Dynamic blinking in the head of hardyhead silverside fish

Masakazu Iwasaka

Hiroshima University, Kagamiyama 1-4-2, Higashihiroshima, Hiroshima 739-8527, Japan

E-mail: miwamas@hiroshima-u.ac.jp

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Dynamic light reflection can be used to provide an efficient tool for biological sensing of micro and nano-organisms. It is therefore interesting that evidence of dynamic light reflection can also be found in the animal kingdom and that there may be alternative methods of light control actuation. In this study, it is discovered that several features in the heads of hardyhead silverside fish, particularly those located around the edges of the iris, caused a blinking phenomenon using environmentally scattered light. Analysis of the blinking using recorded video of the fish iris revealed that circular cells that exist in the iris changed their light intensity at approximately 2 Hz. These cells, which are 5–10 μm in diameter, are normally blue. However, it is found that a distinct light intensity changed in 0.04 s, and additional green and yellow colors then overlapped with the blue.

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1. Introduction

Light reflection is one of the most interesting phenomena in optics and photonics and has also been widely studied in fields including physics,1) geoscience,2–5) optical engineering,6–10) biophysics,11) and medicine.12–17) By detecting scattered light at the periphery of the incident light, dark-field illumination has allowed light particles to be analyzed optically on a nanometer scale.13) Light reflection measurements have also been applied to measurements of larger objects, including our entire planet.2–5) The data collected from the light absorption and reflectance caused by the land and the atmosphere are very useful in sensing of environmental conditions.

Detailed investigations of the light reflected from objects of a similar size to the human body can be enabled by improvements in computer science.6–9) Computer simulation techniques for modeling of light reflection by a wide variety of model objects are now some of the most essential tools in today’s information society. These studies are also aided by the use of advanced semiconducting technologies such as the digital micromirror device, which involves the application of light reflection from a smooth aluminum mirror that can now be attached to the human body.10)

The dark-field illumination technique, which was initially developed in the last century, can now be applied at the microscopic level using contemporary imaging techniques.11–14) Most medical research that concerned light reflection was conducted more than 50 years ago.1,15–20) However, analysis of the light absorption and reflectance phenomena in living tissues near the body surface, e.g., in the skin and peripheral blood vessels, now forms an essential part of medical diagnoses.15–17) Additionally, light reflection by the pupil has been linked to information processing in the brain.18–20) These studies indicate that optical properties such as the light reflectance of living tissue are strongly correlated with signal indication and processing in living creatures.

The skin and scales of fish can be used to study previously unrevealed optical properties of the living body when exposed to external light because most fish have reflecting platelets in their skins.21) Our previous studies have focused on several types of light-reflecting behavior in the reflective guanine platelets found in fish skin.22–25) Examination of fish from sub-tropical regions that show novel photonic properties will offer the chance to discover dynamic and tunable light reflection phenomena. The hardyhead silverside fish (Atherinomorus lacunosus) is an appropriate model fish for investigation of the relationship between mechanical sensing and the bio-photonic properties of the living body because this type of fish can cause light to blink from circular spots aligned along its dorsal trunk.26) This study reveals how the head of the hardyhead silverside fish is able to reflect light dynamically with a blinking frequency that is higher than that observed in the data from previous studies.

2. Materials and methods

2.1. Specimen

Five specimens of the hardyhead silverside fish, Atherinomorus lacunosus, were obtained in Okinawa, Japan. After being transported to Hiroshima, the fish were housed in an aerated seawater aquarium, where the water temperature was thermostatically controlled at 26 °C–29 °C. The five specimens were used in experiments conducted in December 2020 in accordance with the ethics policies of the Hiroshima University Animal Care and Use Committee (approval numbers F19-2 and F16-2, Hiroshima University).

2.2. Observation of fish in aquarium

A fish in the aquarium was observed via a lens (Nikon AF Zoom-Nikkor 24–85 mm f/2.8–4D IF), while the microscopic images of the heads of the anesthetized fish were acquired using a high-resolution Navitar 2.0 × 1-51473 microscopic lens. Both lenses were connected to a CMOS camera (Hozan L-835) via a C-mount adapter during recording of the fish. The white balance and the exposure times were controlled manually and a white Hayashi Repic LA-HDF158A LED light was used as the light source.

The fishes were anesthetized by exposing them to 0.1% 2-phenoxy-ethanol for 10–30 s at approximately 15 °C, which was the water temperature in our experimental room. The anesthetized fish were then placed inside a plastic
aquarium that contained aerated seawater. After a short observation period, the fishes were then returned to the main aquarium.

2.3. Light intensity dynamics measurements on a computer LCD screen during playback of recorded video

Measurements of images on a computer display were collected using an optical fiber system. First, the video playback was paused at the instant at which the point to be measured appeared. One of the ends of the optical fiber was then placed close to the point to be measured and fixed onto the liquid crystal display (LCD) screen. The light intensity data were collected using a CCD spectrophotometer (Unisoku UPC-2000) and video playback then resumed a few seconds after the data collection process began.

3. Results and discussion

Figure 1 shows an image recorded from a video sequence of the blinking eye of a hardyhead silverside fish when swimming in the aquarium. All investigated specimens (four samples) had a common part that demonstrated the blinking phenomenon, which appeared intermittently in seven parts of the head [Parts I–V, VIII, and IX in Figs. 1(a)–1(c)] and in two parts of the body [Parts VI and VII in Figs. 1(a)–1(c)]. The blinking occurred most strongly and frequently at Parts I, II, and III. Parts I and II were located at the edge of the iris and Part III was located at the center of the skull at the midpoint between the eyes. In Part IX, it appeared as though the small circles were blinking. The angles of incident light directing to the fish’s body, the aquarium, and the water surface are illustrated in Fig. 1(d). Because the fish were swimming in an aerated, thermostatically controlled environment, their bodies were always in motion, even when they were floating statically.

We can theorize that the blinking on the body side, observed at Parts VI and VII, is correlated with the inclination of the fish’s body. It was more difficult, however, to distinguish between the effects of body motion and possible actuation occurring in the local tissues. With regard to Part VIII, it was initially believed that the blue blinking shown in Fig. 1(c) was caused by changes in the light reflection related to the movement of the fish’s mouth. However, the experimental video images did not corroborate this speculation.

Figure 2 shows 18 randomly captured images of the eye of a hardyhead silverside fish when swimming in an aquarium. All the individual images have slightly different inclinations. Remarkably, an increase in the light intensity was observed...
to occur at the frontal edge of the iris (Part II), but the timing did not appear to correlate with the eye’s inclination. The blinking phenomena that were observed in Parts I and II were both detectable using the naked eye.

Next, to investigate whether the blinking observed at the edge of the iris occurred independently in local parts of the iris, microscopic observations of the edge of the iris (Part I) were conducted on an anesthetized fish. Figure 3 shows two image sequences obtained from the recorded video, with the magnification used in each case being the only difference between the two sequences. The videos were recorded both while the fish were anesthetized and when they were settled quietly in the main aquarium.

Aggregated circular cells were found on the edge of the iris. The first example of these cells [Fig. 3(a)] shows a rapid increase in light intensity after 0.12 s, with pale-green circular cells being visible on the blue base [left image of Fig. 3(a)]. Among these cells, approximately eight cells changed color to yellow (middle image) after 0.04 s, and the color intensity then increased after 0.08 s (right image). Figure 3(b) shows a similar phenomenon, but with reduced expansion.

The image sequence shows a cycle of increasing and decreasing light intensities observed over a period of 0.44 s. Initially, most of the circular cells were colored blue, but they turned green or yellow after approximately 0.5 s. These results indicate that the condensed circular cells in the iris were blinking but the inclination of the iris did not change. As shown in Fig. 3, the blinking was observed continuously for 1 min, and the frequency of the blinking was approximately 2 Hz.

The blinking of the iris at approximately 2 Hz was confirmed via analysis of the blinking behavior of the iris during video playback, as shown in Fig. 4. The fiber-optics measurements on a local area of the LCD used to project the movie provided time courses for the light intensity, as illustrated in Figs. 4(b) and 4(c).

Figure 4(b) shows that the area covering the four circular cells exhibited blinking for more than 60 s. An expansion of the time course (lower panel) indicates that the blinking occurred at a frequency of 2 Hz. In Fig. 4(c), the light from the measured area was collected within the wavelength range from 400 to 600 nm for approximately 8 s, and the data were then transferred to the time course style. Among the 10 selected wavelengths, the time courses at three wavelengths between 530 and 570 nm (the dark blue, brown, and intense gray lines) showed remarkable oscillations at the same frequency.

Fig. 3. (Color online) Microscopic observation of blinking at the edge of the iris (from Part I, as shown in Figs. 1(a)–1(c) of anesthetized hardyhead silverside fish. (a) First example showing a distinct light intensity increase over 0.12 s. The time indication shows the playback time in supplementary movie B. (b) Second example, showing increasing and decreasing light intensity. The time indication again shows the playback time in supplementary movie C. The scale bar represents 10 μm.
frequency of approximately 2 Hz. The time course at 450 nm (orange line) also shows oscillation at ~2 Hz, although the oscillation pattern was different to those observed between 530 and 570 nm. As shown in the lower panel of Fig. 4(c), the light intensity at the three wavelengths between 530 nm and 570 nm increased dramatically after 0.7 s, while the change that occurred at 450 nm was comparatively small.

A statistical analysis of the blinking in the iris was performed on four hardyhead silverside fish specimens, as shown in Fig. 5. The specifications for these measurements are described in the supplementary data. From the recorded movies of the eyes of the four anesthetized specimens, three to six movies per specimen were selected for the fiber-optics measurements in the LCD used to project the movie. In the analysis, six continuous peaks in the light intensity were observed at 573 nm (yellow color) and the interval between these peaks was then obtained. Figure 5(a) shows the average of the reciprocal number of five intervals between peaks. The horizontal axis in Fig. 5(a) represents the measurement number, which was used to label the data from the four specimens. The vertical axis, which represents the reciprocal number of intervals, corresponds to the approximate blinking frequency. The results show that the blinking at the edge of the iris occurred in three modes: the slow mode (0.5–1 Hz), the fast mode (1–2 Hz), and the fastest mode (over 2 Hz). The time courses of the light intensity for measurement numbers -11 and -16 are shown in Figs. 4(b) and 5(b), respectively.

The blinking circular cells appear to be a type of chromatophore, which is a group of cells that controls light absorption and reflection on the bodies of animals such as fish and amphibians. It is possible that these circular cells, which are less than 10 μm in diameter, are a specific type of...
Fig. 5. (Color online) Statistical analysis of blinking in the irises of four specimens (specimen numbers spm1210-1, -2, and spm1211-1, -2) of hardyhead silverside fish. (a) Averages of the reciprocal numbers of five intervals of the peaks, which were obtained from individual measurements. (The mean + standard deviation is shown.) (b) Example of time course of blinking (measurement number-11). (c) Measured area (marked using the broken black circle) in the movie during playback. The scale bar represents 100 μm.
chromatophore called an iridophore because iridophores often contain reflective platelets inside their cells. The circular cells observed in our study clearly varied their light intensity, probably through use of reflective particles within each cell. However, the movements of the reflective particles inside the cell were unclear in this study. In Fig. 5(b), the light intensity at 573 nm (yellow) caused the opposite change to the intensity at 450 nm (blue). Enhancement of the yellow color reduced the amount of blue color observed. The blue color was provably provided by the guanine platelets in the iridophore. There should also be an additional mechanism that enables rapid change of a mechanical condition relating to the yellow color.

The light intensity on the eye of the hardyhead silverside fish changed after 0.04 s, as shown in Fig. 3. The speed of this change was faster than that observed in the iridophores found in the bodies of other species of teleost fish that show dynamic color changes.28,29) The edges of the iris of hardyhead silverside fish exhibited synchronization of the blinking in the spread regions of iridophore cell aggregation, while the trunk of the same fish showed a localized small circular blinking spot.26)

It was previously reported that the color changes in the iridophores found in chameleon sand tile fish were controlled by the nervous system of the fish.28) In addition, many studies have reported on electric field,30) magnetic field31) and mechanical32) sensing by fish. Most of these fish have a lateral line system, which is a type of sensor network on the body surface.32–35) The lateral line system has canals that are distributed over the head and trunk of the fish. This system can sense hydrostatic pressure and the sensed information is then used by the fish to detect risks or food.36)

The canals contain cilia that are bent by mechanical vibrations in the water stream.36) The locations of the observed blinking parts in the body of the hardyhead silverside fish (as shown in Fig. 1) coincide with the typical distribution of these canals. It will be necessary to verify the relationship between the blinking behavior and this lateral line system.

In previous works on the physical sensing behavior of fish, an Antarctic fish was reported to sense the mechanical vibration from planktonic prey using its lateral line system.33) Fish can localize objects such as predators or prey that exist near their bodies by sensing hydrodynamic signals from distances ranging from one- to two times their body length.33) In this study, the blinking frequency from the head of the hardyhead silverside fish was within the minimum level of the range of reported mechanical vibration frequencies.33)

It has previously been proposed that the lateral line system of some fish (e.g. silversides34) and tuna35) are closely involved with fish school swimming behavior. We can speculate that each fish detects the neighboring bodies of school swimmers when it senses a change in the water flow by using the lateral line system and its eyes. In this situation, the blinking reflectors on the neighboring sensed bodies may improve the visual sensitivity of the sensing fish because the optical signals can reach over longer distances when compared with the hydrostatic mechanical signals. The spatio-temporal patterns of the reflected light in neighboring fish can also inform of the arrival of predators or prey.

The blinking is hypothesized to be a type of frequency modulation of the reflected light that propagates from the light-reflecting tissues of the signal sending fish to the eyes of the signal receiving fish. This hypothesis can be tested in future by verifying the effects of the light illumination angle, the illumination strength, and the swimming conditions for the fish in the aquarium. In future studies, if the proposed ethological hypothesis is denied, the observed phenomenon may be proved to be a product of optical interactions between the hair cells of the lateral line system and the iridophores. Nevertheless, we can open up a new field in which the mechanisms of the dynamic light reflection phenomenon in fish can be applied to various types of imaging technologies.6–25)

4. Conclusion

Hardyhead silverside fish have been observed to have blinking parts on their heads. The blinking behavior was most obvious when the fish were swimming in an aquarium. The most distinctive blinking parts were located on the edges of the iris. Through microscopic observation of the irises of anesthetized fish, we found that the circular cells on the iris were causing the continuous blinking. Fiber-optic measurements on a computer LCD screen during playback of the recorded video confirmed that the blinking occurred at frequencies in the range from 0.5 to 2 Hz.

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Supplementary data

Supplementary data, Supplementary Movies (A–C). Specification of the specimens utilized in the experiments. Examples of raw data shown in FIG. 5.

Authors’ contributions

M. I. performed the experimental design, experiments, measurements and analyses. All parts of the manuscript and illustrations were prepared by M. I.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID iDs

Masakazu Iwasaka @ https://orcid.org/0000-0003-4213-7390

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