in the center of the piston oil film. As the solvent evaporates, the liberated fat spreads as a film against the pressure of the heavy oil film until equilibrium is reached.

A pane of glass is placed on the enameled tray, clearing the water surface by approximately ¼ in. A timer is started as soon as the fat solution is added. The film is allowed to age for 3 min. At the end of this time, by means of a wax pencil, the boundary of the film between the fat and piston oil is traced on the glass surface. A light placed directly above the dish aids in making this boundary distinct. The outline on the glass plate is readily traced on coordinate paper and the area is obtained by counting the squares; or if plain paper is used the outline is traced with a planimeter.

TABLE 1

Amount taken (in g)	Amount spread (in ml)	Spread (in sq in.)	Fat (equiv. in g)	Total fat found (in g)
0.0008	0.0052	3.6	0.0000054	0.00085
0.0018	0.0052	7.24	0.00001096	0.00173
0.0073	0.0052	31.28	0.00004692	0.00739

In the determination of small amounts of fat care must be taken in measuring the petroleum ether solutions. Small pipettes (containing from 0.005 to 0.1 ml) are used, that have narrow constrictions at the tip and at the calibrating mark to slow the flow of the ether. These are calibrated with mercury.

TABLE 2

Molecular weight	Area molecule (sq A)	Theoretical (1 sq in. = μg)	Found
Palmitic acid 256	20.5	1.33	1.35
Stearic acid 284	20.5	1.50	1.46
Cholesterol 390	41.0	1.02	1.05

In use, the pipettes are filled above the mark. The tip is wiped on filter paper and then held against the paper until the solution is brought to the mark. The solution is delivered on the surface with the last droplet forced out under the surface. The pipettes are rinsed with ethyl ether and dried with warm dry air in order to remove traces of moisture that render the measurement inaccurate. Repeated spreading of the same amount of lipid checks within 2%. This covers the pipetting of sample, the tracing, and the planimeter readings. Twenty-one such spreadings at 28° C gave a variation in areas of 1.6 ± .14% with a standard deviation of 0.96.

The sensitivity of the method is shown by Table 1, showing the analysis of skin fat, which was dissolved in 0.82 ml of petroleum ether. Aliquots were drawn with a pipette delivering 0.0052 ml. The factor was 0.0000015 g/sq in.

The important constituents of skin fat are fatty acids and sterols. These should agree with the theoretical spread. Using 20.5 sq A per molecule for fatty acids, and 41 sq A for cholesterol, the agreement is as shown in Table 2.

References

1. Adam, N. K. *The physics and chemistry of surfaces,* London: Oxford Univ. Press, 1941.
2. Blodgett, K. B. *J. Amer. chem. Soc.,* 1934, **56**, 495; and 1935, **57**, 1007.
3. Gorter, E. and Grendel, F. *J. exp. Med.,* 1925, **41**, 439.
4. Langmuir, I. *J. Amer. chem. Soc.,* 1917, **39**, 1848.
5. Rayleigh, Lord. *Proc. roy. Soc.,* Lond., 1890, **47**, 364.

A Preliminary Report on the Role of the Pineal Organ in the Control of Pigment Cells and Light Reactions in Recent Teleost Fishes[1]

C. M. Breder and Priscilla Rasquin

Department of Fishes and Aquatic Biology,
The American Museum of Natural History,
New York City

Although there has been a considerable amount of speculation on the possible functioning of both the pineal and the parietal organ in various fossil vertebrates, very little experimental work has been done on recent forms. Moreover, the precise function of the pineal organ in recent teleosts has never been clearly elucidated, although the structure is evidently present in all. A recent survey of the knowledge of the organ has been given by Gladstone and Wakeley (*5*). In fishes, all that is known concerning the relation of the pineal organ to pigment control is based on the work of von Frisch (*4*), which was confirmed by Scharrer (*7*). They demonstrated that the granules in melanophores of *Phoxinus* would disperse if the pineal region was stimulated by light. Since von Frisch was able to demonstrate that light sensitivity was not completely removed by extirpation of the pineal, and since he considered the lining of the ventricle of the diencephalon to have sensory cells, the exposed portions of the brain that react to light are here referred to as the pineal area. Parker (*6*) considered this fish exceptional. Scharrer demonstrated also that the pineal had an influence on phototaxis as well as on pigmentation, as did Young (*8*) for the very different, nonteleost, *Lampetra.* Breder and Rasquin (*3*) demonstrated an influence of the pineal on phototaxis for the blind cave fish *Anoptichthys,* which is responsible for the sign of the reaction.

Since the last work was published it has been possible to demonstrate that in a variety of eyed fishes dwelling in light, the pineal area has a distinct influence on both the behavior of the melanophores and the reactions of the fishes to light and darkness. It has been found that the fishes studied divide into three groups: those species in which the tissues, including the skull, overlying the pineal are sufficiently transparent or translucent to permit light to enter the cranial cavity freely over this region, as illustrated in Fig. 1; those in which the tissues

---

[1] This study will be reported in full detail at a later time. Most of the work was carried on at the Lerner Marine Laboratory at Bimini, and was in part supported by a grant from the American Cancer Society.

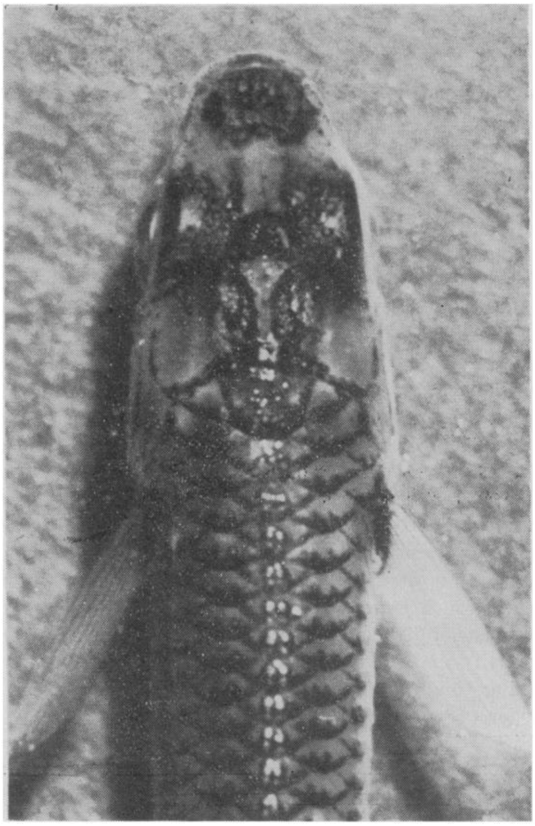

FIG. 1. Photograph of the top of the head of *Atherina* (*Hepsetia*) *stipes* Muller and Troschel, showing the clear median area immediately behind the eyes unprotected by chromatophores directly over the pineal body. Magnification × 3.4.

are apparently too opaque to permit such entry of light; and finally, those in which the behavior of appropriately placed chromatophores control this condition.

To a very large extent, these three morphological conditions are associated with the manner in which the fishes react to light. Species which have the most extremely exposed pineal areas are strongly light-positive, whereas those that are the most protected are among the most light-negative. The fishes whose chromatophores regulate the extent of exposure of the pineal area by the dispersion or concentration of pigment granules present what appears to be an erratic behavior in regard to their reactions to light. This is apparently owing to the functioning of these structures covering the brain, the details of which are as yet to be worked out. Certain fishes with rather complicated structures dorsal to the pineal area do not fit readily into this scheme, but they are under study and it is becoming apparent that each species has its own set of peculiarities. These, however, in no way alter the fundamental fact of pineal control in numerous species in which the effects are clearly apparent.

Light reactions were demonstrated by presenting the fishes with the choice of a black-surfaced and covered compartment or a white-surfaced and lighted compartment, as described by Breder and Rasquin (*3*). The results of these choices were compared with the morphology of the pineal covering. Species showing the three types of behavior are listed here:

Species with exposed pineal areas that are light-positive. The figures give the extent of positivity in terms of percent of time spent in the light or darkness, with zero equal to light indifference.

Jenkinsia lamprotaenia (Gosse)	+ 100.0
Sardinella macropthalmus (Ranzani)	+ 99.8
Leptocephalus larvae Sp.?	+ 26.0
Brachydanio rerio (Ham.-Buchanan)	+ 100.0
Atherina[2] *stipes* Muller & Troschel	+ 100.0
Carapus bermudensis (Jones)	+ 100.0

Species in which chromatophores control the coverage of the pineal areas and show changeable reactions with regard to light. These cannot be given numerical treatment because the methods employed demand a certain amount of swimming by the fishes.

Sphyraena barracuda (Walbaum)
Strongylura notata (Poey)
Hemirhamphus brasiliensis (Linnaeus)
Synodus synodus (Linnaeus)

Species with permanently covered pineal areas that are light-negative. The figures give the extent of negativity in terms of percent.

Astyanax mexicanus (Filippi)	− 58.7
Ameiurus nebulosus (LeSueur)	− 99.3
Haemulon melanurum (Linnaeus)	− 93.5
Thalassoma bifasciatum (Bloch)	− 33.0

Species that for one reason or another do not subscribe to the preceding three simple conditions.

Light-positive but with well-covered pineal area

Eucinostomus gula (Cuvier & Valenciennes)	+ 43.5
Pseudupeneus maculatus (Bloch)	+ 100.0
Apogonichthys stellatus Cope	+ 30.5

Practically light indifferent and pineal area covered

Cyprinodon baconi Breder	+ 9.3

Light negative with exposed pineal area

Mugil trichodon Poey	− 53.5

Covered pineal area but behavior indeterminate
Bathygobius soporator (Cuvier & Valenciennes)

The irregularities shown in the last series should not be surprising when it is recalled that teleost fishes existent today represent long independent evolutionary lines and in many of their numerous aspects show striking variations in practically all their morphological and behavioral features. That better than two out of three of all fishes examined fit into this scheme is probably the most remarkable feature of this study. It is to be noted too that there is a strong semblance of phylogeny inherent in these lists. The exposed pineal group is represented by all nonacanthopterygian fishes except for *Carapus*, which is a highly specialized member of the Ophidioidea, and *Atherina*, one of the Percesoces. The rest are either Isospondyles, Apodes, or Ostariophysi. The group with a controllable cover are also largely nonacanthopterygian, being represented by members of the

---

[2] *Membras* according to the revision of L. P. Schultz, *Proc. U. S. Nat. Mus.*, 1948, **98**, pp. 39–40.

Synentognathi except for *Synodus*, an Isospondyle, and *Sphyraena*, one of the Percesoces. Two of the group with covered pineals are Acanthopterygii, and two, *Astyanax* and *Ameiurus*, represent the Ostariophysi. The inconsistent group is all acanthopterygian except for *Cyprinodon*, one of the Cyprinodontes. This represents a clear reduction of pineal influence as one reaches the spiny-rayed fishes, and their relative independence from that influence may be seen from the following tabulation:

Group	1	2	3	4
% Acanthopterygii	33	25	50	83
Mean of light reactions %	+ 87.6	..	− 71.1	+ 47.4

That there is a somewhat parallel difference in this matter in ontogeny as well, is to be expected, as has previously been suggested by Breder and Rasquin (*3*). It also follows that without special and very elaborate morphological provision, larger fishes have better coverage of the pineal than smaller ones because of the greater thickness, in an absolute sense, of the tissues over the brain. Also a sexual difference in the extent of the negative light reactions of the very sexually dichromatic *Thalassoma* became evident, the females being markedly less light-negative than the more heavily pigmented males. The nocturnal *Apogonichthys* was found to be less light-positive when tested at night than in the daytime. These items indicate some of the numerous factors which must be explored more fully. In cases where duplicate tests were made, only the figures less favorable to the thesis herewith developed have been used.

In addition to the stimuli received by way of the eyes and the pineal area, there is evidently a direct effect on some other receptors, possibly on nerve endings in the skin or deeper in the translucent flesh of some of these fishes. Even with blinding and covering the pineal, it was found that phototaxis in *Atherina* was still light-positive, but to a lesser degree, whereas *Eucinostomus* became light-negative, and *Haemulon* became less light-negative. The numerical values for this experiment follow, the values being expressed in percent of light-positiveness:

Species	Normal	Blind	Blind and pineal covered
A. stipes	+ 100.0	+ 40.6	+ 12.5
E. gula	+ 43.5	+ 31.9	− 17.5
H. melanurum	− 93.5	− 6.9	− 24.0

Statistical analyses of the numerical values are to be fully reported later, but it may be pointed out that they are of high significance, the majority showing no overlap whatever.

The direct influence of the pineal area on pigmentation could be shown by covering with India ink the heads of fishes having exposed pineals. Fishes in the first category so tested all expanded their melanophores, whereas the effect was less extreme in the second category, the fishes showing dark patterns, in some cases not resembling any of the normal ones. Even fishes in the third category showed some effect and seemed to be prevented from entering their lightest phase. This evidently indicated that in the sizes available for experimental purposes there was still some radiant energy impinging on the pineal area through what seemed to be opaque covering.

Because fishes have been shown to react to an injection of adrenalin by concentrating their melanin granules, various species under study were so treated. All showed a reaction in that the meningeal melanophores and the iris melanophores concentrated their granules, but the results here contrasted with work previously recorded in that the dermal melanophores behaved differently in many species. Evidently the melanophores in these species are under strong nervous control which adrenalin, although administered in massive doses, could not override. Some fishes, in fact, showed very dark markings and only three, *Bathygobius*, *Opsanus*, and *Ameiurus*, showed the typical blanching recorded in the literature. Fishes such as *Haemulon melanurum* and *Chaetodipterus faber* (Broussonet), which react to a white background by becoming dark instead of blanching in the more usual manner, were especially marked in this regard. Further details concerning this opposed pigmentary behavior are given by Breder (*1, 2*).

Since the covering of the pineal area contains chromatosomes which consist of melanophores and iridophores, the simple concentration or dispersion of the granules of the melanophores is only one element in the shading of the pineal region. The deflection of light from the guanin crystals carried in the iridophores may have as much to do with shading the pineal area as does the dispersion of black granules in the melanophores. The meninges that cover the brain, exclusive of the pineal area, can be seen to be heavily invested with iridophores as well as melanophores. This is strikingly demonstrated by adrenalin injection in the more or less translucent fishes of the first group. The brain appears white a few seconds after injection, when the pigment granules of the melanophores are concentrated, and the crystals in the iridophores or leucophores either disperse or remain unaffected by the adrenalin. The contents of erythrophores, xanthophores, and iridophores were not concentrated by adrenalin, nor were they seen to be influenced by the pineal area, although they are obviously under nervous control.

References

1. Breder, C. M., Jr. *Bull. Amer. Mus. nat. Hist.*, 1948, **92**, 285.
2. ———. *Ibid.*, 1949, in press.
3. Breder, C. M., Jr. and Rasquin, P. *Bull. Amer. Mus. nat. Hist.*, 1947, **89**, 319.
4. von Frisch, K. *Arch. ges. Physiol.*, 1911, **138**, 319.
5. Gladstone, R. J. and Wakeley, C. P. G. *The pineal organ.* Baltimore: Williams and Wilkins, 1940.
6. Parker, G. H. *Animal colour changes and their neurohumours.* New York: Cambridge Univ. Press, 1948.
7. Scharrer, E. *Z. vergl. Physiol.*, 1927, **7**, 1.
8. Young, J. Z. *J. exp. Biol.*, 1935, **12**, 229, 254.