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## Response of the Retina of Flying Squid *Sthenotiuthis ovalaniensis* (Lesson) to Light Changes\*

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### Abstract

The Hokkaido University training ship Hokusei-Maru has carried out squid investigations in ocean waters near Hawaii since 1981. The most predominant species of squid distributed in this area is the flying squid *Sthenotiuthis ovalaniensis*, as has been previously reported.

The authors has carried out the present study to examine the movement of black pigment in the flying squid retina in response to varying levels of artificial light. Response of retinal pigment is expressed as percentage of the height from the external limiting membrane "b" and the thickness of the retina "a", as  $(b/a) \times 100\%$ .

Results of these experiments can be summarized as follows:

- 1) The black pigment response rose to 100% after from 10 to 30 minutes exposure to between 25 and 170 lux light illumination.
- 2) Black pigment response, up or down, was noted at a critical point of 1.0 lux light illumination.

### Introduction

The Hokkaido University training ship Hokusei-Maru has carried out squid investigations in ocean waters near Hawaii since 1981. The most predominant species of squid distributed in this area is the flying squid *Sthenotuthis ovalaniensis*, as has been previously reported<sup>1)</sup>.

The authors has carried out the present study to examine the movement of black pigment in the flying squid retina in response to varying levels of artificial light.

### Materials and Methods

Squids were collected by use of an automatic jigging machine on board ship. Immediately after capture, flying squids were conditioned to darkness inside a (110cm × 68cm × 39cm) fiberglass reinforced plastic experimental tank. Squids remained in total darkness for three hours prior to exposure to experimental light, except of most lower light intensity of 10 W, 30 V, this case only immediately experiment after capture. Exposure to controlled light levels during experiments varied from five minutes to three hours. Condition of sea water in the experimental tank is every time streamed by overflow, temperature of sea water in the tank is 23.

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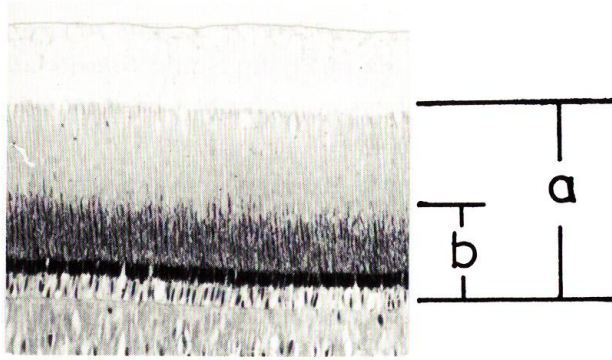


Fig. 1. The state of condition expressed by percentage of the height of black pigment  $b$  to the thickness of retina  $b/a \times 100$ .

5°C to 24.3°C, sampled squid mantle length is 17.0 cm to 26.0 cm.

The light source for these experiments consisted of a standard 100 V, 10 W incandescent light. Light intensity was varied by adjusting the voltage to the light source; full intensity was 100 V, 50 V and 30 V for lower intensities. An additional experiment using relatively high light intensities employed a 100 V 40 W lamp. The lamps were suspended 50 cm above the water surface at the center of the tank. Surface light intensity was measured with a "Tokyo-Kogaku KK" (SP19W) submarine photometer.

At the end of a predetermined time interval, whole eyeballs were excised and preserved in Bouin's fixative. In the laboratory, the central portion of the retina opposite the lens was removed, embedded in paraffin by standard methods, and longitudinally sectioned at thickness of 6 microns. Retinal sections were photographed under light microscope following staining with Dalafield's haematoxylin and eosin.

In squid, visual sensitivity under various intensities of light is altered by the upward migration of black pigment in the retina. The black pigment layer is located between the outer segment of the visual cells and support cells. Support cells lie adjacent to limiting membrane<sup>2-3</sup>. In response to changing levels of light, black pigment migrates through the retinal layers, thus controlling visual sensitivity<sup>4-6</sup>. Adjustment to light intensity within the retina is expressed as:

$$b/a \times 100\%$$

where, "b" is the height of the black pigment from the external limiting membrane, and "a" is the thickness of the retina (Fig. 1). For example to show micrographs to the migrating condition of black pigment in retina of squids *Sthenotiuthis oualaniensis* (Lesson) when exposed to 100 V, 40 W lamps (Fig. 2).

## Results

There is a different of several % of the position of black pigment for left and right eye-balls for an individual squid. Therefore, the moving ratio of the black pigment expressed by the mean value (%) of the both eye-balls.

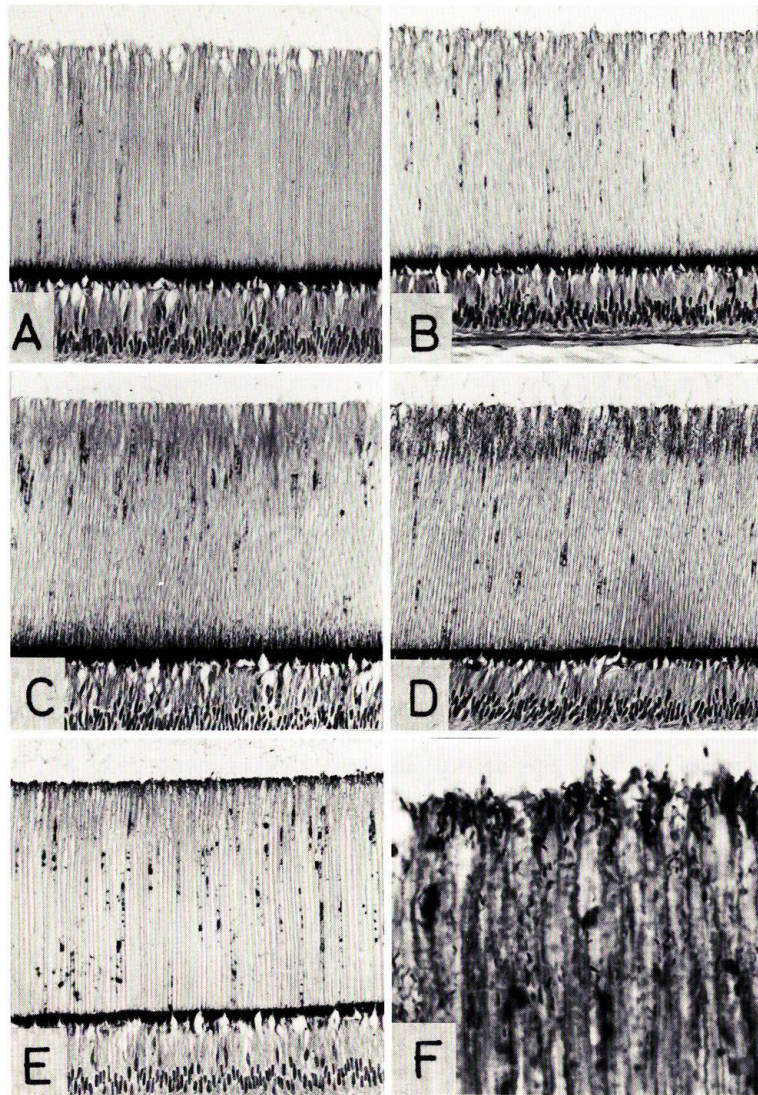


Fig. 2. Micrographs showing the migrating condition of the black pigment in retina of squids *Stenoteuthis oualaniensis* (Lesson) when exposed to 100 V, 40 W lamps (see Fig. 7).

(A) After 3 hours in darkness in the experimental tank. (B) Exposed 5 minutes. (C) Exposed 10 minutes. (D) Exposed 20 minutes, black pigment almost reach to the upper side of the retina. (E) Exposed 30 minutes, black pigment reach to the upper side of the retina. (F) Enlarged micrograph when the black pigment reached the upper side of the retina. Small black dotted point indicates black pigment. (A ~E,  $\times 170$ ; F,  $\times 850$ ).

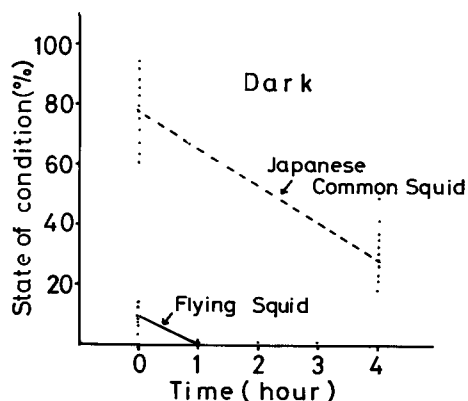


Fig. 3. The conditions of black pigment height before and after 3 hours in darkness in the experimental tank. Time zero indicates the state of retina just after capture. Dotted line shows japanese common squid in similar experiments<sup>7)</sup>.

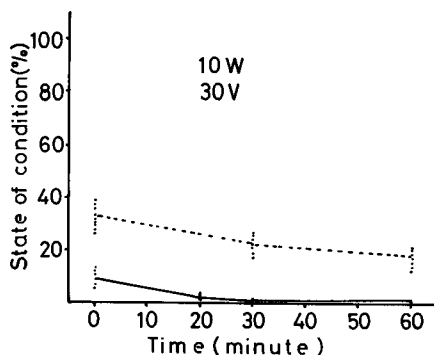


Fig. 4. The results when exposed to reduced voltage of 30 V, 10 W lamp. Time zero indicates the state of retina just after capture. Dotted line shows japanese common squid in similar experiments<sup>7)</sup>.

A notable depression in the black pigment was measured following one hour in total darkness during the three hours acclimation period. The rate of adaptation to darkness in the flying squid differed significantly from that of the japanese common squid (*Todarodes pacificus* Steenstrup) in similar experiments (Fig. 3)<sup>7)</sup>, but in this case of squids (*Todarodes pacificus* Steenstrup) remained less than 5~6 hours under

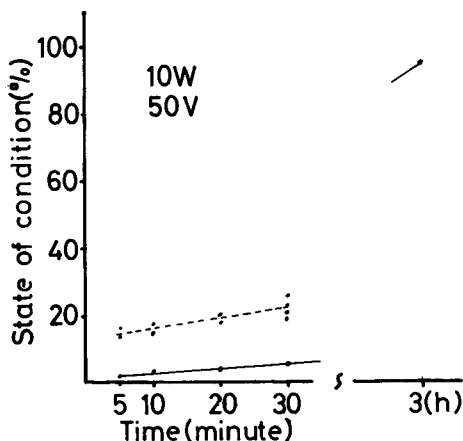


Fig. 5. The results when exposed to reduced voltage of 50 V, 10 W lamp. Time zero indicates the state of retina remained three hours in darkness tank. Dotted line shows japanese common squid in similar experiments<sup>7)</sup>.

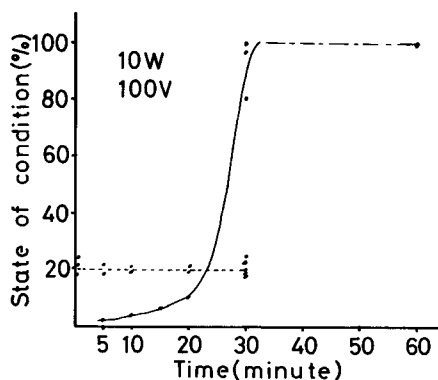


Fig. 6. The results of illumination by the standard voltage 100 V, 10 W lamp. Dotted line showed japanese common squid in similar experiments<sup>7)</sup>.

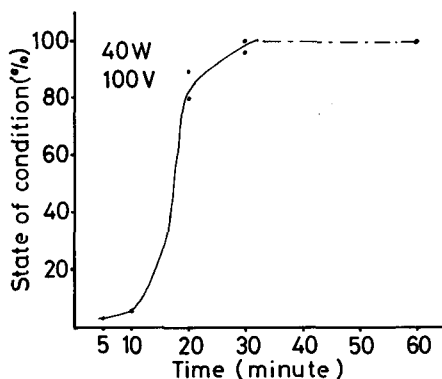


Fig. 7. The results by the standard voltage 100 V, 40 W lamp.

Table 1. Intensity of illumination at the surface of the water by an incandescent light suspended 50 cm directly above.

Incandescent lamp (standard voltage 100 V)	10 W		20 W	40 W
Voltage (V)	30	50	100	100
Illumination (lux)	0.2	1.9	25.3	85

nature light conditions before experiments. Following several minutes exposure to low level light intensity (10 W at 30 V), the black pigment layer descended from about 10% to 0% (Fig. 4). The black pigment became a larger percentage of the retina following up to three hours exposure to mid level light intensity (50 V) of the 10 W light source (Fig. 5). Under exposure of 100 V of the 10 W light source, a pronounced rising of the black pigment was noted after 20 minutes exposure, reaching nearly 100% following 30 minutes exposure (Fig. 6). One hundred percent adaptation was attained between 10 and 20 minutes of exposure to relatively intense light levels of a 40 W light, held at 100 V (Fig. 7).

Changes in adaptation in both the flying and Japanese common squids occurred following changes of 1 lux in light intensity (Fig. 4, 5; Table 1).

### Discussion

The flying squid fully adapted to intense light levels within 30 minutes from initial exposure, much more rapidly than had been noted in the Japanese common squid in a previous study (Fig. 6)<sup>7</sup>. These results suggest that the Japanese common squid is probably adapted to low levels of light intensity in deeper waters during the day and migrates to the surface at night. On the other hand, the flying squid is a pelagic species<sup>8</sup> which is occasionally seen breaching the water surface in daylight hours. This suggests that the flying squid is distributed in the surface layer in the day, and would require a more rapid rate of adaptation to changes in light illumination.

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