RESPONSES TO LIGHT IN CAVE AND SURFACE POPULATIONS OF *ASTYANAX FASCIATUS* (PISCES: CHARACIDAE): AN EVOLUTIONARY INTERPRETATION

By

Aldemaro Romero

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

UNIVERSITY OF MIAMI

Coral Gables, Florida

August, 1984

UNIVERSITY OF MIAMI

A Dissertation submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

REACTIONS TO LIGHT IN CAVE AND SURFACE POPULATIONS OF *ASTYANAX FASCIATUS* (PISCES: CHARACIDAE): AN EVOLUTIONARY INTERPRETATION

Aldemaro Romero

Approved

Steven M. Green
Professor of Biology
Chairman of Dissertation
Committee

Jo Anne K. Hecker
Associate Dean of the
Graduate School

Gene S. Helfman
Assistant Professor of
Zoology, University of
Georgia

Julian C. Lee
Associate Professor of
Biology

John S. Heywood
Assistant Professor of
Biology

Brian L. Partridge
Adjunct Professor of
Biology

ROMERO, ALDEMARO (Ph.D., Biology)

Responses to light in cave and surface populations of _Astyanax fasciatus_ (Pisces: Characidae): an evolutionary interpretation. (August, 1984). Abstract of a doctoral dissertation at the University of Miami. Dissertation supervised by Professor Steven M. Green.

Experimental field studies in Costa Rica reveal that _A. fasciatus_ density in a pool connected to a subterranean cavity is negatively correlated with fishing bat activity. Since fish tend to remain within the cavity when bats are actively foraging, the vulnerability of _A. fasciatus_ to bat predation may be reduced. Fish from the study pool and those living in nearby rivers do not differ in gross morphology. Taking refuge in the cavity may be a first step in cave colonization; this behavioral change can occur prior to gross morphological changes. This result is consistent with Mayr's hypothesis that behvioral changes may precede morphological ones during the invasion of a new niche, and that behavior may act as the pacemaker of evolutionary change.

All _A. fasciatus_ collected from caves in Mexico exhibit scotophilia. Larger (older) fish are more strongly scotophilic than smaller (younger) ones. Scotophilia varies inversely with the degree of reduction in pigmentation and development of the eyes. There is thus a negative correlation between behavior that accompanies cave colonization and the morphology typical of cave-dwelling animals.

Phenotypic intermediacy between surface and cave forms found in one Mexican population is suggested to be the result of introgression that has taken place in less than forty-three years. The contention that cave colonization necessarily requires drastic genotypic and phenotypic changes is thus rejected for _A. fasciatus_.

The concept of "regressive evolution" is evaluated from historical, semantic and conceptual perspectives and is found inadequate on several grounds.

To Ana;

she knows why.

ACKNOWLEDGMENTS

Parts of the field and laboratory studies were supported by grants from the Peter Nikolic Fellowship in Animal Behavior from the T. C. Schneirla Research Fund, the Organization for Tropical Studies, Inc., the National Speleological Society, the Southern Regional Educational Board, and the National Institute of Mental Health (IR03 MH3 7184-01) and by funds from the Department of Biology, University of Miami.

While a graduate student I was supported by a Fundayacucho Scholarship, and by a Maytag Fellowship.

The Organization for Tropical Studies (Costa Rica office) and the Wildlife Service of Costa Rica provided logistic support.

I thank many people for sharing with me vital information (some times unpublished) about their current research and views related to the material included in this dissertation: R. Arai (Japan), K.E. Banister (England), D.L. Bechler (Texas), R. Berti (Italy), S. Bianchi (Italy), M. Boeseman (The Netherlands), M. Lopez (Costa Rica), Xiu-Luo Chu (People's Republic of China), E. De Bellard (Venezuela), J.P. Durand (France), W.R. Elliot (Texas), J.A. Endler (Utah), W. Erckens (Federal Republic of Germany), J. Gery (France), D. Kramer (Canada), G. Longley (Texas), G.S. Myers (California), H. Nomura (Brazil), J.G. Palacios-Vargas (Mexico), J. Parzefall (Federal Republic of Germany), C. R. Robins (Florida), D.E. Rosen (New York), R. Rouch (France), I. Sazima (Brazil), S.A. Smirnov (Soviet Union), R. Vergara (Cuba), S.H. Weitzman (Washington, D.C.), F.H. Whittaker (Kentucky), and H. Wilkens (Federal Republic of Germany).

I was much benefited by discussions with several people I met through the Distinguished Visiting Professor program, Department of Biology, University of Miami; they were: L. Ehrman, P. Marler, P. Regal and M. Rosenzweig.

I thank Gard Otis, coordinator of the OTS (1981-3) summer course, who showed me the pool associated with subterranean waters, W. Bussing for helpful discussion and identification of specimens, T. Fleming and W. Evoy for use of videotape equipment; G. Fernandez, L. Herbst, J. Montanez, J. Moreno, and Ana Romero for technical assistance. W. Evoy, L. Herbst, J. Moreno, T. Poulson, and several anonymous reviewers made valuable suggestions on articles either in press or submitted that are based on this dissertation.

I am grateful to G. Helfman, J. Heywood and J. Lee, members of my research committee for their guidance of my research work and critical reading of this dissertation.

Finally, my deepest gratitude to B. Partridge who first served as chairman of my dissertation committee and S. Green who succeeded him. Their advice and dedication in many matters of my graduate and professional career will never be forgotten.

TABLE OF CONTENTS

PAGE LOCATION OF FIGURES

PAGE LOCATION OF TABLES

CHAPTER 1. THE TOPIC

1.1. Introduction

Behavior has always been envisioned as playing an important evolutionary role. Lamarck (1809, p. 119) believed that the physiological processes initiated by behavioral activity ("use versus disuse"), combined with an inheritance of acquired characters, were the causes of evolution. This explanation was largely followed by Darwin (1859, pp. 137-138) when interpreting disappearance of morphological features. However, when genetics was incorporated into evolutionary thought, mutationists went to another extreme, arguing that major mutations generate new structures, and these "go in search of an appropiate function" (for a historical review see Mayr 1982, p. 611).

Our current knowledge of neuroethology suggests, however, that all behaviors depend on certain structural components in the nervous system (Ewert 1980). So, instead of trying to find a clear-cut answer to the general question "structures first or behavior first?", it would be more meaningful to test hypotheses on the relationship between behavior and the structures involved during evolution. Mayr (1982, p. 612), for instance, advanced the idea that

> "many if not most acquisitions of new structures in the course of evolution can be ascribed to selection forces exerted by newly acquired behaviors ... Behavior, thus, plays an important role as the pacemaker of evolutionary change."

Among the examples he cited to substantiate the suggestion that behavioral changes could occur prior to changes in the structures involved were those of certain parasitic wasps that spend long periods of time underwater without showing the morphological adaptation of other aquatic insects (as initially reported by Darwin 1872, p. 185), and the cases of change in locomotory habits (without structural ones) in fish (based on Westoll 1958) and in large arthropods (based on Manton 1953). However Mayr's (1982, p. 612) foremost evidence is Bock's (1959, pp. 204-207) observation that primitive woodpeckers which have switched to the behavior of climbing on tree trunks and branches still have essentially the ancestral foot structures. Mayr argued that "the new habitat created selection forces in several lines of woodpeckers which led to various highly efficient specializations of foot and tail structures adapted to more efficient climbing."

There is little doubt that the active shift of an animal into a novel niche or entirely new adaptive zone will set up a powerful array of new selective pressures which favor, almost without exception, a change in behavior. In general, the more drastic is the change in the environment, the more far-reaching is the phenotypical reorganization (Mayr 1960, pp. 368-370; Simpson 1953, p. 142). Studying behavioral differences in related species and/or conspecific populations living in different habitats is thus apt to throw much light on the sequence of events that triggers the emergence of evolutionary novelties. An outstanding example of the use of this comparative method is Tinbergen's (1972) study on how environmental pressures impose demands on the behavior of different species of sticklebacks. Comparisons of species that live under the same environmental constraints also provide a further test of hypotheses in such evolutionary studies (see Endler 1982, for an example).

In consequence, the idea that behavioral changes precede morphological ones during the occupation of new niches (adaptive zone, *sensu* Mayr 1982, p. 612) is reasonable, falsifiable in principle, and the examples summarized here suggest that such might be the case. However, few rigorous tests and comparative studies have yet been done. Furthermore, such studies are prerequisite to any attempt to show that behavioral changes produce the selective forces which ultimately trigger changes in the structures involved.

The data and discussion presented in this dissertation are aimed at testing the null hypothesis that changes in behavior and morphology related to the invasion of a new niche or adaptive zone occur simultaneously.

1.2. Study organisms

To address this hypothesis of the relationship between behavioral and morphological changes during evolution, it is essential to find a situation in which meaningful comparisons can be made. To that end the organisms to be studied should meet the following conditions:

a) Conspecific populations (or closely related species) living under very different environmental constraints can be found.

b) Such different related populations and/or species should display differences in morphology which seem to be related to each one's habitat.

c) A specific behavioral trait related to the morphological differences can be studied under both natural and experimental conditions.

d) Comparison between the results obtained by such studies and those of similar behavior-morphology relationships in organisms belonging to unrelated taxa, but displaying similar structural and behavioral modifications, must be feasible. The results of such comparisons will provide insight to the generality of the findings.

As a subject of research I have chosen to examine the evolutionary transitions in behavioral responses to light from an ancestral surface form (eyed and pigmented) to a derived cave form (blind and depigmented) of the freshwater teleost *Astyanax fasciatus*.

1.3. Influence of light in the biology of fishes

The influence of light on the biology of fishes has been abundantly documented. Studies on photoperiodism have shown that many fishes depend on light to control their feeding and locomotory activities (Ericksson & Veen 1980, Godin 1981, 1984, Goudie et al. 1983, Helfman 1981a, and Kavaliers & Ross 1981). Light also affects the daily growth patterns in otoliths (Tanaka et al. 1981), coloration of the integument (Lagler et al. 1977), timing of reproduction (Sundararaj & Vasali 1976), overall rate and pattern of growth (Brett 1979), the behaviors mediated by kinds and amount of food available (Hyatt 1979), and the structure of schools (Hunter 1968, Shaw 1961) (Fig. 1).

Since light is such an important factor in the biology and evolution of most fishes, do fishes which live under total darkness during their whole life still retain photosensory abilities? If so, do they respond to photostimuli? Are their phototactic responses (or lack of them) a consequence of the morphological changes (mainly blindness) commonly found among fishes that live in dark environments, or are

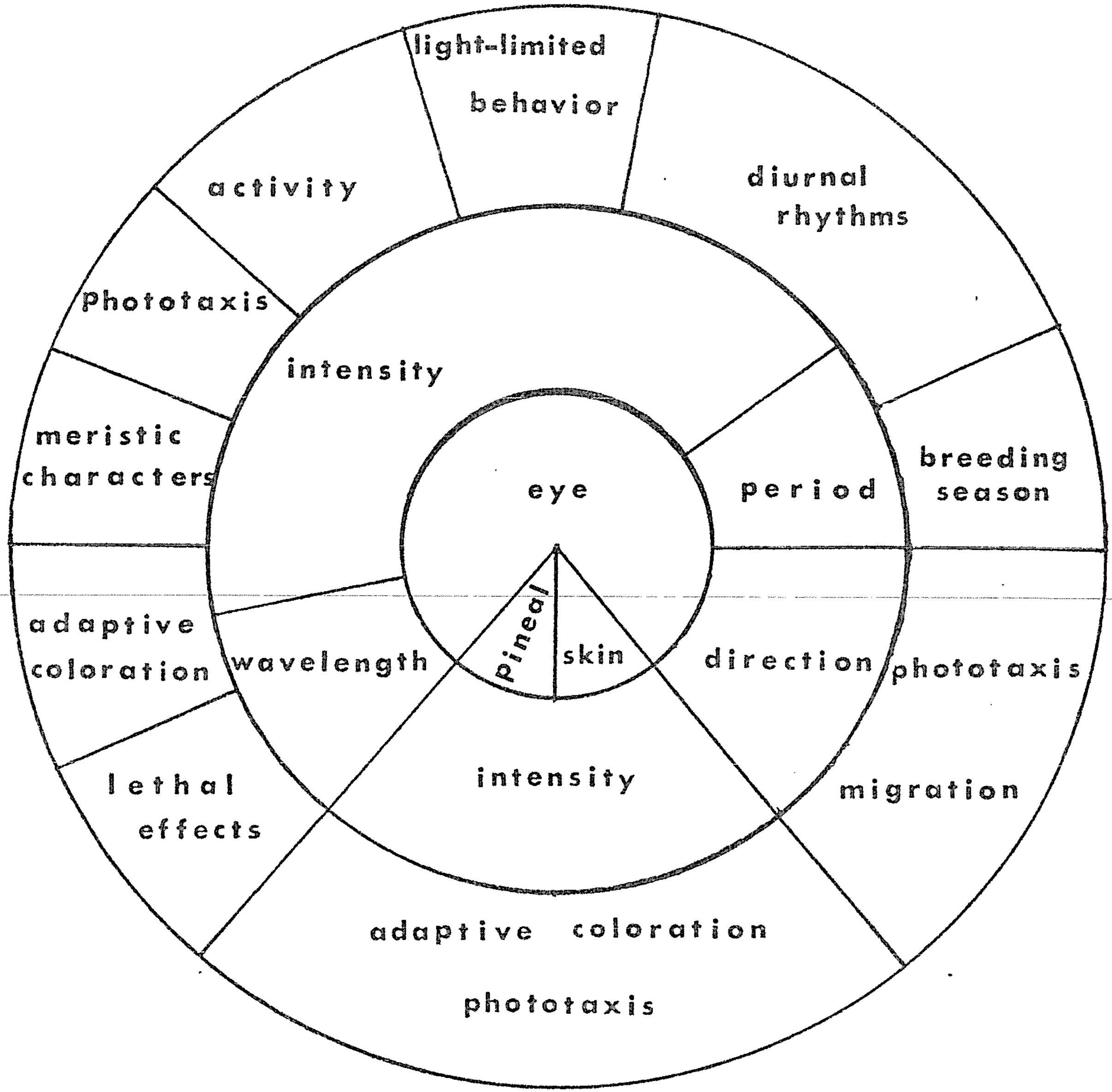


Fig. 1. Schematic diagram of the relationships between photoreceptors (innermost ring), physical properties of light (inner ring), and light's effects on the biology of fishes (outer ring). [Modified from Blaxter 1970, p. 214].

these phenomena independent of each other? How can these phototactic responses be interpreted from an evolutionary standpoint? To obtain answers to these questions, one should look for an absolutely dark natural habitat which sustains populations of species belonging to many different taxa. Deep-sea and cave environments both meet these requirements. Since abyssal organisms present tremendous difficulties for field and laboratory studies, cavernicolous ones are more suitable.

1.4. Caves as natural laboratories

Obligatory cavernicoles (troglobites) have traditionally been of special interest to biologists. These organisms can be found in many localities. In the U.S.A., for example, there are about 200,000 caves where one consistently finds life (Barr 1968, p. 36). Cave environments offer discontinuous habitats which are almost entirely heterotrophic and dependent on input of food energy from the exterior, thus providing places where evolutionary phenomena can be particularly interesting. Troglobites of different taxa display similar morphological, physiological and behavioral features, presumbly adaptations as a result of convergent evolution. They frequently have been a source of controversy in evolutionary issues such as the theory of preadaptation and that of so-called regressive evolution (Barr 1968, pp. 80-96; Culver 1982, pp. 56-76; and chapter 6 of this dissertation).

Blindness and depigmentation --the most common and conspicuous characters among troglobites-- have always been regarded as taxonomic features used to describe new hypogean genera and species (Banister & Bunni 1980).

Usually it has been accepted practice to consider a cave population as generically (and genetically) distinct from its nearest epigean relative (see for example Alvarez 1946, 1947, Boulenger 1921, Cope 1864; Hubbs & Innes 1936, Norman 1926, Pellegrin 1922, Poll 1957). Electrophoretic studies (Avise & Selander 1972), karyotypic descriptions (Kirby et al. 1977), more careful taxonomic analyses (Bannister & Bunni 1980, Greenwood 1976) and detailed osteological studies made while applying phylogenetic considerations (Rosen & Greenwood 1976), have shown that some of the cave "species" are in fact demes or ecotypes of the epigean form. These changes in systematic approach have occurred within a population-based conceptual framework replacing a typological one (Banister & Buni 1980, Gordon & Rosen 1962, Williams & Howell 1979).

There are cases where cave species are clearly valid (see for example Brittan & Bohlke 1965, Cooper & Kuehne 1974, Woods & Inger 1957). In many of these it is possible to infer the epigean ancestor. Among the forty-seven species of hypogean fishes which display structural reduction in their eyes and pigmentary system (Appendix 1), it is likely that some of the descriptions pertain to cave populations of epigean species.

1.5. Hypotheses on the evolution of cave life

Although there are many examples of obligate and facultative cave organisms with putative surface ancestry, the issue has yet to be resolved as to what initiates the structural reductions that characterize cave populations. Barr (1968, p. 80), Poulson (1961) and Vandel (1969, pp. 276-324) have argued that extant troglobites have descended from ancestors preadapted to the cave environment due to either nocturnal habits, the presence of highly developed non-visual sensory systems, or both. Several hypotheses have been suggested to explain structural reductions, such as blindness and depigmentation, that characterize most of the populations that have become isolated in the subterranean environment; whether these features are the product of selection or random drift is still a matter of controversy (Culver 1982, pp. 56-76).

Some theories of the origin of cave populations assume accidental entry into caves resulting in permanent entrapment of the organisms (Barr 1968, pp. 75-76). Other theories propose some directional ("regressive") evolution on the assumption that cave animals represent "dying phylogenetic lines" which seek refuge in caves (see Barr 1968, p. 71 and chapter 6 of this dissertation for review). Neither explanation has experimental confirmation. Thus, how a species changes from epigean to troglobitic existence is still a matter of conjecture and discussion. With the exception of *A. fasciatus*, the amblyopsid fish family (Poulson 1961), and a few other species, we usually have only the ancestral and the blind depigmented forms without any intermediate stages to assist in elucidating how the changes occurred.

1.6. *A. fasciatus* as a subject of research

A. fasciatus is a freshwater fish with a broad distribution in the New World (Fig. 2). It has overcome the Central American filter-barriers that have limited the expansion of almost all the ichthyofauna of South American origin. Unlike other species groups of South American origin, only the *Astyanax fasciatus* species complex and the genus *Synbranchus* and its derivatives (Synbranchidae) have reached the southern United States and southern Mexico, respectively. Despite this wide distribution, neither has undergone extensive speciation

Fig. 2. The general distribution of A. fasciatus is represented by the shaded area; it may be locally absent [based on Eigenmann 1921, p. 228].

(Bussing 1976).

Curiously these two families are the only ones of southern origin that have blind cave representatives in Middle America: *A. mexicanus*, usually considered a subspecies of *A. fasciatus* (Gery 1977, p. 422), in Central Mexico; and *Ophisternum infernale* (Hubbs 1938, Rosen & Greenwood 1976), the blind cave synbranchid species in the Yucatan Peninsula.

Bussing and Lopez (1977) showed that *A. fasciatus* forms schools of large size under widely different ecological conditions, and that its relative biomass when compared to other fish species is very high. This species also shows salt tolerance and faces little ostariophysian competition north to the Panama canal. This high ecological variability and abundance may be due to the high heterozygocity of the *Astyanax* complex group. Avise and Selander (1972) determined that populations of *A. fasciatus* inhabiting rivers and arroyos are among the most polymorphic vertebrates yet studied. The mean value of 11.2% heterozygous loci per individual has been equalled among animals only by *Drosophila pseudoobscura*, *D. willistoni* and *Mus musculus*. Bussing (1976, and pers. comm.) has found high intra- and interpopulation phenotypic variability in *A. fasciatus* of Costa Rica and southern Nicaragua.

Morelli et al. (1983a,b) found that the *Astyanax* species complex is also highly diverse in the number and morphology of the chromosomes, suggesting a model of non-conservative karyotypic evolution for this genus based on chromosomal rearrangements.

It is probable that the uniquely broad distribution of *A. fasciatus* among freshwater fishes of South American origin is due to a combination of generalized ecological requirements, salt tolerance, high phenotypic and genotypic variability and a strong migratory proclivity.

A. fasciatus can be found as a surface (eyed) form and as a cave (usually blind) form. Due to the blindness and depigmentation of the latter, Hubbs and Innes (1936) described the first blind cave population ever found (La Cueva Chica, San Luis de Potosí, Mexico), as a new genus and species (*Anoptichthys jordani*). Alvarez (1946, 1947) later described two other cave populations as new species also (*Anoptichthys antrobius* for La Cueva el Pachón, and *A. hubbsi* for La Cueva de los Sabinos). In addition to the lack of eyes and pigmentation, the cave blind form also displays a larger number of taste buds (Schemmel 1967).

The surface and cave forms interbreed in both natural and laboratory conditions, producing fertile hybrids with a phenotypically intermediate form in the F_1 generation, and with a F_2 generation whose individuals range from an almost completely blind and depigmented form to an almost "normal" eyed pigmented one (Peters & Peters 1973, Sadoglu 1957, Wilkens 1969) (Fig. 3). Breeding, electrophoretic, and karyotypic studies also indicate that the cave and epigean forms are the same species (Avise & Selander 1972, Kirby et al. 1977).

The two forms are not only different in their morphology, but also in their behavior: unlike the surface eyed form, the blind form never schools, is active all the time and is not aggressive, (Boucquey et al. 1965, Breder 1942, Breder & Gresser 1941a, Erckens & Weber 1976). Although the blind form does produce an alarm substance, it does not react to it (Pfeiffer 1966). Schemmel (1980) also reported differences between the two forms in the angle of inclination used when feeding from the bottom. Differences in the level of phototactic responses

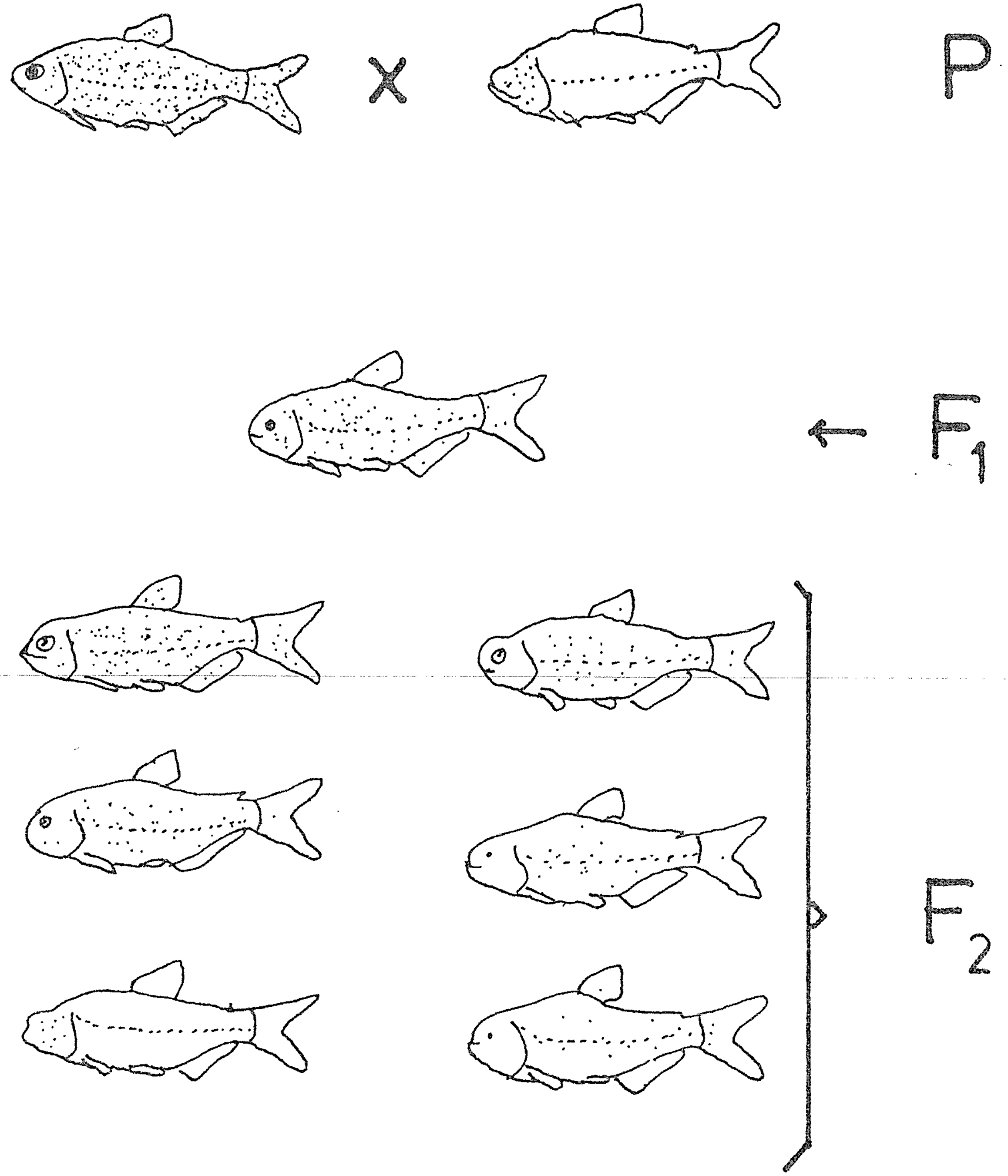


Fig. 3. External morphology of the F_1 and F_2 generations as a result of breeding between the surface (eyed pigmented) form and the cave (usually blind depigmented) one of A. fasciatus [Based on Kosswig 1964 and Peters & Peters 1973].

have also been reported (see chapter 4). These behavioral differences between surface and related cave species and/or populations have also been reported for other cave fishes (Thines 1969).

Although obligatory and facultative cave populations of this fish have been reported for Belize (F. Bonaccorso, pers. comm.), the Yucatan Peninsula (Hubbs 1938), Costa Rica (chapters 2 and 3), and northern Mexico (Reddell 1982, p. 275), the only region in which they have developed into blind depigmented individuals is in the San Luis de Potosí area, central east Mexico, where at least thirty-one caves supporting subterranean populations of this fish can be found (Fig. 4). Not all the cave populations of this area display the same degree of morphological divergence from the surface form, however. Some are fully blind and depigmented while others are only partially so. Three caves contain exclusively individuals showing full eyes and pigmentation. Eleven of these populations contain blind, eyed and phenotypically intermediate forms (Mitchell et al. 1977; chapter 5; and Romero, unpublished observations).

1.7. Previous studies in photobehavior of *A. fasciatus*

Breder and Rasquin (1947a) described the existence of differential phototactic responses among cave populations of *A. fasciatus*: La Cueva Chica population was reportedly scotophilic (i.e., displaying preference to remain in the dark portion of a light/dark choice chamber), La Tinaja population scotophobic (preference to remain in the light portion of the same choice chamber) and the rest "almost" or totally indifferent to light. Hybrid individuals between different populations were not only intermediate in their morphology, but also in the nature

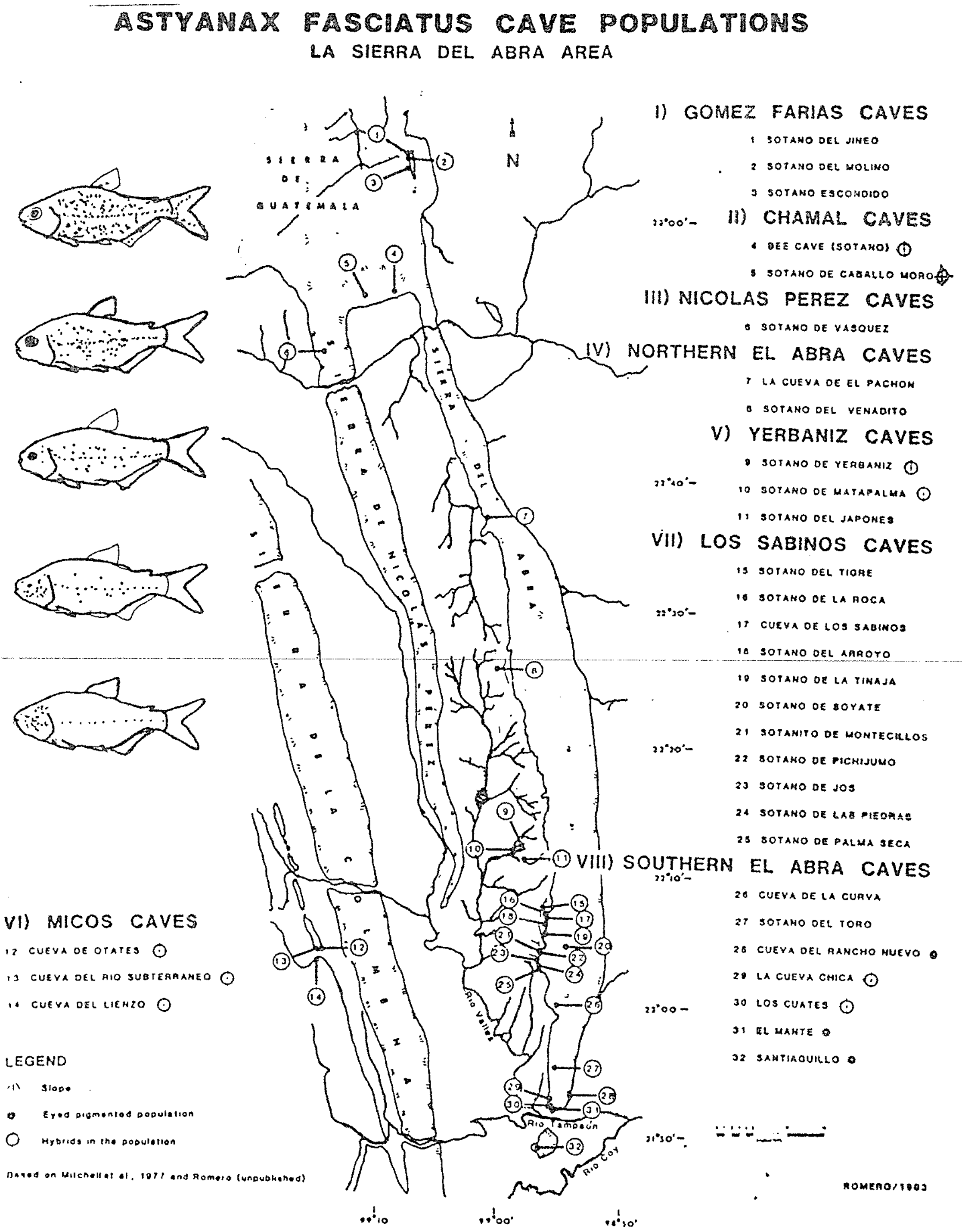


Fig. 4. Distribution of the cave populations of <u>Astyanax fasciatus</u> in La Sierra del Abra area, central east Mexico [Based on Mitchell et al. 1977 and Romero, unpublished observations].

of their phototactic responses. They found two photosensory organs mediating light reception: the pineal organ and the optic cyst. Tabata (1982) has stressed the role of the former as photoreceptor in this fish. Breder and Rasquin (1947a) speculated that no reversal or change in phototactic responses occur during ontogeny. These authors also suggested that scotophilia would inhibit cave fishes from exiting their cave environment toward the exterior. However, no convincing explanation is given for either scotophobia or indifference to light among other populations.

Although further studies (Boucquey et al. 1965, Erckens & Weber 1976, Gertychowa 1971, Kuhn & Kahling 1954, Sadoglu 1967, Schlagel & Breder 1947) have provided additional information about phototactic responses in cave populations of *A. fasciatus*, no study on the ontogeny of these responses has been done nor has a consistent ecological and/or evolutionary interpretation of their variability been offered.

1.8. Approach

Mayr's view of behavioral changes preceding structural ones during evolution was approached in cave fishes by studying the relation between morphological changes, namely structural reductions, and behavioral changes, especially phototactic responses and schooling behavior.

A. fasciatus was the major subject of the present study because:

a) in contrast to other known cave species, this fish does not display the typical cave "preadaptations," namely, nocturnal habits and/or enlargement of non-visual sensory organs. Thus, its adaptation to the subterranean environment may have occurred through drastic behavioral, physiological, and morphological changes;

b) cave and surface populations of the same species can be found, so that comparative studies are feasible;

c) the Mendelian genetics of many of the morphological and behavioral differences is known;

d) this is the only cave organism in which scotophilic, scotophobic, and indifferent responses to light have been reported; and

e) extensive field and laboratory experiments are feasible.

1.9. General questions and specific hypothesis tested

To understand the role played by behavior in evolution, one must address the following questions:

A) How do behavioral predispositions arise and change over time?

B) Can behavior affect the evolution of structural forms?

When applied to the specific case of cave organisms, an additional question can be added:

C) What is the relationship between behavioral and morphological changes during the evolution of the convergent features (structural and behavioral reductions) commonly found in cave organisms?

The specific hypothesis tested is that changes in behavior and morphology related to cave dwelling occur simultaneously in time.

This hypothesis is interesting not only because of the possible examples of behavioral changes preceding morphological ones given in section 1.1., but also because populations of many fish species (including *A. fasciatus*) are obligatory cavernicoles not showing, however, the structural reductions (i.e., blindness and depigmentation) related to cave dwelling (Hubbs 1938). This suggests that changes in morphology are not necessary to achieve cave colonization.

To test the hypothesis of temporal dependence between morphology and behavior, a comparative study of surface (eyed) and cave (both eyed and blind) populations of *A. fasciatus* was carried out under different environmental conditions: in surface rivers and pools, at the entrance of a subterranean source of water in which individuals of *A. fasciatus* can live facultatively outside and inside of a cave, and in the caves of the San Luis de Potosí area in Mexico where populations showing different degrees of blindness and depigmentation can be found. Special attention was given to the study of the phototactic responses among different cave and surface populations of *A. fasciatus* because:

a) total darkness is by far the most important difference between the cave and the surface environments;

b) the major morphological differences found between most cave organisms and their presumed ancestors are light-related (e.g., blindness and depigmentation);

c) phototactic responses have been reported to be variable from one population to another; and

d) this behavior is easy to quantify and the conditions are easy to control.

The results obtained in the studies described throughout this dissertation were compared with those from studies on other species of cave fishes in order to ascertain the generality of the results.

1.10. The predictions

To test the hypothesis of temporal dependence between morphological and behavioral changes in *A. fasciatus*, it was necessary to quantify those changes at different stages of cave colonization. Given that the degree of structural reduction among cave populations of A. fasciatus is variable, I categorized populations of A. fasciatus as follows:

Type 1.- Eyed/pigmented, surface dwelling.

Type 2.- Eyed/pigmented, cave dwelling.

Type 3.- Either reduced eyes and/or pigmentation, cave dwelling.

Type 4.- Blind depigmented, cave dwelling.

If the occurence of specific behavioral traits is independent of these different structural forms, the null hypothesis is rejected. If each form exhibits a corresponding specific behavioral trait, then further analyses of the components of the problem (see below) must be undertaken to provide enough information to decide whether such results appear to be a product of confounding variables or whether the hypothesis of dependence is not rejected.

1.11. Basic assumptions

On the basis of the information provided in section 1.6., I assumed that the eyed surface form that can be found today in the zone surrounding the caves of the San Luis de Potosí area is identical or almost identical to the form that gave rise to the current blind cave form. That the populations of totally blind and depigmented individuals are the product of isolation in cave environments is well accepted. I assumed that populations showing intermediate morphologies between the surface form (Type 1) and the totally blind and depigmented form (Type 4) are also intermediate in terms of cave dwelling, unless hybridization can be proven.

1.12. Components of the problem

The evolution of phototactic responses has at least four components: habitat, ontogeny, photoreceptors, and genetics. The components stressed in the field and laboratory studies developed for the present dissertation were habitat and ontogeny. However, genetical information, especially in cases where hybridization occurs, as well as background on the photoreceptors and sensory bases for other kinds of behavior, were also considered.

1.12.1. Habitat

One component which can affect some of the results, especially in behavioral terms, is that not all the caves represent the same kind of habitat. For instance, the studies on schooling and anti-predator tactics (chapters 2 and 3) were carried out in a pool connected to a subterranean cavity, while most of the other field studies took place in "typical" caves.

1.12.2. Ontogeny

My preliminary studies of a captive pure line of cave fish (probably descended from La Cueva Chica population, see p. 58) suggested that phototactic responses change during development from almost indifferent to light to scotophilia. I therefore undertook comparative studies of the ontogeny of phototactic responses among cave populations, and between these and the epigean ones, to infer the most likely sequence of evolution of phototactic responses from the surface form to the cave one.

CHAPTER 2. BEHAVIOR IN AN "INTERMEDIATE" POPULATION OF *A. fasciatus* IN COSTA RICA: DIURNAL OBSERVATIONS

2.1. Introduction

It is difficult to generate testable hypotheses about how any evolutionary event takes place. With the possible exceptions of experiments on organisms of short generation time where unusual strong selection occurs or can be applied (such as in bacteria and *Drosophila*) and instances in which naturally occurring changes can be documented over a relatively short period of time (such as industrial melanism in Lepidoptera, Kettlewell 1961), evolution can not be easily reproduced and/or hastened and is very difficult to observe within a human life span. Thus any test on organic evolution will generally yield only indirect evidence on how evolution occurs. An additional problem for evolutionary studies is that it is unusual to find intermediate evolutionary situations, i.e., circumstances in which we find not only ancestral organisms and their descendents, but also intermediate forms connecting the former to the latter.

There are many examples of obligate and facultative cave organisms whose surface ancestor can be presumed; however, an important question remains to be answered: How does cave colonization occur? how does a species change from surface to cave existence? We usually have only the ancestral and the cave adapted forms, but not the intermediate stages to indicate how the changes occurred.

In this and the next chapter, the results of the study of behavior and morphology of populations of organisms living in a pool at the entrance of a subterranean cavity (i.e., a possible "intermediate" situation) will be presented in order to address the question of how cave colonization can occur. In this chapter I will specifically test the hypothesis that individuals of _A. fasciatus_ from the population living in this pool differ in behavior but not in morphology from individuals of the same species that live in the surrounding waters of the Tempisque river. I will do so by studying fish density, schooling and feeding behavior and by comparative analyses of eye diameter and pigmentation. Thus, special attention will be given to whether or not fish species can actively colonize a subterranean environment and undergo distinctive behavioral changes without accompanying morphological changes characteristic of cave-dwelling fish.

2.2. Material and methods

During July 1981 and May 1982 I studied an assemblage of fishes numbering about one hundred and twenty individuals consisting of two species, _Astyanax fasciatus_ and the poeciliid _Brachyraphis rhabdophora_ (Regan 1905). Those occurred in a pool close to "La Hacienda de Palo Verde", province of Guanacaste, NW Costa Rica (Fig. 5). This pool is under canopy shade and is supplied by water of subterranean origin (Fig. 6). A containment wall to increase its capacity was made in 1978 at the end of this pool, opposite to the exit of subterranean waters, and a well was placed almost in the center of the pool that same year for the water supply of the nearby field station. The outflow from this pool (henceforth referred to as the "first pool") goes through a canal during the wet season to a second pool in an open area in a depression on a temporary road to "La Hacienda." The second pool dries out during the dry season. The fish in the second pool are all

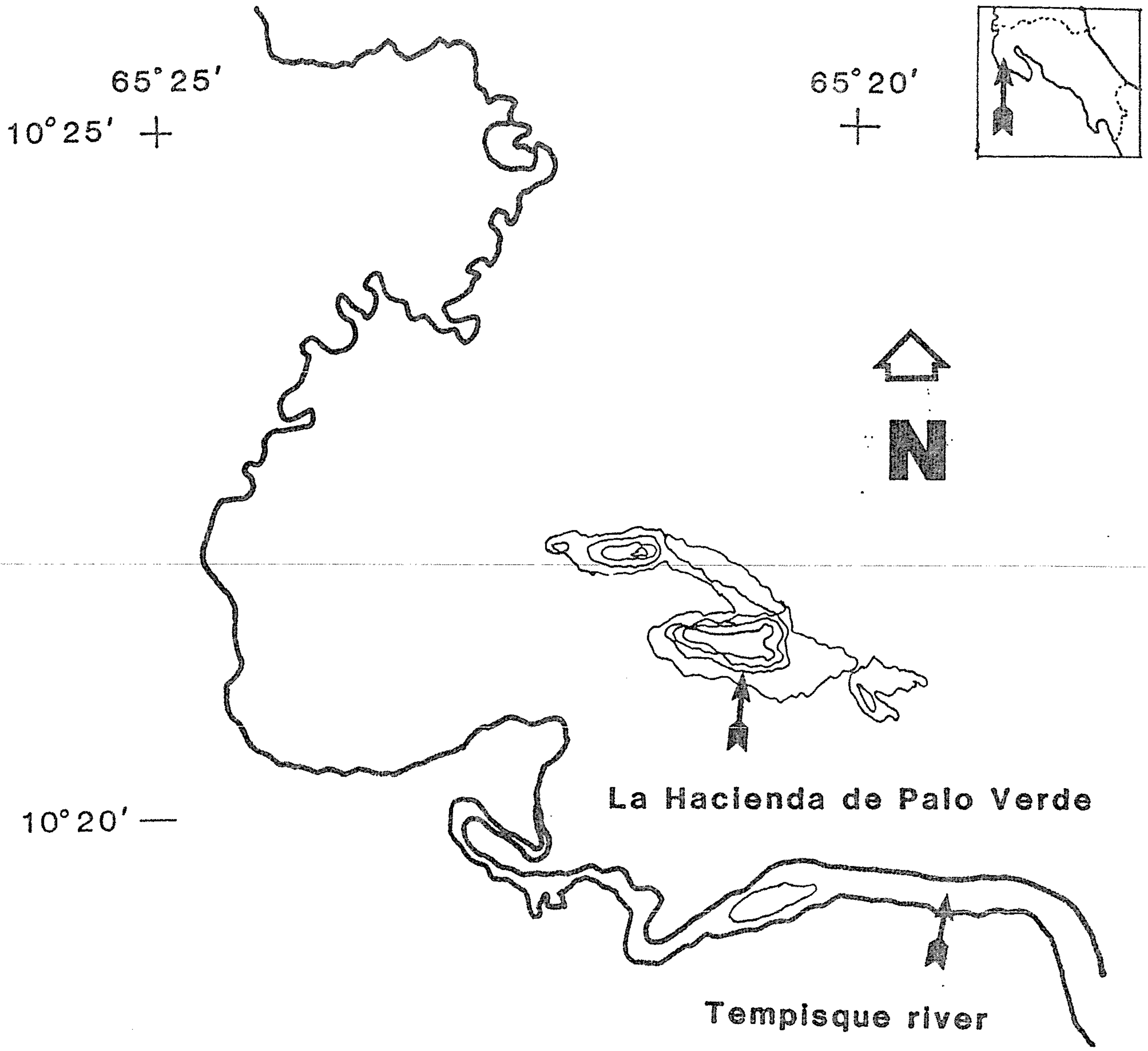


Fig. 5. Map of the location of the study site at Palo Verde, Province of Guanacaste, Costa Rica [Modified from Ministerio 1981, p. 45].

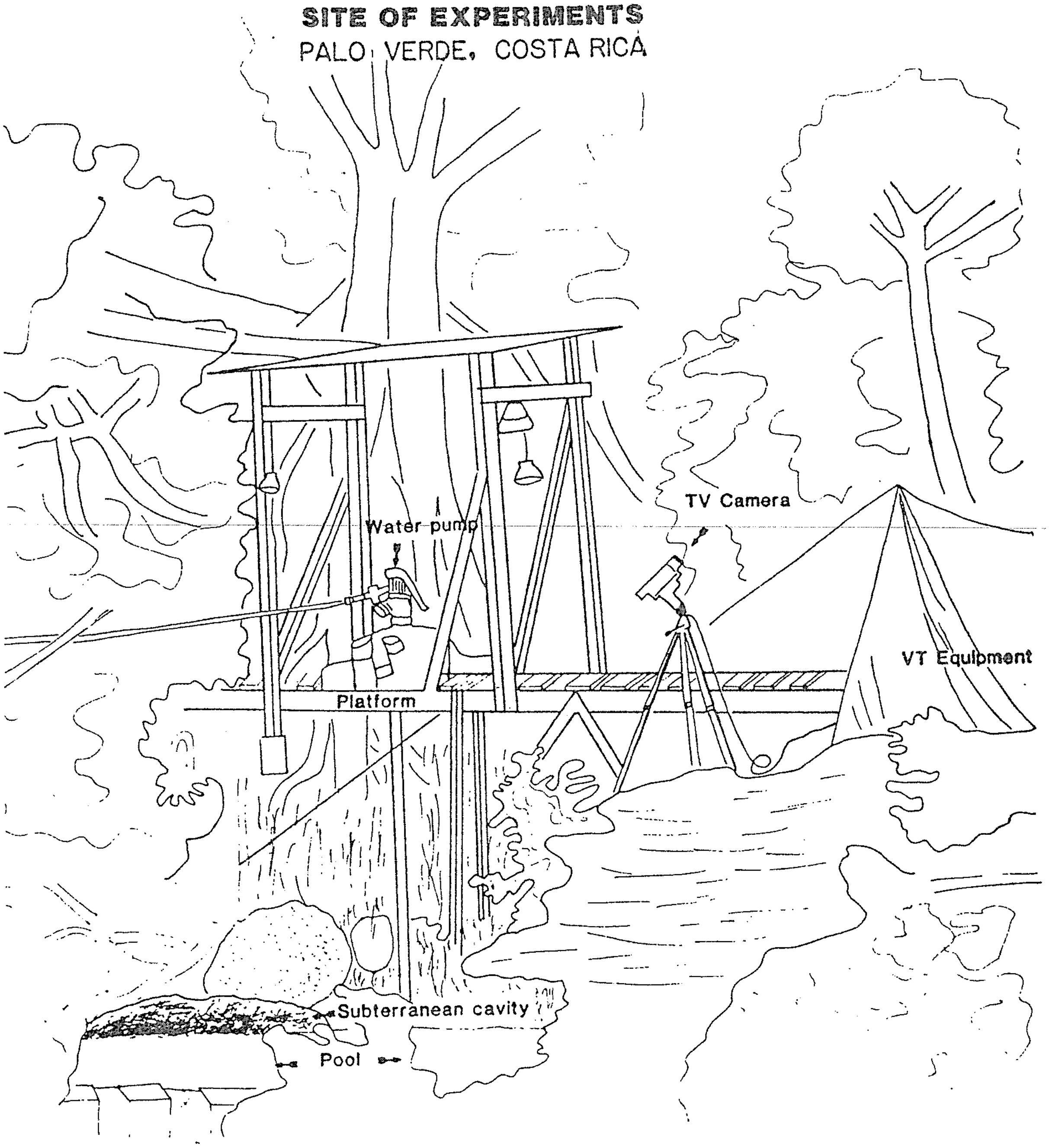


Fig. 6. Site of experiments. For the nocturnal observations described in Chapter 3, the tv camera was placed above the platform.

A. fasciatus. Some individuals of both species can be observed in the canal connecting both pools. Observations made at the beginning of the wet season (May 1982) showed that the individuals in the second pool come from the first one. It is not surprising to find the two species under these conditions since A. fasciatus migrates at the end of the rainy season toward head waters (Lopez 1980), while the other, B. rhabdophora, is a head waters specialist.

Density of fishes was determined by counting the number of fishes per unit of time in 50 X 50 cm grid cells. Grid lines were suspended a few centimeters above the water surface in three different locations in the pool that differed in distance (1, 2.5 and 3.5 m) from the cave mouth. Five minute density counts per grid cell were made over a three days period, four times a day as follows: one in the morning (ca. 0730h) before the drop of the first piece of bread (see below) and another right after the first dropping took place. The same sequence was followed in the afternoon (ca. 1330h). Correlation between density and the distance to the subterranean cavity before and after the first dropping was analyzed using Kendall's correlation coefficient. All statistics for this and next chapters were performed based on Nie et al. 1975 and Hull & Nie 1981, unless otherwise indicated.

Observations on schooling were made in the first pool, in a 1.5 m X 5 m man-made pool also close to "La Hacienda," and in small aquaria (10 gal≃40 l) tanks in my laboratory in Coral Gables. Behavior was recorded using videotape (Sony black and white camera and portable Betamax video recoder). The videotapes of schooling and other behaviors were later analyzed frame by frame in the laboratory using a Panasonic slow motion playback unit.

For the feeding experiments I used pieces of dry bread (ca. 2 cm^3) as a bait. Twenty pieces of bread were dropped less than one meter from the mouth of the cavity each day for three days. Ten were dropped at 10 min. intervals in the morning starting at 0730h, and ten in the afternoon starting at 1330h. The frequency with which the pieces of bread were carried into the cavity and the interval between the time when the bread hit the surface of the water and when it was carried into the cavity were recorded. In addition, sixteen more pieces of bread were dropped more than one meter from the mouth of the cavity at 10 min. intervals after the morning observations, six the first day, four the second and six the third. The frequency with which the pieces of bread were carried into the cavity was also recorded for this experiment.

For the morphological studies, 18 individuals from the first pool and 18 individuals from the nearby Tempisque river were collected by unselectively dip-netting from the surface of the water. Only mature individuals (> 25 mm; W. Bussing personal communication) were used for these comparative studies. Eye diameter and standard length for each individual were measured using vernier calipers. Measurements were recorded to the nearest 1 mm. The number of melanophores on the operculum, the largest of the infraorbital bones, of the left side of the head was also counted for each individual. Morphological differences were examined by comparing two features: 1) the overall ratio of eye diameter/standard length and; 2) the number of melanophores on the left operculum in the two populations. Differences between the two samples were analyzed using a Mann-Whitney U test.

2.3. Results

2.3.1. Fish density

The number of fish counted correlates negatively with distance to the cavity entrance before (Table 1; Kendall's Tau = −.9467, n = 18 counts, $p < 0.001$) and after bread dropping (Kendall's Tau = −.9467, n = 18 counts, $p < 0.001$).

2.3.2. Schooling

Individuals of *A. fasciatus* in the surrounding open-area waters (and under all other open-area conditions) formed large schools, i.e., all or almost all the invididuals of the population (in the hundreds) in a compact group swimming in the same direction at the same time. These compact schools were always present during daytime. In contrast, individuals of this characid in the first pool were rarely observed schooling; such schools were small (never more than six individuals) and of short duration (no more that two min.). *B. rhabdophora* was never observed in schools.

When three groups of six individuals of *A. fasciatus* from the first pool were transferred to the 1.5 m X 5 m man-made pool in an open area they did not show any schooling behavior. Similar results were obtained with two groups of *B. rhabdophora*. However, when an equal number (N = 18) of *A. fasciatus* from river populations were placed in the artificial pool, they retained their schooling behavior. Six individuals of *A. fasciatus* and twelve of *B. rhabdophora* from the first pool were later brought to Coral Gables in May, 1982, and until the end of 1983 (when most of them had died) they did not show any

Table 1. Number of individuals of *A. fasciatus* and *B. rhabdophora* in a grid cell before and after bread is dropped. The number was determined by an initial count then adding number of fishes that entered through all sides of each of the 50 cm X 50 cm grid cells less the number exiting. Each count was conducted over a 5 min. period, two replicates per day over three days

	Mean number of fishes per grid cell ± standard deviation (n = 6 counts for each mean)			
	Before bread dropped	After bread dropped	% increase	Distance from the nearest edge of the grid cell to the entrance of the cavity (m)
GRID CELL I	148 ± 3.162	197 ± 5.253	33.1	1
GRID CELL II	28 ± 2.280	31 ± 1.633	10.7	2.5
GRID CELL III	4 ± 1.672	5 ± 1.265	25.0	3.5

schooling behavior when placed either in small aquaria (40 l) or in a larger (1.5 m X 3 m) tank.

2.3.3. Feeding behavior

Both species reacted to the pieces of bread by pushing them along the surface and into the mouth of the subterranean cavity. This behavior was observed in 59 of 60 instances when the pieces were dropped less than one meter from the mouth of the subterranean chamber but in only one of the 16 instances when the pieces were dropped more than one meter away from the cave entrance (Table 2). The process of taking the pieces of bread, however, was never initiated immediately after the bread was dropped. Videotape analysis of this behavior shows that it takes a mean time of 18 seconds to carry the piece of bread into the subterranean chamber.

When I placed an 8 cm diameter floating log between the dropping point and the entrance of the subterranean chamber, fishes with the bread in their mouths jumped over the log and entered the subterranean cavity on all seven occasions.

2.3.4. Fish morphology

Individuals of *A. fasciatus* from the pool associated with hypogean waters are not different from individuals from river populations in eye diameter vs. standard length ratio (Table 3, Mann-Whitney's $U = 144$, $n_1 = n_2 = 18$, $p > 0.05$) nor in pigmentation (Table 3, Mann-Whitney's $U = 151.5$, $n_1 = n_2 = 18$, $p > 0.05$) , the two major structural reductions among cave organisms.

Table 2. Effect on food carrying of distance from food source to subterranean cavity entrance.

	Day 1	Day 2	Day 3
Number of times bread dropped < 1 m from the mouth of the subterranean cavity	20	20	20
Frequency with which bread was carried into the cavity	20	19	20
Mean interval between the time when the bread hit the surface of the water and when it was carried into the subterranean chamber (seconds ± standard deviation)	17.5 ± 1.4	17 ± 1.3	19.5 ± 1.9
Number of times bread dropped > 1 m from the mouth of the subterranean cavity	6	4	6
Frequency with which bread was carried into the cavity	1	0	0

Table 3. Standard length means, eye diameter/standard length ratio and number of melanophores on the operculum in the left side of the head in individuals of *A. fasciatus* in the population from the first pool and the population of the surrounding areas (N = 18 per population).

	Standard length (mm)		Eye diamater/ standard length ratio		Number of melanophores	
	1st. pool	Tempisque river	1st. pool	Tempisque river	1st. pool	Tempisque river
Mean =	56.611	59.778	0.100	0.095	6.500	6.278
Standard deviation =	12.649	11.958	3.240	0.019	3.240	3.495

2.4. Discussion

Caves are usually limited in food resources due to the lack of primary producers. However, since many taxa occupy caves to some extent (Barr 1968, Poulson & White 1969), active cave colonization is probably advantageous, although such advantages have never been demonstrated. In the present case the affinity that both species of fish show for the subterranean cavity may be explained by at least three factors which could operate in combination or independently as follows:

A) Protection from aerial predators. Piscivorous birds are known to visit Palo Verde in large number, especially during the dry season (Ministerio 1981). However, no predators were observed visiting this pool during daytime.

B) Advantage to fishes of hovering in shade. Most individuals, particularly those of intermediate sizes and large fish of both species, remained mainly in the shaded region created by the mouth of the subterranean cavity. This local overabundance could be explained by the relative visual advantage a shaded fish has over a sunlit fish in detecting approaching objects (Helfman 1981b).

C) Breeding site. The subterranean cavity could be used as a secure breeding site, as has been described by Breder and Bird (1975) for the marine clupeid *Jenkinsia*. Although Breder and Bird observed pigment changes in individuals of *Jenkinsia* that spent some time in caves, the results presented in Table 3 indicate that there was not any statistical difference in the morphologies that usually distinguish cave from surface populations of fishes (i.e., eyes and pigmentation).

2.5. Preliminary conclusions

From these observations and field experiments, some preliminary conclusions can be offerred concerning cave colonization by *A. fasciatus*

1) Cave colonization can take place actively, and not necessarily by accident as some cave-evolution theories claim. Although the construction of the well and the containment wall could have affected fish density in the first pool, the fishes in this pool were not trapped as envisioned by many cave evolutionary hypotheses, since they facultatively enter and exit the subterranean cavity. These fishes can be seen swimming upstream against the flow produced by the subterranean cavity, also supporting the contention that these fishes are not trapped in the subterranean cavity. Evidence presented in the next chapter will also substantiate this assertion.

2) Gross morphology modifications need not to accompany this first stage of cave colonization. No morphological differences were observed in the *A. fasciatus* population of the first pool when compared with individuals from the river populations. The lack of structural reduction in *A. fasciatus* found in the cenotes (a subterranean environment) of the Yucatan Peninsula (Mexico) (Hubbs 1936) further supports the idea that such morphological changes are not necessarily present near the beginning of cave colonization.

The extent to which the behavioral traits in the subterranean-associated population may reflect genetic differences between this and non-subterranean populations has yet to be established. Demonstration would require breeding experiments involving crosses between populations.

This study is probably the first time that behavioral observations have been recorded in "intermediate" populations of a cave dwelling species-complex. Further comparative studies with populations of other species under similar conditions will help to understand any correlation between morphological and behavioral changes during the cave colonization process.

The next chapter will present the results of experimental manipulations performed in the same study site at night. These were designed to ascertain pressures favoring the entrance by *A. fasciatus* and *B. rhabdophora* to the cavity at dusk.

CHAPTER 3. BEHAVIOR IN AN "INTERMEDIATE" POPULATION OF *A. fasciatus* IN COSTA RICA: NOCTURNAL OBSERVATIONS

3.1. Introduction

In the previous chapter I advanced the hypothesis that *A. fasciatus* and *B. rhabdophora* living in the first pool associated with subterranean waters use the cavity for protection against aerial predators, although such predators had not been seen during daytime.

Casual observations made at the beginning of the wet season (May, 1982) indicated that fishes move into the subterranean source of water at dusk, the time at which fishing bats begin foraging (Bloedel 1955), and that they reappear in the pool after the bats cease flying over it.

Such observations were not surprising since it is widely known that several species of tropical bats capture fish from surface waters (Bloedel 1955, Reeder & Norris 1954, Suthers & Fattu 1973). The echolocating *Noctilio leporinus*, for example, is known to prey on *A. fasciatus* and on the cichlid *Cichlasoma urophthalmus* (Simmons et al. 1979, Suthers 1967, Villa-R. 1966). The distributions of *N. leporinus* and *A. fasciatus* are very similar, the former ranging from central western Mexico to Argentina, the latter from southwestern U.S.A. to Argentina.

This situation offered an excellent opportunity to study at least one possible benefit associated with cave colonization. Such study is particularly interesting not only because of the differences in behavior already reported for this fish assemblage, but also because a test of the dogma of cave colonization by entrapment could be performed.

3.2. Methods

To examine whether the subterranean cavity serves to protect fish during the bats' active foraging time, a laboratory experiment and four field manipulations were performed.

In the field all observations and manipulations were made during three non-consecutive nights per set of experiments. The observations were made from 1800 to 2130h since this is the period of time in which bats are most active. Illumination was supplied by six tungsten lamps, two 125 watts red/infrared and four 125 watts "white" floods. Unless otherwise indicated, only the red/infrared lamps were used. These lamps were suspended from the platform at about 1 1/4 m from the water surface.

In addition to direct observations, a tv camera was placed on a platform directly over the center of the pool at about 2 1/2 m from the surface of the water. The field of view covered was ca. 70% of the pool's surface, including the entrance to the subterranean cavity. The camera's output fed a videorecorder yielding videotapes that were later analyzed frame by frame in the laboratory. Two 50 cm x 100 cm matte aluminium ceiling sheets were placed on the bottom of the pool and near the entrance of the subterranean cavity to obtain better video contrast. Relative density was determined by counting the number of fish visible in the camera's field on the videotape at 5 min. intervals. The number of bat flights through the field of the camera was summed over 5 min. periods.

The set of "control" observations consists of recording fish density in the pool and bat activity above it in the evening under red/infrared illumination.

To study the relationship between fish density and bat activity I performed four manipulations. In the first one, white flood lights were turned on after dark from 1900 to 2130h in order to increase fish density at night.

The second manipulation consists only of covering the entrance of the subterranean cavity with a cotton sheet at 1800h, preventing fishes from entering the cavity.

To determine if fishing bats passing over the pool influence fish density, a third manipulation consisted of obstructing the flight path over the pool using opaque plastic sheets, thereby preventing bats from passing over it.

Because fishing bats find their prey by detecting the disturbance that swimming fish create on the surface (Simmons et al. 1979), a fourth manipulation was performed by stretching a cotton sheet across the pool of water so as to cover its surface.

The mean count over the three nights for each 5 min. sample block (instantaneous count at 5 min. intervals for fish, summed count over the 5 min. periods for bats) for the "control" and for each manipulation is one data point or paired data point for determining N for the non-parametric statistical tests.

Laboratory observations were made on light and shade preferences in the evening. Six fishes (two *A. fasciatus* and four *B. rhabdophora*) taken from the study pool and another identical group taken from the surrounding waters of the Tempisque river were brought to my laboratory at the University of Miami. The two fish groups were maintained in separate tanks for a period of 9 mo. The room in which the fish were maintained was under a 12D:12L period (lights on at 0500h; all times for Miami are reported as Costa Rican time). Observations were conducted during three consecutive days between 1730 and 2130h. The fishes were placed in a 2 m x 1 m x 0.6 m arena tank which had one half protected from direct ambient light, thus forming a light/dark choice chamber. The number of fishes present in the light compartment was counted at one min. intervals. The mean count over three days for each sample period is one data point for determining N for the non-parametric statistical tests.

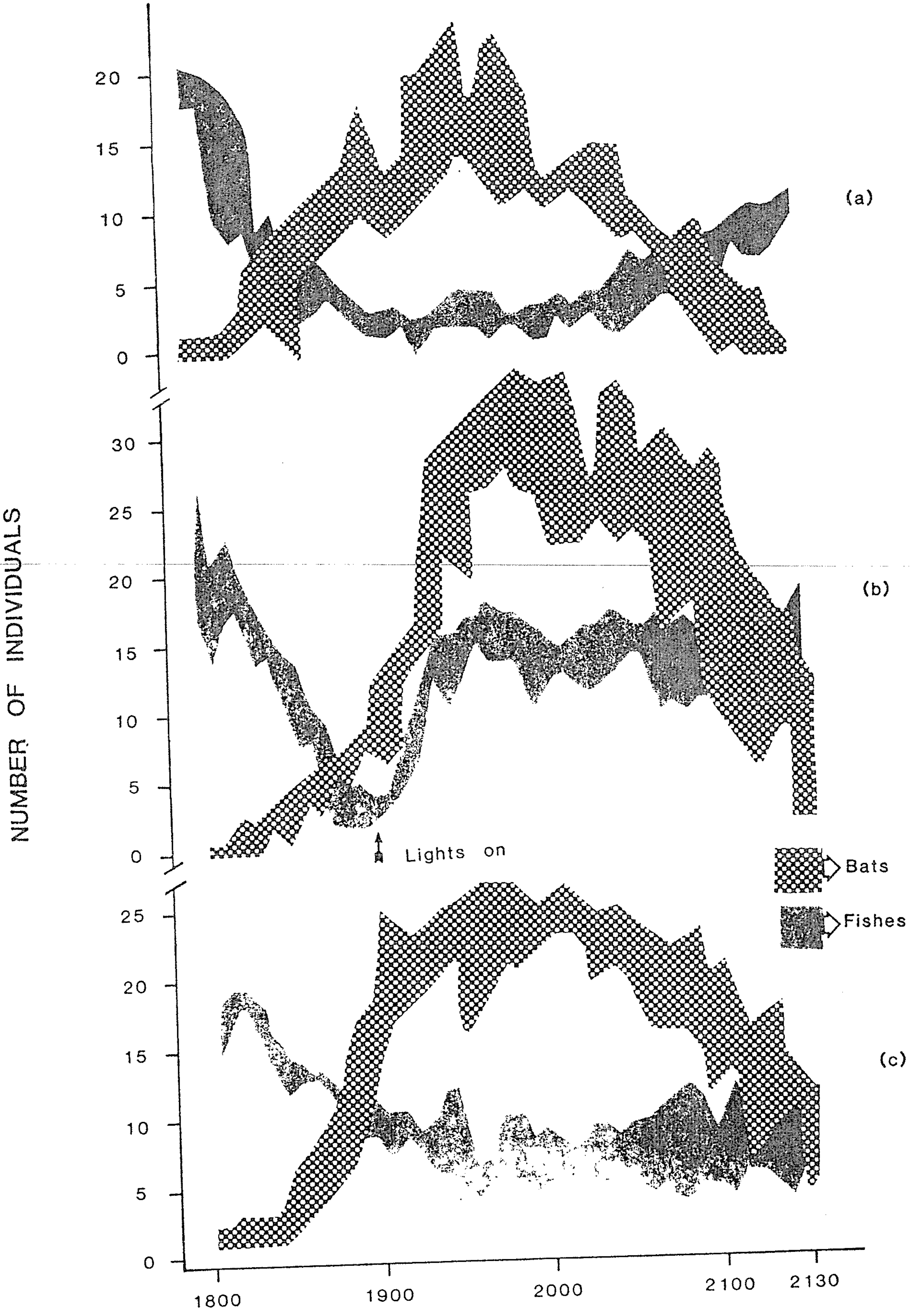
3.3. Results

Fig. 7a shows a decrease in fish density observed in control (red/infrared light) conditions while the number of fishing bats passing above increases. The negative correlation of fish density with bat activity is significant (Kendall's rank correlation coefficient, Tau = -0.8131, $n = 43$, $p < 0.001$).

Fig. 7b shows that when white lights are turned on, fish density increases to nearly earlier levels. Bat activity follows the same temporal pattern as in the control. There is a positive but not significant correlation between fish density and fishing bat activity (Kendall's, Tau = 0.1333, $n = 43$, $p > 0.05$). However, if the correlation is calculated using fish density and bat activity between 1900 and 2100h (i.e., from the time lights were on to just before the decrease in bat activity), the correlation is significant (Kendall's, Tau = 0.3359, $n = 21$, $0.01 < p < 0.05$).

Fig. 7c shows the results of obstructing the entrance of the subterranean cavity with a cotton sheet. Again a significant and negative correlation between fish density and bat activity was found (Kendall's,

Fig. 7. Fish density and bat activity in and above the pool. Fish are counted at 5 min. intervals, bats are counted over 5 min. periods. Numbers of individuals counted in the video field of view on three nights are reported. The maximum and minimum counts for each of the three 5 min. blocks are plotted and the area between them shaded. (a) Control levels observed under red/infrared light. (b) White lights on from 1900 to 2130h. (c) The entrance from the pool to the subterranean cavity blocked with a cotton sheet from 1800 to 2130h; observed with red/infrared light.


NUMBER OF INDIVIDUALS
20
15
10
5
0
(a)
30
25
20
15
10
5
0
(b)
Lights on
Bats
Fishes
25
20
15
10
5
0
(c)
1800
1900
2000
2100
2130
TIME (hrs)

Tau = -0.6640, $n = 43$, $p < 0.001$)

Fig. 8a shows that when the entire area around the pool is covered to exclude bats, there is a significant increase in fish density when compared with the control observation densities (Wilcoxon matched-pairs signed-rank test, $T = 6.0$, $n = 43$ including 1 tie, $p < 0.001$).

Fig. 8b shows that when a sheet is used to cover most of the surface of the pool, a decrease in bat activity is observed when compared with the control observations (Wilcoxon matched-pairs signed-rank test, $T = 3.0$, $n = 43$ including 2 ties, $p < 0.001$).

Fig. 9 summarizes the laboratory observations. The number of individuals situated in the shaded area of the arena tank is greater for fish from the study pool than for fish from the Tempisque river (Wilcoxon matched-pairs signed-rank test, $T = 3.50$, $n = 43$ including 2 ties, $p < 0.001$). Mean standard lengths for both populations were not significantly different (Table 3, p. 30: Mann-Whitney U test, $U = 130$, $n_1 = n_2 = 18$, $p > 0.05$).

3.4. Discussion

Considering the low level of nutrients, cave dwelling as a habit has often been assumed to have little advantage (Barr 1968). Results presented here on the other hand, reveal that entry into a subterranean cavity is linked to fishing-bat activity. Utilizing the underground area of the pool may be advantageous here and wherever bat activity is high, as is the case over most of the range of *A. fasciatus*. It is noteworthy that cave colonization has occurred in the Yucatan peninsula in the two species of fish (*A. fasciatus* and *C. urophthalmus*) on which *N. leporinus* specializes (Hubbs 1938, Villa-R. 1966).

Fig. 8. Changes in fish density and fishing-bat activity when detectability of the other animal group is decreased. Three nights when fish density is examined in the absence of bats alternate with three nights when bat activity is examined while fish are blocked from surfacing. (a) The entire circumference around the pool is blocked using opaque plastic sheets. (b) A cotton sheet is stretched across the pool just covering its surface.

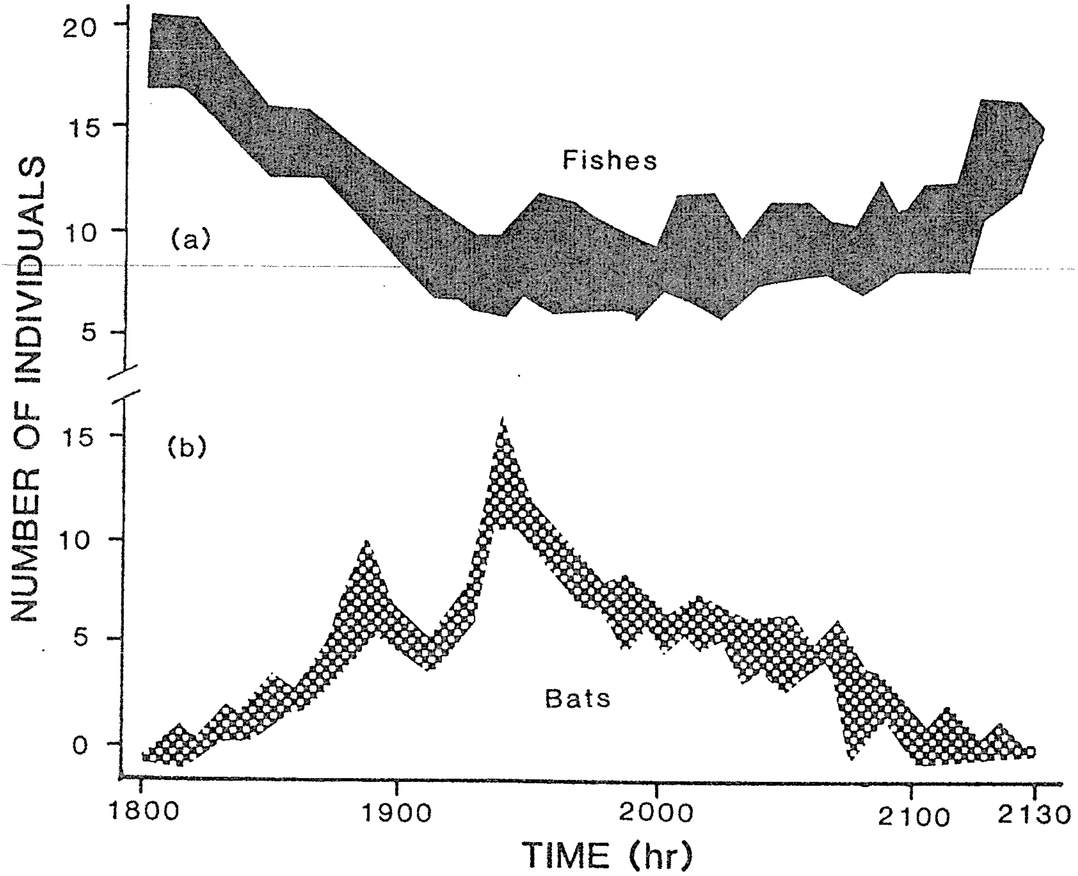
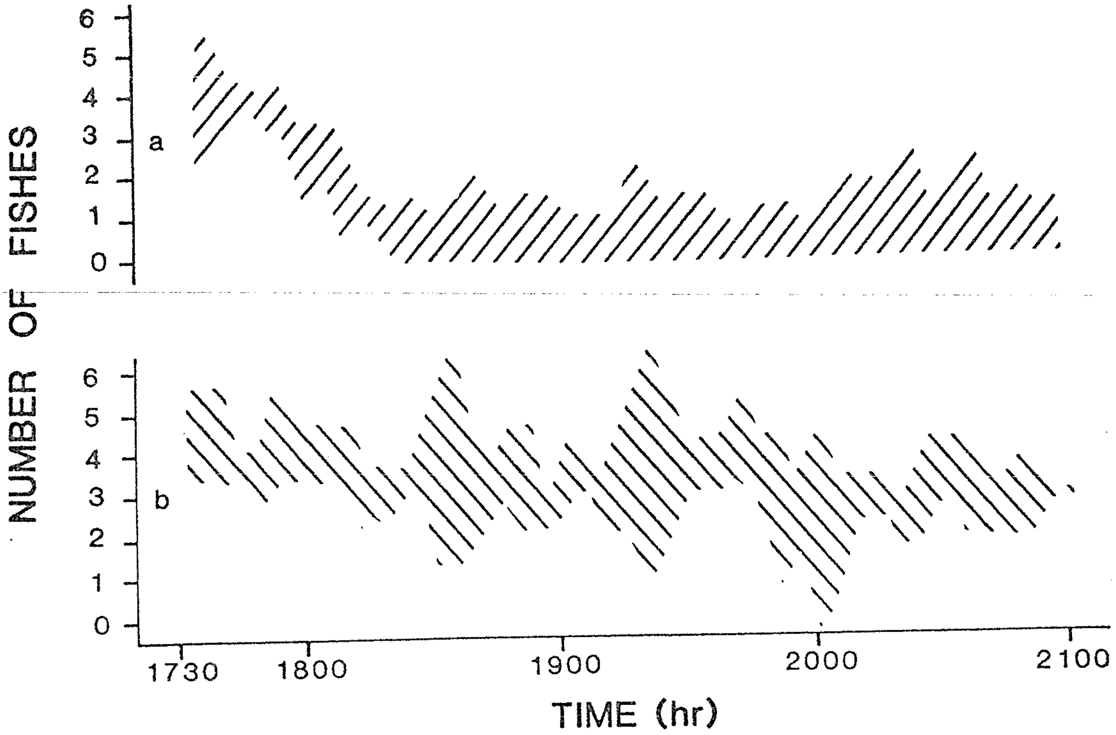

20
15
10
5
(a)
Fishes
15
10
5
0
(b)
Bats
NUMBER OF INDIVIDUALS
1800
1900
2000
2100
2130
TIME (hr)

Fig. 9. Dark and light preferences of fishes from two populations. Number of fish represents the number of individuals counted in the illuminated half of the experimental tank at one min. intervals. (a) Fishes from the study pool; (b) fishes from the Tempisque river.


NUMBER OF FISHES
6
5
4
3
2
1
0
a
b
1730
1800
1900
2000
2100
TIME (hr)

In general these results are in accord with generalizations made on predation and temporal resource partitioning in fishes. Helfman (1978) summarized that "many of the activities of fishes, including behavioral rhythms, can be linked to predator avoidance. The primary concern of an inactive fish is to avoid being eaten ..."

Of course predator avoidance is not the only explanation possible for cave colonization in an animal that has succesfully colonized caves in other regions as well. However, these results suggest that:

1) It is unnecessary to argue for "accidental" or orthogenetic evolution in cave dwelling organisms;
2) It is possible to identify specific potential advantages and test their reasonableness;
3) Cave entry and colonization can occur as a result of a behavioral response to an environmental factor; and
4) Since the cave-invading fish do not show the typical morphological changes associated with cave-dwelling, the overall contention that morphological "preadaptations" are not a prerequisite to invade the subterranean environment is supported.

CHAPTER 4. DIFFERENTIAL PHOTOTACTIC RESPONSES IN SURFACE AND CAVE POPULATIONS OF *A. fasciatus* FROM MEXICO

4.1. Introduction

To better understand the evolution of phototactic responses in *A. fasciatus*, a detailed study of this behavior was undertaken in individuals displaying different degrees of reduction of their sensory bases.

Since ontogeny may illuminate the ecological and evolutionary importance of certain behaviors in fishes (Helfman 1978), and given that fish size and/or age has rarely been taken into consideration in studies of phototactic responses in cave fishes, a description and interpretation of the *A. fasciatus* phototactic responses and its possible changes during development was undertaken.

The ontogeny of behavior in various fish species has been documented for feeding (Grossman 1980, Hairston et al. 1982), migration (Helfman et al. 1982), social behavior (Fernald & Hirata 1979, Noakes 1978), schooling (Shaw 1961), and alarm reaction (Waldman 1982). Development of phototactic responses has been reviewed by Noakes (1978) who concluded that there is a marked transition from scotophilia (photophobia) to scotophobia (photophilia) in some fishes, although other fishes are photophobic in their adult stage (Terami & Watanabe 1980; Ercolini & Berti 1977, 1978).

Only recently, the implications of changes in phototactic responses during ontogeny have been investigated (Carey & Noakes 1981; Helfman et al. 1982). To that end, it is necessary not only to describe such changes, but also to interpret them from an ecological and evolutionary standpoint. This topic is especially attractive when addressed for species which live in total darkness their entire life. It has been shown, for example, that some cave animals exhibit phototactic responses (Thines 1969, Vandel 1969) but the evolutionary significance of such responses is uncertain. Given that for some cave animals it is possible to identify their surface ancestor, it is feasible to infer the evolutionary sequence of change, especially when both surface (epigean, i.e., eyed and pigmented) and cave (troglobitic, i.e., blind and depigmented) populations are conspecific.

Breder and Rasquin (1947a) suggested that scotophilia would inhibit cave fishes from exiting their cave environment, and speculated that either no change, or a reversal of phototactic responses, occurs during ontogeny in some cave populations of *A. fasciatus*. No convincing explanation was given, however, for either scotophobia or unresponsiveness to light among other populations.

Although other studies (Boucquey et al. 1965, Breder 1944, Erckens & Weber 1976, Gertychowa 1971, Kuhn & Kahling 1954, Sadoglu 1967, Schlagel & Breder 1947) have provided additional information about phototactic responses in cave population of *A. fasciatus*, no study on the ontogeny of this behavior has been done.

The aim of this chapter is to test the following hypotheses:

1) Phototactic responses change during development in fish from both cave and surface populations of *A. fasciatus*; and
2) Differences in phototactic responses exist between natural and artificially bred populations of this fish.

I will also present evidence which suggests that scotophilia in the subterranean populations of *A. fasciatus* is a behavioral relic rather than a defensive mechanism to avoid exiting the cave.

4.2. Materials and methods

Four populations of *A. fasciatus* were used in these studies of phototactic responses. The first population consisted of surface (eyed and pigmented) individuals from the Rio Coy (near the cave area of San Luis de Potosí, Mexico, Fig. 4); the second were hybrid fish from La Cueva Chica (pool II, Breder, 1942; see also Fig. 13), all of which showed an incomplete degree of blindness and depigmentation (see chapter 5); the third were morphologically homogeneous (blind and depigmented) fish from La Cueva el Pachon. The fourth population consisted of a commercial stock (captive) bred blind depigmented fish obtained from a Miami pet shop. These fish were imported from a fish farm in the Far East.

4.2.1. Fish from natural populations

Head lamps were used for collecting in caves since preliminary studies had shown that these fish do not react to such light. All fish were caught by unselective netting and their standard lengths were immediately measured using calipers. Measurements were recorded to the nearest 0.1 mm. Fifty-four fish were divided into nine groups of six individuals each, according to size, i.e., one group contained the six smallest fish, another the six largest ones, and seven intermediate groups each contained six fish of very similar size.

A group size of six was selected as the minimum suitable for many non-parametric statistical tests. There were nine groups formed so as to achieve reasonably fine grained size categories given the overall range in fish length.

Each group was placed after capture, measurement, and sorting into a separate opaque plastic container connected to a portable air pump.

The containers were then transported from the caves and rivers to the field laboratory site nearby the collecting areas where the fish were moved into larger opaque containers connected to filters and air pumps. Tests took place in the field laboratory within 15 days of collection. Fish were fed with commercial fish food twice per week, but no experiments were performed on the days that fish were fed.

Tests were performed to ascertain the extent and direction of phototactic responses. A glass aquarium (50 x 25 x 25 cm high) half covered with movable opaque black polyethylene sheeting formed a light/dark choice chamber (Fig. 10). The aquarium was filled to a depth of 20 cm with water from the natural environment (either cave or river) of the fish. Fish were placed into the aquarium using minnow nets.

The light source (either infrared or white) was kept above the aquarium at a distance of 50 cm to avoid heating the water. Lamps used to observe phototactic responses were "white" light General Electric Industrial reflectors, 125-watt (wavelength between 2000 and 3000 nm) and a red/infrared Norelco 2000 hrs red flood, 100-watts. Lamps were turned off between experiments. Nearby, a weak red/infrared lamp (60-watt) was on continuously during the experiments.

Because temperature can affect phototactic responses (Brett 1979), it was maintained at the level of the native habitat (± 1°C) by means of a fan placed about one meter from the aquarium at a level between the aquarium and the light. A thermometer was placed in a corner of the tank. Since chemicals released by fish during an experiment may affect the behavior of the fishes in subsequent experiments, water filters (Dynaflow) with activated charcoal were used during 30 min between

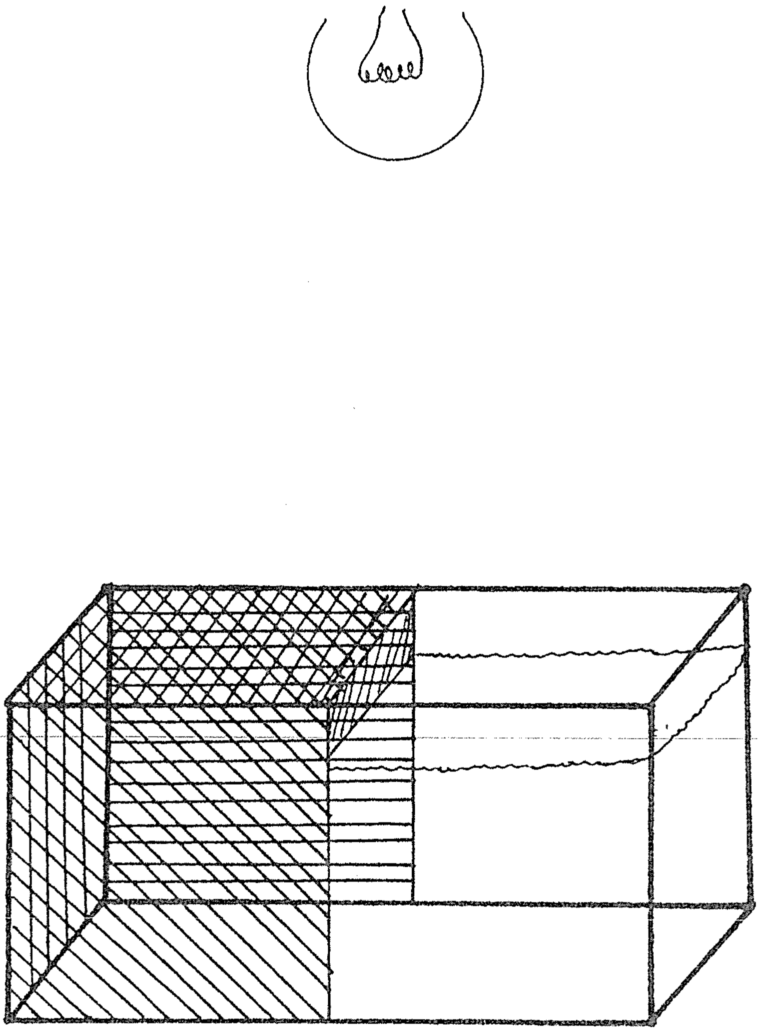


Fig. 10. Experimental set-up for the studies on phototactic responses in the laboratory.

experiments, and the aquaria were throughly rinsed and the water changed after three experiments.

Each group of six fish was tested three times alternatively for red/infrared (control) light (R) and "white" light (W) in the following order: R W W R W R. Tests with red/infrared light were performed before, between, and after tests with white light to detect any preference of the fish for any side of the aquarium as a result of their exposure to light. Every test per group took place alternatively in each side of the aquarium, so at least one test per kind of illumination took place in the opposite side from the other two.

Fish were maintained in the aquarium for 5 min to adjust to the new environment. Fish behavior was either observed directly or videorecorded by placing a videocamera in front of one of the large walls of the tank. For experiments with red/infrared light, a "total darkness-camera" was used; for experiments using white light, a low-light camera was used. Cameras were connected to a portable videorecorder. Tapes were re-recorded on a laboratory slow motion videorecorder for frame-by-frame analysis.

Each test took 30 mins. The number of fish present in the light compartment was sampled at 1 min intervals from the beginning of the test. A fish was counted as in the light compartment if its head was visible.

Preliminary results had shown that there are no significant differences in the total level of phototactic responses when _A. fasciatus_ groups of six are tested individually or together, using either red/infrared light or white light, when following the experimental procedures described above. [Eight tests (four groups in two conditions) comparing counts of all six fish from each group when tested separately vs. together. Counts compared using a one-sample χ^2_{adj} test for each experiment (Sokal & Rohlf 1981, p.711). The maximum difference was observed under red/infrared illumination: 77 counts for six fish tested individually and 90 for that group together. $\chi^2_{adj} = 0.86$ with a critical value for overall $p < 0.05$ of $\chi^2 = 7.441$ for eight replicate tests of one overall hypothesis (Rohlf & Sokal 1981, Table 15).]

For each test of the nine groups from each population, an index of phototactic response was derived by dividing the total count of fish scored in the light compartment by the number of samples per experiment (30) and substracting 3 from the resultant ratio:

$$\text{INDEX} = (\text{counts of fish in the light compartment}/30) - 3$$

So, +3 = maximum scotophobia (all six fish in the light area every sample), −3 = maximum scotophilia (none of the six fish in the light area every sample), and 0 = unresponsiveness to light (a mean of three fish in the light area over the 30 samples). This index was plotted against the mean length of fish in each group.

The one-sided null hypothesis is that these fish under either red/infrared or white illumination will not respond negatively to light. Therefore the lower 90% confidence limit to the null expectation of 90 counts was calculated using the normal approximation to the binomial probability distribution. Indices observed that are above this line mean that the null hypothesis can not be rejected ($p > 0.10$). This value was transformed to the one-sided 90% confidence limit below an index of zero as follows: 30 min X 6 fish scored each minute X

(1/2) probability of scored fish being in lighted half of test aquarium = expected count of 90 = 180 scores X (1/2) = $np = \mu$. $q = 1 - p$ = probability of score not being counted in lighted half. Confidence limits: lower limit = $90 - Z_{90\%}\sqrt{npq} = 90 - Z\sqrt{45}$. From a normal probability area curve $Z_{90\%,1\text{-sided}} = 1.282$. The lower limit is therefore $90 - 1.282\sqrt{45} = 81.400$ which is transformed into an index of -0.287.

4.2.2. The commercial (captive) fish stock

Since most experimental studies on the blind cave form of *A. fasciatus* are performed using either commercially bred stocks of these fish or fish that have been maintained for long periods of time under laboratory conditions (Breder & Rasquin 1947a, Boucquey et al 1965, Erckens & Weber 1976, Gertychowa 1971, Kuhn & Kahling 1954, Sadoglu 1967, Schlagel & Breder 1947), it is important to know if fish subject to such artificial conditions display different phototactic responses. To that end, similar experimental procedures were used for testing the Miami pet shop fish, with the exception that these fish were kept on a 12L:12D photoperiod (lights on at 0600h) until two weeks before the experiments, at which time they were changed to a regime of total darkness to reduce any possible effects of circadian rhythmicity.

To see if growth and changes in phototactic response were correlated, six groups of six fish each were tested. Grouping by length was performed as for the fish from natural populations. Each group of fish was placed in a different tank. The first set of experiments was conducted in April 1982 using the three smallest and the three largest groups. In December 1982 the same experiments were conducted on the three smallest fish groups; these were now designated medium in size due to their growth. So, although only six natural groups of the commercial fish stock were used, results in Fig. 11 for this population show the results of nine length groups because the three smallest ones were tested twice with an eight month interval.

The phototactic responses exibited by each of these three commercially bred fish groups tested three times each and then again after an eight month interval were compared by using a Wilcoxon matched-pairs signed-ranks test on the phototactic index. Matching for test order and combining the results for all groups yields a test of nine pairs of results.

Other results were statistically treated as follows:

1) the phototactic responses of the three smallest groups were compared with those of the three largest groups for each of the four populations using an exact randomization test (Sokal & Rohlf 1981, p. 788 ff.); and,

2) the correlation between phototactic index and size for each of the four populations was analyzed using a Kendall rank-order correlation coefficient. All the indices were negative, so absolute values were used to obtain a scotophilic correlation.

In order to assess the origin of these commercially bred fish, I sacrified six individuals selected at random after the experiments were performed. These fish were stained using the method described by Dingerkus and Uhler (1977) in order to observe bone and cartilage tissue. Circumorbital bones were examined using a dissecting microscope.

4.3. Results

The phototactic index was plotted against mean size for fish in each group for four populations of *A. fasciatus*. When tested under red/infrared light, all fish groups fell within the confidence limit, demonstrating that their distribution in the tank was not scotophilic in this type of light. Under white light, all populations proved to be significantly scotophilic (Fig. 11).

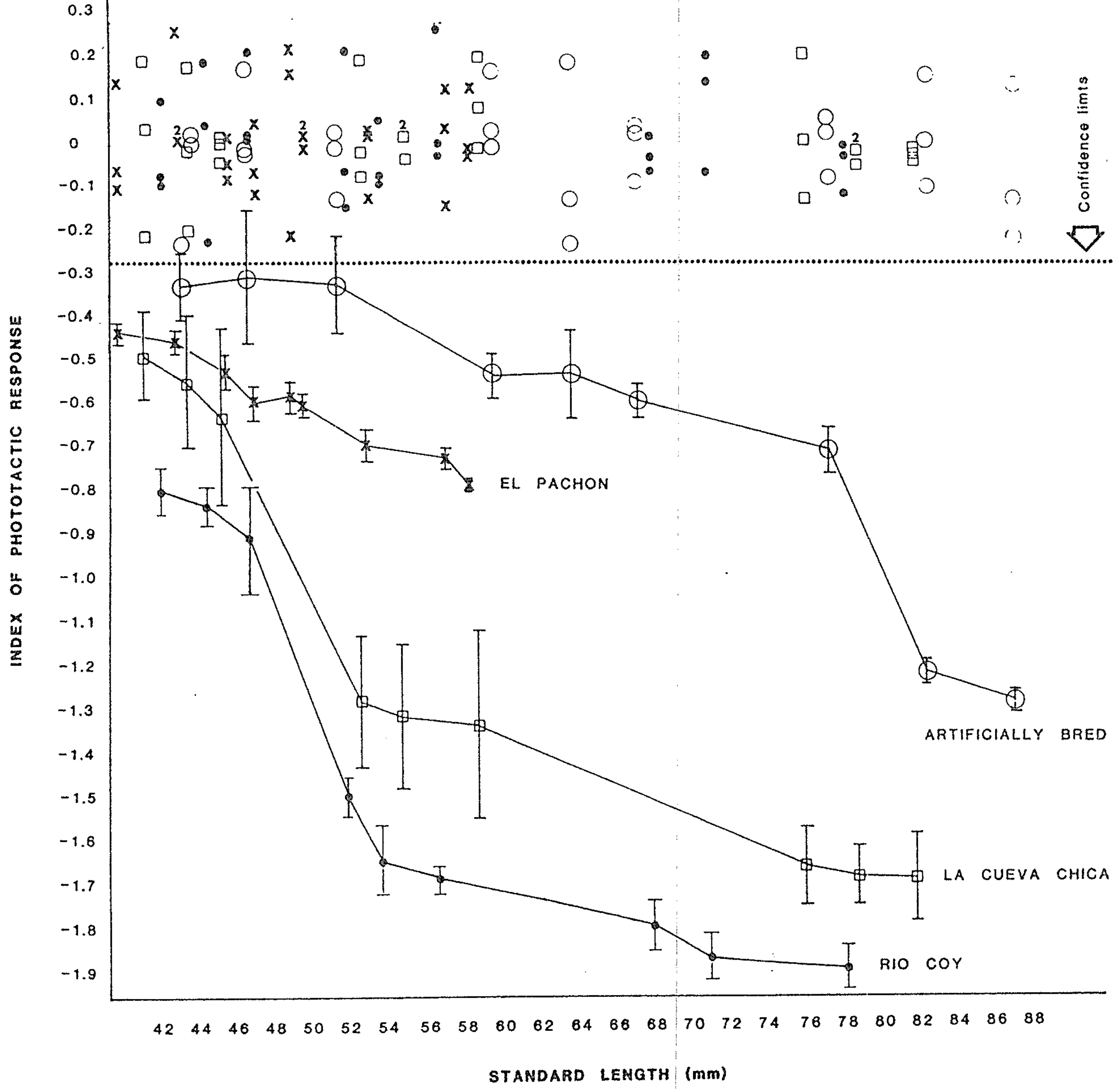
The phototactic index of the three smallest groups of the commercial fish stock when compared with its phototactic index after eight months of captivity, was significantly different (Wilcoxon's $T = 0$, $n = 9$ (no ties), $p < 0.01$).

The groups of the three smallest vs. the three largest fish of each population were compared by performing four independent two sample exact randomization tests on the same phototactic response test results (three tests per three groups of six fish each). The three scores between 0 and 180 for each group were added to form a single score for one group of that size. In each population the three groups of small fish had higher scores than the large fish with no overlap. The randomization test results are $p = 0.10$ that there is no size-related difference for each population. These four probabilities were then combined using Fisher's method (Sokal & Rohlf 1981, p. 799 ff.) to yield a low probability ($0.010 < p < 0.025$) of there being no overall differences in phototactic responses test scores between large and small fish.

Although each population showed a different degree of scotophilia, there was a significant correlation between scotophilia and fish length (Kendall's Tau = 0.7627 for the commercial fish stock, 0.6034 for El

Fig. 11. Phototactic index of each fish group from four populations of *A. fasciatus* plotted against mean length of each group. Symbols: (●) Rio Coy (surface, eyed, pigmented) population, (□) La Cueva Chica (hybrid cave) population, (X) El Pachon (homogeneously blind, depigmented) population, and (○) commercial (blind depigmented) fish stock. Symbols not connected within the 90% confidence limit represent results of tests with red/infrared light (one original and two replicates per fish group per population). Number 2 on symbols means that the same result for the same fish group was obtained twice. Symbols below the confidence limit represent means for an original and two replicate tests per group per population using "white" light. Vertical lines represent standard deviation.

PHOTORESPONSES OF *A. fasciatus* DURING DEVELOPMENT



Pachon population, 0.4845 for La Cueva Chica population, and = 0.7908 for the surface (rio Coy) population; for each population n = 27 [nine groups X three tests each]; $p < 0.001$ for each of the results).

The number, relative size, configuration, and distribution of the circumorbital bones in the commercial fish stock were the same in all the examined individuals (Fig. 12). Among the several cave populations of A. fasciatus that have been described osteologically (Alvarez 1946), only La Cueva Chica population includes individuals with cranial osteologies identical to this artificially bred population. La Cueva Chica is the most accessible and best known source of cave fish to pet shop dealers. Furthermore, given the amount of intrapopulational variation found by Alvarez (1946) (six different cranial osteologies) and the uniformity reported here in the sample from the experimental stock, it is likely that the stock of fish used here is descended from a relatively small original sample, probably from La Cueva Chica.

4.4. Discussion

Several conclusions can be drawn from the present study. First, the level of phototactic responses varies during development in both cave and surface populations of A. fasciatus. This factor was previously neglected (Breder & Rasquin 1947a, Schlagel & Breder 1947, but see Gertychowa 1971), and it may be responsible for the diversity of results reported in these studies on the degree of phototactic responses among the cave populations. For example, Breder & Rasquin (1947a) reported El Pachon population to be "almost" indifferent to light, while the present results indicate that this population is significantly scotophilic. The unique size distribution found in this population

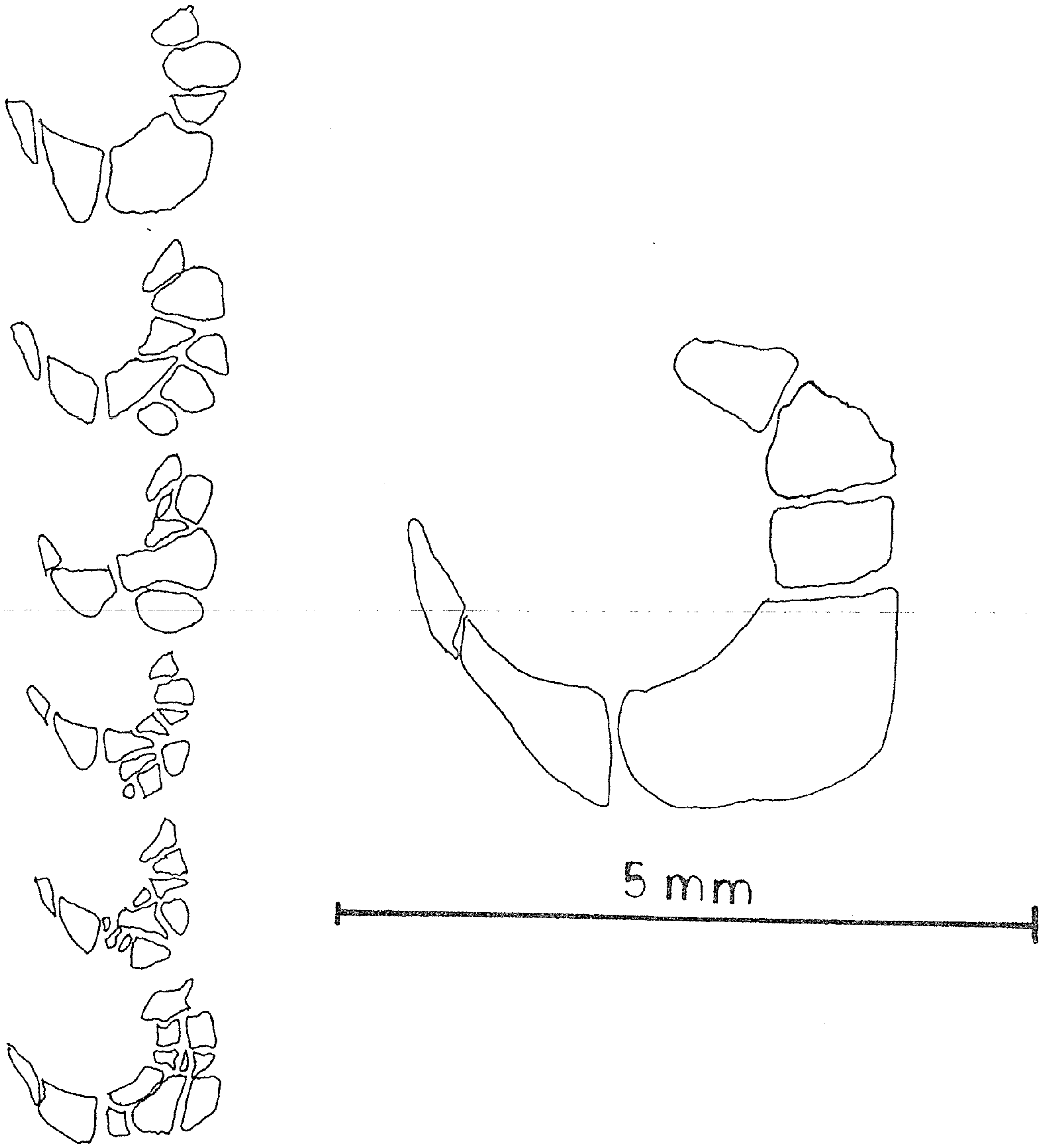


Fig. 12. Number, relative size, configuration and distribution of the circumorbital bones in natural specimens from La Cueva Chica (left column) [from Alvarez 1946, p. 269] and in all specimens examined from the commercial (captive) fish stock [this study].

(no individuals > 60 mm vs. individuals > 88 mm in other populations) demonstates the importance of performing representative sampling on natural populations prior to any study.

Second, the level of phototactic responses differs among the populations. El Pachon population is least scotophilic of the natural populations. La Cueva Chica population is intermediate in the level of its phototactic responses, which is consistent with the interpretation that these morphologically intermediate fish are hybrids between the eyed and the blind forms (see chapter 6). Other kinds of behavior in *A. fasciatus* hybrids are known to be intermediate between those displayed by the parent populations (Schemmel 1980). Finally, the commercial fish stock showed phototactic response levels similar to those of individuals of similar length from El Pachon population; individuals from both populations are uniformly blind and depigmented.

It has frequently been assumed by many authors that the results obtained from commercial blind *A. fasciatus* stock can be extrapolated to natural populations (Durand 1976a, 1978, 1979, Erckens & Weber 1976, Glasser 1966, 1968, Grobbel & Hahn 1958, Humbach 1960, Popper 1970, Quinn 1980, Sadoglu 1979, Tabata 1982, Von Campenhausen et al. 1981, Weissert & Von Campenhausen 1981; Woodhead & Achey 1979, Yew & Yoshibara 1977, and Zaccone 1977). For exceptions see Schemmel 1980, Wilkens 1970, 1971, and Zilles et al. 1983. The differences in phototactic responses found in this study among the populations suggest that the origin of the experimental fish should be considered in subsequent research.

Third, neither El Pachon fish nor the artificially bred fish showed the unresponsiveness to light typical of most troglobites. Their level of phototactic response is intermediate between unresponsiveness and that of the surface population. This result is consistent with the breeding, electrophoretic and karyologic studies that indicate that there is little genetic differentiation between the eyed and blind populations of *A. fasciatus*.

Fourth, these results do not support earlier contentions that cave populations of *A. fasciatus* developed scotophilia as a "defensive" mechanism in order to avoid exiting the cave to a surface environment where they would be disadvantaged relative to eyed fish (Breder & Rasquin 1947a). In fact, the results indicate less scotophilia in cave populations than in the surface form. Furthermore, field observations of the natural habitat of cave *A. fasciatus* in Mexico (Mitchell et al. 1977), indicate that these fish have little, if any, opportunity to be exposed to light, which weakens even further the suggested "defensive mechanism" explanation for scotophilia in these populations.

Finally, because the observed ontogenetic change in phototactic responses is parallel for cave and surface populations of *A. fasciatus*, the presence of this pattern in the cave populations may best be interpreted as a behavioral relic rather than a specific adaptation to the cave environment. This contention is supported by the field studies described in the preceding chapters.

CHAPTER 5. INTROGRESSIVE HYBRIDIZATION IN THE *A. fasciatus* POPULATION LA CUEVA CHICA

5.1. Introduction

Studies on hybridization have contributed substantially to our understanding of evolutionary mechanisms. The analysis of morphology and behavior of natural hybrid populations of *A. fasciatus* provides a unique opportunity for understanding the genetics and evolution of cave-related characters. Given the extreme differences between the blind cave form and the eyed surface one of this fish, some general hypotheses on fish hybrids and genetic differentiation during colonization of a new niche can be tested.

5.1.1. Hybridization in fishes

Natural and artificial fish hybrids, including intergeneric ones, have been reported for more than 50 families, mostly of freshwater fishes (Hubbs 1955, Schwartz 1981). This extensive hybridization is apparently due to the fact that external fertilization is common in these vertebrates (Bianco 1982, Daget & Moreau 1981, Hubbs 1955, Whitmore 1983).

A review of the literature reveals several generalizations frequently made concerning fish hybrids:

1) Hybrids are phenotypically intermediate when compared with the parental characteristics, although the degree of intermediacy has rarely been specified (Bianco 1982, Hubbs 1955, Mayden & Burr 1980; Pethon 1974);

2) Among hybrids there are more males than females (often about 4:1) (Bianco 1982, Daget & Moreau 1981, Hubbs 1955, Pinto 1982);

3) Hybridization is more likely to occur when parental population sizes are substantially unequal (Bianco 1982, Busack & Gall 1981; Butcher 1980, Cashner & Jenkins 1982, Daget & Moreau 1981, Rakocinski 1980, Wallace & Ramsey 1982, Whitmore 1983);
4) Greater survival of hybrids occurs when the parental species and/or populations are closely related (Cashner & Jenkins 1982, Dauble & Buschbom 1981, Rakocinski 1980, Wallis & Beardmore 1980, Whitmore 1983);
5) Hybrids are more successful when the environment of the contact zone of the parental populations is intermediate (Hubbs 1955, Moore 1977);
6) Hybridization is more likely to take place when overlap in both spawning space and spawning periods occurs (Bianco 1982, Dauble & Buschbom 1981, Hubbs 1955, Pethon 1974, 1978, 1981, Rakocinski 1980, Wallace & Ramsey 1982).

5.1.2. Hybridization in cave animals

Hybrids among cave organisms or between cave and surface species and/or populations are rare because most of them are allopatric (Allegrucci et al. 1982). Although hybridization between surface and cave fish populations has been suspected in at least three cases (*Caecorhamdia urichi*, J. Endler, pers. comm.; *Poecilia sphenops*, Gordon & Rosen 1962, Walters & Walters 1965; and *Lucigobius* spp., Tomiyama 1936), the only well-known example is that of the characid *Astyanax fasciatus*.

Despite conspecificity of the cave and surface forms of *A. fasciatus*, their strong morphological and behavioral divergences and the phenotypic intermediacy of the progeny resulting from crosses has led to referring to them as hybrids (Breder 1943a, Peters & Peters

1973, Sadoglu 1957, Wilkens 1974). Hybridization between the surface and the cave populations has been suspected for 9 of the 31 caves supporting subterranean populations of A. fasciatus in the San Luis de Potosi area of east central Mexico (Mitchell et al. 1977).

The first indication of introgression (i.e., stabilization in numbers through time of backcross types) between surface and cave forms was presented by Breder (1943a). He compared specimens of A. fasciatus from La Cueva Chica collected from 1936 to 1942, and based on changes in phenotypic ratios (eyes and pigmentation), he concluded that a recent invasion of eyed and pigmented fish through a subterranean connection with surface waters was changing the genetic structure of the cave population. Further studies support the cave/surface connection hypothesis (Mitchell et al. 1977).

The aim of this chapter is:

a) To test the hypothesis that complete introgression between cave and surface populations of A. fasciatus has occurred at La Cueva Chica, and that it has occurred rapidly in evolutionary time;

b) To compare the characteristics observed in this introgressed population with those commonly attributed to hybrid fishes; and

c) To discuss the implications of introgression regarding our present knowledge of the evolutionary biology of cave organisms.

5.2. Materials and methods

One hundred individuals of A. fasciatus were collected during May 1983 in pool II of La Cueva Chica (Fig. 13). This pool was selected because: a) almost all previous information about morphology of this cave population comes from material collected at that pool (Alvarez

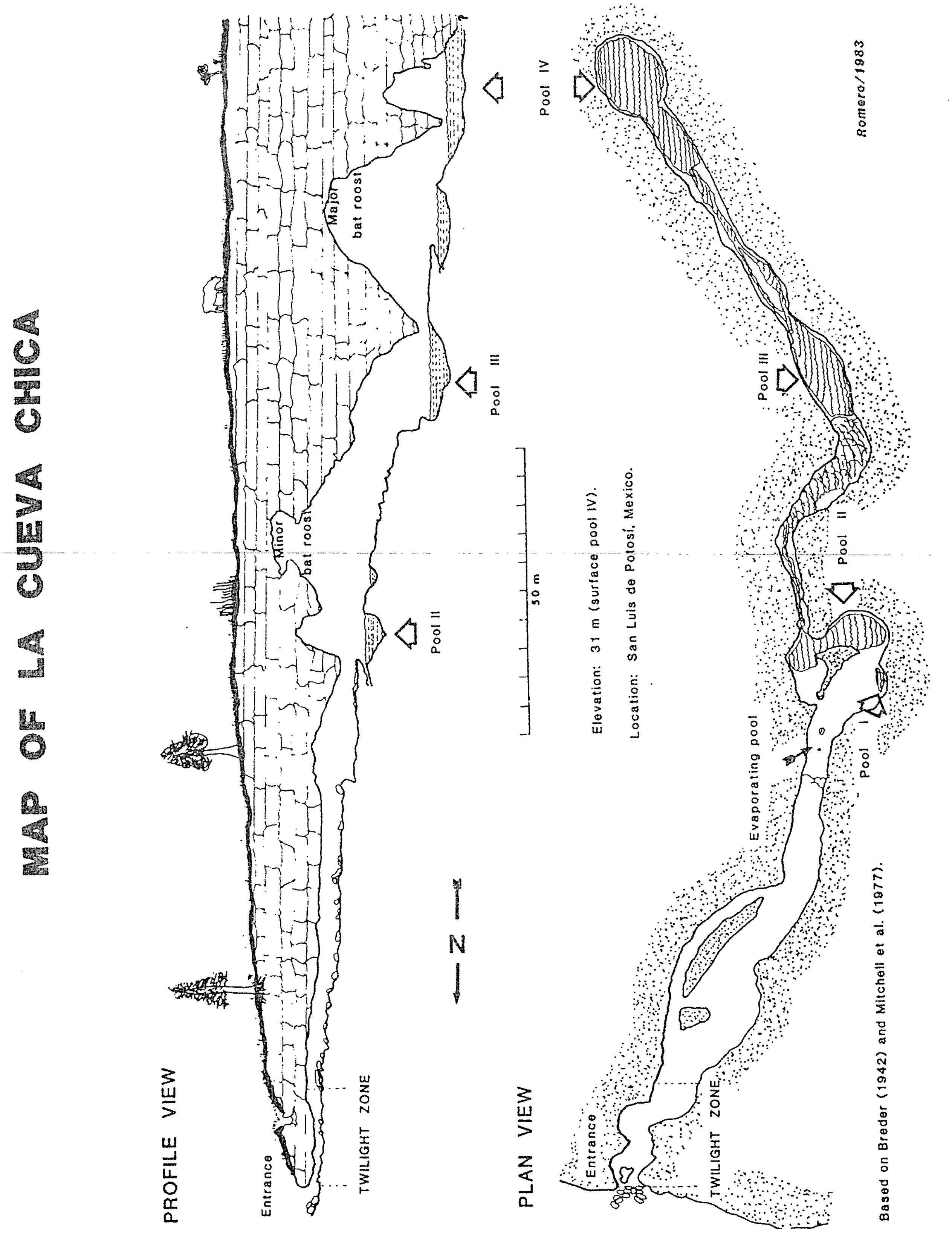


Fig. 13. Map of La Cueva Chica [Based on Breder 1942 and Mitchell et at. 1977]

1946, Breder 1942, 1943a, Hubbs & Innes 1936); b) this cave population is comparatively large for a cave organism (Mitchell et al. 1977), c) this pool is the most remote from the surface eyed A. fasciatus and is isolated from the rest of the pools during the dry season, restricting gene flow.

Collecting was performed as described on p. 48. Sex was determined using sexually dimorphic characters described by Alvarez (1946), Kosswig (1964), and Wiley and Collette (1970). To make comparisons with data previously published, I used the qualitative criteria of Breder (1943a) for eye condition and pigmentation. These criteria are given in Table 4. Individuals (< 35 mm and presumably immature) were impossible to sex and were not included in the 100 individuals used for the present study. Fifty-four fish were retained for behavioral studies; the rest were returned to the pool.

5.3. Results

My results and those of Breder (1943a) are presented in Table 4; they suggest that introgression has occurred since 1940. Table 5 shows that none of the 100 individuals collected in 1983 has the morphological attributes of the parental populations (i.e., either totally blind and depigmented or displaying full eyes and pigmentation simultaneously). Table 6 gives the sex ratio of the individuals collected in 1983, plus the sex ratio obtained in previous studies by other authors at this and other localities for cave and surface forms. The hybrid population at La Cueva Chica contains fewer males than females as is the case for most populations of *A. fasciatus*.

Table 4. Phenotypes of fish from La Cueva Chica. 1936-1942 data from Breder (1943a); 1983 data, this paper.

EYE CONDITION (% of the population)

			Sunken		
Year	N	Blind	Covered	Uncovered	"Normal" eye
1936	90?*	100	0	0	0
1939	90?*	100	0	0	0
1940	38	85	6	0	9
1942	28	61	4	21	14
1983	100	4	83	9	4

PIGMENTATION (% of the population)

			Intermediate			
Year	N	None	Little	Moderate	Considerable	Full
1936	90?*	100	0	0	0	0
1939	90?*	100	0	0	0	0
1940	38	90	2	6	2	0
1942	28	53	11	11	0	25
1983	100	0	28	31	37	4

*Estimate drawn from Breder's (1943a) comment of "something less than 100."

Table 5. Distribution of features in La Cueva Chica fish collected in 1983 (N = 100).

EYE		Reduced but	Sunken		
PIGMENTATION	Normal	external	retina visible	not visible	Absent
Full	0*	0	0	4	0
Considerable	0	5	18	10	4
Moderate	2	2	18	9	0
Little	2	2	15	9	0
None	0	0	0	0	0*

*Parental populations would be classified and placed here.

Table 6. Sex ratios (total number of individuals examined) in cave and surface populations of *A. fasciatus*. Data from Mitchell et al. 1977, unless otherwise indicated.

CAVE FISH

POPULATION	SEX RATIO (male:female)	% OF MALES
Cueva Chica*	3:6	33
Cueva Chica**	42:58	42
Los Sabinos*	5:5	50
Pachon*	6:9	40
Pachon	10:12	45
Montecillos	5:4	56
Arroyo	8:7	53
Curva	6:7	46
Tigre	9:15	38
Jos	6:18	25
Palma seca	6:17	26
Tinaja	4:20	17
TOTAL	110:178	37

SURFACE FISH

POPULATION	SEX RATIO (male:female)	% OF MALES
Tamesi	12:13	48
Arroyo	11:10	52
Panuco	8:15	35
TOTAL	31:38	45

*After Alvarez, 1946.

**This study.

5.4. Discussion

5.4.1. Introgression

The identification of F_2 and/or backcross individuals is the basis for assessing introgressive hybridization (Whitmore 1983). One of the reasons why introgression has been rarely reported in fishes is the difficulty in identifying these hybrid classes (Rakocinski 1980). However, both the clear-cut morphological differences between the cave and surface forms of *A. fasciatus* and the detailed descriptions of F_2 and backcross generations from breeding studies (Kosswig 1964, Peters & Peters 1973) on this fish allow hybrid identification and, consequently, introgression assessment. The data in Table 5 show that: a) no parental forms were found in the current hybrid population; and b) most (if not all) individuals collected correspond to the description of the typical F_2 and backcrossed individuals obtained under laboratory conditions (Peters & Peters 1973). These data strongly indicate that the *A. fasciatus* population at La Cueva Chica is the result of introgression.

Results in Table 4 suggest that introgression took place in about 40 years (or less). The lack of hybrids or eyed pigmented individuals in the first two collections (1936 and 1939) and the absence of parental forms in the last one (1983) support such a contention. The introgression described here could have taken place long before the last collection (1983). Recent data on natural hybrid cichlids of Madagascar, for example, show that introgression can take place in less than 20 years (Daget & Moreau 1981). Unfortunately there is little reliable information on the rate of introgression in other natural fish populations.

A binomial test demonstrated no significant difference ($p > 0.05$) in sex ratio between surface vs. cave populations (excluding La Cueva

Chica, 1983 data) and between either of these two sets of data or the 1983 La Cueva Chica data ($p > 0.05$). These results indicate that neither invasion of the cave environment nor hybridization between surface and cave populations changes the sex ratio from that of the surface population.

5.4.2. Generalizations on fish hybrids

1) Intermediacy of hybrids: Experimental studies have shown that hybrids between the blind form from La Cueva Chica and the eyed form from nearby surface populations are not precisely intermediate in their morphological and behavioral features. Wilkens (1969, 1970), for example, showed that eye size and the number of melanophores and their melanin content are slightly skewed to the surface population characteristics in both F_1 and F_2 generations. Boucquey et al. (1965) found that F_1 hybrids display rhythmical patterns of activity similar to the surface form, while the F_2 showed the same arrhythmic temporal regulation of activity as the cave form. Schemmel (1980) showed that the angle of erection for bottom-feeding in F_1 and F_2 generations is closer to the one of the surface form. Lack of exact intermediacy for various characteristics in the F_1 and F_2 hybrids of this characid is consistent with recent results suggesting that other fish hybrids rarely are exactly intermediate in their characteristics when compared with their parental populations (Bao & Kallman 1982, Kerby 1979, May et al. 1980, Neff & Smith 1979, Ross & Cavender 1981, Sutton et al. 1981, Wallace & Ramsey 1982).

2) Changes in sex ratio: Table 6 does not provide support for the contention that hybrid populations of fish necessarily display a larger proportion of males when compared with the parental populations.

Previous data on sex ratios for other surface and cave populations of *A. fasciatus* (including data for the original blind population of La Cueva Chica) suggest that the sex ratio in most populations including the current population at La Cueva Chica reflects more females (usually 2:3).

3) *Disproportionate size of parental populations*: Although the cave populations of *A. fasciatus* are rather large for a cave species, there is little question that they are outnumbered by the surface form of the same species. However, given the nature of the connection between the cave and surface waters (subterranean and physically impossible to explore), it is difficult to ascertain how frequently and in what numbers surface individuals may invade. The level of introgression may fluctuate through time.

4) *Close relationship of parental species*: Electrophoretic (Avise & Selander 1972), karyotypic (Kirby et al. 1977), and morphologic studies (Hubbs & Innes 1936, Alvarez 1946) suggest that the La Cueva Chica population and the nearby surface population are genetically very similar and differ morphologically only in those characters related to cave-dwelling. This is consistent with the view that close genetic relationship can be considered as a facilitating factor for rapid introgression.

5) *Hybrid success and intermediacy of the environment at the contact zone*: Although introgression has taken place in the native environment of one of the parental populations, the intermediate-environment hypothesis can not be rejected because: A) eyed pigmented populations of *A. fasciatus* can be found in many caves not occupied by blind forms in the San Luis de Potosí area (Mitchel et al. 1977, Romero unpublished

observations), in the cenotes of Yucatan (Hubbs 1938), and in a cave of northern Mexico (Reddell 1982), and as a facultative cavernicole in Costa Rica (see chapters 2 and 3), and; B) unlike other freshwater fishes of the New World, this species can be found in many different surface environments, so it should not be surprising to find it also in the subterranean environment (Bussing 1976, Lopez 1978). In consequence, although La Cueva Chica does not represent an "intermediate" environment, it is clear that caves are not unusual for eyed pigmented *A. fasciatus*, at least for the first stages of cave colonization (see chapters 2 and 3). Mitchell et al. (1977) also suggested that hybridization might be favored by availability of abundant food resources of La Cuave Chica.

6) Overlap in reduced spawning space and spawning periods: La Cueva Chica, whose waters are subdivided into several pools (especially during the dry season), provides a limited area which facilitates contact among the individuals occupying the pools (Breder 1942). Although there is little information on spawning behavior of these fish under natural conditions, no strong differences have been found in laboratory studies other than those related to the lack of vision in the cave form (Breder & Rosen 1966). As for all characids, fertilization is external in *A. fasciatus*, and crosses between the cave and the surface forms are readily obtained under experimental conditions (Sadoglu 1979). Even hybrids between the cave *A. fasciatus* and two other characids (*Ctenobrycon spilurus* and *Moenkhausia oligolepis*) have been reported (Kauffeld 1954), suggesting that the cave *A. fasciatus* displays the spawning behavior common to this fish family. The reduced space in La Cueva Chica facilitates close contact between the cave and

the surface forms. Given the apparent lack of any reproductive isolating mechanism between these forms, there is a high probability of hybridization under these conditions. This is further supported by the existance of eight other hybrid cave populations of this fish (Mitchell et al. 1977).

5.4.3. Introgression and cave evolutionary biology

It has been proposed that there is more genetic differentiation between related cave and surface species and/or troglobitic populations than suggested by morphological characters (Culver 1982, p. 95). This is largely based on Barr's (1968) contention that cave adaptation involves considerable reorganization of the genotype which is, in turn, an application to the evolution of cave organisms of Mayr's (1970) concept of a "genetic revolution" associated with the founder principle

However, the introgression here described, as well as the breeding, electrophoretic, karyotypic, and behavioral studies mentioned above for the *A. fasciatus* population of La Cueva Chica, do not support such a generalization. In fact, all seem to indicate that typical cave features (e.g. blindness, depigmentation, larger number of taste buds, behavioral modifications) can appear in the absence of any major differentiation at electrophoretic and karyotypic levels, and prior to the development of reproductive barriers between the cave and surface populations. It should not be forgotten that comparative morphological studies of cave and surface populations of this fish species suggest that no significant meristic or morphometric differences exist between the forms except for the cave-dwelling attributes (Alvarez 1946, Peters & Peters 1973, Wilkens 1969). Similar conclusions have been reached when related fish species, which are ecologically distinct, are studied from a genetic viewpoint (Avise & Selander 1972, Avise et al. 1975, Bell et al. 1982). More extensive comparisons among obligatory cave organisms, their surface ancestors, and suspected hybrids between the two, will yield additional information about the correlation between phenotypic and genetic changes during the evolution of cave adaptations.

CHAPTER 6. CONCEPTUAL ISSUES: CAN EVOLUTION REGRESS?

6.1. Introduction

The term "regressive evolution" is used to describe the trend toward reduction or disappearance of features. It is frequently applied to the evolution of parasites, deep-sea animals and, especially, cave organisms lacking eyes and pigmentation (Barr 1968, Vandel 1969, Thines 1969). Vestigial characters in termites, flightlessness in insects and birds, and limb loss in tetrapods have also been offered as examples (Byers 1969, Darlington 1943, Emerson 1961, Lande 1978, Olson 1973). The aim of this chapter is to show that from semantic, historic, and descriptive standpoints, the term "regressive" is much more confusing than helpful. I will also argue that there is no evidence for "regressive" evolution as a unique mode of evolution, and that for the kinds of changes to which it refers and the mechanisms involved, current evolutionary terminology provides an adequate conceptual framework.

6.2. Semantics

Although some publications (Culver 1982, Kosswig 1965, Thines 1969) have included detailed treatment of "regressive" evolution, none has concisely stated the meaning of the term from an evolutionary standpoint The earliest uses of this term are by Lamarck (1809) and Weissman (1889). Kosswig (1965) defined it as "le fait que des unites systematiques posedent a l'etat rudimentaire des organes qui sont bien developpes chez leurs ancetres" (the fact that systematic units transform into the rudimentary state of those organs that are well developed in their ancestors), which implies that any loss in structural complexity is "regressive" evolution, a not too helpful definition because all lineages lose (and gain) features during evolution. Other authors, such as Heuts (1953), have been even more obscure by defining "regressive" evolution as "any loss of internal stability in the organism."

The Oxford English Dictionary defines the term regressive to mean "returning, passing back, acting in a backward direction." Similar meanings are given in dictionaries of other languages where this word has been used in the same evolutionary sense. Based on this definition, the question "going back to what?" immediately arises.

The term "regressive" is also problematic in an ontogenetic sense. If the point is that the adult stage reverts to earlier stages of development, then it should be remembered that in those early stages most deep-sea and cave organisms show the same developmental features (including eyes) as their "unregressed" ancestors (Cahn 1958, Durand 1976a,b, Eigenmann 1909, Kuhn & Kahling 1954, Munk 1965, Peters & Peters 1966, Thines 1969, Wilkens 1980a,b).

6.3. Historical background

For the last two centuries, evolution has been largely perceived as something "progressive", leading toward "perfection" (see Bowler 1983, pp. 143-144; Mayr 1982, pp. 323 ff. for review). Lamarck (1809, p. 54) considered evolution a force that "gives to animal life the power of progressively complicated organization." That view was held as a fundamental truth by the first biologists dealing with cave organisms (Agassiz 1853). Later, researchers of parasite and cave animals adopted a neo-Lamarckian view (Cope 1887, Darwin 1859, Packard 1894) and these orthogenetic (directional) views of evolution rapidly became popular among biospeleologists. Lankester (1893a,b), Jeannel (1950) and Vandel (1969) adopted what the latter called an "organicistic" interpretation of orthogenetic evolution in cave animals which held that all phyletic lines pass through four evolutionary stages: rejuvenation, adaptive radiation, specialization, and "phyletic senescence." Vandel (1969, pp. 471-476) rejected any intervention of the environment during the process; he argued that blindness and depigmentation were not features of organisms arising as a result of living in caves, but these animals invaded caves because they were blind and depigmented, and represented a "dying phylogenetic line."

These orthogenetic ideas were extremely popular among European biologists (except in the United Kingdom). Theilhard de Chardin (1959), for example, saw in evolution "only an event, the grand orthogenesis of everything toward a higher degree of imminent spontaneity." Similar orthogenetic views of evolution such as "an unfolding of preexisting rudiments" are still very popular (Berg 1969). However, as Dobzhansky (1970, p. 391) pointed out, "attempts to define what constitutes progressive have met with only mediocre success since mere change is not necessarily progress."

6.4. Descriptive problems

Clearly the structural reductions (such as blindness and depigmentation) that have been used as the descriptive components of "regressive" evolution do exist, regardless of the inaccuracy of the term employed for the phenomenon. When compared with their light-adapted ancestors, deep-sea and cave organisms provide a number of cases in which an increase in the number and/or sophistication of features can be documented.

In addition to the classical examples of an increase in number and extension of sensory buds in amblyopsid fishes (Poulson & White 1969) and in the teleost Astyanax fasciatus mexicanus (Schemmel 1967, 1974, 1980), recent literature offers many examples from other taxa: hypertrophication of chemosensory and tactile organs in cave arthropods (Ahearn & Howard 1982, Dalens 1980), larger antennae in Diplopoda (Conde 1981), development of new sensory organs in Crustacea Mysidacea (Crouau 1978), new eye spots in Arachnida Pseudoscorpionida (Curcic 1980), new amino acid-derived compounds in biochemical pathways of cave sponges (D'Ambrosio et al. 1982), enhancement in the number of neuromasts in synbranchid fishes (Parzefall & Wilkens 1972), hyperdevelopment of bucopharyngeal membranes in cave salamanders (Serra & Stefani 1981), elongation of parts in assellid and isopod Crustacea (Alouf et al. 1981, Carpenter 1981, Schultz 1982), and increase in size of a frog (Brown & Alcala 1982).

Although there are also cases in which neither sensory compensations nor morphological enlargements have been described (Culver 1982, p. 57) there is no question but that the evolution of cave organisms involves much more than simply a few structural reductions. Emerson (1961), for instance, recognized "associated progressive and regressive evolution" and Kosswig (1973) mentions instances of "constructive" evolution during "regressive" evolution.

6.5. The mechanisms

Besides semantic, historic, and descriptive problems, is there a unique mechanism responsible for "regressive" evolution? Experimental studies indicate that known mechanisms are sufficient to explain the reduction in features. Isolation and genetic drift (Barr 1968, Bechler 1983, Culver 1982, Kosswig 1965), selection (Brace 1963, Byers 1969, Culver 1982, Emerson 1961, Lande 1978, Sadoglu 1967, Wilkens 1971, Wright 1964), Regal's (1977) "noise supression" hypothesis, and ontogenetic buffer mechanisms (Katz et al. 1981), have been implicated. Also, conventional behavioral and ecological explanations have been proposed for cave colonization (Sweet 1982, and see chapters 2 and 3). In consequence, no evidence has been provided supporting the assertion that "regressive" evolution is different from other kinds of evolution.

6.6. Is a new term necessary?

If the term "regressive" is inaccurate from many viewpoints, how should we refer to this common trend among many organisms? Previous attempts to solve this problem have not been very successful. The term "degenerative" evolution (Eigenmann 1909) poses not only the same conceptual problems as "regressive" evolution but also adds a pathological aspect (many Darwinists of the end of the 19th century and beginning of the 20th century argued, without evidence, that eyes could be easily infected in caves, with the result that selection would favor the loss of such structures [Anderson 1893, Cunningham 1893]; some recent authors are still concerned about that possibility [Greenfield et al. 1982]). The terms "rudimentation" (Dobzhansky 1970) and "structural reduction" (Brace 1963, Prout 1964), although accurate when applied to specific structures, cannot describe physiological or behavioral changes, much less the evolutionary trend as a whole. The terms "streamlining" evolution (Regal 1977) and "channel" evolution (Katz et al. 1981) present similar descriptive problems.

Gould and Vrba (1982) have pointed out that in biology "unnamed ideas generally remain unconsidered." However, the trend observed in cave animals has been recognized since the discovery of the first cave organisms, regardless of the nomenclature used for it. On the other hand, and for practical purposes, it is convenient to have a term with which one can express concisely a set of ideas or observations. The following attributes characterize obligate cavernicoles (troglobites) and are seen in many lineages: the animals live in a subterranean environment and exhibit at least two common features that are universal (blindness and depigmentation) and others that seem to be very widespread (at least for organisms at upper trophic levels): enlargement, multiplication and/or appearance of new sensory structures, larger size, and lower metabolic rate.

In general, when unrelated species occupying similar environments share a number of characteristics, the term "convergent evolution" is applied. Cave animals provide, in fact, one of the best examples of convergent evolution. Why then is this term not frequently employed by researchers of these organisms? Several reasons can be offered: first, speleology has had its major development in continental Europe (especially in France) where Darwinistic ideas are not very popular and a rather neo-Lamarckian view is still very powerful (Mayr & Provine 1980, but see Vuilleumier 1984). Second, in continental Europe the defense of evolution has been carried out by Catholic priests such as Theilhard de Chardin who envisioned evolution as an orthogenetic perfectionist process in which selection and environment play no role at all. Only in the last 20 years has this orthogenetic and/or neo-Lamarckian tradition been challenged by a few neo-Darwinists and proponents of the neutral mutation hypothesis. These workers object to the term convergent evolution because it may imply directional selection (Barr 1968, Culver 1982, Wilkens et al. 1979).

If we use the term convergent evolution in its strict sense, however, -- independent acquisition of similar features by unrelated organisms that live in similar environments -- then it aptly describes the evolution of cave-dwelling animals. We could then banish "regressive evolution" from our vocabulary. In any event, we should not forget that this convergence is precisely what we are trying to explain in the first place.

CHAPTER 7. DISCUSSION AND CONCLUSIONS

7.1. Introduction

In this chapter I will discuss the results of my studies on schooling behavior and phototactic responses in A. fasciatus in terms of their sensory basis and the evolution of cave adaptations. I will also attempt a synthesis of our current knowledge of the genetics of behavior and related structures in this fish and the data presented in this dissertation. Then, I will offer final conclusions on the general hypothesis of behavioral changes preceding morphological ones during the occupation of the subterranean habitat.

7.2. Schooling behavior

7.2.1. Environmental conditions

From the above results, it is clear that schooling behavior in A. fasciatus differs depending upon the environmental conditions. For example, populations of this fish inhabiting surface waters form large schools as anticipated by previous observations (Breder 1943c, Bussing & Lopez 1977); when groups of individuals from these populations are translocated into experimental conditions (as for example man-made pools or aquaria), they retain their schooling behavior. Also, as expected, no schooling behavior can be observed in the blind cave populations of this fish in Mexico, whether in natural or in experimental conditions.

However, in the pool associated with subterranean waters in Costa Rica (the "intermediate" evolutionary situation), this species occasionally forms small schools (never more than six individuals) of short duration (no more than two min) (p. 27). This is in sharp contrast with the large schools (usually involving entire populations of hundreds of fish) formed by this fish in surface waters all the time (Bussing & Lopez 1977). However, when groups of individuals from this Costa Rican facultative cave-dwelling population were placed under experimental lighted conditions, they also did not school.

7.2.2. The sensory bases of schooling

What is the general applicability of these findings? No obligate cave-dwelling fish has ever been reported to form schools. The possible exception of *Jenkinsia stolifera* mentioned by Breder and Bird (1975), does not apply here because it does not represent a case of an obligate cavernicole, but rather a typical schooler that occasionally uses marine cavities for reproductive purposes.

The case of the facultative cave-dwelling population of *A. fasciatus* from Costa Rica is then particularly interesting since it does not agree with Breder's (1943c) speculation that the cave form was unable to school due to the lack of eyes. He based this suggestion on his observations of lack of schooling behavior in blind cave populations of this characid.

This challenge to Breder's ideas comes not only from these field observations, but also from laboratory studies. Pitcher et al. (1976), for example, showed that a temporarily blindfolded fish can school. Subsequent detailed analyses of the effects on schooling of blindfolding or cutting the posterior lateral line have demonstrated that the role of the lateral line in normal schooling is far greater than had been previously recognized (Partridge & Pitcher 1980).

Lack of schooling behavior in blind *A. fasciatus* cannot be interpreted as the result of a lack of lateral line. Schemmel (1967) showed that when the lateral line system of the eyed surface form and the blind cave one are compared, neither morphological nor histological differences can be found, except that many fragmentations in the intra-orbital canals and more free neuromasts can be seen in the suborbital region of the cave form. These neuromasts are more developed in *A. fasciatus* than in other characids. Other cave fishes also display a well-developed lateral line system (Baker 1972, Schemmel 1977; for exceptions see Angel 1949, and Brittan & Bohlke 1965).

The loss of visual abilities may not have much impact on the orientation and locomotory activities of fishes in the subterranean environment. John (1957) found that artificially blinded surface *A. fasciatus* are as able as the naturally blind form to avoid collision with objects in aquaria. Similar experiments conducted with other fish species have yielded the same results (Timms & Kleerekoper 1970).

7.2.3. Why do cave fishes not school?

Although most cave fishes belong to families in which schooling behavior is rare or absent (see Appendix 1), the question of why the cave form of *A. fasciatus* does not display schooling behavior should be discussed based on the explanations of why fish school at all, and whether aspects of the cave environment obviate the benefits of schooling.

Currently proposed explanations (based on Breder 1959, Cushing & Harden-Jones 1968, and Partridge 1981) for schooling are:

a) Increase in hydrodynamic efficiency. Partridge and Pitcher (1979), have provided evidence against a hydrodynamic function of fish schools; if the hydrodynamic efficiency hypothesis were correct, schooling behavior in cave fishes should be enhanced, not eliminated, since the limitations in nutrients typical of most subterranean environments would compel cave fishes to maximize energy efficiency by adopting behaviors of lower energy costs.

b) Increase in efficiency of food finding. This tactic may be useful in large bodies of waters where large groups of fish would benefit from "sharing" information about the localization of food sources; however, most cave fish usually inhabit small pools where food sources can be located easily by each individual.

c) Increase in reproductive success. Again the small sizes of pools in caves facilitates encounters among fish (see chapter 5). The maintenance of schooling behavior for this purpose is superfluous in locating the mate.

d) Reduce risk of predation. No example of cave fish being significantly affected by predation has been reported. Cave populations are limited by competition for food resources (Poulson 1969). The instance of the cave-facultative population in Costa Rica in which schooling was rare even though it was under bat predation pressure does not favor the anti-predator hypothesis. In this particular instance, a more efficient antipredator strategy was developed: to enter a cave not visited by fishing bats at the time of their predation activity. Finally, although the fish cave localities in Mexico are inhabited by large bat populations, all these Chiroptera are fruit and insect-eaters or vampire species (Martin & Martin 1954, Villa-R. 1966).

7.2.4. Conlusions

The results of these field studies, together with other laboratory ones, lead me to conclude that:

a) Loss of schooling behavior and vision may occur independently of each other during evolution in caves.

b) Even if a highly developed lateral line system remains basically unchanged during the colonization of the cave environment, it does not imply that the same fish species that behaves as an obligatory schooler in surface conditions will display schooling behavior in the subterranean environment.

c) The lack of schooling behavior is a convergent feature among cave fishes, and could have been either facilitated or maintained by both the lack of fish predators in caves and the reduction in space availability.

7.3. Phototactic responses

7.3.1. Pattern of change

My studies show a decreased level in scotophilia from the surface to the cave form of *A. fasciatus* and an increasing level of scotophilia with age or size in both cave and surface populations.

These results suggest that the existence of scotophilia in the cave populations of this species is not an evolutionary innovation (contrary to Breder & Rasquin 1947a), but rather a behavioral relic.

Among the cave fishes in which phototactic responses have been experimentally studied, nine species are scotophilic (usually weakly scotophilic), three show a variation between weak scotophilia and phototactic unresponsiveness, and one is totally phototactically unresponsive (see Appendix 1). Among other cave taxa, a larger proportion of scotophilic than phototactically unresponsive species have also been reported (Vandel 1969), and except for the report by Breder and Rasquin (1947a) (see discussion in chapter 4), no scotophobia has ever been reported for cave organisms.

7.3.2. Relationships between phototactic responses and their sensory bases

When the extent of eye development and the kinds of phototactic responses found among species of cave fishes are compared (Table 7), no clear indication of a relationship can be found.

There is, however, evidence suggesting that the epiphysis (pineal organ or gland) plays a major role as a photoreceptor. The first indication that phototactic responses in blind cave *A. fasciatus* are mediated (at least in part) by the epiphysis was given by Breder and Rasquin (1947a). They reported that phototaxis in this fish is abolished after pinealectomy. Grunswald-Lowestein (1956) observed histological and histochemical changes in the epiphysis of this fish following prolonged exposure to continuous darkness or illumination. This was the first indication of ambient light influencing the functional morphology of this organ.

But it was not until the late 1970's that the functional morphology of the epiphysis in *A. fasciatus* and other blind fishes was a focus of studies. Herwig (1976) found high morphological similarity between the pineal organs of the blind and eyed form of this species, pointing out that the only difference was a reduction of the organization of the outer segments in the epiphysis of the blind form, and that this reduction was age-dependent and occurred gradually. Herwig (1976) also pointed out that these morphological differences are genetically determined but that the degree of structural reduction could be

Table 7. Relationships between phototactic responses and eye development in cave fishes (based on information provided in Appendix 1 and by Breder [1944, 1941a,b,c], Breder & Rasquin [1947a,b], Durand [1976a], Eigenmann [1909], Ercolini & Berti [1977,1978], Gresser & Breder [1940], Kuhn & Kahling [1954], Marshall & Thines [1958], Poulson [1961], Yew & Yoshibara [1977] and Zilles et al. [1983]). Notation for eye development: A= absent (no eye structure visible), C= cryptophthalmic (remaining eye structures under the skin), M= microphthalmic (superficial eyes of reduced size).

	NUMBER OF CAVE FISH SPECIES THAT ARE:	
EYE DEVELOPMENT	SCOTOPHILIC	PHOTOTACTICALLY UNRESPONSIVE
M	1	1
C	2	2
A	4	1
A,C	1	0
M,C,A	3	1

N = 16

influenced by the exposure of this organ to light. Other comparative studies have also shown little morphological differentiation between the pineal organs of blind fishes and those of their surface ancestors (Marshall & Thines 1958, Van de Kamer, unpublished observations cited by Herwig 1976).

In a series of papers, McNulty showed that the epiphysis of many other dark-adapted fishes such as deep-sea fishes (McNulty 1976, McNulty & Nafrakfitis 1976), the California blind goby *Typhlogobius californiensis*, and the cave facultative amblyopsid *Chologaster agassizi* (McNulty 1978a,b) retain photoreceptive abilities. The epiphysis is absent, however, in another amblyopsid, *Typhlichthys subterraneus* (McNulty 1978c), one of the very few blind fishes which does not display phototactic responses (Romero & Green unpublished results).

Thus, the question of the relative importance of the eye and the epiphysis as photoreceptors in fishes should be addressed. Recent experimental studies suggest the answer: Goudie et al. (1983) showed that in the channel catfish (*Ictalurus punctatus*), normal, blinded, and pinealectomized fish exhibit a nocturnal activity pattern which corresponds with the exogenous photoperiod; however, pinealectomized-blinded fish did not entrain to the photoperiod, suggesting that this fish was able to perceive light in the absence of either organ, but not in the absence of both.

Since the epiphysis in the blind *A. fasciatus* has nearly the same photosensitivity as the one in the normally eyed form, why has this organ and its photoreceptive function been maintained almost intact during the colonization of the cave enviromnent? Tabata (1982) suggested that many of the electrophysiological functions of the epiphysis depend not only of the presence of light, but also on the absence of it: in darkness, the photoreceptor membrane potential is sustained at a certain decreased level (resting membrane potential), and its ganglion cells continue to fire, so that excitation of these cells depends on darkness. Also, the epiphysis carries out endocrine functions which must be maintained regardless of the amount of light in the environment (Fenwick 1970).

7.3.3. Conclusions

My results on the blind cave form of *A. fasciatus* can be interpreted as follows:

a) The blind cave form of *A. fasciatus* displays phototactic abilities.

b) The gradual ontogenetic changes in degree of phototactic responses in all the forms of this species might be related also to the gradual changes observed in the functional morphology of the epiphysis during development.

c) Despite little morphological differentiation of the epiphysis between eyed and blind forms, there are differences in levels of phototactic responses. These behavioral differences are probably due to the lack of eyes in the blind form, the effects of light in the development of the epiphysis in fishes exposed to light, differences elsewhere in the sensory-response system, or a combination of these factors.

d) The epiphysis shows little differentiation in cave fishes which are genetically or phylogenetically close to their surface ancestors, probably because, contrary to what happens to the eye, the epiphysis has endocrine functions which remain effective regardless of the presence or absent of light.

7.3.4. Confounding variables

The problem of the evolution of phototactic responses presents many confounding variables, namely, that at least two organs are involved and one of them, the epiphysis, has other functions besides photoreception and its functional morphology can be influenced by environmental factors as well as by ontogenetical changes.

The null hypothesis was, however, that changes in behavior and changes in gross morphology are not independent; the results presented in chapters 2, 3 and 4 reject this hypothesis.

Finally, a distinction can be made between schooling and phototactic responses as behaviors: while the former is a type of social behavior which may or may not be displayed independently of the presence of one of its sensory bases, phototactic responses do not require social interaction and can be triggered by a gland with non-photoreceptive functions, which helps to explain why they persist in fishes living under complete darkness.

7.4. Other kinds of behavior

In order to better understand the general context of the pattern of change in the two kinds of behaviors described above, it would be worthwhile to take a look at the evolution of other kinds of behaviors in cave fishes.

The loss of constant circadian activity is another of the common features of cave fishes, but the loss is not permanent since it can be triggered under light conditions (Boucquey et al. 1965, Erckens 1981a,b, Erckens & Martin 1982a,b, Erckens & Weber 1976).

Although loss of aggressive behavior has been reported for cave fishes in large populations living under high energy input conditions (Breder 1943b,c for A. fasciatus and Parzefall 1969, 1973, 1979 for Poecilia sphenops), in many other species in energy poor environments, such behavior still remains (Eigenmann 1909 for Amblyopsis spelaea, Thines 1969 for Caecobarbus geertssi and Berti & Ercolini 1979 and Ercolini et al. 1981 for Uegitglanis zammaranoi). These latter cases have been linked to territoriality, a behavioral characteristic which is usually maintained under conditions of limited resources and mates (Gould 1982).

Differences in aggressive behavior may also be related to intraspecific recognition systems. In A. fasciatus and P. sphenops, recognition is mediated by visual contact. Although the surface form produces and reacts to an alarm substance, it has been demonstrated that blind A. fasciatus are able to produce the same alarm substance but do not react to it (Parzefall 1973, Pfeiffer 1966). On the other hand, all available data indicate that when aggressive behavior is present in cave species, it is triggered by the same kind of chemical stimuli as that of their surface ancestor (Ercolini et al. 1981).

A reduction in the complexity of agonistic displays has been reported for members of the Amblyopsidae fish family, in which the species that display the most profound structural reductions (Typhlichtichys subterraneus and Amblyopsis rosae) engage in simpler, less intense, and considerably shorter bouts than their presumed ancestors (Bechler 1983).

7.5. Genetics

In addition to the description of patterns of change in behavior from the surface to the cave fish forms and their relationships with the structures involved, the last necessary element is the genetic basis of these phenotypic differences.

Schemmel (1980) showed that the blind cave form of A. fasciatus when searching for food swims with the body at an angle of about 45° to the ground. The river fish, however, whether in full light conditions, in complete darkness or blinded, stands vertically on its head. Since the presence or absence of eyes does not affect this behavior, one could argue that the behavioral differences can be related to differences in the gustatory apparatus: in the river fish the taste buds are restricted to the mouth and lips, whereas in the cave fish, taste buds are spread all over the head and are very numerous on the lower jaw and the ventral side of the head. However, the genetics of both angle of feeding (headstanding) and the gustatory apparatus was also investigated by Schemmel (1980) who concluded that the reduction of headstanding and the increase in the development of the gustatory equipment are genetically independent and that both traits have been achieved by small genetic steps.

Parzefall (1973, 1979) also found that the aggressive behavior of the surface ancestor of Poecilia sphenops was controlled by a polygenic system. The presence of this type of behavior was independent of the presence of eyes.

The data on the introgressed population of A. fasciatus at La Cueva Chica provided in chapters 4 and 5, together with the results of others' work mentioned in this section, suggest that not only phototactic responses, but also other kinds of behaviors showing some degree of reduction during the process of cave colonization are polygenically based. Peters and Peters (1973) showed that the morphological traits which are reduced during the cave adaptation process (eyes and pigmentation) were polygenic and, more importantly, inherited independently of each other and from other behavioral traits.

7.6. Final conclusions

Manipulative field studies in Costa Rica suggest that use of subterranean cavities by *A. fasciatus* reduces vulnerability to bat predation, as evidenced by the significant negative correlation between fishing-bat activity and fish density in a pool connected to subterranean waters. When bats are actively foraging, fish tend to remain within the subterranean cavity. This behavior was found only in the study pool population, not in others nearby. Morphological examination showed that there are no significant gross differences in the structures usually reduced in cave animals, eyes and pigmentation, comparing fish from the study pool with those from surrounding areas. This indicates that behavioral changes can precede morphological ones during what may be the first steps of cave colonization. Since use of a subterranean refuge by *A. fasciatus* apparently occurs as a behavioral response to an environmental factor (bat predation), the invasion of this new habitat takes place actively, not as a result of "entrapment" or orthogenetic evolution, as some theories of the evolution of cave organisms claim.

Studies on phototactic responses of several populations of *A. fasciatus* demonstrate that the intensity of the scotophilic responses varies inversely with the degree of structural reduction of the eyes and directly with the size (or age) of the fish; larger fish are more scotophilic than the small ones. These results contradict the hypothesis that scotophilia in cave animals is a defensive mechanism for avoiding exiting the cave. Instead, I suggest that the weak scotophilia found among cave animals is a behavioral relict. These results also warn against the indiscriminate use of fish from commercial stocks for experimental studies, because their phototactic responses are different from those of fish obtained from natural populations. Also, fish size (age) should be considered because the level of phototactic responses is size dependent.

Morphological studies on a cave population of _A. fasciatus_ phenotypically intermediate between the eyed and the blind forms show that the intermediacy is the result of introgression that took place in less than 43 years. These results, together with genetic data collected by others, do not support the contention that cave colonization necessarily implies drastic changes in genotypes and/or phenotypes, but instead suggest that the classical features displayed by cave animals can be acquired without major reorganization of the genotype.

A historical analysis of the ideas pertaining to the evolution of cave organisms suggests that they should be rephrased within a modern biological conceptual framework. Most of the terminology presently used (e.g., "regressive" evolution) is vague, ambiguous, and misleading.

Finally, comparison of my results on _A. fasciatus_ with published studies on other cave fish species indicates that these animals share the same set of morphological and behavioral features. One plausible explanation for such convergence is that these common characteristics are the result of adaptation to the cave environment (specifically to the lack of light, since deep-sea fishes also display similar convergences). This is consistent with the allozyme data obtained for these organisms which show, in general, little differences when the ancestral and the derived population and/or species are compared (Culver 1982, Chapt. 4). However, other hypotheses that do not invoke selection cannot be excluded.

My results are consistent with Mayr's hypothesis that behavioral changes precede morphological ones during the invasion of a new niche or habitat. Future studies should examine the possibility that changes in behavior exert the selective pressures ("evolutionary pacemaking") which modify the structures involved.

APPENDIX 1: MAJOR CHARACTERISTICS OF THE MICROPHTHALMIC, CRYPTOPHTHALMIC AND ANOPHTHALMIC SUBTERRANEAN FISHES OF THE WORLD

Explanation and justification of features compared and symbols used.

Species: Families are arranged following Greenwood et al. (1966). Within families, genera and species are arranged alphabetically.

Locality: Unless information is incomplete, province or state is cited prior to country.

Habitat: E = epigean (surface), C = cave, P = phreatic (includes wells, springs, and cisterns), ? = not described.

Eyes: N = normal (when compared with closest surface ancestor) , M = microphthalmic (reduced in size and external), C = Cryptophthalmic (reduced in size and under the skin, remanents of eye structures in the optic cavity), A = absent (no eye structures at all). ? = reported, but appears doubtful.

Pigmentation: N = normal, P = partial (at least traces of melanophores present), A = absent (no traces of pigment in the skin). ? = information missing or doubtful.

Intermediate forms/hybrids: Refers to the presence of morphological variation due to either gradients or hybridization with the surface fish.

Presumed ancestor: The closest relative that can be inferred based on current information.

Preadapted?: The ancestor of the cave form exhibits well-developed non-visual system and/or nocturnal/dark-adapted habits.

Phototactic responses: - = photophobic, 0 = phototactic unresponsive.

Phylogenetic age: Old and recent means that those characteristics of the species which are not related to the adaptation to the subterranean habitat appear to be primitive or derived from the ancestral stock of its family.

Authorities: First citation refers to the description of the species (or the cave population in the case that the surface form was already known). The second citation is for a major revision and/or addition to the knowledge of the species.

Species	Locality	Habitat	Eyes	Pigmentation	Intermediate forms/hybrids?	Presumed ancestor	Preadapted?	Phototactic responses	Phylogenetic age	Authorities
CHARACIDAE:										
Astyanax fasciatus	NE Mexico	E,C	N,M,C,A	N,P,A	YES	Astyanax complex	NO	-,0	Recent	Hubbs & Innes 1936, Mitchell et al. 1977
Stygichthys typhlops	Minas Gerai, Brazil	P	A	A	NO	Primitive characidae	?	?	Old	Brittan & Bohlke 1965
CYPRINIDAE:										
Barbopsis devecchii	Eil, Somalia	P	N,M	N,P	NO	Barbus sp.	?	-,0	Recent	Di Caporiaco 1926, Ercolini et al. 1982
Caecobarbus geerrtsi	Thysville, Zaire	C	C	P,A	NO	Barbus sp.	?	-	Old?	Boulenger 1921, Heuts 1951
Caecocypris basimi	Haditha, Iraq	P	C	A	NO	?	?	- (?)	Old	Banister & Bunni 1980
Iranocypris typhlops	Kaaje-Ru, Iran	P	C,A	P,A	NO	Barbus sp.	?	?	Old	Brunn & Kaiser 1943
Phreatichthys andruzzii	Bud-Bud, Somalia	P	A	A	NO	Barbus sp.	?	-	Old	Vinciguerra 1924
Puntius microps	Djocja, Java	C	N,M?	N	?	P. binotatus	?	?	?	Gunther 1868, Thines 1969
Typhlobarbus nudiventris	Yunnan, China	C	C	P	NO	Primitive cyprinid	?	?	Old?	Chu & Chen 1982
Typhlogarra widdowsoni	Haditha, Iraq	C	C	P	NO	Garra rufa	?	-	Old	Trewavas 1955, Marshall & Thines 1958
COBITIDAE:										
Noemacheilus evezardi	?, India	C	N,C?	N,P,A	?	?	?	?	?	Thines 1969, Greenwood 1976
Noemacheilus gejiuensis	Yunan, China	P (mine)	A	A	NO	?	?	0?	?	Chu & Chen 1979
Noemacheilus smithi	Kaaje-Ru, Iran	P	C	A	NO	Noemacheilus sp.	?	0?	Old	Greenwood 1976
Noemacheilus sp.	Assam, India	C	N,M?	N,P	?	?	?	?	Recent	Hora 1924
Noemacheilus starostini	Turkmenistan, USSR	C	A	A	NO	?	?	?	Old	Parin 1983
ICTALURIDAE:										
Prietella phreatophila	Coahuila, Mexico	P	A	A	NO	Noturus sp.	YES	- (?)	Old	Carranza 1954
Satan eurystomus	Texas, USA	P	A	A	NO	Pilodictis sp.	YES	?	Old	Hubbs & Bailey 1947, Longley & Karnei 1979b
Trogloglanis pattersoni	Texas, USA	P	A	A	NO	Ameirus sp.	YES	?	Old	Eigenmann 1919, Longley & Karnei 1979a
[illegible]						[illegible]	YES	?	Recent	Trewavas 1936

LITERATURE CITED

Agassiz, L. 1853. Recent researches of Prof. Agassiz. Amer. J. Sci. Arts. 16: 134-136.

Ahearn, G.A. & F.G. Howard. 1982. Physiology of cave arthropods in Hawaii. J. Exp. Zool. 222: 227-238.

Allegrucci, G.; A. Caccone, D. Cesaroni, M.C. Sbordoni, E. De Matthaeis & V. Sbordoni. 1982. Natural and experimental interspecific hybridization between populations of *Dolichopoda* cave crickets: Experientia 38: 96-98.

Alouf, N.; J.-P. Henry & G. Magniez. 1981. Faune souterraine rejeteè par une exsurgence temporaire du Liban: *Proasellus bardaunii* n. sp. Vie Milieu 31: 93-99.

Anderson, A. 1893. Blind animals in caves. Nature 47: 439.

Alvarez, J. 1946. Revision del género *Anoptichthys* con descripción de una especie nueva (Pisc., Characidae). Anal. Esc. Nac. Cienc. Biol. México 4: 263-282.

Alvarez, J. 1947. Descripción de *Anoptichthys hubbsi* caracínido ciego de la Cueva de los Sabinos, S.L.P. Rev. Soc. Mex. Hist. Nat. 8: 215-219.

Angel, F. 1949. Contribution a l'etude du *Typhleotris madagascariensis* poisson aveugle, cavernicole, du Sud-Ouest de Madagascar. Bull. Mus. Natn. Hist. Nat. Paris 21: 56-59.

Arnoult, J.C. 1959. Une nouvelle espece de poisson aveugle de Madagascar: *Typhleotris pauliani* n. sp. Mem. Inst. Sci. Madagascar Ser. A, 13: 133-138.

Avise, J.C. & R.K. Selander. 1972. Evolutionary genetics of cave-dwelling fishes of the genus Astyanax. Evolution 26: 1-19.

Avise, J.C.; J.J. Smith, & F.J. Ayala. 1975. Adaptive differentiation with little genic change between two native California minnows: Evolution 29: 411-426.

Baker, C.D. 1972. The cephalic lateral line system of Amblyopsid blind cavefishes and other percopsiform fishes. Ph. D. thesis, University of Louisville, 122 pp.

Banister, K.E. & M.K. Bunni. 1980. A new blind cyprinid fish from Iraq. Bull. Brit. Mus. Nat. Hist. 38: 151-158.

Bao, I.Y. & K.D. Kallman. 1982. Genetic control of the hypothalamo-pituitary axis and the effect of hybridization on sexual maturation (Xiphophorus, Pisces, Poeciliidae): J. Exp. Zool. 220: 297-309.

Barr, T.C. 1968. Cave ecology and the evolution of troglobites. pp. 53-102. In: T. Dobzhansky, M. Hecht & W. Steere (Eds.) Evolutionary Biology, vol 2. Amsterdam: North Holland Publishing Co.

Bechler, D.L. 1983. The evolution of agonistic behavior in amblyopsid fishes. Beh. Ecol. Sociobiol. 12: 35-42.

Bell, L.J.; J.T. Moyer, & K. Numachi. 1982. Morphological and genetic variation in Japanese populations of the anemonefish Amphiprion clarkii. Mar. Biol. 72: 99-108.

Berg, L.S. 1969. Nomogenesis, or evolution determined by law. Cambridge: M.I.T. Press.

Berti, R. & H. Ercolini. 1979. Aggressive behavior in the anophthalmic phreatic fish Uegitglanis zammaranoi (Claridae, Siluriformes). Monit. Zool. Ital. (n.s.) 13: 197

Bianco, P.G. 1982. Hybridization between *Alburnus albidus* (C.) and *Leuciscus cephalus cabeda* R. in Italy: J. Fish Biol. *21*: 593-603.

Blaxter, J.H.S. 1970. Light. pp. 213-320. *In*: O. Kinne (Ed.) Marine Ecology, Vol. 1, pt. 1. London: Wiley-Interscience.

Bloedel, P. 1955. Hunting methods of fish-eating bats, particularly *Noctilio leporinus*. J. Mammal. *36*: 390-399.

Bock, W. 1959. Preadaptation and multiple evolutionary pathways. Evolution *13*: 194-211.

Borodin, N.A. 1927. A new blind catfish from Brazil. Amer. Mus. Novit. (263): 1-4

Boucquey, C.; G. Thinès & C. Van Der Borght. 1965. Étude comparative de la capacité photopatique et de l'activité chez les poisson cavernicole *Anoptichthys antrobius*, chez les hybrides F1 (*Astyanax* x *Anoptichthys*) et F2. pp. 79-103. *In*: J. Medioni (Ed.). La distribution temporelle des activites animales et humaines. Paris: Masson et Cie.

Boulenger, G.A. 1921. Description d'un poisson aveugle decouvert par M.G. Geerts dans la grotte de Thysville (Bas-Congo). Rev. Zool. Afr. *9*: 252-253.

Bowler, P.J. 1983. The eclipse of Darwinism. Anti-Darwinian evolution theories in the decades around 1900. Baltimore:John Hopkins University Press.

Brace, C.L. 1963. Structural reduction in evolution. Amer. Natur. *97*: 39-49.

Breder, C.M. 1942. Descriptive ecology of La Cueva Chica, with especial reference to the blind fish, *Anoptichthys*. Zoologica *27*: 7-15.

Breder, C.M. 1943a. Apparent changes in phenotypic ratios of the characins at the type locality of *Anoptichthys jordani* Hubbs and

Innes. Copeia 1943: 26-30.

Breder, C.M. 1943b. A note on erratic viciousness in Astyanax mexicanus (Phillipi) Copeia 1943: 82-84.

Breder, C.M. 1943c. Problems in the behavior and evolution of a species of blind cave fish. Trans. N.Y. Acad. Sci. 5: 168-176.

Breder, C.M. 1944. Ocular anatomy and light sensitivity studies on the blind fish from Cueva de los Sabinos, Mexico. Zoologica 29: 131-143.

Breder, C.M. 1959. Studies on social groupings in fishes. Bull. Amer. Mus. Nat. Hist. 117: 393-482.

Breder, C.M. & P.M. Bird. 1975. Cave entry by schools and associated pigmentary changes of the marine clupeid, Jenkinsia. Bull. Mar. Sci. 25: 377-386.

Breder, C.M. & E.B. Gresser. 1941a. Correlations between structural eye defects and behavior in the Mexican blind characin. Zoologica 26: 123-131.

Breder, C.M. & E.B. Gresser. 1941b. Further studies on the light sensitivity and behavior of the Mexican blind characin. Zoologica 28: 289-296.

Breder, C.M. & E.B. Gresser. 1941c. Behavior of Mexican cave characins in reference to light and cave entry. Anat. Rec. 81: 112

Breder, C.M. & P. Rasquin. 1947a. Comparative studies in the light sensitivity of blind characins from a series of Mexican caves. Bull. Amer. Mus. Nat. Hist. 89: 25-351.

Breder, C.M. & P. Rasquin. 1947b. Evidence for the lack of a growth principle in the optic cyst of Mexican cave fish. Zoologica 32: 29-33.

Breder, C.M. & D.E. Rosen. 1966. Modes of reproduction in fishes. New York: Natural History Press.

Brett, J.R. 1979. Environmental factors and growth. pp. 599-675. In: W.S. Hoar, D.J. Randall, and J.R. Brett (Eds). Fish Physiology, vol 8 New York: Academic Press.

Brittan, M.R. & J.E. Bohlke. 1965. A new blind characid fish from Southeastern Brazil. Notulae Naturae (380): 1-4.

Brown, W.C. & A.C. Alcala. 1982. A new cave Platymantis (Amphibia: Ranidae) from the Philippine islands. Proc. Biol. Soc. Wash. 95: 386-391.

Bruun, A.F. & E.W. Kaiser. 1943. Iranocypris typhlops, the first true cave fish from Asia. Danish Scient. Invest. Iran. Part IV. Copenhaguen: Ejnar Munksgaard.

Busack, C.A. & G.A.E. Gall. 1981. Introgressive habridization in populations of Paiute cutthroat trout (Salmo clarki selenris): Can. J. Fish. Aquat. Sci. 38: 939-951.

Bussing, W. 1976. Geographical distribution of the San Juan Ichthyofauna of Central America with remarks on its origins and ecology. pp. 157-175. In: T.B. Thorson (Ed.). Investigations of the Ichthyofauna of Nicaraguan Lakes. Lincoln: School of Life Sciences, University of Nebraska-Lincoln.

Bussing, W. & M. Lopez. 1977. Distribución y aspectos ecológicos de los peces de las cuencas hidrográficas de Arenal, Bebedero y Tempisque, Costa Rica. Rev. Biol. Trop. 26: 13-37.

Butcher, G.A. 1980. Multivariate analysis of hybridizing Cyrpinidae from the Kananaskis Reservoirs, Alberta. Can. J. Zool. 58: 1664-1673.

Byers, G.W. 1969. Evolution of wing reduction in crane flies (Diptera: Tipulidae). Evolution 23: 346-354.

Cahn, P.H. 1958. Comparative optic development in Astyanax mexicanus and in two of its blind cave derivatives. Bull. Amer. Mus. Nat. Hist. 115: 69-112.

Carpenter, J.H. 1981. Bahalana geracei n. gen., n. sp., a troglobitic marine cirolanid isopod from lighthouse cave, San Salvador Island, Bahamas. Bijdr. Dierk. 51: 259-267.

Carey, W.E. & D.L.G. Noakes. 1981. Development of photobehavioural responses in young rainbow trout, Salmo gairdneri Richardson. J. Fish. Biol. 19: 285-296.

Carranza, J. 1954. Descripcion del primer bagre anoftalmo y depigmentado encontrado en aguas mexicanas (Pisc. Ameiur.) Ciencia 14: 129-136.

Cashner, R.A. & R.E. Jenkins. 1982. Systematics of the Roanoke Bass, Amblopites cavifrons. Copeia 1982: 581-594.

Chu, S.L. & Y.R. Chen. 1979. A new blind cobitid fish (Pisces, Cypriniformes) from subterranean waters in Yunnan, China. Acta Zool. Sinica 25: 285-287.

Chu, S.L. & Y.R. Chen. 1982. A new genus and species of blind cyprinid fish from China with special reference to its relationships. Acta Zool. Sinica 28: 383-388.

Cohen, P.M. & C.R. Robins. 1970. A new ophidioid fish (genus Lucifuga) from a limestone sink, New Providence Island, Bahamas. Proc. Biol. Soc. Wash. 83: 133-144

Conde, B. 1981. Un penecillate cavernicole de Majorque (Diplopoda, Penicillata). Arch. Sc. (Geneve) 34: 313-318.

Cooper, J.E. & R.A. Kuehne. 1974. Speoplatyrhinus poulsoni, a new genus and species of subterranean fish fauna from Alabama. Copeia 1974: 486-493.

Cope, E.D. 1864. On a blind silurid from Pennsylvania Proc. Nat. Sci. Phil. 1864 (oct.): 231-233.

Cope, E.D. 1887. The origin of the fittest: essays on evolution. New York: D. Appleton Co.

Crouau, Y. 1978. Organes sensorialles d'un Mysidace souterrain anophthalme, *Antromysis juberthiei*: etude ultrastructurale des aesthetascs. Bull. Mus. Natn. Hist. Nat. Paris. 3rd series, (513): 165-175.

Culver, D.C. 1982. Cave life. Evolution and ecology. Cambridge: University Press.

Cunningham, J.T. 1893. Blind animals in caves. Nature 47: 439.

Curcic, B.P.M. 1980. A new species of cave-dwelling pseudoscorpion from Serbia (Arachnidae:Pseudoscorpiones:Neobisiidae). Senck. Biol. 60: 249-254.

Cushing, D.H. & F.R. Harden-Jones. 1968. Why do fish school? Nature 218: 918-920.

Daget, J. & J. Moreau. 1981. Hybriditation introgressive entre deux espèces de *Sarotherodon* (Pisces, Cichlidae) dans un lac de Madagascar. Bull. Mus. Natn. Hist. Nat. Paris. 4e. ser., 3: 689-703.

Dalens, H. 1980. Une nouvelle espèce de *Trichoniscoides* provenant des Asturies: *T. chapmani* n. sp. (Crustaces, Isopodes terrestres). Bull Soc. Hist. Nat. Toulouse 116: 64-67.

D'Ambrosio, M.; A. Guerriero, P. Traldi, & F. Pietra. 1982. Cavernicolin-1 and Cavernicolin-2, two epimeric dibromalactams from the Mediterranean sponge *Aplysina* (*Verongia*) *cavernicola*. Tetrah. Lett. 23: 4403-4406.

Darlington, P.J. 1943. Carabidae of mountains and islands: data on the evolution of isolated faunas, and on atrophy of wings. Ecol. Monogr. 13: 37-61.

Darwin, C. 1859. On the origin of species. Cambridge: Harvard-University Press.

Darwin, C. 1872. Origin of Species. 6th ed. Oxford: Oxford University Press. Reprint (1956).

Dauble, D.D. & R.L. Buschbom. 1981. Estimates of hybridization betweeen two species of catostomids in the Columbia river: Copeia 1981: 802-810.

De Kay, J.E. 1842. Zoology of New York or the New York Fauna. Part IV. Fishes. Albany: W. & A. White & J. Visscher.

Di Caporiaco, L. 1926. Un nuovo genere ciego du cirpinidae somalo delle acque di pozzo. Monit. Zool. Ital. 37: 23-25.

Dingerkus, G. & L.D. Uhler. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demostration of cartilage. Stain Tech. 52: 229-232.

Dobzhansky, T. 1970. Genetics of the evolutionary process. New York: Columbia Univeristy Press.

Durand, J.P. 1968. Étude des poissons récoltés dans la grotte de Umayalanta (Bolovie), Trichomycterus chaberti sp. n. Ann. Speleol. 23: 343-353.

Durand, J.P. 1976a. Sur la structure oculaire, l'ultrastructure des muscles intrinsèques, des enceloppes de l'oeil et de l'épithélium pigmentaire rétinien de l'Anoptichthys (form aveugle de l'Astyanax mexicanus -Characidae, Pisces). Ann. Speleol. 31: 149-161.

Durand, J.P. 1976b Recherches sur les mécanismes de la rudimentation oculaire chez les vertébrés. Coll. Inst. CNRS (266): 149-155.

Durand, J.P. 1978. Phénomènes de convergences tissulaires et cytologiques, lies aux processes dégénératifs quiu affectent l'oeil chez deux téléostéens cavernicoles Astyanax (Anoptichthys) mexicanus (Characidae) et Lucifuga (Stygicola) dentatus (Ophidiidae). Bull. Soc. Zool. France 103: 269-274.

Durand, J.P. 1979. Aspects ultrastructuraux des mécanismes de la rudimentation rétinienne chez l'Anoptichthys adulte, forme cavernicole aveugle de l'Astyanax mexicanus (Characidae, Pisces). Can. J. Zool. 57: 196-205.

Eigenmann, C.H. 1909. Cave vertebrates of America, a study in degenerative evolution. Washington: Publ. Carnegie Inst.

Eigenmann, C.H. 1918. The Pygidae, a family of South American catfishes. Mem. Carnegie Mus. 7: 259-398.

Eigenmann, C.H. 1919. Trogloglanis pattersoni, a new blind fish from San Antonio, Texas. Proc. Amer. Phil. Soc. 58: 397-400.

Eigenmann, C.H. 1921. The American Characidae. Mem. Mus. Comp. Zool. Harvard. 43: 209-310.

Emerson, A.E. 1961. Vestigial characters of termites and processes of regressive evolution. Evolution 15: 115-131.

Endler, J.A. 1982. Convergent and divergent effects of natural selection on color patters in two fish faunas. Evolution 36: 178-188.

Erckens, W. 1981a. Analyse von Versuchen zur Erforschung des Grundlagen der tagesperiodischen Aktivitatsverfeilung einer oberivdischen und einer unterivdischen Population des Astyanax mexicanus (Characidae, Pisces). Dissertation am Zoology. Institut Munster.

Erckens, W. 1981b. The activity controlling time-system in epigean and hypogean populations of Astyanax mexicanus (Characidae, Pisces). Proc. 8th. Int. Congr. Speleol. 2: 796-797.

Erckens, W. & W. Martin. 1982a. Exogenous and endogenous control of swimming activity in Astyanax mexicanus (Characidae, Pisces) by direct light response and by a circadian oscillator. I. Analyses of the time control systems of an epigean river population. Z. Naturforsch. 37c: 1253-1265.

Erckens, W. & W. Martin. 1982b. Exogenous and endogenous control of swimming activity in Astyanax mexicanus (Characidae, Pisces) by direct light response and by a circadian oscillator. II. Features of time-control behavior of a cave population and their comparison to an epigean ancestral form. Z. Naturforsch. 37c: 1266-1273.

Erckens, W. & F. Weber. 1976. Rudiments of an ability for time measurements in cavernicoles fish Anoptichthys jordani Hubbs & Innes (Pisces: Characidae). Experientia 32: 1297-1299.

Ercolini, A. & R. Berti. 1977. Morphology and response to light of Uegitglanis zammaranoi Gianferrari, anophthalmic phreatic fish from Somalia. Monit. Zool. Ital. Supp. 9: 183-199.

Ercolini, A. & R. Berti, 1978. Morphology and response to light of Barbopsis devecchii di Caporiacco (Cyprinidae) microphthalmic phreatic fish from Somalia. Monit. Zool. Ital. Suppl. 10: 199-314.

Ercolini, A.; R. Berti, L. Chelazzi & G. Messana. 1982. Researches on the phreatobic fishes of Somalia: achievements and prospects. Monit. Zool. Ital. Suppl. 17: 219-241.

Ercolini, A.; R. Berti & A. Gianferrari. 1981. Aggressive behavior in Uegitglanis zammaranoi Gianferrari (Claridae, Siluriformes), anophthalmic phreatic fish from Somalia. Monit. Zool. Ital. Suppl. 14: 39-56.

Eriksson, L.-O. & T.V. Veen. Circadian rhthms in the brown bullhead, Ictalurus notatus (Teleostei). Evidence for an endogenous rhythm in feeding, locomotor, and reaction time behavior. Can. J. Zool. 58: 1899-1907.

Ewert, J.-P. 1980. Neuroethology. An introduction to the neurophysiological fundamentals of behavior. New York: Springer-Verlag.

Fenwick, J.C. 1970. The pineal organ. pp. 91-108. In: W.S. Hoar & D.J. Randall (Eds.). Fish Physiology, Vol. IV. New York: Academic Press.

Fernald, R.D. & R. Hirata. 1979. Ontogeny of social behavior and body coloration in the African cichlid fish Haplochromis burtoni. J. Comp. Ethol. 50: 180-187.

Garman, S. 1889. Cave animals from southwestern Missouri. Bull. Mus. Comp. Zool. 7: 225-240.

Gertychowa, R. 1971. Heliotaktyzm mlodych rybek jaskiniowych Anoptichthys jordani Hubbs et Innes. Przeg. Zool. 15: 66-69.

Gery, J. 1977. Characoids of the world. Neptune City: T.F.H. Publications.

Gianferrari, L. 1923. Uegitglanis zammaranoi, un nuovo Siluridae cieco Africano. Atti Soc. Ital Sci. Nat. Milano 62: 1-3

Girard, C.F. 1859. Ichthyological notices. Proc. Acad. Nat. Sci. Philad. 1859: 223-225.

Glasser, D. 1966. Untersuchungen uber die absoluten geschmachsschweellen von fischen. Z. Vergleich. Physiol. 52: 1-25.

Glasser, D. 1968. Zum verhalten blinder fische. Z. Tierpsychol. 25: 648-658.

Godin, J.-G. J. 1981. Circadian rhythm of swimming activity in juvenile pink salmon (*Oncorhynchus gorbuscha*). Mar. Biol. 64: 341-349.

Godin, J.-G. J. 1984. Temporal variations in daily patterns of swimming activity and vertical distribution in juvenile pink salmon (*Onchorhynchus gorbuscha*). Can. J. Zool. 62: 72-79.

Goeldi, E.A. 1904. Novo zoologica aus den Amazonas-Region. Neue Wirbeltieri. Comt. Rend. 6e. Congr. Int. Zool., Berna, pp. 542-549.

Gordon, M.S. & D.E. Rosen. 1962. A cevernicolous form of the poecilid fish *Poecilia sphenops* from Tabasco, Mexico. Copeia 1962: 360-368.

Goudie, C.A.; K.B. Davis & B.A. Simco. 1983. Influence of the eyes and pineal gland on locomotor activity patterns of channel catfish *Ictalurus punctatus*. Physiol. Zool. 56: 10-17.

Gould, J.L. 1982. Ethology. The mechanisms and evolution of behavior. New York: W.N. Norton & Company.

Gould, S.J. & E.S. Vrba. 1982. Exaptation- a missing term in the science of form. Paleobiology 8: 4-15

Greenfield, D.W.; T.A. Greenfield & R.L. Woods. 1982. A new subspecies of cave-dwelling pimelodid catfish, *Rhamdia laticauda typhla* from Belize, Central America. Brenesia 19/20: 563-576.

Greenwood, P.H. 1976. A new and eyeless cobitid fish (Pisces, Cypriniformes) from the Zagros Mountains. J. Zool. 180: 129-137.

Gresser, E.B. & C.M. Breder. 1940. The histology of the eye of the cave characin, *Anoptichthys*. Zoologica 25: 113-116.

Grobbel, G. & G. Hahn 1958. Morphologie und histologie des Seitenorgane des augenlosen hohlenfisches anderen Teleosteern. Z. Morph. Okol. Tiere. 47: 249-266.

Grossman, G.D. 1980. Ecological aspects of ontogenetic shifts in prey size utilization in the bay goby (Pisces: Gobiidae). Oecologia (Berl.) 47: 233-238.

Grunewald-Lowenstein, M. 1956. Influence of light and darkness on the pineal body in *Astyanax mexicanus* (Fillipi). Zoologica 41: 119-128

Gunther, A. 1868. A catalogue of the fishes of the British Museum. London: British Museum.

Hairston, N.G.; K.J. Li & S.S. Easter. 1982. Fish vision and the detection of planktonic prey. Science 218: 1240-1242.

Helfman, G.S. 1978. Patterns of community structure in fishes: summary and overview. Env. Biol. Fishes. 3: 129-148.

Helfman, G.S. 1981a. Twilight activities and temporal structure in a freshwater fiscommunity. Can. J. Fish. Aquat. Sci. 38: 1405-1420.

Helfman, G.S. 1981b. The advantage to fishes of hovering in shade. Copeia 1981: 392-400.

Helfman, G.S.; J.L. Meyer & W.N. McFarland. 1982. The ontogeny of twilight migration patterns in grunts (Pisces:Haemulidae). Anim. Behav. 30: 317-326.

Herwig, H.J. 1976. Comparative ultraestructural investigations of the pineal organ of the blind cave fish, *Anoptichthys jordani* and its ancestor, the eyed river fish, *Astyanax mexicanus*. Cell. Tiss. Res. 167: 297-324.

Heuts, M.J. 1951. Ecology, variation and adaptation of the blind African cave fish Caecobarbus geerstsii Blgr. Ann. Soc. Roy. Belg. 82: 155-392.

Heuts, M.J. 1953. Regressive evolution in cave animals. Symp. Soc. Exper. Biol. 7: 290-309.

Hora, S.L. 1924. Fishes of the Siju cave, Garo Hills. Rec. Ind. Mus. 26: 27-31.

Hubbs, C.L. 1936. Fishes of the Yucatan Peninsula. pp. 157-287. In: A.S. Pearse, E.P. Creaser & F.G. Hall (Eds.). The Cenotes of Yucatan. Washington: Carnegie Institution.

Hubbs, C.L. 1938. Fishes of the caves of Yucatan. pp.: 261-295. In: A.S. Pearse (Ed.). Fauna of the caves of Yucatan. Washington: Carnegie Institution.

Hubbs, C.L. 1955. Hybridization between fish species in nature. Syst. Zool. 4: 1-20.

Hubbs, C.L. & R.M. Bailey. 1947. Blind catfishes from artesian waters of Texas. Occ. Pap. Mus. Zool. Univ. Mich. (499): 1-15.

Hubbs, C.L. & W.T. Innes. 1936. The first known blind fish of the family Characidae: a new genus from Mexico. Occ. Pap. Mus. Zool. Univ. Michigan (342): 1-7.

Hull, C.H. & N.H. Nie. 1981. SPSS Update 7-9. New York: McGraw-Hill.

Humbach, I. 1960. Geruch und Geschmack bei den Augenlosen hohlenfischen Anoptichthys jordani, Hubbs und Innes und Anoptichthys hubbsi, Alvarez (Abstr.) Naturwiss. 47: 551-552.

Hunter, J.R. 1968. Effect of light on the schooling and feeding of jack mackerel. J. Fish. Res. Bd. Can. 25: 393-407.

Hyatt, K.D. 1979. Feeding strategy. pp. 71-119. In: W.S. Hoar, D.J. Randall, & J.R. Brett (Eds.). Fish Physiology, vol 8. New York: Academic Press.

Jeannel, R. 1950. La marche de l'evolution. Publ. Mus. Natn. Hist. Nat. Paris. no. 15.

John, K.R. 1957. Observations on the behavior of blind anmd blinded fishes. Copeia 1957: 123-132.

Katz, M.J.; R.J. Lasek & I.R. Kaiserman-Abramof. 1981. Ontophyletics of the nervous system: eyeless mutants illustrate how ontogenetic buffer mechanisms channel evolution. Proc. Natl. Acad. Sci. USA. 78: 397-401.

Kauffeld, J.E. 1954. Ctenobrycon spilurus fathers strange young. Aquarium (Philadelphia) (3): 140-141.

Kavaliers, M. & D.M. Ross. 1981. Twilight and day length affects the seasonality of entrainment and endogenous circadian rhythms in a fish, Cuesius plumbeus. Can. J. Zool. 59: 1326-1334.

Kerby, J.H. 1979. Meristic characters of two Morone hybris. Copeia 1979: 513-518.

Kettlewell, H.B.D. 1961. The phenomena of industrial melanism in the Lepidoptera. Ann. Rev. Entom. 6: 245-262.

Kirby, R.F.; K.W. Thompson & C. Hubbs. 1977. Karyotypic similarities between the Mexican and blind tetras. Copeia 1977: 578-580.

Kosswig, C. 1964. Problems of polymorphism in fishes: Copeia 1964:65-75.

Kosswig, C. 1965. Génétique et évolution régressive. Rev. Quest. Sci. Bruxelles 136: 227-257.

Kosswig, C. 1973. The role of fish research on genetics and evolution. pp. 3-16. In: J.H. Schroeder (Ed.) Genetics and mutagenesis of fish. New York: Springer-Verlag.

Kuhn, O. & J. Kahling. 1954. Augenruckbildung und lichtsinn bei Anoptichthys jordani Hubbs und Innes. Experientia 10: 385-388.

Lagler, K.F.; J.E. Bardach, R.R. Miller & D.R.M. Passino. 1977. Ichthyology. New York: John Wiley & Sons.

Lamarck, J.B. 1809. Philosophie Zoologique. Paris (English translation by Hugh Elliot, The Zoological Philosophy, 1914). London: Macmillan.

Lande, R. 1978. Evolutionary mechanisms of limb loss in tetrapods. Evolution 32: 73-92.

Lankester, E.R. 1893a. Blind animals in caves. Nature 47: 389.

Lankester, E.R. 1893b. Blind animals in caves. Nature 47: 486.

Longley, G. & H. Karnei. 1979a. Status of Trogloglanis pattersoni Eigenmann, the Toothless Blindcat. Endangered Species Report 5 (Part 1), Special Publication, U.S. Fish and Wildlife Service, Albuquerque, N.M. 54 pp.

Longley, G. & H. Karnei. 1979b. Status of Satan eurystomus Hubbs and Bailey, the Widemouth Blindcat. Endangered Species Report 5 (Part 2), Special Publication, U.S. Fish and Wildlife Service, Albuquerque, N.M. 48 pp.

Lopez, M. I. 1978. Migración de la sardina Astyanax fasciatus (Characidae) en el rio Tempisque, Guanacaste, Costa Rica. Rev. Biol. Trop. 26: 261-273.

Manton, S.M. 1953. Locomotory habits on the evolution of the larger arthropodan groups. Symp. Soc. Exp. Biol. 7: 339-376.

Marshall, N.B. & G.L. Thines. 1958. Studies of the brain, sense organs and light sensitivity of a blind cave fish (Typhlogarra widdowsoni) from Iraq. Proc. Zool. Soc. London 131: 441-456.

Martin, M. & P.S. Martin. 1954. Notes on the capture of tropical bats at Cueva el Pachon, Tamaulipas, Mexico. J. Mammal. 35: 584-585.

May, B.; M. Stoneking & J.E. Wright. 1980. Joint segregation of biochemical loci in salmonidae. II. Linkage associations from a hybridized salvelinus genome (S. namaycush X S. fontinalis). Genetics 95: 707-726.

Mayden, R.L. & B.M. Burr. 1980. Two natural darter hybrids involving members of the genus Etheostoma (Pisces:Percidae): Amer. Midl. Natur. 104: 390-393.

Mayr, E. 1960. The emergence of evolutionary novelties. pp. 349-380. In: S. Tax (Ed.). Evolution after Darwin. Vol. I. The evolution of life: its origins, history, and future. Chicago: University of Chicago Press.

Mayr, E. 1970. Populations, Species, and Evolution. Cambridge: Harvard University Press.

Mayr, E. 1982. The growth of biological thought. Diversity, evolution, and inheritance. Cambridge: The Belknap Press of Harvard University Press.

Mayr, E. & W.B. Provine (Eds.). 1980. The evolutionarty synthesis. Perspectives on the unification of biology. Cambridge: Harvard University Press.

McNulty, J.A. 1976. A comparative study of the pineal complex in the deep-sea fishes Bathylagus wesethi and Nezumia liolepis. Cell. Tiss. Res. 172: 205-225.

McNulty, J.A. 1978a. A light and electron microscopic study of the pineal in the blind goby, Typhlogobius californiensis. J. Comp. Neurol. 181: 197-212.

McNulty, J.A. 1978b. The pineal of the troglophilic fish, Chologaster agazzisi: an ultrastructural study. J. Neural. Trans. 43:47-71.

McNulty, J.A. 1978c. Fine structure of the pineal organ in the troglobytic fish, Typhlichthys subterraneous (Pisces: Amblyopsidae) Cell. Tiss. Res. 195: 535-545.

McNulty, J.A. & B.G. Nafpaktitis. 1976. The structure and development of the pineal complex in the lanternfish Triphoturus mexicanus (Family Myctophidae). J. Morph. 150: 579-606.

Mees, G.F. 1962. The subterranean freshwater fauna of Yardie Creek Station, North West Cape, Western Australia. J. R. Soc. West. Austr. 45: 24-32.

Menon, A.G.K. 1951. On a remarkable blind siluroid fish of the family Clariidae from Kerala (India). Rec. Indian Mus. 48: 59-66.

Miller, R.R. 1984. Rhamdia reddelli, new species, the first blind pimelodid catfish from Middle America, with a key to the Mexican species. Trans. San Diego Soc. Nat. Hist. 20: 135-144.

Ministerio de Agricultura y Ganaderia. 1981. Plan de manejo y desarrollo del refugio de fauna silvestre Dr. Rafael Lucas Rodriguez Caballero, Palo Verde-Bagaces. San José: Ministerio de Agricultura y Ganaderia (Mimeograph).

Miranda-Ribeiro, A. 1907. Una novidade Ichthyologica. Kosmos 4: 21-22.

Mitchell, R.W.; W H. Russel & W.R. Elliot. 1977. Mexican eyeless characin fishes, genus Astyanax: environment, distribution, and evolution. Sp. Publ. No. 12. Lubbock: Texas Univ. Press.

Moore, W.S. 1977. An evaluation of narrow hybrid zones in vertebrates. Quart. Rev. Biol. 52: 263-277.

Morelli, S.; L. Bertollo, F. Foresti, O. Moreira & S. de Almeida Toledo. 1983a. Cytogenetic considerations on the genus Astyanax (Pisces, Characidae). I. Karyotypic variability. Caryologia 36: 236-244.

Morelli, S.; L.A.C. Bertollo & O. Moreira. 1983b. Cytogenetic considerations on the genus Astyanax (Pisces, Characidae). II. Occurrence of natural triploidy. Caryology 36: 245-250.

Munk, O. 1965. Ocular degeneration in deep-sea fishes. Galathea Rep. 8: 1-21.

Neff, N.A. & G.R. Smith. 1979. Multivariate analysis of hybrid fishes: Syst. Zool. 28: 176-196.

Nie, N.H.; C.H. Hull, J.G. Jenkins, K. Steinbrenner & D.H. Bent. 1975. Statitical Package for the Social Sciences. 2nd. Edition. New York: McGraw-Hill.

Noakes, D.L.G. 1978. Ontogeny of behavior in fishes: a survey and suggestions. pp. 103-123. In: G.M. Burghardt and M. Bekoff (Eds). Development of Behavior. New York: Garland Press.

Norman, J.R. 1926. A new catfish from Trinidad, with a list of the blind cave fishes. Ann. Mag. Nat. Hist. Ser. 9, 18: 324-331.

Olson, S. 1973. Evolution of the rails of the South Atlantic Islands (Aves: Rallidae). Smith. Contrib. Zool. (152): 1-53.

Packard, A.S. 1894. On the origin of the subterranean fauna of North America. Amer. Natur. 28: 727-751.

Parin, N.V. 1983. Noemacheilus (Troglocobitis) starastoni sp. n.

(Osteichthyes, Cobitidae), a new blind fish from subterranean waters of Kugitangtau (Turkmenia). Zool. Zh. 62: 83-89.

Partridge, B.L. 1981. Lateral line function and the internal dynamics of fish schools. pp. 515-521. In: W.N. Tavolga, A.N. Poper & R.R. Fay (Eds.). Hearing and sound communication in fishes. New York: Springer-Verlag.

Partridge, B.L. & T.J. Pitcher. 1980. The sensory basis of fish schools: relative roles of lateral line and vision. J. Comp. Physiol. 135: 315-325.

Parzefall, J. 1969. Zur vergleichende ethologie verschriedener *Mollinesia* arten einschliesslich einer Hohlenform von *M. sphenops*. Behaviour 33: 1-37.

Parzefall, J. 1973. Modifications du comportament de *Poecilia sphenops* (Pisces, Poecilidae) consécutives a son passage a la vie cavernivole. Ann. Speleol. 28: 283-290.

Parzefall, J. 1979. Zur Genetik und biologischen Bedeutung des Aggressions-verhaltens von *Poecilia sphenops* (Pisces, Poecilidae). Untersuchungen an Bastarden ober- und unterirdisch lebender Populationen. Z. Tierpsychol. 50: 399-422.

Parzefall, J. & H. Wilkens. 1972. Artbildung bei hoelenfischen; verlichende untersuchungen anzuei amerikanisches synbranchiden (Pisces, Teleostei). Z. Morph. Tiere 73: 63-79.

Pavan, C. 1946. Observations and experiments on the cave fish *Pimelodella kronei* and its relatives. Am. Natur. 80: 343-361.

Pellegrin, J. 1922. Sur un noveau poisson aveugle de eaux douces de l'Afrique occidental. C. R. Acad. Sci. Paris 164: 854-855.

Peters, N. & G. Peters. 1966. Das auge zuier hohlenformen von Astyanax mexicanus (Philippi) (Characidae, Pisces). Wilhelm Roux's Arch. Entwickl.-Mech. Org. 157: 393-414.

Peters, N. & G. Peters. 1973. Genetic problems in the regressive evolution evolution of cavernicolous fish. pp. 187-201 In: L.H. Schroeder (Ed.) Genetics and mutagenesis in fish. New York: Springer-Verlag.

Pethon, P. 1974. Naturally occurring hybrids between whitefish (Coregonus lavaretus L.) and cisco (Coregonus albula L.) in Orrevann: Norwegian. J. Zool. 22: 287-293.

Pethon, P. 1978. Age, growth, and maturation of natural hybrids between roach (Rutilus rutilus [L.]) and beam (Abramis brama L.) in Lake Øyeren, SE Norway. Acta Hydrobiol. 20: 281-295.

Pethon, P. 1981. Four natural cyprinid hybrids recorded from Lake Vansjø, SE Norway. Fauna Norv. Ser. A, 2: 28-33.

Petit, G. 1933. Un poisson cavernicole aveugle des eaux douces de Madagascar: Typhleotris madagascariensis gen. et. sp. nov. Compt. Rend. Acad. Sci. Paris 197: 347-348.

Pinto, L.P. 1982. Hybridization between species of Tilapia. Trans. Amer. Fish. Soc. 111: 481-484.

Pfeiffer, W. 1966. Uber die Vererbung der Schreckreakion bei Astyanax (Characidae, Pisces). Z. Vererbungel 98: 97-105.

Pitcher, T.J.; B.L. Partridge & C.S. Wardle. A blind fish can school. Science 194: 963-965.

Poey, F. 1856. Peces ciegos de la Isla de Cuba, comparados con algunas especies de sistintos géneros. pp.: 45-114. In: Memorias sobre la Historia Natural de la Isla de Cuba, Vol. 2. La Habana.

Poll, M. 1957. Redescription du Gymnalabes tithoni Poll, 1944, Clariidae microphthalme du Stanley-Pool (Congo-Belge). Rev. Zool. Bot. Afr. 55: 237-248.

Popper, A.N. 1970. Auditory capacities of the Mexican blind cave fish (Astyanax jordani) and its eyed ancestor (Astyanax mexicanus). Anim. Behav. 18: 552-562.

Poulson, T.L. 1961. Cave adaptation in Amblyopsid fishes. Amer. Midl. Nat. 70: 257-290.

Poulson, T.L. 1969. Population size, density, and regulation in cave fishes. Act. IVe Congr. Int. Speleol.: 4-5.

Poulson, T.L. & W.B. White. 1969. The cave environment. Science 165: 971-981.

Prout, T. 1964. Observations on structural reduction in evolution. Amer. Natur. 93: 239-249.

Putnam, F.W. 1887. Presentation of specimens of Chologaster agassizi Putnam, from Mammoth cave, Kentucky. Proc. Boston Soc. Nat. Hist. 19: 297-280.

Quinn, T.P. 1980. Locomotor responses of juveniles blind cave fish, Astyanax jordani, to the odors of conspecifics. Beh. Neur. Biol. 29: 123-127.

Rakocisnki, C.F. 1980. Hybridization and introgression betweeen Campostoma ologolepis and C. anomalum pullum (Cypriniformes: Cyprinidae). Copeia 1980: 584-594.

Reddell, J.R. 1982. A checklist of the cave fauna of Mexico. VII. Northern Mexico. IN J.R. Reddell, Ed. Further studies in the cavernicole fauna of Mexico, and adjacent regions. Ass. Mex. Cave Stud. Bull. 8: 249-283.

Reeder, W.G. & K.S. Norris. 1954. Distribution, type locality, and habits of the fish-eating bat, Pizonyx vivesi. J. Mammal. 35: 81-87.

Regal, P.J. 1977. Evolutionary loss of eyeless features: is it molecular noise supression? Amer. Natur. 111: 123-133.

Regan, C.T. 1905. A revision of the american cichlid genus Cichlosoma and of the allied genera. Ann. Mag. Nat. Hist. Ser. 7, 16: 60-77, 225-243, 316-340, 433-445.

Regan, T. 1940. The fishes of the genus Lucigobius Gill. Ann. Mag. Nat. Hist. 11: 462-465.

Rohlf, F.J. & R.R. Sokal. 1981. Statistical tables. San Francisco: W.H. Freeman & Co.

Rosen, D.E. & P.H. Greenwood. 1976. A fourth neotropical species of synbranchid eel and the phylogeny and systematics of synbranchiform fishes. Bull. Am. Mus. Nat. Hist. 157: 1-69.

Ross, A.R. & T.M. Cavender. 1981. Morphological analyses of four experimental intergeneric cyprinid hybrid crosses. Copeia 1981: 377-387.

Sadoglu, P. 1957 Mendelian inheritance in the hybrids between the Mexican blind cave fishes and their overground ancestor. Verh. dtsch. zool. Ges. Graz. 1957: 432-439.

Sadoglu, P. 1967. The selective value of eye and pigment loss in Mexican cave fish. Evolution 21: 541-549.

Sadoglu, P. 1975. Genetics paths leading to blindness in Astyanax mexicanus. pp. 419-426. In: M.A. Ali (Ed.). Vison in Fishes: New approaches in reserach. New York: Plenum Press

Sadoglu, P. 1979. A breeding method for blind *Astyanax mexicanus* based on annual spawning patterns. Copeia *1979*: 369-371.

Schemmel, C. 1967. Vergleichende Untersuchungen an den Hautsinnesorganen ober- und unterirdisch lebender *Astyanax* -Formen. Ein beitrag zur evolution der Cavernicolen. Z. Morph. Tiere *61*: 255-316.

Schemmel, C. 1974. Genetische Untersuchungen zur Evolution des Geschmackspparates bei cavernicolen Fischen. Z. Zool. Syst. Evolutionsforsch. *12*: 196-215.

Schemmel, C. 1977. Zur Morphologie und Funktion der Sinnesorgane von *Typhliasina pearsei* (Hubbs) (Ophidioidea, Teleostei). Zoomorphology *87*: 191-202.

Schemmel, C. 1980. Studies on the genetics of feeding behaviour in the cave fish *Astyanax mexicanus* f. *Anoptichthys*. An example of apparent monofactorial inheritance by polygenes. Z. Tierpsychol. *53*: 9-22.

Schlagel, S.R. & C.M. Breder. 1947. A study of the oxygen comsumption of blind and eyed cave characins in light and darkness. Zoologica *32*: 17-27.

Schultz, G.A. 1982. *Amerigoniscus malheurensis*, new species, from a cave in western Oregon (Crustacea:Isopoda:Trichoniscidae). Proc. Biol. Soc. Wash. *95*: 89-92.

Schwartz, F.J. 1981. World literature to fish hybrids with an analysis by family, species, and hybrid: supplement 1. NOAA Tech. Rep. NMFS SSRF-750, 507 pp.

Serra, G.P. & R. Stefani. 1981. La vascolarizzazione della cute e della mucosa buccofaringea nel geotritone. Arch. Ital. Anat. Embriol. *86*: 55-64.

Shaw, E. 1961. Minimal light intensity and the dispersal of schooling fish. Bull. Ocean. Monaco (1213): 1-18.

Simmons, J.A.; M.B. Tenton, & M.J. O'Farrel. 1979. Echolocation and pursuit of prey by bats. Science 203: 16-21.

Simpson, G.G. 1953. The major features of evolution. New York: Columbia University Press.

Sokal, R.R. & F.J. Rohlf. 1981. Biometry. San Francisco: W.H. Freeman & Co.

Sundararaj, B.I. & S. Vasali. 1976. Photoperiod and temperature control in the regulation of reproduction in the female catfish *Heteropneustes fossilis*. J. Fish. Res. Bd. Can. 33: 959.

Suthers, R.A. 1967. Comparative echolocation by fishing bats. J. Mammal. 48: 79-87.

Suthers, R.A. & J.M. Fattu. 1973. Fishing behavior and acoustic orientation by the bat (*Noctilio labialis*). Anim. Behav. 21: 61-66.

Sutton, D.L.; J.G. Stanley, & W.W. Miley. 1981. Grass carp hybridization and observations of a grass carp X bighead hybrid: J. Aquat. Plant Manag. 19: 37-39.

Sweet, S.S. 1982. A distributional analysis of epigean populations of *Eurycea neotenes* in central Texas, with comments on the origin of troglobitic populations. Herpetologica 38: 430-444.

Tabata, M. 1982. Persistence of pineal photosensory function in blind cave fish, *Astyanax mexicanus*. Comp. Biochem. Physiol. 73A: 125-127.

Tanaka, K.; Y. Mugiya & J. Yamada. 1981. Effects of photoperiod and feeding on daily growth patterns in otoliths of juvenile *Tilapia nilotica*. Fish. Bull. 79: 459-466.

Terami, H. & M. Watanabe. 1980. Contrastive movement under bright and dark conditions in the cichlid fish Pelmatochromis pulcher. Zool. Mag. 89: 201-205.

Theilard de Chardin, P. 1959. The phenomenon of man. New York: Harper & Row.

Thinès, G. 1969. L'évolution régressive des poissons cavernicoles et abyssaux. Paris: Masson et Cie.

Timms, A.M. & H. Kleerekoper. 1970. Locomotor responses of blinded goldfish (Carassius auratus) to remote perception of barriers. J. Fish. Res. Bd. Canada 27: 1103-1107.

Tinbergen, N. 1972. The animal in its world: explorations of an ethologist, 1932-1972. 2 vols. Cambridge: Harvard University Press.

Tomiyama, I. 1936. Gobiidae of Japan. Jap. J. Zool. 7: 37-112.

Trewavas, S. 1936. Dr. Karl Jordan's expedition to S.W. Africa and angola: the fresh-water fishes. Novit. Zool. 40: 70-71.

Trewavas, E. 1955. A blind fish from Iraq related to Garra. Ann. Mag. Nat. Hist., ser 12, 8: 551-555.

Vandel, A. 1969. Biospeleology. New York: Pergamon Press.

Villa-R., B. 1966. Los Murciélagos de Mexico. Univ. Nac. Autón. México, Mexico.

Vinciguerra, D. 1924. Descrizione de un ciprinide cieco proveniente della Somalia italiana. Ann. Mus. Civ. Stor. Nat. Genova 51: 239-243.

Von Campenhausen, C.; J. Riess & R. Weissert. 1981. Detection of stationary objects by the blind cave fish Anoptichthys jordani (Characidae). J. Comp. Phys. 143A: 369-374.

Vuilleumier, F. 1984. Evolutionary biology in France: A review of some recent books. Quart. Rev. Biol. 59: 139-159.

Waldman, B. 1982. Quantitative and developmental analyses of the alarm reaction in the zebra danio, *Brachydanio rerio*. Copeia 1982: 1-9.

Wallace, R.K. & J.S. Ramsey. 1982. A new cyprinid hybrid, *Notropis lutrensis* X *N. callitaenia* from the Apalachicola drainage in Alabama. Copeia 1982: 214-217.

Wallis, G.P. & J.A. Beardmore. 1980. Genetic evidence for naturally occurring fertile hybrids between two goby species, *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae). Mar. Ecol. Prog. Ser. 3: 309-315.

Walters, L.H. & V. Walters. 1965. Laboratory observations on a cavernicolous poecilid from Tabasco, Mexico. Copeia 1965: 214-223.

Weissert, R. & V. Von Campenhausen. 1981. Discrimination between stationary objects by the blind cave fish *Anoptichthys jordani* (Characidae). J. Comp. Phys. 143A: 375-382.

Weissman, A. 1889. Essays upon heredity. Oxford: Clarendon Press.

Westoll, S.T. 1958. The lateral fin-fold theory and the pectoral fins of ostracoderms and early fishes. pp.: 180-211. *In*: T.S. Westoll (Ed.), Studies on fossil vertebrates. London: Athlone Press.

Whitley, G.P. 1945. New sharks and fishes from Western Australia. Part 2. Austr. Zool. 11: 1-42.

Whitmore, D.H. 1983. Introgressive hybridization of smallmouth bass (*Micropterus dolomieui*) and Guadalupe bass (*M. treculi*). Copeia 1983: 672-679.

Wiley, M.L. & B.B. Collette. 1970. Breeding tubercles and contact organs in fishes: their occurrence, structure, and significance: Bull. Amer. Mus. Nat. Hist. 143: 143-216.

Wilkens, H. 1969. Beitrage zur Degeneration des Auges bei Cavernicolen, Genzahlk und Manifestationsart (Untersuchungen an mexikanischen Hohlenfischen). Zool. Anz. 180:454-464.

Wilkens, H. 1970. Beitrage zur Degeneration des Auges bei cavernicolen, genzhal und Manifestationsart (Untersuchungen an mexikanischen Hohlenfische). Z. Zool. Syst. Evolutionsf. 8: 1-47.

Wilkens, H. 1971. Genetic interpretation of regressive evolutionary process: studies on hybrid eyes of two Astyanax cave populations (Characidae, Pisces) Evolution 25: 530-544.

Wilkens, H. 1973. Phylogenetic age and degree of reduction of cave animals. In: J.H. Schroeder (ed.). Genetics and mutagenesis of fish. New York: Springer-Verlag.

Wilkens, H. 1980a. The problem of rudimentation, studied in the troglobite eye during ontogeny. Z. Zool. Syst. Evolutionf. 18: 232-238.

Wilkens, H. 1980b. Zur Problematik des Rudimentation, untersucht un des Ontogenie des Auges von Hohlenfischen (Astyanax mexicanus). Z. Zool. Syst. Evolutionf. 18: 232-238.

Wilkens, H.; N. Peters & C. Schemmel. 1979. GesetzmaBigkeiten der regressiven Evolution. Verh. Dtsch. Zool. Ges. 1979: 123-140.

Williams, J.D. & W.M. Howell. 1979. An albino sculpin from a cave in the new river drainage of West Virginia (Pisces: Cottidae) Brimleyana (1): 141-146.

Woods, L.P. & R.F. Inger. 1957. The cave, spring, and swmp fishes of the family Amblyopsidae of Central and Eastern United States. Amer. Midl. Nat. 58: 232-256.

Woodhead, A.D. & M. Achey. 1979. Photoreactivity enzymes in the blind cave fish *Anoptichthys jordani*. Comp. Bioch. Phys. B Comp. Bioch. *63*: 73-76.

Wright, S. 1964. Pleiotropy in the evolution of structural reduction and of dominance. Amer. Natur. *98*: 65-69.

Yew, D.T. & H.M. Yoshibara. 1977. An ultrastructural study on the retina of the blind cave fish (*Astyanax hubbssii*). Cytologica (Tokyo) *42*: 175-180.

Zaccone, G. 1977. Histology, innervation and histochemestry of the UB gland in the Mexican cave fish *Anoptichthys jordani* Hubbs & Innes (Teleostei: Characidae). Acta Histochem. *58*: 31-38.

Zilles, K.; B. Tillmann & R. Bonnemann. 1983. The development of the eye in *Astyanax mexicanus* (Characidae, Pisces), its blind cave derivative, *Anoptichthys jordani* (Characidae, Pisces), and their crossbreds. A scanning- and transmission-electron microscopic study. Cell. Tiss. Res. *229*: 423-432.

VITA

Aldemaro Romero was born in Caracas, Venezuela on September 11, 1951. His parents are Aldemaro Romero (senior) and Margot Diaz Saavedra. He received his elementary and secondary education in the San Agustin and the Alexander von Humboldt German schools of Caracas. In September 1971 he entered the School of Biological Sciences of the University of Barcelona (Spain) from which he was graduated with a Licenciatura en Ciencias Biologicas (Zoology) degree in June 1977. During that time he worked as a scientific journalist, writer and producer for the press, the broadcasting and TV industries.

In 1981 he was admitted to the Graduate School of the University of Miami. In August 1984 he was granted the degree of Doctor of Philosophy by the University of Miami, Coral Gables, Florida.

Permanent address: Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124, USA.