

Change in Phototactic Behaviour with Growth of Milkfish, *Chanos chanos* (FORSSKÅL)

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Abstract

Phototactic behaviour of milkfish larvae and juveniles was observed in the sea and laboratory, and their retinæ were observed histologically. Larvae collection by using a lamp at night and tank experiments revealed that the larvae have strong positive phototaxis. Throughout their growth, there were three shifts in phototactic behaviour in a tank. The photopositive behaviour of the larvae became weak by degrees through a metamorphic stage, and juveniles showed photonegative behaviour. On the 39th day from capture, their behaviour became photopositive again. After they were transferred into a larger tank, on the 139th day, any significant photopositive or photonegative behaviour could not be observed. The larvae on capture, have duplex retinæ. The rods were found to be a few in number, and a clear retinomotor response was observed on the day of capture. The first change in behaviour from photopositive to photonegative seemed to relate with the development of the rods.

INTRODUCTION

It seems that the behaviour of fish larve in relation to light originally was observed with special reference to depth distribution or vertical migration of the larvae. Based on the larger catch of night hauls by silk tow net rather than that of day hauls, it was assumed that the larvae avoided the surface waters when illumination was high. It now seems that diurnal migration of many larval fishes is not as extensive as previously surmised, and it has been shown that much of the difference between day and night catches is due to the ability of many species to avoid the slow-speed sampler in daylight (WOODHEAD, 1966). Based on the catch of a high-speed sampler, it was suggested that young herring avoided intensive surface light (BRIDGER, 1958). On the other hand, it had been suggested that the depth of vertical distribution of the larvae of herring and *Sardinops caerulea* varied with larval size (BRIDGER, 1958). KELLY and BARKER (1961) similarly showed the vertical distribution of the larvae of *Sebastes marinus*. Although it might have been expected that light would play a dominant role in governing the depth distribution of different species or stages, the critical studies on fish larval distribution are still few.

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From the experiments with larval fish, it was revealed that their reactions to light changes with time (HARDER and HEMPEL, 1954). The change in reaction to light probably relate to ontogenical development of the visual system. In this paper, we report the change in phototactic behaviour with growth of the milkfish, *Chanos chanos* (FORSSKÅL), and relationship between the change and the histological development of the retina.

MATERIALS AND METHODS

The experiments were done in the field and laboratory during 1979~1980.

FIELD EXPERIMENT Larvae collection was carried out along the shore by using a fish lamp. The collection ground was located over a shallow, gently sloping, sandy shore in Kumano Bay, Tanega Island in Kagoshima Prefecture, which had been reported earlier by SENTA *et al.* (1980) as a ground wherein numbers of milkfish larvae can seasonally be captured. A kerosene lamp (350cp) was hung at about 50cm above the water surface from a pole fixed in waist-deep water, and its position was shifted to shore or off shore depending on the tide. After dusk, the larvae were attracted by light and were collected with a bagnet of 1mm mesh size netting with a frame, 62cm deep by 136cm long, attached to the net-mouth. This gear was hand-operated by two wading men for four minutes in the illuminated area (test site) and in a dark area 50-75m away from the lamp (control site). The towing speed was about 70cm/s. The milkfish larvae thus captured in the bagnet were selected from the other larvae and were counted on the beach. The number of the larvae captured was compared statistically between the test and control site.

LABORATORY EXPERIMENT 250 milkfish larvae captured near shore in daylight on July 30, 1979 were transported to the laboratory of the Faculty of Fisheries, with 50% sea water. After starving for two days from capture they were fed on cooked egg yolk, carp pellets, and algae which grew naturally in the tanks. One hundred larvae were kept and reared in an experimental tank (170cm long, 34cm wide, 20cm water deep) for the phototaxis experiment. The two long sides of the tank were transparent; the other walls and bottom were opaque and grey in color, of which the inside surface was coarsely ground to prevent a mirror light reflection.

As the animals were found to respond more strongly to a vertical light beam than to a horizontal one, a miner's type flashlamp with a 1.5W bulb was fixed vertically at a point 15cm from a side wall and 20cm above the water surface. In the evening the room was darkened and the animals were acclimated to the darkness for more than two hours prior to switching on the lamp. At 5, 10, 15 min after the onset of illumination, the number of animals in the circle of 30cm diameter beneath the lamp was counted. At 20min after the onset, the lamp was moved horizontally toward the opposite wall and was returned to the initial point at the speed of 1.4m/min, and the response of the animals to the moving light beam was observed and the number of animals which followed the lamp was counted.

A quantitative indication of the phototactic behaviour was given as the amplitude of response to the light (ARL) which is defined thus :

$$ARL = \frac{N_5 + N_{10} + N_{15}}{3} + \text{Score}$$

where, N_5 , N_{10} , and N_{15} are the number of animals which stayed in the circle beneath the lamp at 5, 10, and 15min after the on set of illumination, Score is the response of the animals to the horizontally moving light and fixed light which was scored thus:

Response	Score
Animals were strongly attracted to the fixed light and followed well to the moving light	20
Significant but weak positive response to the fixed light was observed and the durability of the following reaction to the moving light was weak	10
No remarkable positive or negative response to the fixed and moving light was observed	0
Animals did not respond to fixed light but showed remarkable negative response to the moving light	-10
Animals responded negatively to both fixed and moving light	-20

Therefore the value of ARL can vary between 100 and -20. On December 16, on the 139th day from capture, all animals in the experimental tank were transferred into a larger experimental tank (4m long, 44cm wide, 20cm water deep) made of opaque vinyl chloride plate, and observations were made until January 11, 1980.

When animals died in the experimental tank, they were replaced by animals from the rearing tanks. The water temperature in the experimental tank was kept constant, but ranged from 28.0 to 30.7°C.

On the day of capture and every two or four days after capture, light adapted and dark adapted animals were preserved for histological observation of the retinae. The light adapted animals were randomly sampled from the rearing tanks in daylight. The dark adapted samples were the specimens kept in a dark tank for more than two hours in the evening, and killed and fixed in BOUIN'S solution. Generally, the eyes were sectioned at 6 microns with the lens intact and stained by the H. E. or Azan method for light microscopy.

It was very difficult to resolve the rods by light microscopy if they existed. Therefore, the appearance of the rods was observed by calculating the ratio of countable visual cells (which will be cone) to the visual cell nuclei in the outer nuclear layer.

The retinae of adult fish, which were obtained at the Aquaculture Department of Southeast Asian Fisheries Development Center in the Philippines, were fixed in Orth solution for 24h, washed in running tap water for 24h, and were preserved in 70% ethanol.

RESULTS

LARVAE COLLECTION The total number of all fish larvae captured by 21 operations was 2256 individuals in the test site and 3117 in the control site. The total number of milkfish larvae captured was 69 specimens; 62 from the test site, 7 from

the control. The statistical test of the difference of the catch between the test and control, shown in Table 1, reveals that more larvae were captured in the test site than in the control (average of the difference, 2.75; confidence limit of the average at 99.9% level, 0.84–4.65). Although the catch was poor, it is evident that the larvae can be

Table 1. Result of larvae collection.

Date	Number of catch		
	Test site	Control	Difference
July 5-6	4	1	3
	3	0	3
6-7	1	0	1
	1	0	1
7-8	5	0	5
	0	1	-1
	4	0	4
	0	0	0
8-9	2	2	0
	4	0	4
	1	0	1
	1	1	0
	2	0	2
	2	0	2
	4	0	4
30	2	1	1
	5	1	4
31	8	0	8
	6	0	6
	2	0	2
	5	0	5
Total	62	7	55
		Average	2.75
		S. E.	± 0.50
Confidence limit of the average of the differences at 99.9% level : 0.84–4.65			

attracted by an artificial light at night. They have positive phototaxis.

GROWTH IN TANK When the larvae were introduced into the tank on the first day of August, they swam continuously, forming one or two loose schools very close to the bottom, and showed no preference for any particular area in the tank. In the evening, they dispersed horizontally and vertically under the illumination of the room lamp. Next day, they showed strong feeding activity on cooked egg yolk and seemed very healthy in the tank.

The larvae on capture had densely pigmented eyes, transparent bodies, and no pelvic fins. On the seventh day from capture, all specimens had pelvic fins. On the 18th day, the finfold completely disappeared in all specimens. Following KAWAMURA and HARA (1980a), the authors here named this period, from the appearance of the pelvic fins until the complete disappearance of the finfold, the "metamorphic stage". In the Philippines, the metamorphic stage starts on the 5th day from capture and lasts until the 15th day (KAWAMURA and HARA, 1980a), but delayed by stunting (KAWAMURA and HARA, 1980b).

The metamorphosis which took place in our laboratory delayed by the two-day starvation period following capture. After the metamorphic stage they showed strong synchronized movements. Although their feeding was very active, they grew with extremely lower growth rates than the published data (SCHUSTER, 1952 ; RABANAL *et al.*, 1953)(Fig. 1). According to HUNTER (1977) and HUNTER and KIMBRELL (1980), the growth of

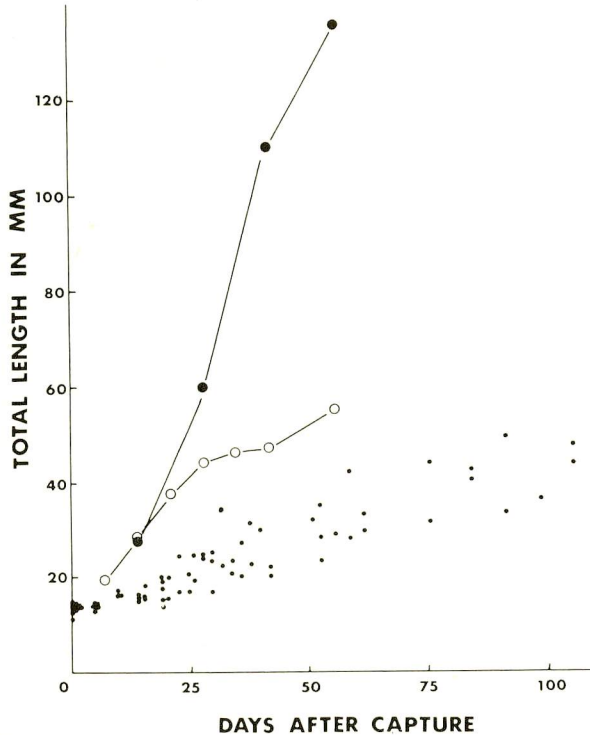


Fig. 1. Growth of the milkfish reared in different conditions. Large closed circle ; average growth at a rate of one fish/m² (from SHUSTER, 1952), open circle ; at a rate of 10–100 fish/m² (from RABANEL *et al.*, 1953) ; small closed circle ; present data at a rate of 173 fish/m².

northern anchovy and Pacific mackerel is accelerated by high temperatures, while metamorphosis is delayed at lower temperatures. This seems general in other temperate fishes as well. Because there is no available data on the growth of milkfish under natural conditions, we could not compare the growth of reared milkfish with wild ones. Hence we here used the day of capture and metamorphosis as a reference stage to show age of the animals used.

PHOTOTACTIC BEHAVIOUR IN TANK On the fourth day from capture, when the lamp was turned on, the larvae rushed into the illuminated area and stayed there for a long period. They so seldom or slowly moved in the light spot that it was very easy to count the numbers of the animals attracted in the area. When the lamp was slowly moved, the animals in the bright area obediently moved with the moving light spot, and the ARL became around 50. This strong photopositive behaviour could be observed for three consecutive days.

From the seventh day from capture, the photopositive behaviour became weak day

by day and disappeared on the 11th day. On the 14th day, which the lamp was turned on, the animals immediately swam away from the bright area. When the lamp was moved, the animals swam forward keeping a certain distance from the moving light spot. Finally, a part of them were driven by the moving light to the opposite wall. The strong phototaxis of the larvae completely turned to negative through the metamorphic stage, and on the 18th day, the ARL showed minimum value. A similar photonegative behaviour lasted until the 37th day.

On the 39th day, they showed photopositive behaviour again. They darted into the bright zone and moved with the moving light spot taking very high ARL values. This strong photopositive behaviour lasted until the 136th day, although they responded negatively to the light on the 43rd day.

On the 139th day, all animals in the experimental tank were transferred into a larger experimental tank. In the larger tank, they seldom formed a school. At night, individual animals scattered and were resting motionless on the bottom and the strong photopositive behaviour shown in the smaller tank completely disappeared. Immediately after the onset of illumination, the animals showed negative responses. A few minutes after that, some of them came into the bright zone, stayed there for a moment, then swam away again. They repeated such behaviour. No remarkable response to the moving light could be observed. In the larger tank, the ARL largely fluctuated between 11.0 and -10.0.

The over-all results of the phototactic behaviour observed in the tanks are illustrated in Fig. 2.

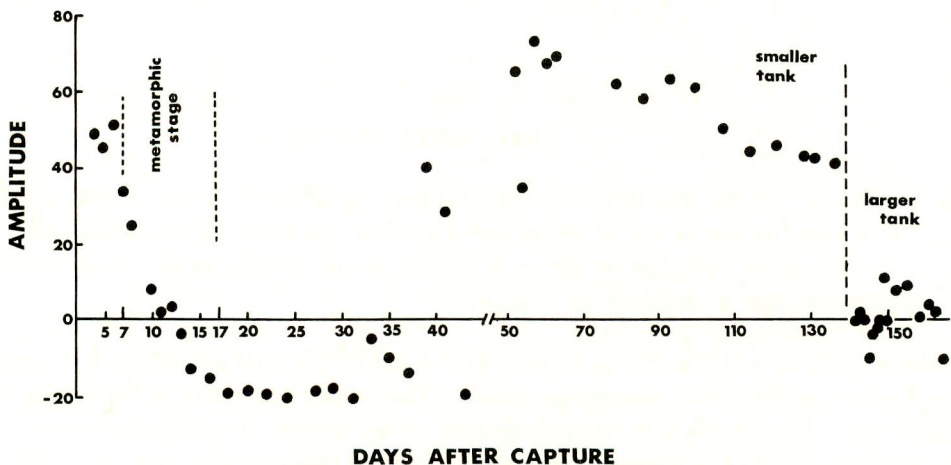


Fig. 2. Change in the amplitude of phototactic response in the experimental tank with days after capture.

HISTOLOGY OF THE RETINA The larvae on capture had dense ganglion cells and cells in the inner nuclear layer, but the number of nuclei of the visual cells was very small. For the adults, on the other hand, the former two cells distribute in very low density, while the density of the nuclei of visual cells was very high (Fig. 5). It seems, therefore, that the cells in the inner nuclear layer and the ganglion cells are already formed in the larval stage and disperse tangentially with retinal growth.

In the larvae on capture, a clear retinomotor response could be observed. In a

dark adapted retina, the masking pigment retreated to the periphery of the retina, the cone myoids extended and the rod myoids contracted (Fig. 6). The pigment index (ALI, 1959) for the dark adapted retinae gradually decreased with growth of the layer of pigment epithelium and visual cell layer, but for the light adapted retinae, after the seventh day, it was almost constant (Fig. 3).

All retinal elements, except for the cells in the outer nuclear layer, did not seem to undergo large changes in development for two months after capture. The density of the cells in the outer nuclear layer underwent a large change within 12 days. The ratio between cone elipsoid and the nuclei ranged from 1.4 to 2.1 in the larvae on capture, 1.6 to 2.3 on the seventh day, 2.3 to 2.9 on the 10th day, and 2.6 to 3.3 on the 12th

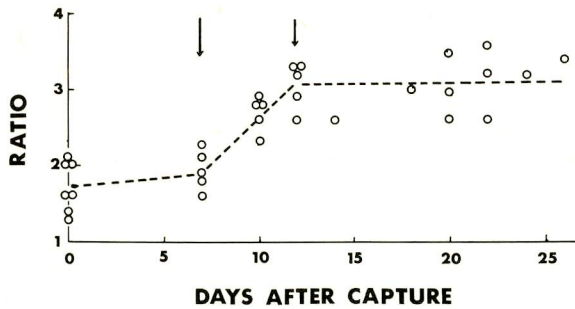


Fig. 3. Ratio between the cone elipsoids and the uncles with days after capture. Arrows show metamorphic stage.

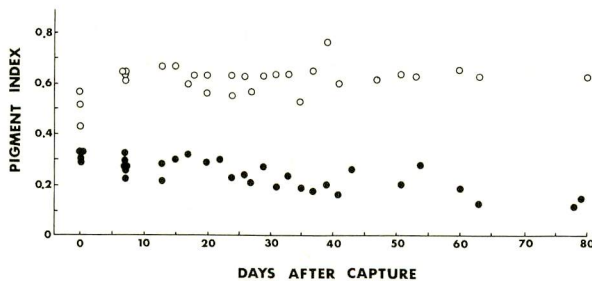


Fig. 4. Pigment index with days after capture. Open circle ; light adapted, closed circle ; dark adapted.

day. After the 12th day, the ratio did not largely increase until the 63th day (Figs. 4, 6-9).

DISCUSSION

The larvae collection by use of fish lamp and the tank experiments revealed that the larvae which appear near the shore exhibit a strong positive phototaxis. This is supported by the fact that, in the Philippines, the larvae collectors use kerosene lamps at night for more efficient larvae collection. KUMAGAI *et al.* (in press) carried out a tank experiment using the milkfish larvae and observed strong photopositive behaviour of the larvae.

According to BLAXTER (1975), the larvae of herring, pilchard, haddock, sand goby, plaice, and lemon sole have pure-cone retinæ, and the rods appear at metamorphosis. The milkfish larvae, on the other hand, already have rods. The milkfish is not only one species which has rods in larval stage. The Japanese anchovy have developed grouped rods and dense retinal tapetum in their larval stage (KAWAMURA *et al.* in press).

The ratio of cones to the visual cell nuclei underwent large changes through the metamorphic stage. This increase in the ratio coincides with the first change of photopositive to photonegative behaviour of the larvae. Therefore, it seems that the rod development relates to the first change in phototactic behaviour. However, the second and the third changes in phototactic behaviour can not be explained by the rod development.

YUSA *et al.* (1971) described a shift from photopositive to photonegative through metamorphosis in *Limanda yokohama*. A similar shift from photopositive to photonegative occurs in *Acanthurus triostegus sandivicensis* when they settle in tide pools (MALIAVE, 1977), and *Anguilla vulgaris* before they enter rivers (DEELDER, 1958). The change in phototaxis is probably a sort of adaptation to a new habitat. According to SCHUSTER (1960), milkfish larvae enter creeks, backwaters or estuaries, and the juveniles remain there for an uninterrupted period of four years before trying to return to the sea; but the period seems to vary depending on the capacity of the backwaters.

MARLIAVE (1977) noted that yolk-sac larvae of *Anoplarchus purpurescens* will become negatively phototactic if starved, but remained photopositive if fed from the time of hatching; and warned that observation of behaviour of larvae in the laboratory must be interpreted cautiously if those larvae do not feed or grow. Even though the growth rate of the milkfish in the laboratory was very low, they actively fed on the foods supplied and appeared to be very healthy. Thus we cannot believe that the second change from photonegative to photopositive will be attributed to very slow growth of the juveniles in the tank. Their photopositive behaviour was very strong. It might be resolved by ecological observations whether the change was a natural behavioural change or an induced one caused by the rearing condition.

BURI (1981) observed wild juvenile milkfish in a mangrove swamp in the Philippines, and reported that during night schooling habit was more or less abandoned and individual fish were found scattered and resting motionless over the substrate. These fish did not react to light or shadow movements. As he did not explain the size or age of the juveniles, we can not compare the behaviour observed in the tank directly with the wild ones observed in the mangrove swamp. Although the animals in the larger tank were a little more active, their behaviour resemble that of the wild ones. Therefore the behaviour observed in the larger tank seems reliable, but we can not reject the effect of the size of tank on fish behaviour.

Only from the tank experiments, it would be very difficult to reveal the phototactic behaviour of fishes. We should always refer the observations of wild fish in the field. We desire more ecological observations in the field. Moreover, the behaviour through vision may relate not only to development of the eyes, but also to development of the optic tectum. The optomotor reaction undergoes a change through the metamorphic stage. The optomotor reaction is also somewhat weak in the larvae, but becomes strong and almost perfect in the juveniles (KAWAMURA and HARA, 198a). This change cannot be attributed only to the development of the retina. The relationship, if it exists, between the development of the behaviour by vision and the optic tectum is a point of interest which arises from the developmental study.

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Plate 1

- Fig. 5. Gross sections showing the retinal elements of an adult milkfish. C ; cone, G ; ganglion cell, N ; visual cell nuclear. 5A ; ventral retinal region, 5B ; dorsal retinal region. Scale ; 50 microns.

Plate 2

- Fig. 6. Retinae of larvae on capture. 6A ; light adapted, 6B ; dark adapted. C ; cone, B ; bipolar and amacrine cells, C ; ganglion cell, N ; visual cell nuclear.
- Fig. 7. Retinae of larvae on the seventh day from capture. 7A ; light adapted, 7B ; dark adapted. Scale ; 50 microns.

Plate 3

- Fig. 8. Retinae of juveniles on the 15th day from capture. 8A ; light adapted, 8B ; dark adapted.
- Fig. 9. Light adapted retina of a juvenile on the 63th day from capture. 9A ; dorsal retinal region, 9B ; ventral region. Scale ; 50 microns.

Plate 1

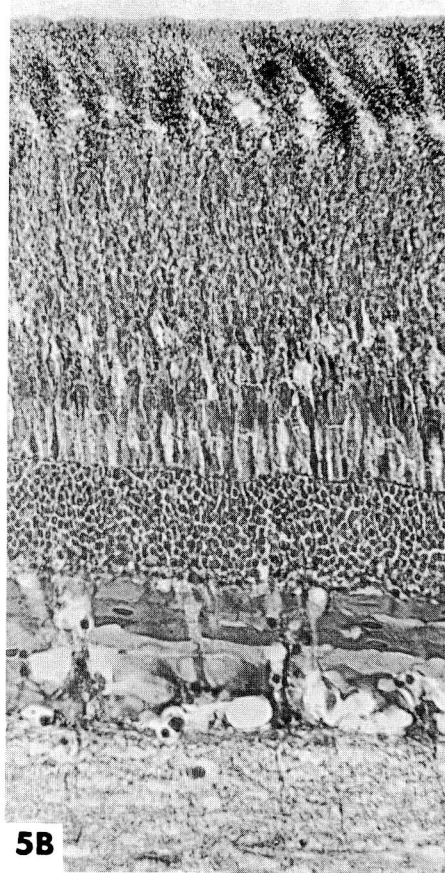
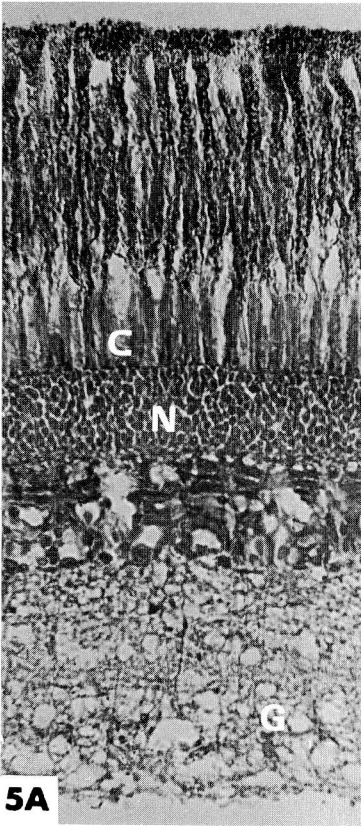


Plate 2

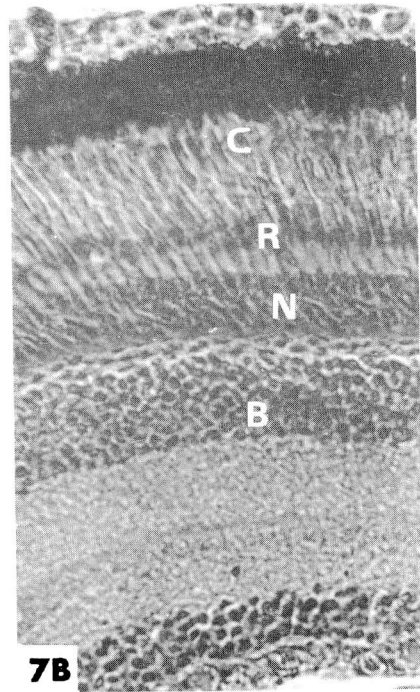
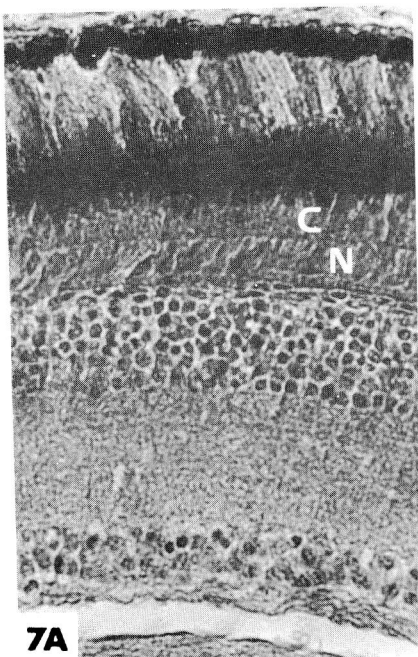
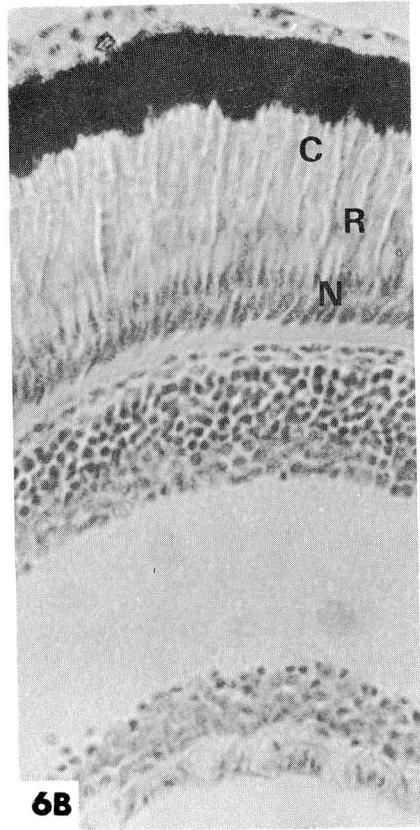
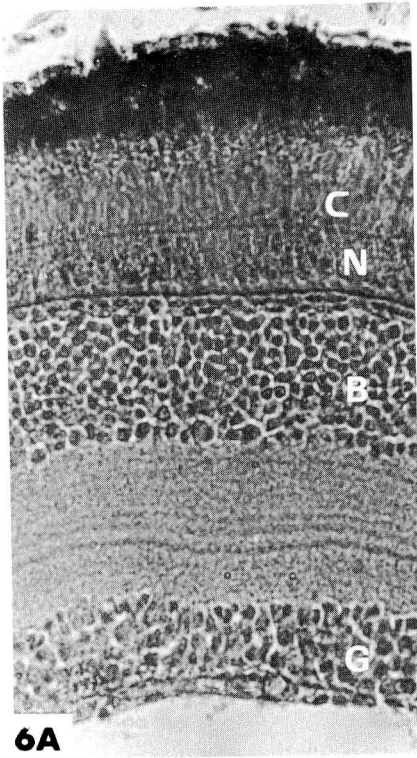
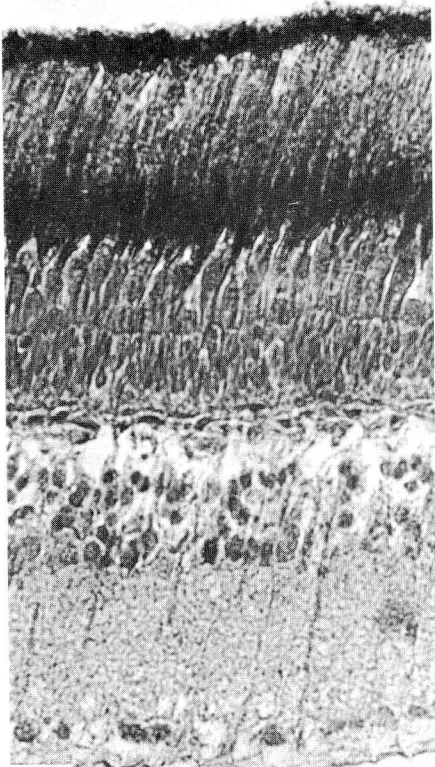
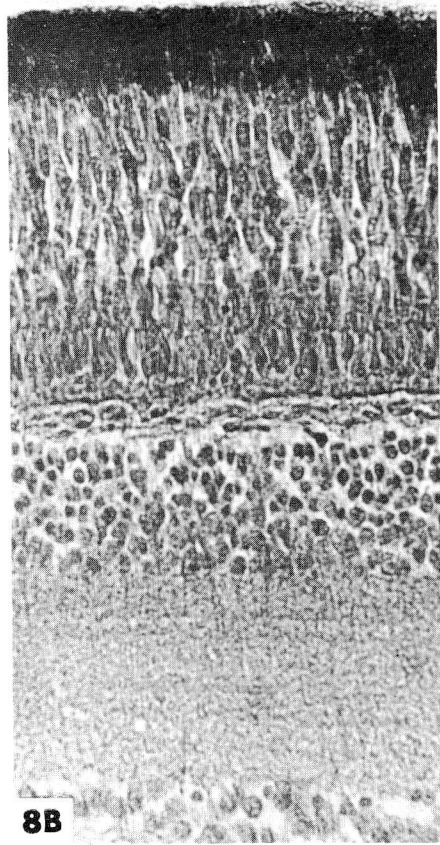


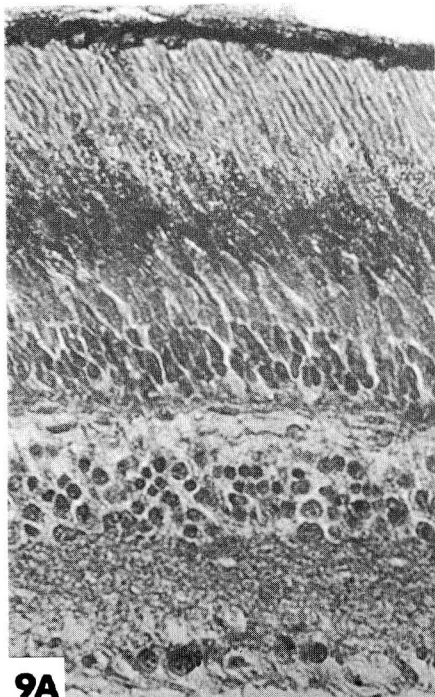
Plate 3



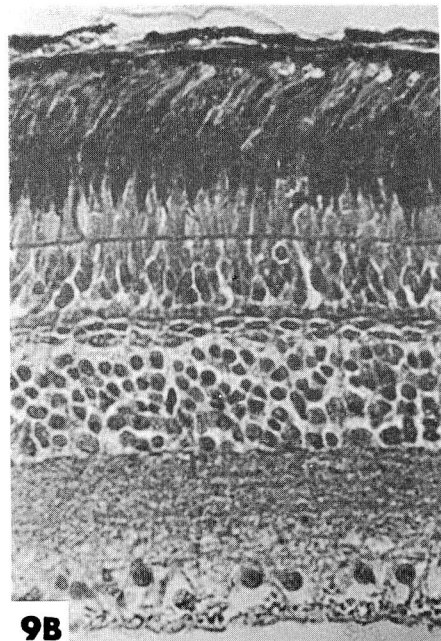
8A



8B



9A



9B

