Notes on Retino-motor Phenomena in Some Fishes under the Various Light Conditions※

By

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Since BOLL (1877)[2] and KÜHN (1877)[9] found that the rods, cones and retinal pigment of vertebrate exhibit marked changes of position in light and darkness, the shifting of these visual elements of fish in light and dark adaptation was measured by v. FRISCH (1925)[5], WUNDER (1926)[11], STUDNITZ (1933)[10] and others; and their researches contributed to the physiological studies of fish vision such as "Duplicity Theory", visual acuity, etc.. However, these works were the experiments using fresh water fish, and moreover there are no systematic and quantative measurements on the relation between the moving ratio of visual element and the intensity of the illumination, though BRUNNER (1934)[3] observed the elongation and contraction of the cone in lower intensity. Therefore, from the viewpoint of fundamental investigation regarding the effects of fish gathering lamp, the author made experiments in some fishes including the marine fish in order to investigate the differences between fish species, and it seemed necessary that he should consider these results as compared with the relation between the moving ratio of visual element and visual acuity curve of *Phoxinus* measured by BRUNNER. And thus the moving ratio of the visual element in relation to the intensity of the illumination and the time relation of light and dark adaptation in their elements are explained in present paper.

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Material and Method

Materials used in this work are silver mackerel, *Trachurus japonicus*, crusian fish, *Carassius auratus*, loach, *Misgurnus auguillicaudatus*. Their body length is about 10—12 cm, 3—5 cm and 10—12 cm, respectively. Experiment was made in a dark room in the day-time. Four fishes are held in glass tank which is 35 cm in diameter and 15 cm in depth. Water in the tank was not running during

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the experiment. From the consideration of individual variation, the moving ratio was measured with four fishes. But the number of the fish in the tank was in point judging from the respiratory condition in the tank during the experiment. The lamp, MATSUDA 2 c.p., used as light source, was suspended on the straight line up from the tank. This illumination was adjusted by varying the distance between the lamp and the tank, and atso by using of the tracing papers as filters. For the light intensity was measured by YOKOGAWA's Lux meter using a selenium photo-cell, but the value in dim light was calculated by Lambert's formula from the value measured in short distance. The retina of a fish was carefully removed following the cut and fixation of the head after various light conditions. Before the head was cut, the cornea of the eye was injoured in order that fixation solution could be enough penetrated. Fixation of the retina was rapidly taken in use of Carnoy's solution and imbeded into paraffine. The section was made in 7—8 μ thick and was stained by iron-haematoxylin and eosin. The ratio in the movement of the visual element was measured in adjacent part to center forvea by the horizontal section passing the optic nerve.

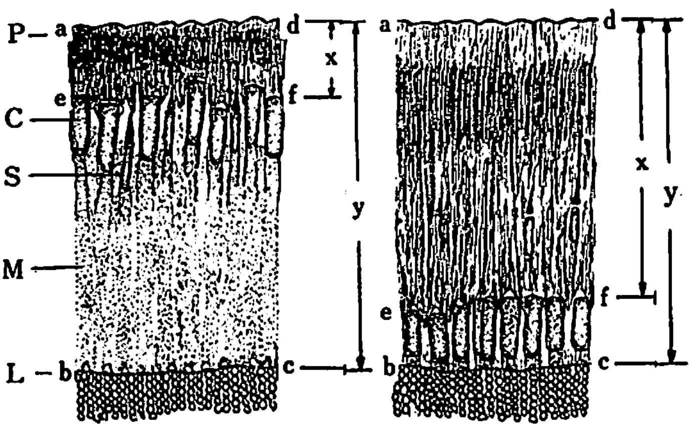

Fig. 1. Schema shows the positions of cones and pigment in the section of *Trachurus* in light (right) and dark (left) adaptation. The moving ratio of cone and pigment is indicated by $\frac{x}{y}$ and $\frac{\text{area aefd}}{\text{area abcd}}$, respectively. P, pigment; C, cone; S, small cone*; M, myoid of cone; L, external limitting membrane.

As shown in Fig. 1, the change in the position of the cone was indicated with percentage of the distance from the base of the pigment epithelium to the distal point of the cone ellipsoid to the distance from the base of the pigment to the external limitting membrane. Thus, the shifting of the cone was measured by about twenty ones per one individual and the average was put out. As for the moving ratio of the pigment, a certain part of the retina was made in a sketch by Abbe's drawing apparatus and then the area of pigment layer was measured by means of a planimeter. Thus, the moving ratio of pigment was calculated by the percentage of the area of the pigment layer to the total area from the base of the pigment epithelium to the external limitting membrane. All the experiments were carried out from noon to four o'clock p.m., considering the occurrence of the phenomena as described in KAWAMOTO & KONISHI's report (1955), or from the persistent diurnal rhythm in the movement

* It is so called temporarily by the author.

of the retinal element as shown by AREY & MUNDT (1941).

Experiments were carried out from September to November of 1953.

Experimental Results

Relation between the moving ratio and light intensity: The fishes were illuminated with the various intensity of the illumination for 2 hours after 2 hours of dark adaptation and then the head was cut and fixed. The range of light intensity used in this experiment was the degree from 0.008 to 25 Lux on the surface of the water.

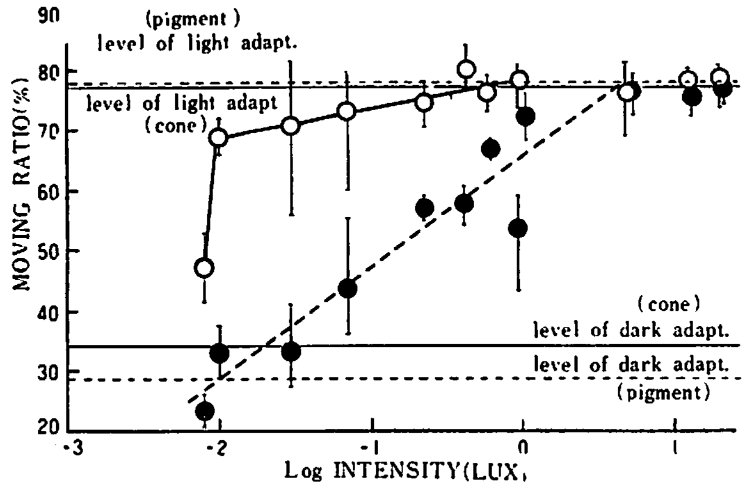

Fig. 2. Showing the moving ratio of cone (○) and pigment (●) in relation to the intensity of the illumination in the retina of *Trachurus*. The length of the vertical line at each point (average) shows the variation of measurement values. Water temperature is 17—20°C

The result obtained in *Trachurus* is shown in Fig. 2. When the light intensity decreases to 0.008 Lux, the cone shows elongation, and its moving ratio is 47 %. However, the cone takes contraction rapidly in 0.01 Lux of the illumination, and then it takes slowly contraction in proportion to the intensity of the illumination in the range of the more large intensity than 0.01 Lux. Thus at the intensity of more than 1 Lux, the cone shows the state of light adaptation. As for the pigment migration, it begins to expand at length from the state of dark adaptation when light intensity is given to 0.03 Lux, and in the intensity of 5 Lux, the expansion shows the state of light adaptation.

In *Carassius*, the cone does not show the complete elongation in 0.008 Lux, and then it becomes to the state of the light adaptation at the intensity of 0.23 Lux. Concerning the pigment migration, it shows the contraction in 0.008 Lux as well as dark adaptation, but the ratio of the pigment migration for the increasing of the illumination is very slow comparing with that in *Trachurus*, and it could

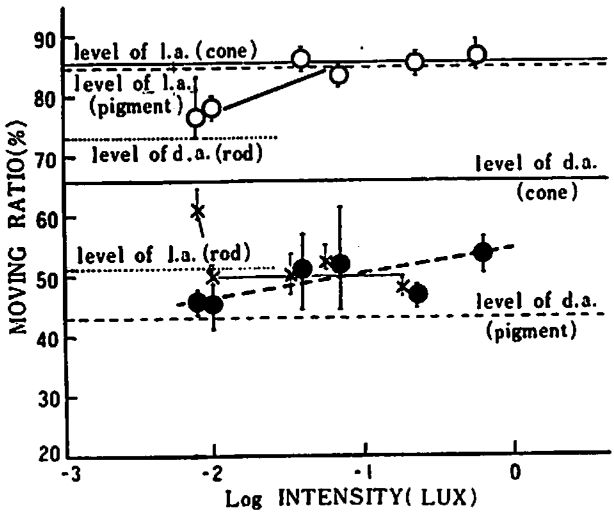

Fig. 3. Showing the moving ratio of cone (○), rod (×) and pigment (●) in the retina of *Carassius* in relation to the intensity of the illumination. Water temperature is 14—17°C.

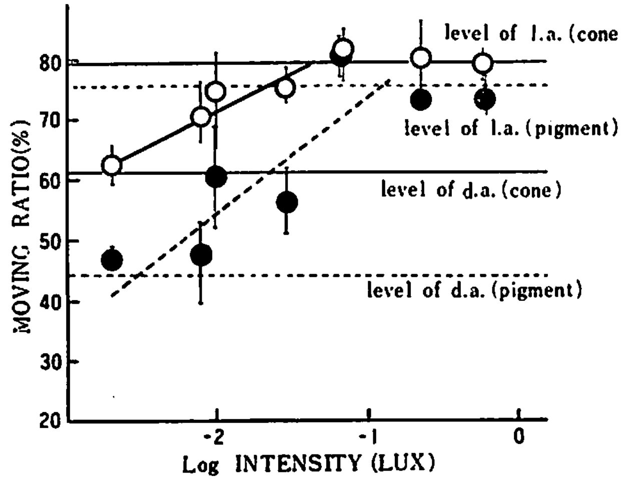

Fig. 4. Showing the moving ratio of cone (○) and pigment (●) in the retina of *Misgurnus*. Water temperature is 20—23°C.

not reach to the state of the light adaptation in the range of the illumination shown with present experiment. The extension in which the visual elements change the positions according to the intensity of the illumination, is more small than that of *Trachurus*, particularly so in the cone. The rod elongates rapidly at 0.008 Lux, but it does not yet reach to the level of dark adaptation. Therefore, it is thought that the intensity, with which the rod alternates the position with the cone, is more less than 0.008 Lux. I am sorry to cannot find the alternate intensity owing to the circumstances of material.

The results in *Misgurnus* is simillar to that in *Carassius*, but the movement of pigment reaches to the level of light adaptation in 0.07 Lux, and also in 0.002 Lux both cone and pigment reach to the dark adaptation.

It is clearly seen that these visual elements move in proportion to the logarithm of the light intensity in certain range of the intensity, and moreover these shifting is not applied to the all-or-none principle.

Time relation of the movement in dark adaptation : It seems that there are no reports concerning the changes of visual elements which occur during dark adaptation of marine fish. Present experiments were made in order to investigate these morphological changes directly within the living organism. Still this experiment was made by the same method as in the former one. *Trachurus* were adapted in light of 50 Lux and 0.23 Lux for 2 hours respectively, and then retina of the fish was fixed about every 10 minutes. These results were shown in Figs. 5 and 6.

These figures indicate that if the illumination of pre-adaptation is more intense, the time course of dark adaptation proceeds more slowly. The moving ratio was observed in central and marginal part(back)of the retina. However, the ratio of the visual element in latter part seems to be of high value ; namely, the movement of that element tends to be carried out rapidly. But, the time course seems to be the same in each course. Moreover, it should be noted that pre-adaptation to a light of high intensity is followed by a gradual dark adaptation course, and at the time of about 90 minutes it reaches to a stationary state, and a pre-adaptation to a light of low intensity is followed by a rapid course, and it reaches to the stationary state in about 40 minutes. It is interesting to note also that in 5—10 minutes after dark adaptation, the migration of the reversible direction is seen.

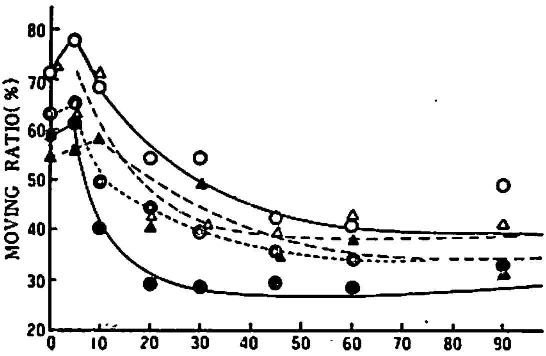

Fig. 5. Showing the time relation of dark adaptation of the moving ratio of the visual element in *Trachurus*. The fish was pre-adapted for 2 hours with the illumination of 0.23 Lux. The marks are as follows. ○, cone in the central part of the retina; △, cone in the marginal part; ◎, Small cone; ●, pigment in the central part; ▲, pigment of the marginal part. Water temperature is 17—20°C.

Time relation of the movement in light adaptation : Time relation of the cone and the pigment during light adaptation was observed after 2 hours of dark adaptation (Figs. 7 and 8). *Trachurus* was illuminated with light of 50 Lux and 0.23 Lux. In these results it is seen that the state of the light adaptation is formed more rapidly than that of the dark adaptation, and in the illumination of 0.23 Lux for one minute the cone already shows the contraction, but after 5—10 minutes it elongates slowly, and then it takes contraction again. This process agrees with the re-

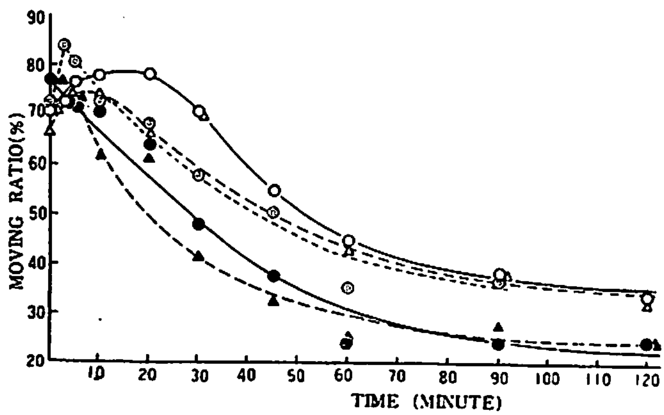

Fig. 6. Showing the time relation of dark adaptation of the moving ratio of the visual element in *Trachurus*. The fish was pre-adapted for 2 hours with illumination of 50 Lux. Water temperature is 17—20°C. The marks are the same as those of Fig. 5.

sult of STUDNITZ (1933).[11] However, on the contrary, the continuous process of light adaptation was obtained in the illumination of 50 Lux and reached to the state of light adaptation in about 30 minutes.

Discussion

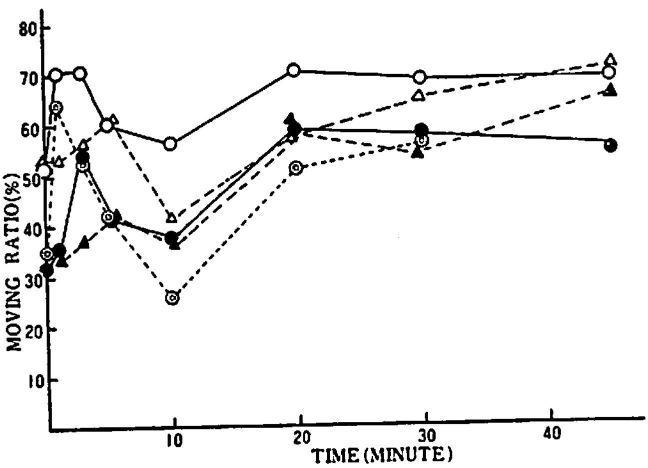

Fig. 7. Showing the time process of light adaptation of the moving ratio of the visual element in *Trachurus*. The fish was adapted for 2 hours with the illumination of 0.23 Lux. Water temperature is 17–20°C. The marks are the same as those of Fig. 5.

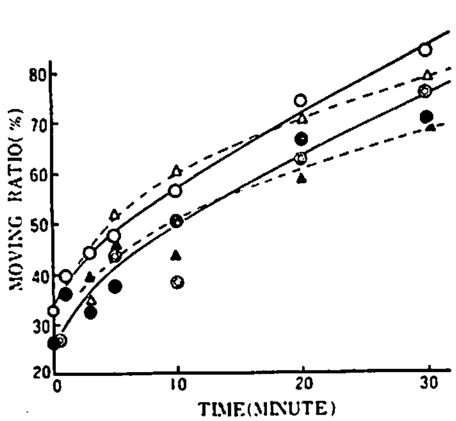

Fig. 8. Showing the time process of the moving ratio of the visual element in light adaptation in *Trachurus*. The fish was adapted for 2 hours with the illumination of 50 Lux. Water temperature is 17—20°C. The marks are the same as those of Fig. 5.

As described by PARKER (1932)[10] and DETWILER (1943),[4] the significance in the function of the retinal pigment exists in the absorption of light by the pigment rather than the support in the regeneration of visual purple; namely, the pigment is eliminating the light that would otherwise be disturbing to the sharpness of the visual image or overpowering in stimulation. And then, it is thought that the moving of the retinal pigment is also important for the investigation of fish vision. In the present work, marine fish was used as one of experimental materials. Therefore, the results of this experiment will give a fundamental thing to the studies regarding the effect of fish gathering lamp. And a few considerations will be described below as compared with the results of fresh water fish.

As shown in Figs. 2, 3 and 4, it is applied to the Law of Weber-Fächner that the moving ratio is linear in relation to the logarithm of the light intensity in certain range of the intensity.

It is assumed that white light used in this experiment may be changed in the composition of wave length in the lower intensity of the illumination, because the neutral filter could not be used for lowering of the intensity. However, from HONJO's date[6] the range of the shifting of visual element in relation to wave length is about 10 %, particulary it is remarkable in yellow-orange light, and the direction of the moving shows the light adaptation. Therefore, the excess over the value observed in the present experiment will not occur in truth ; then the present results are thought to be due chiefly to the effect of the light intensity than that of the wave length.

The moving of the visual elements is different from fish species ; for instance, in *Trachurus*, the cone moves from 35% in dark adaptation to 85% in light adaptation; in *Carassius*, it moves from 65 % to 85 %. After a fast contraction of the cone in low intensity, the velocity of the cone-movement for the intensity is not different from each other, but the velocity of the pigment migration in *Trachurus* is larger than that in *Carassius*. It is also seen that the intensity with which these retinal movements reach to the stationary state is different in fish species.

Above mentioned fact may be in consequence of adaptation due to the ecological difference ; namely, *Trachurus* moves the long distance vertically. In other words, the fish moves up and down rapidly from dark place to light place. *Carassius* moves in the place where dark and light conditions are considerabl stable. Also this suggests that the role of the retinal pigment migration may be in the regulation of the ray of light incident upon the eye slit because of the fixed pupil in fish.

It is very interesting to note that the range of intensity of $1—10^{-2}$ Lux with which the movement of retinal element occurs remarkably agrees with the fact that the lower limitting intensity in which the *Trachurus* meet together to the fish gathering lamp is $1—10^{-2}$ Lux in open sea. [12]

Moreover, BRUNNER[3] investigated the relation between the visual acuity of *Phoxinus* and the light intensity, and found that the transition of the function between rods and cones occures in the intensity of 0.002—0.008 Lux. When this intensity is compared with the results obtained in the present work, it is noted that the intensity produceing the elongation of cones in *Trachrus* is very similar to this intensity. However, since the rods of *Trachurus* can not be found in the present writer's collections, it may be thought that the intensity mentioned above does not show the transition of the two functions, but shows the limitting intensity of photoperception. On the other hand, this interpretation seems to be confirmed from the fact that a-wave of electroretinogram of *Trachurus* disappears in this intensity[8]. Those intensities in *Carassius* and *Misgurnus* are lower than that of *Trachurus* (Figs. 2, 3 and 4) ; therefore this fact shows that their retina may be more sensitive as described in *Cyprinus* from ecological viewpoint by TAMURA.[13]

And also YAMANOUCHI [16] observed in *Microcanthus* that the site of transition between rods and cones occures in the intensity of 0.4—5 Lux. But, fishes used in the present work still show the decrease of the moving ratio of the cones and pigment below this intensity, and so the alternate of the two elements will occur below this intensity. Therefore, it is assumed that these fishes have more fine visual acuity than that of *Microcanthus*.

The correlation was not found between the intensities where visual acuity becomes stationary and the shifting of visual element reaches to the stationary state.

Although time relation of dark adaptation is different from the light intensity and the duration of pre-adaptation light, it appears that the time of complete dark adaptation in fish is longer than that which has been generally considered. At 5—10 minutes after dark adaptation, the movements of the retinal element in reverse direction were seen ; however, these physiological significances were not considered. STUDNITZ [11] described that the contraction of the cone and expansion of the pigment in light adaptation does not show the continuous process, but in the present writer's experiment with 50 Lux, the continuous process was seen. This increpancy is not understood too. Moreover, in experiment of time relation, visual element was measured in both central and marginal part of the retina. Therefore, judging from these results (Figs. 5, 6, 7 and 8), it may be thought that the marginal part (back) of the retina is more sensitive than the central part, and the time relation of the central part is more rapid. This supports the TAMURA's results which the visual axis of *Trachurus* shows the fore direction at least.

However, the movement of these visual element does not always proceed in parallel with the decomposition and regeneration of photosensitive substances. Accordingly, though the function of photoperception could not be discussed on the data in the present report, it is thought that fish conduct for light stimulus is assumed to a certain degree from the retinal state under the various light conditions. Therefore, not only these morphological changes, but also electrophysiological or biochemical comparative researches of retina will be expected in the foreseeable future.

Summary

1) The movements of cone, rod and pigment in retina of fish, *Trachurus japonica*, *Carassius auratus* and *Misgurnus auguillicaudatus*, were investigated in relation to the intensity of the illumination.

2) The moving ratio of the cone and pigment is shown in proportion to logarithm of the light intensity in certain range of the low intensity, and these relations are applied to the Law of Weber-Fächner.

3) Movement of the cone is not applied to all-or-none principle.

4) The intensities of the illumination in which the cone contracts rapidly and the shifting of these retinal elements reaches to the stationary state, are different from each other in fish species. This seems to be the consequence of the ecological adaptation.

5) It was found that the intensity of alternative function in visual acuity curve

obtained in *Phoxinus* by BRUNNER, agrees with the intensity which occurs in the elongation of the cone in *Trachurus*. And in *Carassius* and *Misgurnus*, these intensities were lower than that of *Trachurus*.

6) It is seen that the degree of the illumination in which the cone and pigment move, coincides with the illumination where *Trachurus* comes to the fish gathering lamp in the open sea.

7) Time process of dark and light adaptation is observed in *Trachurus*. Dark adaptation is formed in 40—90 minutes by pre-adapted light of 0.23—50 Lux, respectively. Light adaptation is formed more rapidly than dark adaptation. Light adaptation with 50 Lux does not show a none-continuous process as described by STUDNITZ. The marginal part of the retina seems to be more sensitive than the center.

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