COMPARATIVE STUDIES IN THE LIGHT SENSITIVITY OF BLIND CHARACINS FROM A SERIES OF MEXICAN CAVES

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INTRODUCTION

THE CAVE DERIVATIVES of the eyed and river dwelling *Astyanax mexicanus* (Filippi) are now known to inhabit a series of caves which occupy a single valley in the state of San Luis Potosi, Mexico. The first form was found by C. B. Jordan in La Cueva Chica, near the village of Pujal on the banks of the Rio Tampaon. This form was described by Hubbs and Innes (1936) as *Anoptichthys jordani* Hubbs and Innes. A second form was found in Cueva de los Sabinos near the village of Sabinos by one of Dr. Hubbs' correspondents and is under taxonomic study by him. A third form was found by Dr. J. Alvarez (1946) in Cueva del Pachon and has been called *Anoptichthys antrobius* Alvarez.

In order to further our own studies of the biology of these cave forms, Mr. B. Dontzin and Mr. E. Ruda were commissioned to explore these caves further and acquire both living and preserved material for the Museum. These gentlemen carried on their field work in the spring of 1946 and occupied two months in making the collections, surveying and photographing the caves. In so doing they discovered two more caves which contained representatives of this type of troglodyte. Their ecological field data and the taxonomic status of these forms are under study by Mr. Dontzin, and he intends to report on this aspect of the study separately.

Cueva del Pachon was unknown to us at the time of the above-mentioned expedition. It is actually located in the state of Tamaulipas, just over the line from San Luis Potosi. Mr. C. E. Mohr of the Academy of Natural Sciences of Philadelphia, who subsequently visited this cave, determined that it was actually in the same drainage valley as the others, the available maps of the region being inadequate to determine this point. Mr. C. M. Bogert of the American Museum and Dr. and Mrs. Clarence Goodnight of the Department of Biology of Purdue University also have visited this cave. From each of these visits we obtained living specimens with the cooperation of Dr. Alvarez and wish at this point to express our thanks to the above-mentioned persons for their valuable aid in collecting this material. It completed the series of living specimens in our laboratory and made it possible to make comparative studies on specimens from each of the localities known to be inhabited by these fishes.

The underground drainage of this dry valley is into the Rio Tampaon, and the caves so far found to contain blind fish range up the valley from the river in the following order, a listing which also indicates the present taxonomic status of the various forms:

Rio Tampaon	*Astyanax mexicanus* (Filippi)
La Cueva Chica	*Anoptichthys jordani* Hubbs and Innes
Cueva de los Sabinos	Under study by Hubbs
Sótano de la Tinaja	Under study by Dontzin
Sótano de la Arroya	Under study by Dontzin
Cueva del Pachon	*Anoptichthys antrobius* Alvarez

The geographical locations of these caves are shown in figure 1. This map is based on data obtained by Mr. Dontzin, Mr. Mohr, and the earlier (1940) expedition. Cueva de los Sabinos, Sótano de la Tinaja, and Sótano de la Arroya have been mapped interiorly by Mr. Dontzin, and a map of La Cueva Chica is given by Breder (1942), leaving only Cueva del Pachon to be adequately surveyed.

Whatever the eventual taxonomic disposition of these forms may be, or what further caves may be found to be inhabited by such fishes, or what isolation or communication may or may not exist between these caves is not the direct concern of the present communication but comes under the purview of that part of the whole study being undertaken by Mr. Dontzin.

The present study is confined to an investigation of the reactivity to light displayed by these various fishes. It has already been shown that the blind white type of fish, the typical *Anoptichthys jordani* from La Cueva Chica, is measurably photo-negative (Breder and Gresser, 1941a, 1941b) and that the Sabinos fish are not photo-negative (Breder, 1944). The present paper extends those studies to include fishes from all the caves now known to contain blind populations.

The experimental portions of the work were carried out in one of the laboratories of the Department of Birds, which has been placed at our disposal for these studies through the kindness of Dr. R. C. Murphy. We are grateful to Mr. J. W. Atz who was kind enough to read the manuscript critically.

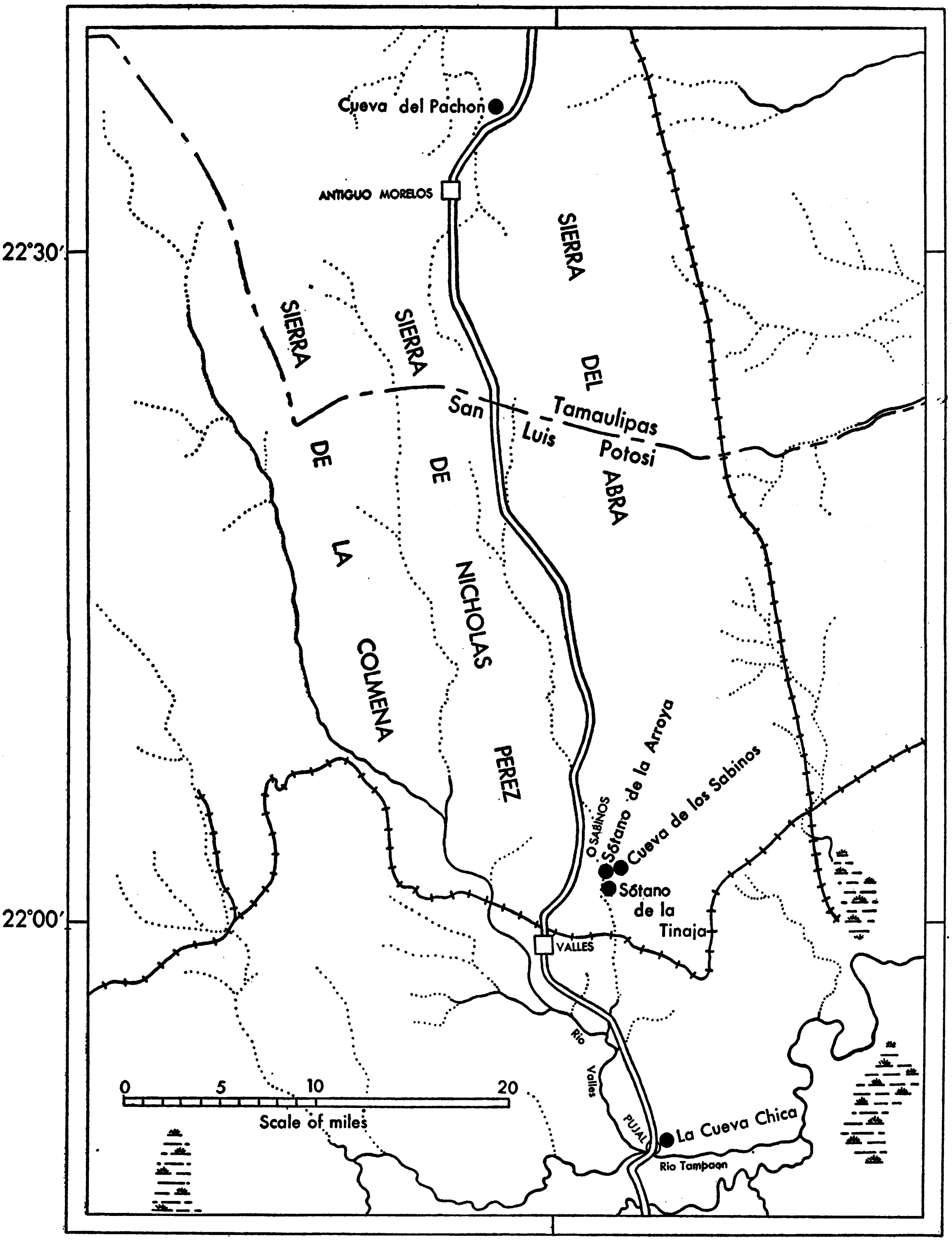

FIG. 1. Geographical location of the five caves known to be inhabited by blind characins. Based on data obtained by Breder, 1940; Dontzin, 1942, 1946; and Mohr, 1946.

MATERIALS AND METHODS

TO DETERMINE THE REACTIONS to light the method first used on these fishes by Breder and Gresser (1941a) was employed. It was improved by the use of a specially constructed aquarium which measured 20 by 40 inches and was 4 inches deep. This was of standard angle iron frame construction with all sides and the bottom of clear glass. One half was covered by black cardboard and roofed and floored by a black board. The other half was left open on the sides, while the under side was floored with white. This side was covered by a removable clear glass, above the center of which hung a 60-watt bulb at a height of 3 feet. Water was placed in the tank to a depth of 3 inches and the temperature held from 72° to 75° F.

The fish to be tested were placed in this container and allowed to accustom themselves to the surroundings for 10 minutes. The number in the lighted half of the tank were then noted every five seconds for 100 times. Each such experiment was repeated five times. Thus 500 observations form the basis of each full test. The justification of such a procedure is indicated in Breder and Halpern (1946) and Breder and Roemhild (1947). In most cases four such tests were combined to make one of 2000 observations in order to increase the statistical certainty to still greater refinement. The intervals were ticked off by a pendulum-actuated electric counting device which produced a barely audible click in a remote corner of the room. Four fish at a time were mostly used, for it was found that this was a convenient and statistically useful number with which to deal. Later tests on single fish were made when this appeared to be necessary or desirable.

The means and frequency distributions resulting from these studies in most cases required no statistical examination for significance as they are clearly, from inspection alone, of obviously different means and parameters. However, some do approach each other so closely that a detailed knowledge of the statistical significance becomes important.

Since the experiments to be compared were purposely made up of the same number of observations, it is possible to use the simple formula

$$\sigma_d = \sqrt{\sigma_{M_1}^2 + \sigma_{M_2}^2}$$

for the derivation of the value of d/σ_d instead of some of the more complicated formulas. When the value of this expression is >3 the difference is almost surely significant, when it is >2.5 it usually is, but when <2 it is not significant. The justification for the use of this formula in the present connections may be found in Simpson and Roe (1939).

ANALYSIS OF DATA

FOUR EXPERIMENTS each were performed on fishes from each of the five caves and combined so that $N=2000$, a number four times as great as was found necessary in the work done on other fishes by Breder and Halpern (1946) and Breder and Roemhild (1947) as well as the already noted earlier work done on these cave fishes. In addition, tests were made on the eyed river fish and on two other species of Ostariophysi, one known to be light positive and the other light negative.

The statistical data so obtained are given in table 1 and graphically in figure 2. In this graph the fishes are arranged according to an ascending order of their mean values. A solid line connects the means of the blind fish and a dotted line those fish that have vision. It is at once apparent that *Ameiurus* and *Brachydanio* stand nearly perfectly at either end of this scale. That is to say, the first hardly ever left the dark chamber, and the second refused to enter it at all. The cave fish, as a whole, hovered about the line of neutrality, but it is also clear that nearly each cave contains fishes that have a reaction to light which shows a statistically significant difference. For example, the Chica fish are negative, as had already been established, but the Tinaja fish are just as clearly positive, a result that was not anticipated. The rest are closer to the line of light indifference. The statistical values of comparison are given in the lower part of table 1. From this it is clear that there is no statistical difference between the Sabinos and Pachon fish, but that all the rest show a high degree of statistical significance. The Arroya

TABLE 1

LIGHT SENSITIVITY EXPERIMENTS ON FISHES FROM EACH CAVE

Each experiment is composed of 2000 observations, which are made up of four sets of 500 each. Four fish have been used in each case

Locality of Fish	Frequency Distribution					Mean	Standard Deviation	Standard Error
	0	1	2	3	4			
Rio Tampaon	1485	87	59	31	338	0.825	1.524	0.0341
La Cueva Chica	350	670	646	277	57	1.510	1.023	0.0228
Arroya	180	557	656	475	132	1.911	1.064	0.0237
Pachon	120	434	655	555	236	2.176	1.084	0.0242
Sabinos	113	448	644	543	252	2.186	1.411	0.0315
Tinaja	51	259	494	733	463	2.649	1.050	0.0235
Binomial distribution	125	500	750	500	125	2.000	1.000	0.0223

Each experiment below is made up of 500 observations and is presented as a basis of comparison with other fishes

Species								
Ameiurus	495	5	0	0	0	0.010	0.013	0.0000
Brachydanio	0	0	0	0	500	4.000	0.000	0.0000

Statistical significance of the closer values. Those not given are obviously of high significance

Pair Compared	d/σ_d	
Pachon-Sabinos	0.2	No significant difference
Arroya-Binomial distribution	2.7	Probably significant
Pachon-Arroya	8.3	Highly significant

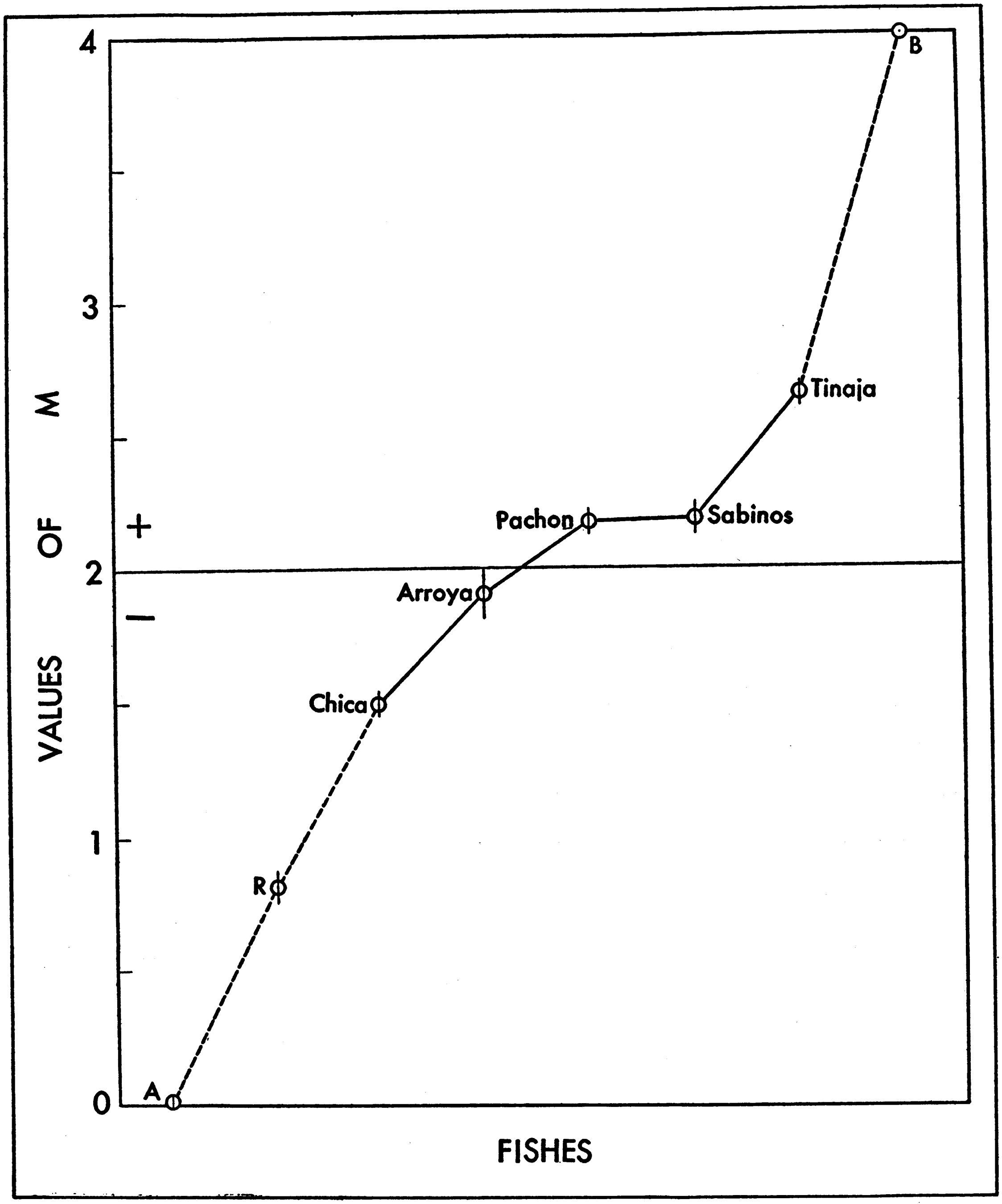

FIG. 2. Comparison of reactions to light of blind fishes from five caves, their river ancestor, and two other species of Ostariophysi. A mean value of 2 indicates randomness with reference to light, above that value positive photokinesis and below it negative photokinesis. The fishes are arranged in increasing order of light positiveness. The blind fishes are connected by a solid line and those with vision by a dotted line. Each point represents 2000 observations. The short vertical lines at each point indicate twice the standard error in both plus and minus directions. A. *Ameiurus nebulosus*. B. *Brachydanio*. R. River fish.

fish are close to, but on this basis probably distinct from, simple random movement. The actual conditions found in the population from this cave are further analyzed in a later connection.

In an effort to find the reason why some of these blind fishes are light negative and the others light positive, further experiments were undertaken, the data of which are given in table 2.

fish lose their light negativeness and become light indifferent when their optic cysts have been removed. This also happens when the cysts are removed from the Tinaja fish. Thus while one is light negative and the other light positive, the removal of these cysts reduces them to an identical light indifference.

The possibility that the pineal or other associated areas are involved was considered in an attempt to understand this difference

TABLE 2

SPECIAL LIGHT SENSITIVITY EXPERIMENTS

Fish and Condition	Frequency Distribution					Mean	Standard Deviation	Standard Error
	0	1	2	3	4			
Each experiment is composed of 2000 observations, which are made up of four sets of 500 each. Four fish have been used								
Chica × Sabinos	116	530	712	484	158	2.059	1.433	0.0320
Chica in dark	154	523	702	477	144	1.967	1.046	0.0234
Each experiment is composed of 2000 observations, which are made up of four sets of 500 each. One fish has been used. To compare means with those of four fish divide the latter by four or multiply the former by four. For purposes of the present graphs the latter has been done								
River fish with no lenses	1140	860				0.430	0.495	0.0111
Chica with pineal exposed	840	1160				0.580	0.498	0.0111
Tinaja with blackened head	895	1105				0.552	0.497	0.0111
River fish from dark	1994	6				0.003	0.054	0.0012
Each experiment composed of 500 observations. Four fish have been used								
Tinaja without capsule	30	141	166	125	38	2.000	1.037	0.0424
Ameiurus	435	61	4	0	0	0.138	0.324	0.0140

Hybrids between Chica fish and Sabinos fish, which physically appear as intermediate between the two parent forms, were tested. Since the one parent form is light negative and the other light positive, as shown in table 2, it is interesting to note that the mean of the hybrid offspring is likewise intermediate between those of the parent forms and all show good statistical differences. They form the series 1.510–2.059–2.186. The value for the hybrids is not statistically different from randomness, the value for d/σ_d being 1.5.

It has already been shown that the Chica

in reactivity to light. The need for undertaking such a study has already been indicated by Breder (1944) who was somewhat concerned about the possible effects of radiant heat in the lighted area as influencing the fish to some extent. It was this possibility that led him to suppose that the slight light positivity of the Sabinos fish might be a response to radiant heat on a light indifferent form. The general pineal area of a Chica fish was exposed by removing the dermal layers over it which carry a heavy investment of guanine. This fish then became light positive. Since the Tinaja fish have no such protective layer the alternate experiment was tried—of covering the top of the head. For this purpose India ink was injected subdermally. Because of the delicacy of the the integument and danger from pressure on the mid-brain, considerable difficulty was encountered in producing satisfactory covers. Consequently the results were not complete in all cases but

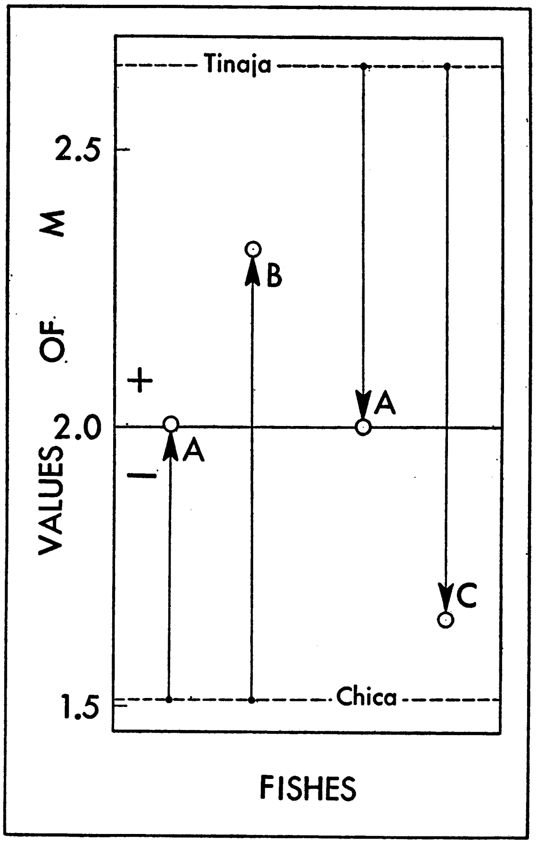

FIG. 3. Influence of the blind optic cysts and the pineal complex on light reactions in the most light negative cave fish (Chica) and the most light positive cave fish (Tinaja). The mean position of the intact fishes is shown by dotted horizontal lines. Their retreat from it is indicated by the vertical arrows. With the optic cyst removed both forms reach the line of light indifference. With the cyst intact but the pineal region exposed the Chica fish becomes light positive, while when the normally exposed pineal of the Tinaja fish is covered it becomes light negative. A. Fish with optic cysts removed. B. Chica fish with guanine cover on head removed. C. Tinaja fish with India ink cover on head.

nevertheless are statistically significant as is evident from table 2. If the individual fish is considered, it will be noted in table 3 that the specimen with the most completely black head showed the largest change, having a mean value of 0.4114. This establishment of the involvement of some sensitive area of the the brain as the site of the control of the sign of photokinesis in these fishes is indicated graphically in figure 3. Here it is clearly shown that the removal of the optic cyst reduces both types of fishes to movements which cannot be separated from pure randomness-and that when these cysts are allowed to re, main intact, the exposure of the brain to incident light causes both forms to show positive phototaxis. The normal fishes from the two caves show opposite signs of reaction, while those that have been experimentally reversed by respectively covering and uncovering the light sensitive area accordingly change their response to light.

In addition to showing the role played by the pineal area in the nature of the light reactions, this experiment also unequivocally demonstrates that any possible effect of radiant heat has nothing to do with observed behavior. The removal of a small patch of guanine or the presence of a drop of ink could hardly be imagined as having any bearing on the response of the fish to warmth, especially as all direction is eliminated in fishes normally with and without a brain shade by removing the optic cysts.

Since all but two populations have been shown to be well separated statistically in table 1 and it is possible to break down each experiment into its original components of sets of 500 observations each, further details concerning the behavior within each group are available, as is shown in table 3. These data are most easily grasped by reference to figure 4 in which the four components of each experiment are indicated, together with their combined recalculation. The lowest reading of all was given by a river fish which had been kept in total darkness for two and one-half years. This, no doubt, had more to do with a new element in the environment and probably some shock effect on the long unused retina. It has been pointed out previously (Breder, 1943b) that such fishes show no reflexes to motion and behave as do the blind

TABLE 3

VARIATION WITHIN GROUPS IN REGARD TO LIGHT SENSITIVITY

A breakdown of the experiments involving 2000 observations into their four component sets

Fish and Condition	Mean	Standard Deviation	Standard Error	Remarks
Rio Tampaon	0.030	0.192	0.0086	Aquarium-reared fish
	0.608	1.347	0.0602	The same
	0.956	1.567	0.0701	The same
	1.506	1.885	0.0887	The same
All	*0.825*	*1.524*	*0.0341*	4 fish used
Chica	1.284	0.952	0.0425	Unpigmented. 1946 collection
	1.514	1.026	0.0458	11th aquarium-reared generation
	1.844	1.095	0.0489	Pigmented. 1946 collection
	1.384	0.923	0.0413	Unpigmented. 1946 collection
All	*1.510*	*1.023*	*0.0228*	12 fish used
Arroya	2.100	1.024	0.0457	Unpigmented. 1946 collection
	2.050	1.026	0.0458	Pigmented. 1946 collection
	1.456	0.982	0.0439	Pigmented. 1946 collection
	2.038	1.090	0.0487	Unpigmented. 1946 collection
All	*1.911*	*1.064*	*0.0237*	12 fish used
Pachon	2.158	0.958	0.0428	1946 collection
	2.552	1.052	0.0470	1946 collection
	2.286	1.002	0.0448	1946 collection
	1.902	1.051	0.0470	1946 collection
All	*2.176*	*1.084*	*0.0242*	16 fish used
Sabinos	1.972	1.052	0.0420	Kept in dark. 1946 collection
	2.746	1.007	0.0440	1946 collection
	2.300	0.981	0.0439	1946 collection
	1.714	0.947	0.0424	1946 collection
All	*2.186*	*1.411*	*0.0315*	12 fish used
Tinaja	3.154	0.866	0.0387	1946 collection
	2.254	1.097	0.0490	1st aquarium-reared generation
	2.412	1.003	0.0448	1946 collection
	2.770	0.980	0.0438	1st aquarium-reared generation
All	*2.649*	*1.050*	*0.0235*	12 fish used
Chica × Sabinos	1.800	1.003	0.0448	Parents from 1942 collection
	1.972	1.050	0.0474	The same
	2.252	0.979	0.0438	The same
	2.064	1.017	0.0454	The same
All	*2.059*	*1.433*	*0.0320*	8 fish used

TABLE 3—*Continued*

Fish and Condition	Mean	Standard Deviation	Standard Error	Remarks
River fish without lenses	0.326	0.489	0.0219	One operated fish
	0.448	0.497	0.0220	The same
	0.446	0.497	0.0222	The same
	0.460	0.498	0.0227	The same
All	*0.430*	*0.495*	*0.0111*	1 fish used
Chica with pineal exposed	0.503	0.499	0.0223	One operated fish
	0.562	0.496	0.0222	The same
	0.580	0.493	0.0220	The same
	0.672	0.469	0.0210	The same
All	*0.580*	*0.498*	*0.0111*	1 fish used
Tinaja with a blackened head	0.414	0.491	0.0219	Most successful blackening
	0.568	0.495	0.0221	Intermediate
	0.576	0.494	0.0222	Intermediate
	0.652	0.472	0.0221	Least successful blackening
All	*0.552*	*0.497*	*0.0111*	4 fish used
Chica from dark room	2.242	1.073	0.0480	Aquarium-reared fish (set 1)
	1.872	1.011	0.0452	The same
	1.750	1.040	0.0465	Aquarium-reared fish (set 2)
	2.004	0.993	0.0441	The same
All	*1.967*	*1.046*	*0.0234*	8 fish used
River fish from dark room	0.000	0.000	0.0000	Kept in dark for 2½ years
	0.000	0.000	0.0000	
	0.000	0.000	0.0000	
	0.012	0.109	0.0049	
All	*0.003*	*0.054*	*0.0012*	1 fish used

fish towards food for a period of about a month in light, at which time their eyes have returned to normal functioning.

Since the above general observation was made it has been checked repeatedly, and the following detailed records were made on two individuals which had been kept in total darkness two years, two months, and two years, eight months, respectively.

The normal Chica fish are a very variable

Reactions	Days in Light: First Fish	Days in Light: Second Fish
No reaction to shadows or moving objects; collided with aquarium fitting	1	1
Reacts to shadow	2	5
Chased another fish	7	6
Excited at feeding time but does not strike at particles	7	8
Strikes inaccurately at food	13	—
Strikes accurately at food	14	26

group of fishes, as has been previously shown. The two unpigmented sets of 1946 show no significant difference with a value of 1.7, while those of the eleventh generation of aquarium-reared fish show a significant difference of 3.6 from the lower value of the former. This is not surprising since these fish have been long inbred non-selectively and show an obviously greater spread of cyst variation than the pigmentless wild fish which were chosen from the mixed population. However, when the pigmented forms from the caves with their generally less degenerate cyst are used the significance becomes prominent.

A river fish that has had its lenses removed is clearly shown to be close to this condition in figure 4, as well as in table 3.

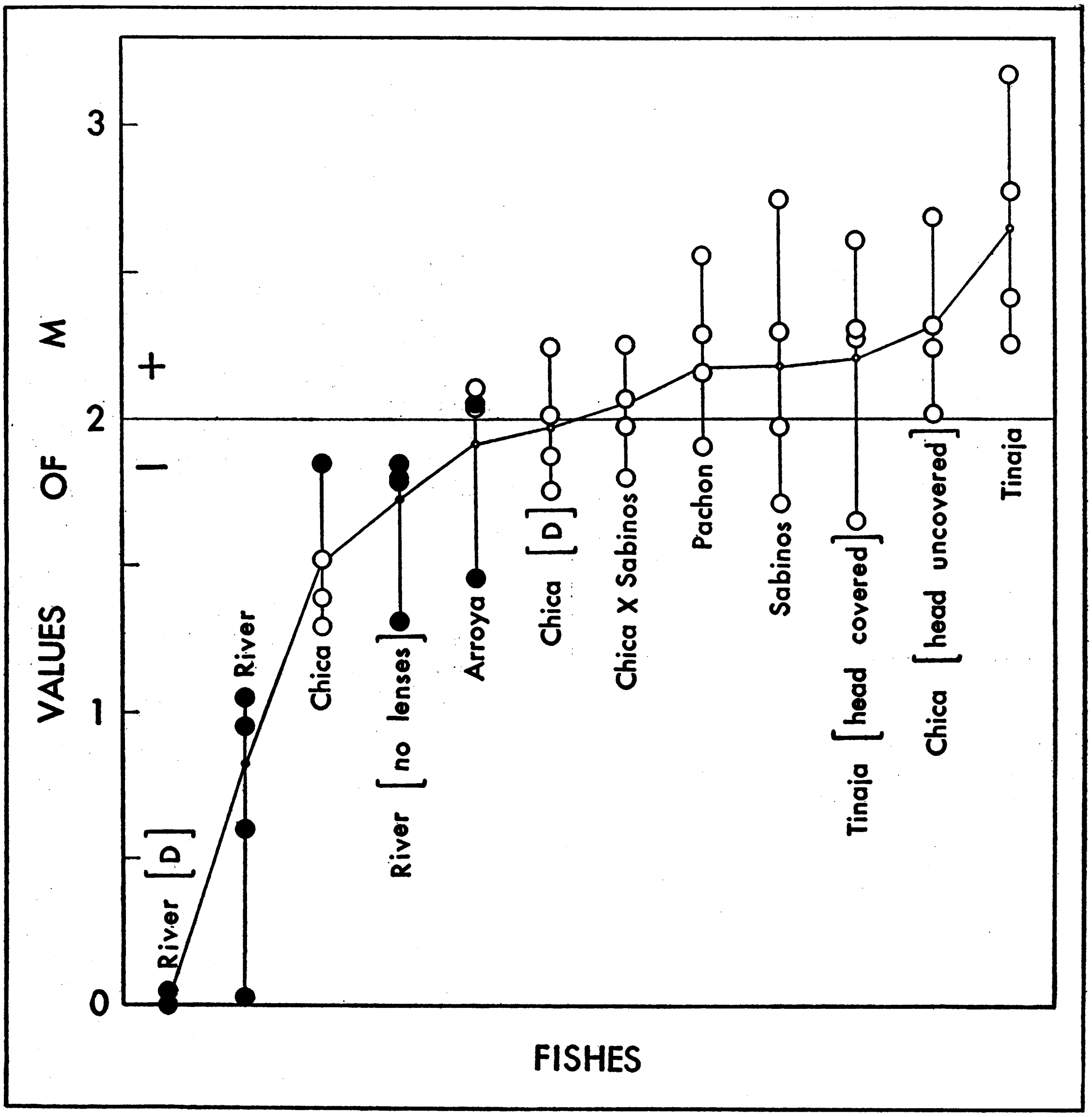

FIG. 4. Variations in light reactions of cave and river fish and of those under special conditions. The data are given in table 3. See text for full explanation. [D]. Fish from darkness. Solid circles indicate pigmented fishes. Open circles indicate unpigmented fishes.

The physical condition and behavior in the Arroya fish might at first be thought to constitute a puzzle until the ecological conditions under which these fish occur are considered. In this cave two evident types of fish are found, those without pigment and those with a fairly prominent amount. Two sets of each

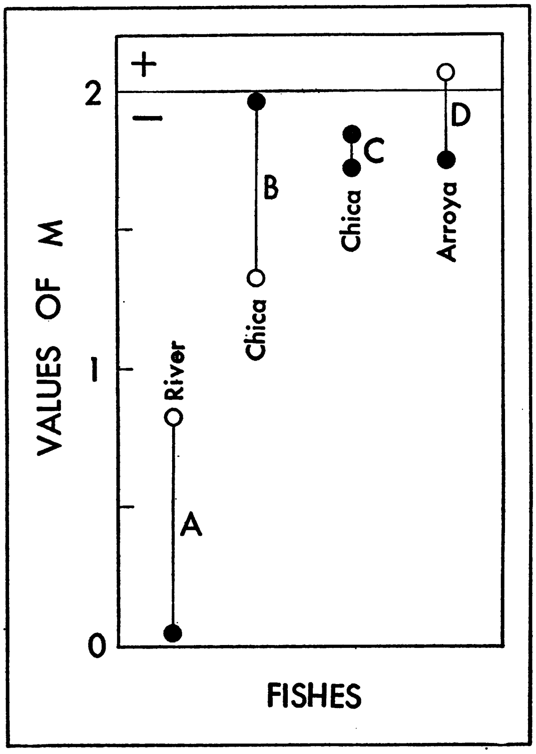

FIG. 5. Variations in light reactions associated with differences in condition. A. River fish from a lighted aquarium (open circle) and from a totally dark one (solid circle). B. Chica fish from a lighted aquarium (open circle) and from a totally dark one (solid circle). C. Pigmented Chica fish (upper) and lenseless river fish (lower). D. Arroya fish, pigmented (solid circle), and Arroya fish, unpigmented (open circle).

kind were tested. If the two pairs be treated separately, it is clear that the pigmented ones would have their mean close to that of the pigmented Chica fish and the lensless river fish, while the pigmentless ones would have a significant difference about the line of randomness. The actual values of the means taken this way are as follows: pigmented Arroya fish, 1.753; unpigmented Arroya fish, 2.069. The two show a strong significant difference, 6.7, but the latter shows no significant difference from random distribution, 0.1.

Chica fish that had been reared in light and then placed in darkness for three and one-half months show a mean value not significantly different from random distribution, 0.1. These were young fish in a rapidly growing condition, and Rasquin (1947) has shown that under such conditions the Chica fish do not continue to lay down guanine but do retain what they already have. If these fish had never been in the light at all, or if fish were still available from the last expedition which had not had considerable exposure to light, a still higher mean value would be expected, approaching but probably not reaching the Chica fish which had its guanine removed. Compare figure 5 with figure 3.

The Sabinos-Chica hybrids straddle the neutral line, and it will be noted that two sets are significantly above and below this line. Since their parents are respectively light positive and light negative, it may be fairly inferred that varying phenotypic amounts of guanine, the extent of the optic cyst and the optic nerve in these intermediate fishes are responsible for the conditions found.

The Pachon and Sabinos fishes, which represent the pair of populations which are not statistically separable, may be discussed together. They show little difference in figure 4 except that the Sabinos fish have a slightly greater spread, but one that is just about equal in both directions. It is the Sabinos fish which Breder (1944) thought to be light indifferent, based on fewer specimens because of the fact that the specimen he sectioned had no nervous connection between brain and optic cyst. The availability of more material has shown that they are not all thus light indifferent or all lacking an optic connection with the brain. In this group some are light positive, some indifferent, and some negative, as may be noted from figure 4. The change of sign within this group of fishes can only be ascribed to these various structural differences which are more variable than first thought. An attempt to check on the possible influence of past conditioning was attempted in this fish by using for one of the tests individuals that had been kept in the dark since brought from the cave. The values obtained from this test in reference to the other tests, as well as other considerations which are discussed in another connection, cannot be used to support the idea that the individual past history of this form modifies its reactions to light to an extent necessary to override the reactions here under study.

The Tinaja fish that had been treated with India ink appear next in this series and show graphically the effects associated with an increasing amount of coverage, the only entirely successful case, being far below the line of indifference.

The Chica fish with the exposed brain is significantly above the line of neutrality, since there was no difficulty in exposing comparable to that of covering the area. This case alone, with the exception of the normal Tinaja fish, is the only one that is completely above the line of indifference.

The Tinaja fish finally are all significantly above the neutral line, and each set shows a significant difference from the others, indicating a considerable amount of variability among these fishes, a feature marking all these groups, which while well separated from one another show the kind of variability among themselves that is usually to be associated with processes involving evolution of loss. Two sets of these fish were brought from the cave and two sets were of their first generation of aquarium-reared offspring. Compared in this way the following extent of significance is found. The mean of the fish brought from the cave is 2.787 and that of the aquarium-reared fish 2.512. A significant difference of 5.9 is found. The *Ameiurus* which had been kept in darkness for five months, indicated in table 2, may be compared with those kept in light, as a further check on the possible effect of past history in regard to light. This yields a value of 9.1 which again shows a significant difference. In other words, those that had been kept in darkness were less light negative than those that had been exposed to it.

A notable feature appears in connection with all the experiments which may be paired in reference to extent of pigmentation, whether originally from the caves or aquarium reared, or whether the fish have been recently kept in darkness or in light. The means of all such pairs, with four special exceptions to be discussed later, show the same direction of change in reaction to light and may be listed as follows:

Fish and Conditions	Corresponding Values of Reactions to Light	d/σ_d
Ameiurus, in light and dark	0.010→0.138	9.1
Tinaja, from aquaria and caves	2.512→2.787	5.9
Chica, in light and dark	1.510→1.967	sig.
Chica, pineal covered and exposed	1.510→2.400	sig.
Arroya, pigmented and unpigmented	1.753→2.069	6.7
Tinaja, pineal covered and exposed	0.414→2.400	sig.

It will be noted that all show statistically significant differences, only those that might appear to be at all close having been calculated. This is interpreted to mean that the reduction of pigment by genetic cause and by the evironmental or experimental influences noted conspires to produce this rather remarkable uniformity of result in widely differing fishes. The roles of conditioning of behavior as apart from simple physiological reactions to the uncovering of a sensitive area are not easily separable in any case and certainly not determinable from the above table, but it is clear from the experiments on covering and uncovering of areas and extirpation of optic cysts that the effects of conditioning in the blind fish must play a very minor role as compared with automatic responses controlled by direct selective stimulation.

The four exceptions to the conditions discussed concern two sets of Chica fish and one set of Sabinos and the normal river fish, as follows:

Fish and Conditions	Corresponding Values of Reactions to Light	d/σ_d
Chica, pigmented and unpigmented	1.844←1.334	sig.
Chica, from aquaria and caves	1.514←1.334	sig.
Sabinos, in light and dark	2.280←1.972	sig.
River fish, in light and dark	0.825←0.003	sig.

In the case of the Chica fish that are pigmented it is not simply a matter of how much the pineal area is covered, since these fish have less reduced optic cysts and as already indicated are morphologically comparable to to river fish without lenses as has been illustrated by Breder and Gresser (1941a).

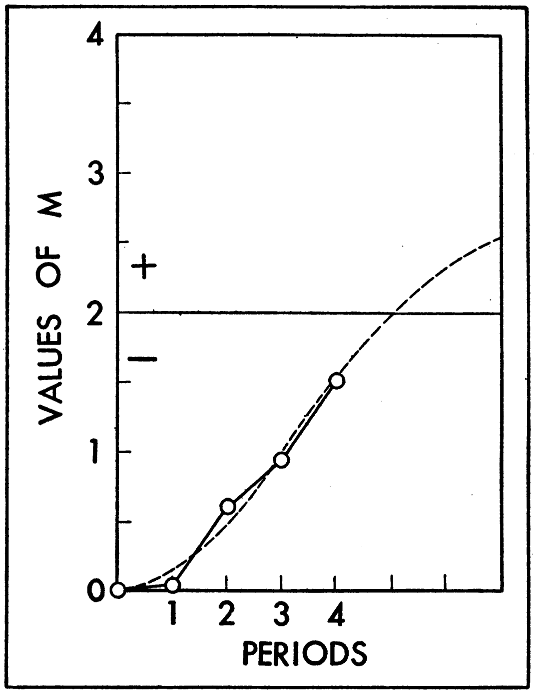

FIG. 6. Light reactions of river fish with vision in successive test periods.

The Chica fish reared in aquaria have passed their eleventh generation of closely inbred stock and show evident external variations in the optic cysts greater than those of the extreme forms from the cave from which they were derived. The two sets calculated separately show a significant difference, 4.7, which is evidently a measure of this variation.

The Sabinos inversion cannot be so neatly disposed of, but since this group shows a much greater spread of variation than any of the other natural populations, as is indicated in figure 4, with individuals light negative, individuals light positive, and some indifferent, it can be assumed only that the four individuals kept in the dark had more that tended to be light negative than had the sample kept in light. It is perhaps more than accidental that the group that shows the greatest spread of variation in this regard should be the only one out of 10 comparisons that is contrary to expectations.

Moreover, in the case of the river fish the role of conditioning is clearly apparent and gives a measure of the qualitative differences between vision and mere light sensitivity. If the data on the river fish in table 3 be examined it will be noted that the four mean values represent a graded series, a feature not displayed by any of the other comparable series of mean values for blind fish. In order to take this series of readings it was necessary for the recorder to be established behind a screen so that any motions of the hand would not be seen, for the slightest movement would cause immediate retreat into the dark compartment. These data are plotted against time in figure 6. The fish were allowed a half hour to adjust themselves to the new environment, and then all four sets of readings were run consecutively. It would not have been practical to run such a series much longer in the situation available as noises, such as the slamming of a door, would cause the behavior to recommence. However, by an extrapolation, as shown in the dotted line, these fish if undisturbed should spend most of their time outside the dark compartment. This is, of course, just what they do in either an aquarium or the state of nature. That is, their life consists largely of swimming about in a lighted place not far from as good a dark retreat as the environment permits. Incidentally, these individual fish had been reared in an aquarium in which the only shelter offered was that provided by the aquarium plants present. They had never before encountered such a "cave," but nevertheless their acceptance of it was immediate. This is in clear contrast to the *Ameiurus* which would scarcely leave the retreat at all on the one hand and *Brachydanio* on the other which could be forced into the retreat only by vigorous chasing with a dip net.

Table 4 gives data from earlier work on some of these fishes, recalculated in order to make them comparable to the present data. Although the experiments listed were all done for other purposes and in differently shaped troughs, it is clear that all this work is in excellent agreement with the present and reinforces the whole proposition. For example, observations made on pigmented Chica fish with vision are between those of the river fish that had been kept in light and those that had been kept in darkness. The latter had been kept in a lighted aquarium several months after having been brought from the cave. The values of the blind, pigmented individuals of this same collection are higher than the river fish kept in light but considerably below those of the pigmented fish tested for the present work. This is to be expected because, as already noted, these Chica fish show a large amount of individual variation. Thus in the large number of tests run on the unpigmented Chica fish a very considerable spread is notable, but all significantly below 2 and above 0. The Chica fish with the cysts removed are not significantly different from 2 and were incorporated in the previous remarks. The Sabinos fish are within the range and not far from the mean values later obtained on further material. Even in those cases in which only one-quarter of the trough was lighted the Chica fish fall within the range of those measured with a half-and-half condition of the trough. There is thus clearly a very considerable constancy in these results within the limits of variability inherent in the material.

TABLE 4

EARLIER DATA ON LIGHT REACTIONS

A recalculation of all earlier data taken on these fishes and brought into uniformity with the present usage

Type of Condition of Fish	Number of Fishes	*N*	Mean	Standard Deviation	Standard Error
Pigmented Chica with sight,[a] 1940[b]	1	1000	0.063	0.243	0.0077
Pigmented Chica, blind,[a] 1940	1	1600	0.300	0.459	0.0115
Unpigmented Chica,[a] F_5[c]	1	2400	0.381	0.485	0.0099
Unpigmented Chica,[d] F_5	1	4175	0.341	0.341	0.0074
Unpigmented Chica,[e] F_7	1	1000	0.378	0.489	0.0155
Unpigmented Chica,[a] F_5	2	300	0.270	0.431	0.0249
Unpigmented Chica,[a] 1940	3	200	1.470	0.816	0.0578
Unpigmented Chica,[a] F_5	4	200	1.320	0.859	0.0607
Chica with cysts removed,[d] F_5	1	4200	0.503	0.499	0.0078
Sabinos,[e] 1942	1	1000	0.490	0.499	0.0157
Sabinos,[e] 1942	4	1000	2.117	1.046	0.0331
Unpigmented Chica,[f] 1940	2	200	0.515	0.636	0.0500
Unpigmented Chica,[f] 1940	4	1000	0.755	0.811	0.0256

[a] From Breder and Gresser (1941a).
[b] Years indicate dates of expedition on which taken.
[c] F numbers indicate number of generations in aquaria.
[d] From Breder and Gresser (1941b).
[e] From Breder (1944).
[f] Trough with one-quarter of area lighted. From Breder and Gresser (1941a).

CORRELATED STRUCTURAL MODIFICATION

THE PROGRESSIVE REDUCTION of pigmentation in these fishes has already been discussed by Rasquin (1947). In general terms it leads to a decrease of opacity and a consequent increase of exposure of the internal parts of these fishes, including among other things the pineal complex. As already indicated, because this body, together with its associated areas, has been shown by others to be light sensitive, e.g., von Frisch (1911a, 1911b), Scharrer (1927), and Gladstone and Wakeley (1940), its possible connection with the present behavior studies made necessary an investigation of its morphological relationships within the head of the fish.

The fish under study all agree in having a much reduced surface to the roof of the skull. A large frontoparietal fontanelle leaves a central strip of the brain protected only by soft tissues. The extent of this exposure is indicated in figure 7. In some of the blind specimens this opening is somewhat wider. A very considerable number of characins in this as well as other subfamilies have similar, if not so large, fontanelles, as do a variety of other non-characin Ostariophysi; see, for example, Sagemehl (1885), Eigenmann (1916), Gregory (1933), and Gregory and Conrad (1938). Thus light does not have to penetrate any osseous tissue in order to reach the brain. Near the center of this fontanelle there is a cross bar, the so-called interfrontal bar, evidently a strengthening brace of considerable importance to a skull practically split down its middle. This fontanelle is clearly a secondary opening of the skull, as the ancestors of these fishes had solidly covered heads, the parietal or pineal openings of early fishes having been long since closed.

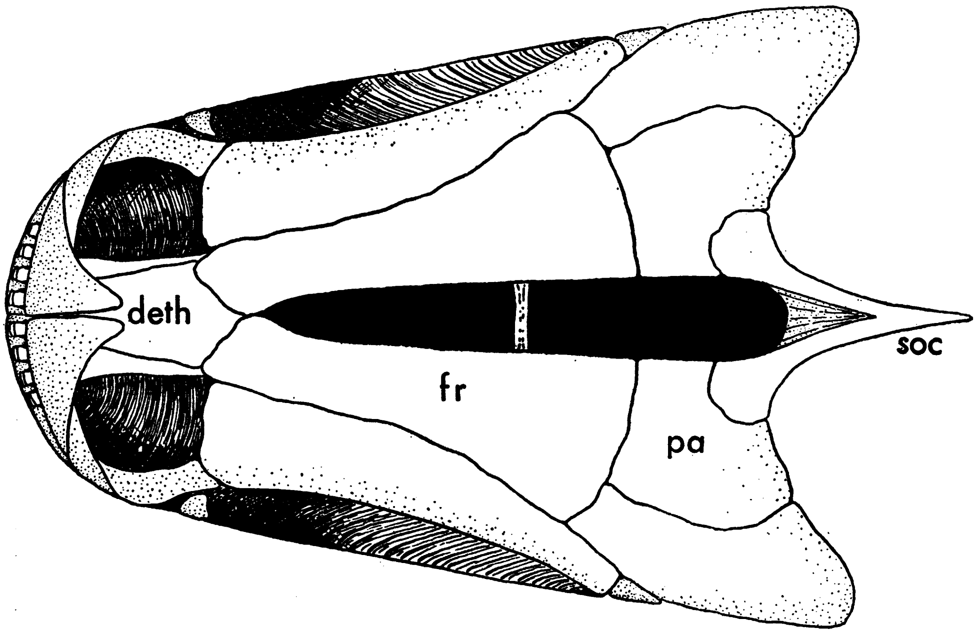

FIG. 7. Dorsal view of skull of river fish showing extent of the frontoparietal fontanelle (semi-diagrammatic). *Abbreviations:* deth, dermethmoid; fr, frontal; pa, parietal; soc, supra-occipital.

A study of sections shows that a considerable area of the brain is exposed by the fontanelle in the skull. The aperture appears first at the level of the olfactory lobes. The skull remains open, proceeding posteriorly, until two bones from opposite sides of the skull fuse again over the extreme posterior part of the pineal. This fusion takes the form of a small bridge, the above-mentioned interfrontal bar. The opening appears again immediately posterior to the pineal. The opening continues posteriorly, leaving most of the top of the brain exposed, and the bones fuse again over the level where the auditory nerves enter the medulla.

In order that the morphological relationships of pineal areas and optic nerves in the various forms could be studied, serial sections were made of the heads of all the types under consideration. The heads of each were fixed in formalin, mordanted in 3 per cent potassium dichromate, decalcified and sectioned at 15 microns. They were stained with Weigert's technique to facilitate tracing of the optic nerve remnants. In addition hemotoxylin- and eosin-stained sections of each were studied.

In all instances some remnants of the optic nerve were found. The size of the blind capsules and numbers of fibers present in the optic nerves varied not only among the different populations but within one cave. In some cases not more than half a dozen fibers of each optic nerve were seen to enter the brain, and in other cases a reasonably large nerve was present. The river fish alone presented a normal optic nerve and chiasma.

The pineal body is situated between the forebrain and midbrain, directly beneath the aperture caused by the fontanelle of the brain case. The bones bordering the fontanelle are connected by a heavy band of collagenous tissue, and the pineal lies directly beneath this connective tissue, as indicated in figure 8. In the case of most of the blind fishes adipose tissue is present in great quantities between the bone and the epidermis and between the bone and the meninges covering the brain, but at the site of the pineal body no fat is seen in either place. The epidermis is immediately adjacent to the collagenous tissue connection of the bones dorsally, and the pineal is immediately adjacent to the same structure ventrally.

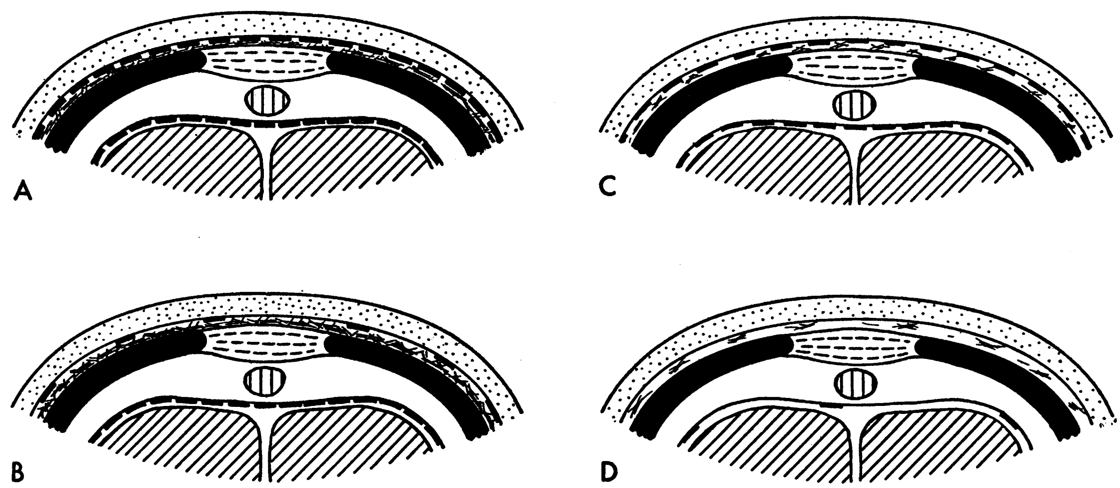

FIG. 8. Diagrammatic section through the plane of the pineal, indicating progressive reduction of its protection from light. A. River fish. B. Chica fish. C. Pigmented Arroya fish. D. All others: unpigmented Arroya, Sabinos, Tinaja, and Pachon fish. Epithelium, stippled area. Bone (skull roof), solid black. Connective tissue, dashed horizontal lines. Pineal body, vertical hatching. Brain, diagonal hatching. Melanophores, black rectangular areas (subdermal and meningeal). Guanine, criss-cross lines (subdermal).

Protecting the pineal dorsally in the river and Chica fishes are the black pigment cells and the heavy deposition of guanine characteristic of the river fish and the Chica fish reared in the light. To a lesser extent this is also true of the pigmented Arroya fishes which show a considerable number of melanophores beneath the epidermis, although they lack much of the guanine deposition seen in the other two types of fishes. Heavy pigmentation is seen in the meninges of the river

Chica and pigmented Arroya forms, but in no case does this cover the pineal body, as this protrudes through and lies over the pigmented membrane. The only pigmentary difference therefore between those fish that react positively to light and those that react negatively is the layer of melanin and guanine which prevents the light from striking this exposed area of brain and pineal area in those fishes that are negatively phototropic.

The conditions above described and indicated in figure 8 may be tabulated in reference to the four main differences of pigmentation.

It is to be noted that the first three forms, river, Chica in light, and pigmented Arroya, are the light negative forms, while "all others," unpigmented Arroya, Sabinos, Tinaja, and Pachon, are light positive. Chica fish in dark lack the heavy investment of guanine as already noted. Evidently in this type of reduction of pigment and the ability to form deposits in response to light the dermal melanophore system is the first to go and is then followed by the guanine layer, while the meningeal melanophores appear to be the last to be affected.

From this it is evident that little if any ordinary light can penetrate to the river fish pineal and probably little if any in a Chica fish which has laid down a deposit of brilliantly reflective guanine as a consequence of having lived in light for some time.

Structures	River	Chica (In Light)	Arroya (Pigmented)	All Others
Dermal melanophores	Heavy investment	Very few	Moderate investment	Virtually absent
Dermal guanine	Heavy investment	Heavy investment	Scattered investment	Little investment
Meningeal melanophores	Heavy investment	Heavy investment	Moderate investment	Very few

DISCUSSION

The comparison of the behavior of these fishes drawn from different populations, together with their optic morphology and the geographic relationships of the five caves in which they are known to occur, makes possible the development of some rather interesting points concerning the possible origin and evolutionary sequences inherent in an ecological situation of this kind.

If reference be made to the map (fig. 1), it will be found that the various cave openings are arranged in the following sequence from the nearest point on the Rio Tampaon, which is the closest body of water and which is well populated with the eyed river form.

Cave	Miles from River	Light Reaction
La Cueva Chica	0.75	Negative to positive
Sótano de la Tinaja	14	Positive
Sótano de la Arroya	15	Mixed
Cueva de los Sabinos	15	Slightly positive
Cueva del Pachon	54.5	Slightly positive

These range up the dry valley between the Sierra del Abra and Sierra de Nicholas Perez in very nearly a straight line. We may assume for purposes of discussion that river fish entered La Cueva Chica, the while undergoing optical and pigmentary reduction, and worked on up the valley under ground, and that all the waters of this underground system in the valley are or were at some time connected, the "caves" representing merely incidental openings that make the fishes locally accessible to man. The distance in miles may be plotted against the mean light reaction with due regard for sign, as is shown in figure 9. Thus the eyed river fish have a nearly 0 value and actually in the trough mostly take up a stationary position in the dark end and peer out. The blind forms all show a continual wandering motion, which in part at least is due to the impossibility of optical fixation. Although none of these show an obvious response when entering light or dark, their average movements in light and dark respectively give the results obtained. On a basis of oxygen consumed Schlagel and Breder (1947) have shown that the Chica fish move faster in the light than in the dark, which would give, on a mechanical basis alone, the results noted, as Young (1935a, 1935b) found to be the case with *Lampetra*. Although oxygen consumption data have not been assayed from the other cave forms as yet, on the above basis the fish from the other caves should move faster in dark, most notably those from Tinaja.

In terms of optic capsule and nerve reduction the series runs from the normally eyed river fish to the negative Chica fish and from the somewhat further reduced but positive Tinaja fish and thence on to the still further reduction found in the other three caves. Although in individual cases this amounts to complete reduction so that some individuals are as light indifferent as those that have been operated upon, in no case is any detected response in these fishes other than positive.

Another way in which one might suppose these caves became populated is to assume that before the waters sank below ground the now dry river bed was a permanent stream well populated with the rather ubiquitous river form and that as the caves were formed various local populations sank into the ground in substantially their present location, the survivors giving rise independently to the present blind populations. There are, however, several objections to such a supposition. One is that there is a progressive reduction in optical equipment up the valley. Another is that hybrids between Chica and Sabinos fish are intermediate in both morphology and behavior, indicating that the two stocks are related. Such would certainly not be expected from two separate populations that have both undergone morphologically and behavioristically parallel changes associated with similar environments.

Alvarez (1946), in comparing Chica, Sabinos, and Pachon fish taxonomically, shows that fin ray counts and various body proportions form overlapping series as one passes up the valley through these three caves, another item that agrees with the supposition that the fish worked their way up the valley underground and one that strongly militates against the possibility of their having worked their way *down* the valley.

Another way to consider this problem is to assume that the progress in reduction ran from Chica to Sabinos or Arroya and thence to Pachon. Tinaja would then be in the nature of a little isolated spur in which for some reason associated with population isolation developed further towards a light positive condition. The development of any such argument is mitigated against by the fact that all four caves show positiveness to light.

It might even be presumed that the present underground populations have arisen from a successful establishment of an underground population far up the valley, perhaps near Cueva del Pachon. Then these would be conceived of as gradually working down stream and finally hybridizing with fresh entrants from the river in La Cueva Chica. The conditions in Sótano de la Arroya should then be the working back of river fish genes. This is considered very unlikely for reasons already indicated. Incidentally, the blind white fish of La Cueva Chica breed true to type when inbred for many generations. A single successful spawning was obtained between such a blind white individual and an eyed and pigmented individual with evident perfect vision from the same place. All the offspring resembled the eyed parent and none showed any variation from this type. As already noted, it must be recalled that the Arroya cave actually differs from all the rest in admitting light to an area inhabited by fishes. It would seem from this to be much more likely that the conditions in the Arroya cave represent a reëstablishment of a pigmented condition, presumably in response to a long-continued exposure to a low light intensity of one fraction of the population and that the pigmented and unpigmented forms are actually phenotypes of this mixture.

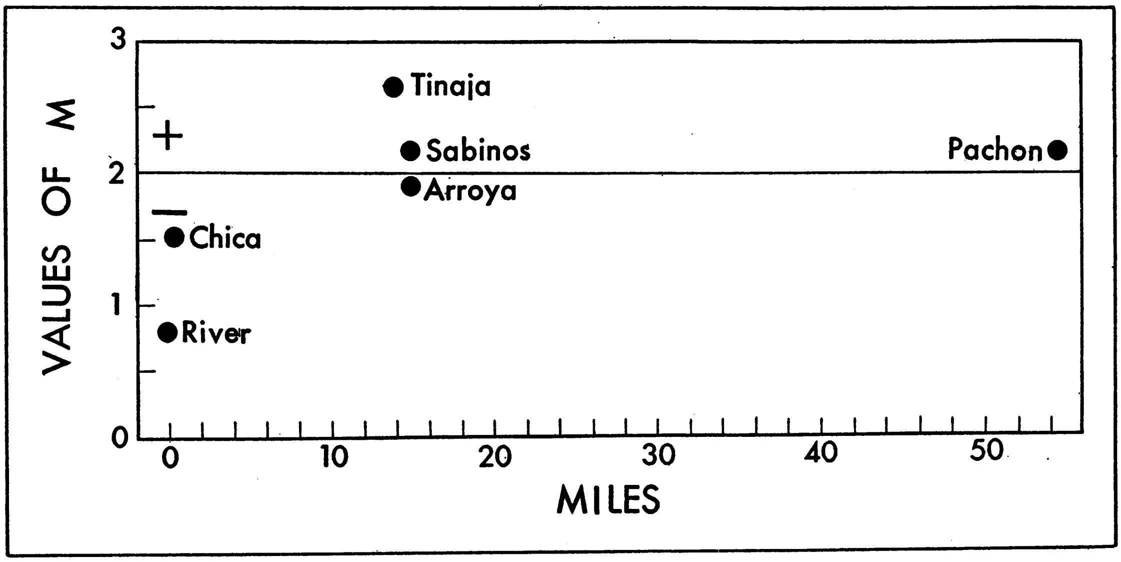

FIG. 9. Comparison of the light sensitivity of the various cave populations with the distance of the cave from the nearest surface water.

It is noteworthy that the offspring of the two types of Chica fish, although presenting a uniform group, changed radically as time went by. About one out of four had their eyes involute at an age of approximately one and one-half years, some becoming fully blind and others remaining shadow sensitive. At about three years one of them out of a remaining five lost its pigment but retained its vision. These fish resembled closely the various phenotypes from La Cueva Chica discussed by Breder and Gresser (1941a) and Breder (1943a).

The data on the optic morphology and light sensitivity of these fishes agree nicely as do the changes induced by operation. However, the matter of reversal of sign of the phototaxis presents a number of puzzling problems. While the abundant literature on phototaxis and tropism contains many references to reversal in sign associated with environmental change and ontogeny, there appears to be no parallel case where two otherwise similar populations in closely similar environments undergo such a change or indeed where two similar species show opposite reactions. See, for example, the extensive data of Fraenkel and Gunn (1940).

The term skoto-taxis proposed by Alverdes (1930) for what is usually thought of as a negative phototaxis has been discussed by Dietrich (1931) and Fraenkel and Gunn (1940) as to the philosophical implications in considering the absence of a stimulus, in this case darkness, as a positive influence. The present case would seem to have some bearing on such considerations.

As indicated, these fishes show a continual wandering motion irrespective of the condition of the optic cysts or the brain cover. There is evidently just as much locomotion when the cysts are removed as when intact, but it is then clearly without reference to light or darkness. Consequently, the "taxis" is not initiated through the intermediation of the cysts but exists independently of them, evidently because of some non-exteroceptive condition. Furthermore, it is only when such fishes have vision that other behavior than this continual wandering is possible, for it was demonstrated by Breder and Rasquin (1943) that river fish with cut optic nerves immediately took on such wandering activity. Since it is shown here that the presence of these cysts is essential for light reactivity, whether the reaction is towards or away from light depending on the impingement of light on the brain, it would seem that the need for such a term as skoto-taxis retreats into the realm of metaphysics.

Whatever the mechanism involved in the reversal of the sign of the phototaxis, its existence implies some rather curious biological implications. A negative response to light on the part of cave fish should contribute towards keeping them within their cave and thus safe from a lighted river environment where they could not compete with fishes provided with normal vision. Furthermore, this feature should be of general utility to cavernicolous animals, for as Walls (1942) points out, "There is some point to a retention of a sense of light and darkness by subterranean forms so that they may be aware when their burrows have been broken into by weather or by other animals." This, of course, implies that the animals will react appropriately for their own safety. Pure light indifference in itself should be a considerable danger under such conditions, while any slight degree of positiveness in reaction would probably be disastrous unless the conditions were of a very special nature. Actually in the case of Arroya there is just such a condition in the area where the cave roof has broken in. This hole is very deep and a place unlikely for a heron or other fish-eater to wander, but a population of this sort certainly could be depleted with ease if the break became large enough to expose these light attracted blind fish to any marked extent.

Lest it be thought that the continued presence of these light sensitive capsules has some other function bearing on the physiology of the animal but in no way connected with any utilitarian light sensitivity, it may be pointed out that Breder and Rasquin (1947) extirpated the cysts in a large number and were unable to find any measurable difference in growth or development. Since then many of these individuals have attained full maturity and are indistinguishable from fish with intact cysts.

The much further specialized cave fish, *Amblyopsis*, has been shown by Eigenmann (1909) to be much more violently light negative than the forms here under study. Although evidently having dwelt in caves for a much longer evolutionary period they immediately retreat from even the light of a match. This fact, as well as similar reactions by other cave fishes and salamanders, leads to the inevitable conclusion that the reversal of light negative reactions to positive ones is not a common attribute of animals that have lived in caverns for many generations, but is a peculiarity of these characins and so far as known is unique.

In La Cueva Chica this could be interpreted to mean that if some of the population became light positive and wandered those that came into lighted areas would be lost while those that wandered farther up the valley under ground would not be troubled by this light positive reaction which would have no opportunity to be brought into play. In other words, these fish that remained near the river outlet possessed a positive selective advantage in the retention of an ability to reform a guanine shield on exposure to light. It has been shown that these fish when reared in darkness lack this deposit to a notable degree and are light positive. It thus follows that they would first be light positive, followed by becoming light negative after sufficient exposure to light to induce the deposition of guanine. This could safely take place only in an environment in which this operated on the fish before they reached any considerable exposure to active predation. Such a situation might be difficult to visualize were it not for the fact that just such a situation is present in Sótano de la Arroya where part of the roof has collapsed, as previously described.

In this cave, as has been discussed, there are fishes that are about as pink as the Sabinos fish and some that have enough dermal melanophores to make them comparatively dusky. When first brought from the cave, only the collection made from the pool near enough to the fallen roof to make possible their capture by daylight contained these dusky individuals. Later it was discovered that some of the individuals from pools in completely dark parts of the cave were becoming dusky and that as time went on all the dusky ones were becoming darker. This is evidently owing to the operation of light on blind fish which still hold enough melanophores, as has been shown by Rasquin (1946) in the case of yellow goldfish experimentally blinded which in less than a year became dusky. These Arroya fish thus differ from all the rest in that they alone have enough responsiveness retained in their melanophore system to make possible such a reaction to light. The Chica fish attain the same result by means of a still responsive guanine system, but all the rest, including the majority of the Arroya fish, show neither of these responses and remain light positive even after long-continued residence in a well-lighted laboratory.

The condition may be expressed as follows:

Form	Light: Negative	Light: Positive
River fish	←——o	
Chica fish, pigmented	←——o	
Chica fish, unpigmented		o——→
(guanine increases in light)	← - - - -	- - - -
Arroya fish (two phenotypes)	←——o	o——→
Tinaja fish		o——→
Sabinos fish		o——→
Pachon fish		o——→

Thus the river fish and pigmented Chica fish tend to enter dark places, but after they lose their melanin and guanine, phylogenetically, they become light postive, only to reverse this response again when light induces a development of guanine. The pigmented Arroya fish are light negative, which pigment increases on exposure to light. All the rest are light positive and remain that way even after prolonged exposure to light. These forms alone have been found in places where no light at all is evidently available to them. This chromatic behavior is evidently the physiological counterpart of what Pavan (1946) describes for Brazilian cave derivatives of *Pimelodella*.

The work of von Frisch (1911a, 1911b) showed that the cyprinid *Phoxinus laevis* was light sensitive in the pineal and adjacent areas. He measured this sensitivity, not by locomotor activities but by responses of the pigmentary system. He found that the melanophores expanded on exposing the pineal region to light in blinded individuals and contracted when not so exposed.

Since the Chica fish lay down guanine in the light and certain individuals of the Arroya fish increase the number of melanophores on similar exposure while all the others show no such reactivity, the phenomenon as here discussed would seem to be one of an evolution of loss of this type of reaction, those forms which show no such reaction simply having reached a stage in degenerative evolution which will no longer operate in this manner in response to light.

Park (1941) pointed out that with increasing socialization organisms tended to become increasingly arhythmic in their activity patterns. This is apparently the case, and we have no disagreement with his views but take this opportunity to point out that such an increase in arhythmicity does not necessarily establish an increase in social integration. In the case of the fishes here under discussion exactly the reverse is found. The eyed river fish are a closely aggregating, semi-schooling form whose rhythm of social activity is necessarily an optically intermediated affair, the diurnal pattern of which is obliterated below the visual threshold. It is obvious on inspection that the river fish are "paying much more attention" to one another than are the blind cave fish, which of course they can only do in light, as is evident from the experiments of Breder and Rasquin (1943) and Schlagel and Breder (1947). Since the cave fishes are not acting in any kind of unison and night is permanent with them, they are evidently fully arhythmic in their caves. Despite the fact of light sensitivity as herein discussed, in ordinary lighted aquaria they are found to show similar locomotor patterns throughout 24 hours. It thus may be that an increasing uniformity of environment, socially induced in bees, ants, and men, but tropistically and geologically induced in these fishes, may be more the cause of arhythmicity in them all than anything peculiar to an increase in complexity of social order.

Since it has been shown that the exposure of the pineal area of the brain to light has various effects involving both the pigmentary systems and locomotor behavior of teleost fishes which have lost their vision in one way or another, there appears a direct invitation to explore the matter further and in more general terms. It is not the purpose of this contribution to go into such matters, but certain general considerations are very suggestive of possible approaches. Appropriate material is not immediately available to the authors for the carrying out of various natural continuations of which the present contribution may be considered a starting point.

Since vision plays such an apparently dominating role in many fishes, it might be thought that any influence induced by exposure or covering of the pineal would be negligible. In other words, does this effect operating through the pineal find expression only in the absence of vision? Bearing on this is the curious fact that many of the small fishes that are very positively phototropic are often nearly transparent, translucent, and very lightly pigmented, while many, if not all, the photo-negative forms of small size are heavily pigmented, especially over the head. In the first group come many minnows, atherinids, and characins, while in the latter come many silurids, gobies, and blennies. *Fierasfer*, a nearly transparent creature that resides in the cloacal chamber of holothurians, might be thought of as an exception until it is realized that the one place it is heavily pigmented is on the top of the head where a patch of dark pigment sits very like the India ink caps provided for the cave fish. On the other hand, the highly transparent young of the silurid *Cryptopterus* shun dark places and seek lighted areas.

Longley and Hildebrand (1940), in discussing the behavior of the chiefly translucent and little pigmented *Emblemariopsis diaphana* Longley, write: "It is usually found fully exposed but sometimes sheltered in empty tubes. . . .

"In life the female is largely translucent. Only the head and trunk to the pelvic base inclusive have pigment in quantity and distribution approaching the usual in fishes. Even the head is more or less translucent brownish olive with minute white points in an external color pattern, through which in dorsal view there may be seen an internal pattern of white lines upon the pale brown of the meninges."

Continuing in another place they write:

"The adult males are more heavily pigmented than the females. Their black heads, protruding from the holes in which they are usually taken, are unmistakable. When these fish, however, are placed in white dishes, in a short time they become as light as the females. This lighter phase is that in which both sexes are usually seen in the open."

The other blennies of more or less closely related genera which are discussed in the same paper are all heavily pigmented and are noted by the authors as normally found in holes and not in the open. One cannot but wonder if this differential behavior of the two sexes of this lightly pigmented species may be accounted for by a differential exposure of the pineal area, especially as compared with the behavior of the heavily pigmented species.

Obviously this effect, if actually present at all, can operate only in fishes of relatively small size, because in large fish the mass of tissue and overlying bone makes for a uniform light tight cover over the pineal area.

In the prolarvae of most species, before pigmentation is developed to any notable extent, positive phototropism is the rule which, as pigmentation develops, gives way to a reversal of reaction. Whether this has to do with the functioning of the primitive pineal or not should be checked.

Dolley and Golden (1947), in connection with a study on the reversal in reaction to light among insects, reviewed the literature on factors involved in this change of sign. They indicated that in no case was the mechanism involved in such reversal known. Consequently the present report evidently represents a first description of such a sensory mechanism. As this mechanism includes two different forms of light receptors, which are essential to the observed behavior, it would seem possible that some analogous mechanism, involving more than one type of sense organ, may be operating in insects as well as other forms.

Since the role that the pineal plays in blind fish in regard to light sensitivity has been demonstrated and considering the above-mentioned features of other fishes, a question can properly be raised as to the present day importance of this body in reference to behavior patterns in fishes generally. Does it have behavioral significance in phylogeny, in ontogeny, and in the direction of appropriate behavior patterns? What of light intensity in reference to season and appropriate behavior in response to diurnal variations in light? It would seem that the whole problem needs attack from several quarters before such day-to-day importance of the pineal body, or its lack, can be demonstrated for non-cave dwelling modern teleosts with good vision.

SUMMARY

1. FIVE CAVES in a Mexican valley in the state of San Luis Potosi contain related blind characins differing slightly in morphology and reactivity to light.

2. The deriving river form and the blind cave forms still somewhat pigmented near the river connection are light negative, while the unpigmented are light positive.

3. The unpigmented forms in the cave nearest the river became light negative after having been kept in light for a time, concomitantly developing a heavy layer of guanine, and some of those from the next cave in a similar manner developed melanophores, while those from the remaining three caves showed no such changes in behavior or pigmentation following exposure to light.

4. The phototaxis exhibited, either positive or negative, is mediated through the remnant optic cyst, since both types become light indifferent on removal of the cysts. However, some fish in all the more advanced populations are light indifferent and have no nervous connection between the cyst and the brain.

5. The sign of the phototaxis is determined by whether the pineal complex is exposed to light or not, the fish in which it is exposed being light positive. Thus with the elimination of pigmentation and the reduction of other overlying tissues, these fishes, in an evolutionary sense, pass from a condition of being light negative to that of being light positive.

6. The sign of the light reaction may be reversed experimentally by covering the normally exposed pineal area or exposing the normally covered area in the two types of fishes.

7. Hybrids between light positive and light negative fishes are found to be intermediate in morphology and reactivity to light.

8. All these fishes have a secondary "pineal opening," which is well covered with pigment and guanine in the river fish and the less advanced cave forms. However, these structures are progressively reduced in the more advanced cave forms, thus accounting for the exposed area.

9. Genetic and morphologic data suggest that these populations worked up the now dry valley under ground and did not simply "sink" into the ground from local surface populations when the caves were formed to develop independent but parallel blind fish populations.

10. The survival value of a light negative reaction of blind fishes near a cave outlet is obvious, and it would appear that the reversal of sign in the more advanced forms can be tolerated only because it occurs in caves too remote from surface outlets to permit the fishes from draining out into an unsuitable lighted environment.

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