *A. spelaea* from 417 per hectare in Upper Twin Cave, Indiana ( $130 \times 2.5$  in  $3600 \text{ m}^2$ ), to 4199 per hectare in Under the Road Cave, Kentucky ( $84 \times 1.5$  in  $300 \text{ m}^2$ ).

For *A. rosae* the top three caves by maximum number of fish per census account for 55% of the 417 fish in 44 caves (G.O. Graening, unpublished data). These three caves have been regularly censused over a period of 20–50 years (Graening unpublished data, Poulson 1960, Brown and Johnson 2001). The top three censuses were 100, 122, and 139 for Cave Springs Cave, Arkansas, 48, 50, and 51 for Logan Cave, Arkansas, and 27, 26, and 33 for Ben Lassiter Cave, Missouri. The densities for the two best caves for the prime habitats with > 80% of all fish in each cave are 2134 per hectare for Cave Springs Cave ( $0.8 \times 139 \times 2.5$  in  $1300 \text{ m}^2$ ) and 932 per hectare for Logan Cave ( $0.8 \times 51 \times 2.5$  in  $1100 \text{ m}^2$ ).

*Speoplatyrhinus poulsoni* may be the rarest fish in North America, restricted to Key Cave in Lauderdale Co., Alabama. Alabama cavefish have only been observed in five pools within the cave and is extremely difficult to capture because of difficulty in sampling in deep pools in which it resides. Accordingly, its rarity and difficulty in capture make mark-recapture studies to estimate population size infeasible. Therefore, abundance has been inferred from sight observations during pool surveys. Kuhajda and Mayden (2001) summarized their survey efforts and those of past researchers since the 1970s. Although the numbers of fish observed are comparable from the 1970s to the 1980s and the most recent surveys by Kuhajda and Mayden in the 1990s, abundance is extremely low. Only two of the five pools have been routinely surveyed since the 1970s. A total of 25 survey trips of these two pools have been conducted in the last 30 years (reviewed by Kuhajda and Mayden 2001). Up to nine fish have been observed between these two pools during a single visit with the majority



of observations occurring in the mid-1980s. In the most recent surveys that included examination of five pools, Kuhajda and Mayden (2001) observed from 5 to 10 fish with an average of 7.8 fish per survey. Although low in numbers, three different broad size classes were observed indicating that recruitment is occurring. In this cave we can clearly only access a very small amount of habitat and the geology indicates that there is considerable inaccessible habitat.

## VII. FORAGING EFFICIENCY AND METABOLIC EFFICIENCY

### A. Past and New Studies on Metabolic Efficiency

Poulson (1960, 1963, 1985) has documented a trend of increasing metabolic efficiency from the epigean *C. cornuta*, to the troglophile *F. agassizii*, to *Typhlichthys* and *A. spelaea*, to *A. rosae* to *S. poulsoni*. Earlier in this chapter, we have reviewed the old and new evidence, from eyes and pigment, which corroborates Poulson's early inference that the four trogllobites show an increasing period of evolutionary time in caves from *T. subterraneus* to *A. spelaea* to *A. rosae* to *S. poulsoni*. To summarize the original data and new data (see Metabolic Rates) there is a trend of about a five-fold decrease in routine metabolic rates but with a five to ten-fold increase in daily body lengths swimming (Table 2 in Poulson 1985).

Poulson (1985) hypothesized that part of the increased swimming activity is due to longer and inferred bigger relative areas of pectoral and caudal fins that results in greater distance moved per coordinated caudal-pectoral fin stroke. However, only *A. spelaea* has average body lengths moved per fins stroke (0.62–1.25) that is higher than the overlapping body lengths per stroke of *F. agassizii* (0.32–0.72), *T. subterraneus* (0.35–0.67), and *A. rosae* (0.42–0.81).

The spontaneous swimming levels, measured at the same time as metabolic acclimation studies in a 0.5 liter torus respirometer, shows that the lowered metabolic rates of at least *T. subterraneus* and *A. spelaea* is not at the expense of lowered swimming activity. The ranges of swimming activity indices are *C. cornuta* 1–14 <<< *F. agassizii* 19–195 < *T. subterraneus* 70–320 = *A. spelaea* 19–300 >> *A. rosae* 6–55. These studies were done only over about a 20–30-hour period.

A more realistic measure of activity is to follow spontaneous activity in a 0.5-liter torus container with continuously renewed water over a period of 6–12 days. These data show that activity changed in different ways for the Amblyopsid species over the study. In general, over time *Forbesichthys*

activity declined drastically (n=2), *Typhlichthys* activity decreased slightly (n=4), *A. spelaea* activity actually increased on average (n=3), and *A. rosae* activity remained the same (n=3). The patterns were consistent within species.

An even more realistic measure of activity is in a 50-liter aquarium. Poulson used an elongate aquarium with a bank of infrared sensors at mid-point to bias for swimming over spontaneous activity. The percent of hours with at least some swimming was measured for two fish of each species for 7 days. The percent of hours swimming were as follows: *C. cornuta* 51 & 53% = *F. agassizii* 52 & 58% < *T. subterraneus* 76 & 86% < *A. spelaea* 92 & 99% = *A. rosae* 86 & 100%. Along with data on swimming speed, this gave estimates of body lengths swum per day while foraging (Table 2 in Poulson 1985) of: *C. cornuta* 1000 body lengths per day < *F. agassizii* 1854 << *T. subterraneus* 5454 << *A. spelaea* 10980 > *A. rosae* 8082.

And a still more realistic measure is provided by Bechler (personal communication) for fish in a 6.3 × 1.5 m artificial stream with rock shelters. Using videotape he followed four individuals at a time periodically for 30 days. The average cm moved per 15 minutes was *F. agassizii* 141 << *A. rosae* 408 < *T. subterraneus* 647 << *A. spelaea* 1274.

## B. Efficiency of Searching for and Capturing Prey

Poulson and Michael Barnett (unpublished data from 1967–1968) studied the searching patterns of Amblyopsids and the efficiency of finding and eating two types of prey at different densities. The prey were ten large cladoceran (*Daphnia magna*) and or five small isopods (*Lirceus*) in a 10-liter aquarium or one cladoceran or one isopod in a 100-liter aquarium. Fish sample sizes were 3–5 individuals of each species.

Poulson made *a priori* predictions of capture efficiency using simulated foraging of average-sized fish for each species (Table 8). He calculated the volumes of water that foraging fish could search in the following way. The width and depth (mm) across which prey could be detected was predicted based on head dimensions, plus projection of neuromast ridges and cupulae above head skin plus distance at which neuromast cupula move to moving prey. This was multiplied by two for head width and for head depth. These two sensitivity distances (horizontal and vertical) were multiplied to get a sensitivity area in mm<sup>2</sup> and this area was multiplied by body lengths swum per minute while foraging length to get a volume of sensitivity in mm<sup>3</sup> per minute. The swimming speed used was that observed when ten *Daphnia* or five *Lirceus* were first introduced to the aquarium with one fish.

**Table 8** Predictions of foraging effectiveness. We start by calculating the surface area (in  $\text{mm}^2$ ) of sensitivity to detection of prey around the head. Then, we convert this to volume (in  $\text{mm}^3$ ) searched per time by multiplying surface area by body lengths swum per minute. Compare to [Table 9](#) Time to capture prey and [Table 9](#) % of hours some swimming in 7 days.

Species	<i>C. cornuta</i>	<i>F. agassizii</i>	<i>T. subterraneus</i>	<i>A. spelaea</i>	<i>A. rosae</i>
Habitat	Swamp	Spring	Cave	Cave	Cave
1. Body length (mm)	42	60	50	65	45
2. Neuromast cup + cupula length (mm)	0.3	0.66	0.84	1.14	0.96
3. Distance (mm) at which cupula moves to moving prey	10	16	40	35	30
4. Head width + 2 + 3 × head depth + 2 + 3 ( $\text{mm}^2$ )	283	646	2536	1944	2076
5. Body length × $\text{min}^{-1}$ × Body length (mm)	105	222	425	423	320
6. Volume searched ( $4 \times 5$ ) × $\text{min}^{-1}$ × $10^{-3}$ = $\text{ml min}^{-1}$	40	143	1078	822	664
7. Time (min) to search a 10-l aquarium	249	70	9.3	12.2	15.1
8. Time (min) to search a 100-l aquarium	2487	699	93	122	151

The volume of sensitivity per minute in  $\text{mm}^3$  was converted to  $\text{cm}^3$  to compare to the average volume occupied by different densities of the two prey types in the 10,000  $\text{cm}^3$  or 100,000  $\text{cm}^3$  aquarium to get a measure of how long it would take for the fish to encounter one prey if it did not repeat searching any volume. This gave predictions of how the species would differ. This prediction was tested for each species by comparing the times to capture of each prey until all were gone. In tests with multiple prey of each type, data were time at which successive prey were captured along with swimming speed in body lengths per minute and turning rate (right or left by 90 degrees) in turns per minute.

With increasing troglomorphy among the Amblyopsids, there is a trend of increasing head size, increased projection of the neuromast cups and cupulae above the head, and increased distance at which cupulae move in response to moving prey. This trend is striking when comparing *Forbesichthys* to any troglobite but the differences among the troglobites are subtle (Table 8; Table 2 in Poulson 1985). There was no trend in swimming speed except that *Forbesichthys* swam slower than any of the troglobites and *Typhlichthys* was the fastest swimming species.

The same relative differences among species were predicted for volume potentially searched in  $\text{cm}^3$  per minute ( $73 \text{ cm}^3$  per minute for *Forbesichthys*  $\lll 1300 \text{ cm}^3$  for *Typhlichthys*  $> 864 \text{ cm}^3$  for *A. spelaea*  $\geq 839 \text{ cm}^3$  for *A. rosae*). Thus, all of the  $10,000 \text{ cm}^3$  test aquarium could be searched, if the fish used no overlapping paths, in 137 minutes for *Forbesichthys*  $\ggg 8$  minutes for *Typhlichthys*  $< 12$  minutes for *A. spelaea*  $= 12$  minutes for *A. rosae*. How does this compare to the time taken to actually find and eat different densities and kinds of prey? The answer is not well. There were unexpected differences in time to capture ten vs. one *Daphnia magna* and the species best at finding and eating *Lirceus* isopods was not the same species best at foraging for *Daphnia* cladocerans (Table 9).

A single *Daphnia* is the foraging challenge that most closely mimics the extremely low prey densities of zooplankton in caves where the maximum ever recorded is  $\sim 5 \times 10^{-2}$  per  $10,000 \text{ cm}^3$ ! With this test, *Forbesichthys* did worse than predicted as it took 34–2100 minutes to find and eat the single *Daphnia*. *Amblyopsis rosae* was like the other troglobites and better

**Table 9** Comparison of computed volume searched (Table 8) with time to capture one isopod and one water flea in a 100-l aquarium and first isopod of five total and first water flea of ten total in a 10-l aquarium. Ranges are reported in parentheses where available.

Species	<i>C. cornuta</i>	<i>F. agassizii</i>	<i>T. subterraneus</i>	<i>A. spelaea</i>	<i>A. rosae</i>
Habitat	Swamp	Spring	Cave	Cave	Cave
Time to search 100-l aquarium (min)	2487	699	93	122	151
Time to catch one water flea in 100-l (min)	2160	300	48	25	18
Time to catch one isopod in 100-l (min)	620	50 (45–74)	6.2 (1.5–12)	2.8 (1.5–4)	14 (4–36)
Time to catch first of 10 water fleas in 10-l (min)	na	$6.8 \pm 9.1$	$1.5 \pm 0.5$	$5.6 \pm 5.4$	$11.0 \pm 2.0$
Time to catch first of 5 isopods in 10-l (min)	na	4.4 (3.7–5.0)	3.6 (2.2–5.0)	1.8 (0.5–4.0)	9.0 (7.0–11.0)
Hrs (%) swimming over one week	52	55	81	96	93
Body lengths $\times \text{hr}^{-1}$ in 100-l with no rocks	1000	1854	5454	10980	8000
Body lengths $\times \text{hr}^{-1}$ in 6.3 m stream w/rocks (4 fish over 30 days)	na	94	557	784	362

than predicted as it took 3–42 minutes to find and eat the single *Daphnia*. What accounts for these departures from prediction? It is not differences in searching speed or turning rate.

All the Amblyopsid species showed behavioral changes as soon as prey were introduced though no controls were used adding inanimate objects. That they were reacting to live prey is shown by sustained increases in swimming speed. *Forbesichthys* increased its speed 20% from 3.8 to 6.1 body lengths per minute. *Typhlichthys* increased its speed 30% from 6.6 to 8.6. *Amblyopsis spelaea* increased its speed 49% from 4.1 to 6.1 and *A. rosae* increased its speed 51% from 4.7 to 7.7 body lengths per minute. All species increased their turning rates by three-fold which helped keep them in the same area where they had caught a prey when multiple prey were in the test aquarium. This behavior would be even more adaptive in caves with much lower densities and much higher patchiness of prey.

When a fish detects a living prey there are also similarities among the species. If a large food item like an isopod or white worm is dropped into an aquarium. Most individuals of all species move toward the area of disturbance (Eigenmann 1909, Hill 1966, Barnett and Poulson, unpublished data). Also when a fish approaches a prey on the bottom, it will pause and back up if the prey stops moving. Then it will “jerk and grab” as the prey starts moving again. All species use a lunge and grab motion as they capture a prey item; none use gape and suck to catch prey.

We have especially detailed observations of the changes in swimming rate, turning rate, and general behavior of *A. spelaea* while foraging for and eating ten *Daphnia magna*. In Michael Barnett’s words the fish initially show “lots of turning and jerking” and one fish was “furious at four near misses”. After the fish had reduced the number of *Daphnia* to 4–6 their swimming and turning rates increased from 0–3 to 4–18 body lengths per minute and turning increased from 0–2 to 2–5 turns per minute. These rates slowed some when they had reduced prey number to 2–4 and the fish seemed “much less frustrated” if they missed a first strike. One interpretation is that they were becoming “full” since the interval between captures increased.

The relative success of Amblyopsid species (Table 9) was different with ten *Daphnia* in a 10-liter aquarium than with only one in a 100-liter aquarium and this more closely matched the predictions from simulated volume searched per time. Though *Forbesichthys* did better and *A. rosae* worse than predicted, all species were effective foragers. They showed statistically but not ecologically significant differences. The times to catch the first prey ranged in minutes from  $1.5 \pm 0.5$  SD *T. subterraneus* to  $11.0 \pm 2.0$  SD *A. rosae*, with  $T. subterraneus < A. spelaea = F. agassizii < A. rosae$ . The relative differences were the same for the 5<sup>th</sup> and 10<sup>th</sup> prey captured

though *F. agassizii* and *T. subterraneus* showed no decline in intervals between prey capture and *A. spelaea* and *A. rosae* capture intervals increased after the 5<sup>th</sup> prey was captured. Poulson believes that *T. subterraneus* was best at capturing *Daphnia* once detected because its neuromast cupulae are knobbed at the tip and seem to be especially sensitive to slight water movements. We cannot explain why *A. rosae* was the slowest at eating *Daphnia* since it was the fastest by far at finding and eating a single *Daphnia* (above).

Interestingly, *A. spelaea* was fastest at finding and eating isopods whether with five or one per test aquarium. We think this is because isopods are its principal prey in nature (see Food Habits) and its search patterns in nature suggest they are concentrating on finding isopods (see Mohr and Poulson 1966). For sample sizes of three for each species, the range in minutes for capture of the first of five isopods was 0.5–4 for *A. spelaea* to 7–11 for *A. rosae* with  $A. spelaea < T. subterraneus = F. agassizii < A. rosae$ . For capture of the third of five isopods, *A. spelaea* was still fastest but now *Forbesichthys* was slower than *A. rosae*:  $A. spelaea \leq T. subterraneus < A. rosae < F. agassizii$ . These differences were accentuated with capture times of a single isopod. Times to capture were 2–4 minutes for *A. spelaea* and 7–74 minutes for *F. agassizii* with  $A. spelaea < T. subterraneus \ll A. rosae \ll F. agassizii$ . Part of the explanation is that both *A. spelaea* and *T. subterraneus* search almost only at the bottom where the isopods are located, whereas *F. agassizii* and *A. rosae* continue to search the entire tank. This is a more effective strategy for finding *Daphnia* that swim throughout the water column.

Poulson's perspective from these foraging experiments is that his predictions based on morphological troglomorphy missed some interesting differences in effectiveness when foraging for different prey types. These differences seem to be due partly to the types of prey most common in each species habitat. We have just mentioned this for *A. spelaea*, which lives in caves that have many isopods. We predict that *Forbesichthys* from Pine Hills will outperform even the troglobites with amphipods as prey because it eats only amphipods in the field. Also, *Forbesichthys* from Rich Pond eats mainly chironomid larvae and oligochaetes and so will also be effective foraging for amphipods with their strong thigmotaxis and rapid grabbing during prey capture. We have watched them eat white worms in the lab and they are especially effective. With worms, midge larvae, and amphipods the thigmotaxis used in prey capture complements lateral line detection of prey. We also predict that *A. rosae* and *Typhlichthys* may be most effective at foraging for copepods that are their main prey. This should also be true for small *A. spelaea* < 40 mm SL that eat mainly copepods and few isopods in the field. We think *Typhlichthys* will do the best with copepod prey because our lab observations show that it is especially sensitive to

slight water movement, even 'excitedly' moving towards a previously quiescent isopod when it started to wave its antennae!

### C. Hypothesized Tradeoffs of Living in Caves

Despite the negative (–) tradeoff of low food supply in caves, it is clear that *Forbesichthys* spawns in caves. Hill's (1966) study of *Forbesichthys* was much more complete than Weise's (1957) though both provide excellent laboratory observations of feeding and behavior. In both sites, the fish reproduce in the cave since adults disappear from the springs for a few months starting in late fall and then adults and small young appear outside in the spring. Unlike Weise, Hill was able to study the fish in both the spring run and in the cave and the contrasts provide important insights into the tradeoffs of the cave and spring run for feeding, refuge, and reproduction.

The Rich Pond Cave may be a refuge from parasitism and predation for newly hatched young but it has 3–4 orders of magnitude less food than Hill sampled outside and the new hatched fish of 6 mm SL stop growing and die if they do not leave the cave. If they leave the cave, they grow to about 35 mm SL their first year eating > 80% chironomid larvae by volume. When fish then overwinter in the cave their guts are 99.9% empty. When fish leave the cave the following spring, they grow to about 50mm SL with a diet of approximately 20% *Forbesichthys* and 78% chironomid larvae. When fish return to the cave again, about 82% of their guts have *Forbesichthys* and the rest are empty. This indicates that the spring cavefish is especially food-limited in caves and may not be a good model for the way other Amblyopsids became isolated in caves.

*Forbesichthys* may only be a marginal troglophile because there is no documentation of it reproducing in caves. The best-studied populations emerge nightly or seasonally from food-poor caves to feed in spring runs that have abundant live prey. If the surface spring runs dried up with climatic warming and drying then these populations might well go extinct. So a better habitat type to allow gradual evolution of food finding and metabolic efficiencies would be a sinking stream. These would have reliable allochthonous organic input and so much higher food supplies than in caves that exit to springs. *Forbesichthys* is occasionally found in such caves, in the Mammoth Cave Region, but these populations have not been studied to see if they have incipient adaptations to low food supply.

## D. Matching Amblyopsid Adaptations to Cave Type

Poulson has had a bit of an epiphany as he has spent three months reviewing old data, working up old unpublished data, and carefully re-reading what he thinks are the best studies available. He agrees with Noltie and Wicks (2001) that *A. rosae* and *T. subterraneus* occupy very different kinds of caves and karst in Missouri and here extend their suggestions about how cave type relates to all troglobitic Amblyopsid biology.

*Typhlichthys* occurs over such a wide geographic area with such a great difference in cave and karst type that it is difficult to make generalizations. If the hypothesis of some match of adaptations to cave type is correct then we predict differences between Missouri populations and Kentucky–Tennessee populations. The Missouri *Typhlichthys* are in caves that are far below the surface, have extensive underground watersheds, and have relatively low input of allochthonous organic matter due to often great distances from surface inputs and low currents. One prediction is that they will have more efficient metabolic patterns and foraging with longer lives and larger sizes than reported thus far for *T. subterraneus*. The studied *Typhlichthys* east of the Mississippi River occur in caves that are close to the surface, have small underground watersheds, and often have streams that flood and bring in allochthonous organic matter. For these *Typhlichthys*, we may expect to find local adaptation of populations that have been isolated in caves independently. Local adaptation is especially likely since genetic evidence indicates less gene flow than among cave populations of other Amblyopsid troglobites.

*Amblyopsis rosae* occur in caves that are shallow with small underground watersheds (Noltie and Wicks 2001) and small rocky streams that never flood severely. The shallow caves often have extensive allochthonous organic input and guano from gray bat maternity colonies in the habitats with the largest populations of fish. Poulson (1985) has previously suggested that this high food supply is related to the relatively short life and relatively frequent reproduction of *A. rosae* compared to other Amblyopsid troglobites. He argued that small maximum size (smallest of all cave Amblyopsids) is associated with less food needed to get the extra energy needed to allow reproduction. Here we extend the connection of high food supply to relatively fast growth rates allowed by an especially energy efficient metabolic pattern. This along with an inferred small clutch size (see Fecundity) allow for more frequent reproduction that is manifested in a fairly even size-frequency distributions in the best habitats.



*Amblyopsis spelaea* is the largest Amblyopsid and occurs in large streams that have the highest input of allochthonous organic matter and often strong currents. The extreme of input of coarse particulate organic matter is in Penitentiary Cave, Kentucky (Pearson and Boston 1995), with 519 fish censused in 3702 meters of rimstone pools with 10–30 cm of leaf, twig, and acorn fragments. And the extreme for live prey input is in Twin Cave, Indiana, with 130 fish censused in 3600 m<sup>2</sup> of riffles and pools with 70 ml of plankton including hundreds of cladocerans and copepods and 500 ml of fine silt and organic matter in an 800 liter sample during one flood (Scott 1909); this cave also has very high densities of troglobitic isopod prey. Poulson now hypothesizes that even those cave stream *A. spelaea* habitats now predominately silt-bottomed and slow-flowing still have high food supplies and once had higher stream velocities and greater flooding. His reasoning is that the range of *A. spelaea* is just south of the farthest glacial advances where there would have been much greater flooding and faster cave enlargement than at present. He thinks that the dense populations of isopods in many *A. spelaea* caves are explained by cave stream character and that the efficient foraging for isopods by fish > 35 mm SL is associated with isopods as the most frequent and abundant prey larger than copepods. Regular flooding with strong currents has selected for large size and abundant isopods have allowed large size and a large clutch size with branchial incubation. Consonant with this hypothesis is Bechler's observation (personal communication) that in an artificial stream *A. spelaea* was the most active swimmer of the four Amblyopsids studied.

An interesting corollary of large size of *A. spelaea* may be that it allowed syntopic coexistence with the much smaller *Typhlichthys* in the Mammoth Cave System (Poulson 1992). Recall that *T. subterraneus* is in the more food-rich small shaft drain streams and is replaced by *A. spelaea* as you census downstream to the larger and more food-poor base level streams. These *A. spelaea* in the Mammoth Cave System grow to much larger sizes (115–130 mm SL) than farther north (80–90 mm SL) and this may reflect character displacement. As a final note, Poulson thinks that he missed the significance of large size in *A. spelaea* because he always reported traits at the same 45 mm SL size. This was a kind of control when comparing troglomorphic trends (Table 2 in Poulson 1985). Poulson (2001b) has discussed the + and – tradeoffs of large body size for cavefish and for *A. spelaea* the positives are much greater than the negatives.

What kinds of specializations to the character of Key Cave might we look for in *S. poulsoni*? Key Cave is the only locality for *S. poulsoni* and its character may have changed due to anthropogenic impacts (see below Threats). Another complication is that we cannot access much of its habitat even using SCUBA. Nonetheless, Key Cave is a maze system developed at

and well below the water table. It does not flood and the only allochthonous organic input seems to be bat guano. The predominant prey is undoubtedly copepods though a large fish might be able to eat a very small crayfish. The maximum size estimated for visual sightings is around 60 mm SL. They are apparently very slow growing based on a conservative scale mark age estimate of 5 years for a 42 mm SL fish. If Alabama cavefish are like other Amblyopsids, we can multiply this estimate by 2–3 to get a probable age of 10–15 years. Also, since adults of other Amblyopsids slow down greatly in growth rate a 60 mm SL fish (estimated size of largest observed fish) may be 20–30 years old. This suggests extremely low available food supply and is consonant with estimates that its metabolic rate is even lower than for *A. rosae*. It is also consonant with the extreme neoteny inferred from its huge relative head size and unbranched fin rays in adults. These extremes may represent both adaptation to its very low food supply and the inference that it has been isolated in caves the longest of all the troglobitic Amblyopsids based on pigment and optic system rudimentation (Table 4; Table 2 in Poulson 1985). Whether we will be allowed to test the predicted genetic, physiological, and behavioral correlates of these morphological trends is problematic because the species is listed as ‘critically endangered’. Poulson would at least love to see his namesake alive before he dies (but see Poulson, 2009a)!

## VIII. CONSERVATION

The conservation status of subterranean fishes has received increasing attention in the past few years. According to Proudlove (2006), 63 of the 104 known species of subterranean fishes are listed by the International Union for the Conservation of Nature and Natural Resources (IUCN 1996, 2000). All four troglobitic Amblyopsids are included on the list. Three are considered Vulnerable and the other, *S. poulsoni*, is considered Critically Endangered. Two species, *A. rosae* and *S. poulsoni*, are listed by the United States Fish and Wildlife Service (USFWS) under the Endangered Species Act.

In this section, we review the conservation status of Amblyopsid fishes including the two nontroglobitic species, examine the major threats facing each species, and conservation measures that have either been implemented or proposed. This section largely follows that of Proudlove’s (2006) chapter titled “*The Conservation Status of Subterranean Fishes.*” We encourage readers seeking more information about the conservation of other species of troglobitic fishes to examine the works of Proudlove (2006) and the ‘Threatened Fishes of the World’ series in the journal *Environmental Biology of Fishes*.

## A. Conservation Status

All troglobitic Amblyopsids are considered vulnerable or endangered across their respective distributions (Table 10). In general, there is trend towards increasing threat status with increased cave adaptation. The non-troglobitic species are apparently secure throughout their respective ranges, although disjunct populations of *F. agassizii* in southeast Missouri are listed as endangered (Missouri Natural Heritage Program 2008). *Typhlichthys subterraneus* (as currently recognized) is the most widely distributed and least cave-adapted (Poulson 1963) of the cave Amblyopsids. As such, it is considered the most secure (although it is afforded protection in several states) and is considered endangered only in Georgia where it ranges only into the extreme northwest corner of the state. *Amblyopsis spelaea* is afforded protection at the state level and is a species of concern by USFWS (USFWS 1996). *Amblyopsis rosae* is listed as endangered throughout its range and was listed as threatened by USFWS in 1984 with a recovery plan written in 1989 (USFWS 1989). The most cave-adapted Amblyopsid, *S. poulsoni*, has been described as “the rarest American cavefish and one of the rarest of all freshwater fish” (USFWS 1996) and is found only in Key Cave, Lauderdale Co., Alabama. Because of its extremely limited distribution and suspected low population size, *S. poulsoni* is designated as Critically Endangered by IUCN and was listed as threatened by USFWS in 1977 and later elevated to endangered in 1988.

## B. Threats

Proudlove (2006) listed five broad threats that subterranean fishes can face. This list includes: (1) habitat degradation, (2) hydrological manipulations, (3) environmental pollution, (4) overexploitation, and (5) impacts of introduced aquatic animals. Many of the threats discussed below are interrelated because of their wide range of potential effects. For example, dam construction can result in direct destruction and degradation of cavefish habitat, alter hydrological patterns, and allow surface species to colonize and either compete or prey on existing cavefish populations. Here we generally follow the broad classification of threats listed by Proudlove (2006) and review the threats to Amblyopsid populations and focus the majority of our discussion on three troglobitic species, *A. rosae*, *A. spelaea*, and *S. poulsoni*, for which aspects of conservation have been most thoroughly examined. We focus on the first four of Proudlove’s list as little work has investigated the effects of introduced species on cavefish populations.

**Table 10** Conservation status of each Amblyopsid species throughout its respective distribution

<i>Listing</i>	<i>C. cornuta</i>	<i>F. agassizii</i>	<i>T. subterraneus</i>	<i>A. spelaea</i>	<i>A. rosae</i>	<i>S. poulsoni</i>
Federal Listing	Not listed	Not listed	Not listed	Not listed	Threatened	Endangered
Global Status	G5	G4G5	G4	G4	G3	G1
IUCN Red List Category	Not listed	Not listed	Vulnerable	Vulnerable	Vulnerable	Critically Endangered
Alabama			Protected (S3)			Endangered, Protected (S1)
Arkansas			Inventory Element (S1)		Endangered (S1)	
Georgia	Not listed (SU)		Endangered (S1)			
Illinois		Not listed (S1S2)				
Indiana				Endangered (S1)		
Kentucky		Not listed (S4S5)	Special Concern (S2S3)	Special Concern (S3)		
Missouri		Endangered (S1)	Not listed (S2S3)		Endangered (S2)	
North Carolina	Not listed (S4)					
Oklahoma					Endangered (S1)	
South Carolina	Not listed (SNR)					
Tennessee		Not listed (S4)	Deemed in Need of Management (S3)			
Virginia	Not listed (S3)					

*Sources:* Alabama Natural Heritage Program 2007, Illinois Endangered Species Protection Board 2006, Indiana Department of Natural Resources 2007, Kentucky State Nature Preserves Commission 2005, Missouri Natural Heritage Program 2008, Oklahoma Department of Wildlife Conservation 2008, Romero and Conner 2007, Withers *et al.* 2004.

## **Habitat Degradation and Alteration**

Habitat degradation and alteration can result from the direct destruction or manipulation of habitat during quarrying and mining operations, highway construction, and urban development. Keith (1988) reported two *A. spelaea* localities were either partly or completely destroyed by quarrying operations. However, the majority of habitat degradation and alteration threats are indirect resulting in loss of habitat because of siltation, sedimentation, and alteration of hydrological flow patterns and levels. Many caves in the eastern Interior Low Plateau have massive silt banks along streams that are likely associated with farming that began in the 1800s (Poulson, personal observation). However, some caves contain cavefish populations that are found entirely on silt substrates and have high population sizes and frequent reproduction. Likewise, on a much longer time scale, huge changes in habitat composition and food availability must have occurred with glacial cycles during the Pleistocene. Studies are needed to assess the actual rather than the potential impacts on cavefish populations from increased siltation and sedimentation.

Land development within cave recharge zones can alter surface runoff patterns or even block or destroy major recharge points. This can result in dramatic habitat alteration because of increased or decreased water volume, water velocity, sedimentation, or stream scouring depending on local hydrological patterns. In forested areas, increased erosion and production of sediment because of logging can result in increased siltation and sedimentation or the complete blockage of a cave passage. For surface Amblyopsids, loss of forested areas can cause the decline or loss of local populations. The installation of an electric transmission line has been cited as the cause of population decline at a *C. cornuta* locality in Virginia (de Rageot 1992). Removal of the surrounding forest caused increased insolation and drying of aquatic habitat.

Reduced input of surface runoff in recharge zones could have dramatic impacts on reproduction of Amblyopsid cavefishes. Cavefish are believed to rely on increased flow and small temperature changes associated with cave flood events during winter and spring to coordinate reproduction and spawning (Poulson 1963, 1969). Because most populations of cavefish are reportedly small (*S. poulsoni* in particular), the importance of successful timing of sexual maturation and spawning is magnified (Kuhajda 2004). Reductions in surface runoff may disrupt the environmental cues necessary for successful reproduction leading to greater susceptibility to extirpation.

Impoundments have resulted in many problems and threats for cavefish populations. A primary example is the construction of Lock and Dam #6 constructed on the Green River below Mammoth Cave in 1906. Although the Green River naturally back-floods into the cave system, flood levels

have increased since dam construction (Lisowski and Poulson 1981). The Styx and Echo River areas in Mammoth Cave experienced an apparent decline in cave biota, including cavefish, from the late 1800s to the 1920s (Elliott 2000). From the 1950s to the 1970s, cavefish were still observed but were only large in size and low in abundance. The present rarity of *A. spelaea* in the Echo and Styx River parts of the system likely is related to flooding and silting associated with deforestation and construction of Lock and Dam #6 and other impoundments along the Green River and the Nolin River, a tributary to the Green River, in the 1970s (Poulson 1969, 1996, Elliott 2000). In addition to alteration and loss of habitat, river impoundments may have resulted in decrease in within cave downstream transport of particulate organic matter by hydraulic damming when releases from the upstream Green River dam keep the river level at Mammoth Cave from declining completely after flood peaks. This exacerbates the decline of cave biota in the base-level stream of Mammoth Cave (Poulson 1996). This is discussed in more detail by Poulson (1992).

### **Hydrological Manipulations**

Hydrological manipulations can include underground water removal for human consumption, irrigation, or industry. However, some hydrological manipulations, such as impoundments or increased surface runoff, can raise water tables and alter habitats (see above). Lowering of the water table resulting from direct human consumption, irrigation, or industrial use may threaten cavefish populations. Because *S. poulsoni* lives in a zone of seasonal oscillation of the water table (Trajano 2001), lowering of the water table (and drainage alterations) may isolate fish in these pools exposing them to decreased oxygen levels if decomposable organic matter is present and increased concentrations of contaminants and death if pools dry out during the dry season (Kuhajda 2004). Accordingly, lowering of the water table because of a proposed industrial park for the city of Florence, Alabama, within the recharge area of Key Cave has been cited as a threat to *S. poulsoni* (USFWS 1977, Kuhajda and Mayden 2001).

### **Groundwater Pollution**

Groundwater pollution has been listed as factor negatively affecting populations for all cave Amblyopsids. This threat includes eutrophication and contamination from agricultural and industrial runoff containing pesticides, fertilizers, and heavy metals, sewage effluent, spills and illegal dumping of hazard materials, and thermally altered runoff. Although few studies have examined the direct effects of groundwater pollution on cavefish populations in detail, several studies implicate this threat in population declines.

Groundwater pollution may be acute in nature, such as a toxic spill resulting in a large impulse of contaminants, or chronic occurring over several months to years (Proudlove 2001). Both forms have been attributed to cavefish declines or extirpations from cave systems. Nearly 1,000 dead or dying *T. subterraneus* were expelled from Meramec Spring in Missouri after a fertilizer pipeline rupture in November 1981 caused acute, catastrophic deoxygenation of groundwater (Vandike 1984, Crunkilton 1985). In contrast, several decades worth of gross pollution by decomposable organic matter (creamery waste) and heavy metal contamination (electroplating waste) is the suspected cause of the apparent extirpation of *T. subterraneus* along with other cave life at Hidden River Cave in Kentucky (Lewis 1996). However, *T. subterraneus* and other cave life have re-colonized areas previously affected from far upstream refuges.

Several sources have been implicated in heavy metal and hazardous chemical contamination of groundwater throughout the distributions of cave Amblyopsids. These sources include drilling related to oil and gas development, inactive wells, industrial effluents, accidental spills, underground storage tank leaks, sinkhole dumping, and runoff from roads (road salt) and agricultural fields (pesticides and herbicides) (Keith 1988, Pearson and Boston 1995, Kuhajda and Mayden 2001, Kuhajda 2004). Heavy metal runoff from a local landfill may threaten populations of *T. subterraneus* in Pulaski Co., Kentucky (Tercafs 1992). Increased contaminant levels may cause increased susceptibility to disease. Pesticides were attributed as the cause of "broken back syndrome" that affected perhaps 10 percent of a population of *A. spelaea* in Indiana (Keith and Poulson 1981).

At least four of these threats, industrial effluents, underground storage tank leaks and sinkhole dumping, have been connected to the decline of *T. subterraneus* and other cave life from Hidden River Cave (Pearson and Boston 1995, Lewis 1996). Organic enrichment from sewage treatment plant effluents and septic tank leaks also have been implicated at Hidden River Cave and other caves with Amblyopsids. Organic enrichment can increase nutrients in an otherwise low-nutrient environment and drastically alter food web dynamics, increase risk of disease, and dramatically decrease dissolved oxygen levels. Brown *et al.* (1998) attributed a 30% decline in a population of *A. rosae* to increased levels of inorganic and organic compounds.

Groundwater pollution is a major threat to *S. poulsoni* (Kuhajda 2004) and has been considered as one of the factors likely to cause the decline of the species. Herbicide and pesticide application to cotton fields in the recharge zone of Key Cave have been shown to have direct access into the cave. Moreover, a sewage sludge disposal operation also lies within the recharge zone of the cave (Aley 1986). Thermal pollution in

the form of thermally altered runoff may alter reproductive cycles of Alabama cavefish (USFWS 1982, Kuhajda 2004) and potentially reduce or eliminate recruitment. These and other threats associated with either the current or potential alteration and degradation of the Key Cave recharge area and aquatic habitat, along with the perceived small population size and extremely small distribution have resulted in the listing of *S. poulsoni* as 'Critically Endangered' because of its high risk of extinction (Hilton-Taylor 2000). These threats have been addressed by Kuhajda (2004) and outlined in detail in three different versions of the USFWS Recovery Plan for *S. poulsoni* (USFWS 1982, 1985, 1990).

### **Collection and Cave Visitation**

The collection of cavefish, illegal or otherwise, for the aquarium trade or scientific purposes may pose a threat to all cave Amblyopsids. Because of their uniqueness to hobbyists and the ease at which individuals can be captured, cave Amblyopsids can be easily exploited. Over-collection of fish can reduce or even eliminate local populations. The rarity of *A. spelaea* in the Echo River and River Styx sections of Mammoth Cave system and its presumed absence from adjacent caves to the north have led some to speculate that the species was either introduced or decimated during the 1800s when it was sold as a novelty (Poulson 1968, Elliott 2000). Over-collection by both the scientific community and amateurs is thought to be a concern for populations of *A. rosae* (Culver 1986, USFWS 1989).

Commercial exploitation of caves can either alter or even destroy considerable amounts of cavefish habitat. Commercial caves increase human traffic and disturbance in addition to increased light levels. At least five populations of *A. spelaea* are indirectly affected by commercial cave tours including Blue Springs Caverns and Upper Twin Cave in Indiana and Hidden River Cave and Mammoth Cave in Kentucky (Pearson and Boston 1995). However, the exact impacts and long-term effects of commercial cave operations remains to be examined. At Upper Twin Cave, no differences in apparent abundance of *A. spelaea* exist between times when tours are conducted and times when tours are not in operation (Poulson, personal observation). And the continued abundance of fish in Upper Twin Cave since Eigenmann's studies suggests that pole boat tours in the downstream part of the cave, starting in the 1950s, have not compromised the populations.

Human disturbance caused by increased traffic is more of a concern than commercial exploitation. The activities of even the most cautious caver may have serious impacts on cave organisms in shallow, silt-bottomed streams. Disturbance caused by substantial cave visitation may alter breeding of cavefish populations, disturb food sources, and unknowingly



stress individual fish by increasing fish activity. However, no evidence has been obtained for any of the above.

Increased disturbance caused by human disturbance is thought to negatively affect grey bat (*Myotis grisescens*) colonies by increasing bat mortality or the eventual abandonment of a cave. If bat colonies are extirpated, cavefish populations may lose an important source of food and nutrients (USFWS 1989). This could be a very serious threat given that the caves with the largest *A. rosae* populations are also grey bat maternity colony caves. However, more studies are needed to assess the impacts and lasting effects of intense cave visitation on cave fauna. The huge decline in the rich aquatic fauna of Shelta Cave, Alabama, including *Typhlichthys*, has been attributed to the loss of a major grey bat roost (Hobbs, personal communication).

### **Mechanisms of Impact: Community Signatures**

Poulson (1992) has hypothesized community signatures both as early warning signs and to identify the four most common kinds of pollution impact. These are meant to supplement Indices of Biological Integrity (IBIs) developed by William Pearson and his students to detect any changes in aquatic cave communities over time. As with IBIs, community signatures require un-impacted baselines. And impacted caves serve as inadvertent experiments to test the community signature hypotheses. Poulson (2009b) proposes that aquatic cave communities will show different responses to siltation, eutrophication, acute toxicity, and chronic toxicity. These signatures are based both on first principles of ecology and toxicology and the differences in life history and metabolic rate of different aquatic cave fauna. The obvious caveat for using community signatures is that several kinds of pollution may occur in the same cave. In these cases, there should be extremely low IBIs. Historically, Hidden River Cave, Kentucky, lost all its troglobites in the main stream. The normal species were replaced by sheets of colonial sewage bacteria and by dense mats of sludge worms that were not killed by the massive inputs of decomposable organic matter with attendant low dissolved oxygen and/or high concentrations of heavy metals.

Differences are expected with chronic toxicity at low levels and acute toxicity at high levels. Heavy metals, like lead and mercury, and organic compounds, like chlorinated hydrocarbons, are usually toxic because they are not naturally occurring and so organisms have not evolved physiological ways of sequestering or detoxifying them. As with herbicides used on weeds as a model, the species with fastest metabolic and growth rate will be most quickly and seriously affected by a pulse of heavy metals or pesticides. Also, species with low metabolic and growth

rates may not be affected by pulsed toxicity but are at particular risk with chronic low levels of toxins due to continued bioaccumulation over a long lifetime. If they are also top predators they are in double jeopardy due to biomagnification of toxins along food chains. With acute toxicity their prey may be killed.

Aquatic cave communities impacted by acute toxicity may be missing the organisms at the beginning of the food chain. Troglaphiles at the base of food chains will be more affected than troglobites because the troglaphiles have the highest growth and metabolic rates. If there is a pulse of toxin input, as in a railroad car derailing or truck accident on a highway near a sinkhole, then *Forbesichthys* is most at risk and *A. rosae* and *Speoplatyrhinus* at least risk.

The community signature for low-level toxic pollution will be different with opposite vulnerabilities than for high-level toxic pollution. Among Amblyopsids, troglobites will be most affected due to both biomagnification and bioaccumulation. Size frequency distributions will show that the largest and oldest fish are missing or under-represented compared to smaller fish. Even troglobitic crayfish may be at risk if the food supply is low and since longevities are documented to be as long as a century in food-poor caves! Short-lived species like copepods and isopods may be unaffected even if they are troglobitic. They may even increase in density if predatory fish and crayfish are reduced in numbers due to chronic toxicity.

It is difficult to find an unimpacted control cave to provide a comparison for the expected community signature for siltation. The problem is that a great number of caves have had increases in silt levels associated with land clearing for agriculture over the past 200 years. Nonetheless, the expected impact of siltation is homogenization of the stream bottom habitat. At the extremes, silt can cover rock and gravel refuges for isopods and amphipods in riffles and so there will be fewer prey washing into pools and shoals deep enough for fish and crayfish. Silt is also likely to cover or mix with fine particulate organic matter that copepods, isopods, and amphipods graze or ingest. Also, at low levels it may foul the biofilms on rocks that are grazed by everything except predators. Poulson (1992) has provided detailed examples for the lower levels of the Mammoth Cave where siltation is due to the combined effects of downstream and upstream dams on the hydropattern of Green River into which the cave streams flow.

The community signature for organic enrichment (eutrophication) is the most clear since it is sensitive to the level of pollution. The mechanism is that decomposition of organic matter provides increased food at low levels but uses up dissolved oxygen. At low levels of organic enrichment

species at the beginning of the food chain increase in numbers and their size frequency distributions are skewed more to smaller size classes with increased rates of reproduction. This is especially true if they are troglaphiles. Over time the faster reproducing troglaphiles simply outproduce the troglobites even though more energy efficient troglobites cannot be outcompeted. See Poulson (this volume) for an explanation of how this may lead to demographic swamping of troglobites by the faster reproducing troglaphiles.

At high levels of organic enrichment, especially if pulsed in time, all the normal aquatic fauna is replaced by species tolerant of extremely low dissolved oxygen. At the extreme there are only stringy mats of colonial sewage bacteria, like *Sphaerotilus*, and tubificid worms. The red tubificids have a hemoglobin that can bind oxygen at very low concentrations and so these worms can be seen as waving mats at the stream edge where a little bit of dissolved oxygen remains.

### C. Conservation Measures

Several conservation measures have been proposed or implemented for populations of cave Amblyopsids. Fencing or gating of cave entrances have been proposed or implemented to reduce and control human visitation to sensitive cave ecosystems including Amblyopsid caves. Special bat gates are needed to allow entry and exit by bats but stop human entry. Bat Conservation International and The National Speleological Society have been leaders in the improvement and installation of such bats on an increasing number of bat caves.

Protection of cave surface and subsurface watersheds is probably the most important intervention for cavefish caves. Thomas Aley (Ozark Underground Laboratory, Protom, Missouri) is one of the best practitioners of state-of-the-art water tracing that is critical to delineating cave watersheds. Among others his studies have led to the protection of watersheds of at least Key Cave, Alabama, the only locality for *Speoplatyrhinus poulsoni*, and for the best *Amblyopsis rosae* cave, Cave Springs Cave in Arkansas. Watershed protection has included establishing preserves as well as institution of best land management practices around sinkholes and sinking creeks that includes reforestation. In other cases water tracing has identified the source of pollutants and so allowed legal action that remedied the situation. Hidden River Cave in Kentucky is one example.

We suggest that what we have called source caves deserve complete protection of their watersheds. Recall that a few caves for each species have the vast majority of all individuals ever censused. To us, attention to protecting these caves is a number one priority for the near future.

Despite the fact that the population of *S. poulsoni* appears to have remained stable over the past 30 years, perturbations within the recharge basin could alter the status of this species (Kuhajda and Mayden 2001). Accordingly, USFWS purchased 1060 acres within the recharge basin in January 1997 and established the Key Cave National Wildlife Refuge. Likewise, the Logan Cave population of *A. rosae* is protected by the 123-acre Logan Cave National Wildlife Refuge, and another population is protected by the 40-acre Ozark Cavefish National Wildlife Refuge.

Introduction of cavefish to new localities or to caves that were historic localities is worth considering. Until we learn to breed Amblyopsids the only source for introductions is existing caves with thriving populations. To protect genetic integrity these source caves should only be in watersheds that include the recipient cave. In the case of *Speoplatyrhinus*, there is only one cave so spreading of risk of extinction by introductions to adjacent caves will require very careful consideration.

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