Tokyo salamanders (*Hynobius tokyoensis*) may possess the capability to distinguish between red and blue

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ABSTRACT

Color vision is a fundamental trait in many animal species that enhances survival and reproduction. Despite the importance of this ability for survival, there have been few examples of the use of knowledge about color vision to improve rearing environments. This study examined color vision in the endangered Tokyo salamanders (*Hynobius tokyoensis*). By learning about the color vision of this creature, for which conservation efforts are increasingly needed, this study encourages the use of knowledge about the cognitive functions of animals in captivity. The aim of the current study was to elucidate whether the Tokyo salamanders have color vision. To evaluate their ability to distinguish two colors, salamanders were placed in a two-colored box, split evenly into red and blue sections, and their behaviors in response to the color differences were keenly observed. As a result, their stay in the red areas was markedly longer in comparison to the blue areas, suggesting that they appeared to exhibit a stronger affinity for the color red. This study indicates the potential existence of color vision within Tokyo salamanders.

KEYWORDS: Tokyo salamander (*Hynobius tokyoensis)*, color vision, salamander

INTRODUCTION

Color vision, playing a crucial role in enhancing survival and reproduction, is an essential characteristic common to a lot of animal species. Although there is a substantial amount of research that focuses on the color vision of diverse animal species, there is a noticeable lack of research specifically investigating color vision in salamanders, with the exception of the fire salamanders (*Salamandra salamandra)* (Rozenblit & Gollisch, 2020). Rozenbilt and Gollisch built upon the work of fire salamanders of Przyrembel and Neumeyer (1995), which estimated that fire salamanders possess trichromatic color vision, as evidenced by their behavioral responses to monochromatic lights with different wavelengths. However, previous studies have not investigated the color vision of other salamanders.

Tokyo salamanders (*Hynobius tokyoensis*), a member of the *Hynobiidae* family of salamanders, have been endangered in recent years (IUCN SSC Amphibian Specialist Group, 2022). About half of all salamanders in the *Hynobiidae* family are endemic to Japan and many are threatened with extinction. Because of their low mobility and narrow geographic distribution, the Tokyo salamanders, which are distributed around the Tokyo metropolitan area, are in particular danger of extinction by human encroachment (Hayase & Yamane, 1989). Therefore, it is anticipated that a more detailed understanding of their vision and ecological conditions will aid in their preservation of natural environments and in enhancing artificial breeding techniques.

The objective of this study was to investigate whether the Tokyo salamanders possess color vision. This research sheds new light on the color vision of *Hynobiidae*, filling a gap in the literature on the color vision of salamanders in many species and contributing to their conservation efforts. Although there were no cases studied in the past, I thought Tokyo salamanders have color vision because they were more likely to approach a red-colored smartphone, which is usually the same color as their bait.

METHODS

Study Design

In order to confirm whether the salamanders modified their behavior in response to color stimuli, they were placed in a black box (serving as a controlled environment) and a two-colored box (with the left half being blue and the right half red). Red was chosen because salamanders have been observed to approach the area on a daily basis, and blue was selected because it is the wavelength in the visible light spectrum farthest from red. The duration of time they spent on each side was compared.

Animals

A pair of Tokyo salamanders, born in the spring of 2022 in Chiba, Japan, was obtained from the Kannonzaki Nature Museum, Kanagawa, Japan. Each salamander was housed individually under controlled conditions, with a temperature of 20℃ ±4℃, appropriate nourishment, and a proper environment in accordance with guidelines laid out by the Japanese Association of Zoos and Aquariums (2022a; 2022b). They were fed with sludge worms (*Tubifex tubifex*) and *Propsilocerus akamusi* every other day.

Procedure

Make a Black Box and a Two-colored Box (the left half area is red and the right half area is blue) with two transparent plastic boxes (Daiso Industries Co., 30 × 20 × 18 cm) as tanks, and black, blue, and red colored papers (CAN DO Co., Ltd). Put the Black Box right under the LED desk light (Nitori Co. Ltd., YP410DL) and the USB camera as no shadow exists in the box. Move a Tokyo salamander from a living tank to the Black Box with the fish net after the water (160 ml, 20℃ ±2℃), which chlorine was removed with WATER SUPPLE for amphibians (Axis Co.), was added to the Box. Ten minutes later, a Tokyo salamander was moved back into the living tank, and a Black Box was rinsed in tap water. Then similarly, a Tokyo salamander was moved in a Black Box two more times. After all Black Box experiments, the same test was done with the Two-colored Box instead of the Black Box with a Tokyo salamander. Then, the same procedure was done with another Tokyo salamander in both Boxes. As the second Tokyo salamander was sick after the first ten minutes in the Two-colored Box, the experiment was discontinued to protect its health. The length of the Tokyo salamander stayed on the left and right sides and four areas (up-left, down-left, up-right, and down-right) in the box were counted per second. The location of the Tokyo salamander was decided based on the center of their heads. The average ratio of the Tokyo salamanders stayed on the left and right sides and was compared between the Black Box and the Two-colored Box.

Statistical Significance

Given the nature of the dataset, conducting statistical tests was deemed inappropriate. This conclusion was drawn from the fact that the identical Tokyo salamanders were used repetitively for both black box and bi-colored box tests.

RESULTS

The primary purpose of this experiment was to determine the presence of color vision in Tokyo salamanders by comparing the length of time they spent in a black box versus a two-colored box. It was hypothesized that if they can recognize the difference in colors, their behavior in the two-colored box would be different from that observed in the black box. The results of this experiment are shown in Figure 1. A striking feature of these graphs is the notably extensive high rate of the length of time in the red area. In the Black box trials, the time spent by salamanders on either side was nearly identical, with an average difference of merely 0.88%. However, in the two-colored box trials, the average percentage of time they spent in the red area exceeded that in the blue region by 19.5%. This outcome suggests that Tokyo salamanders can distinguish between red and blue.

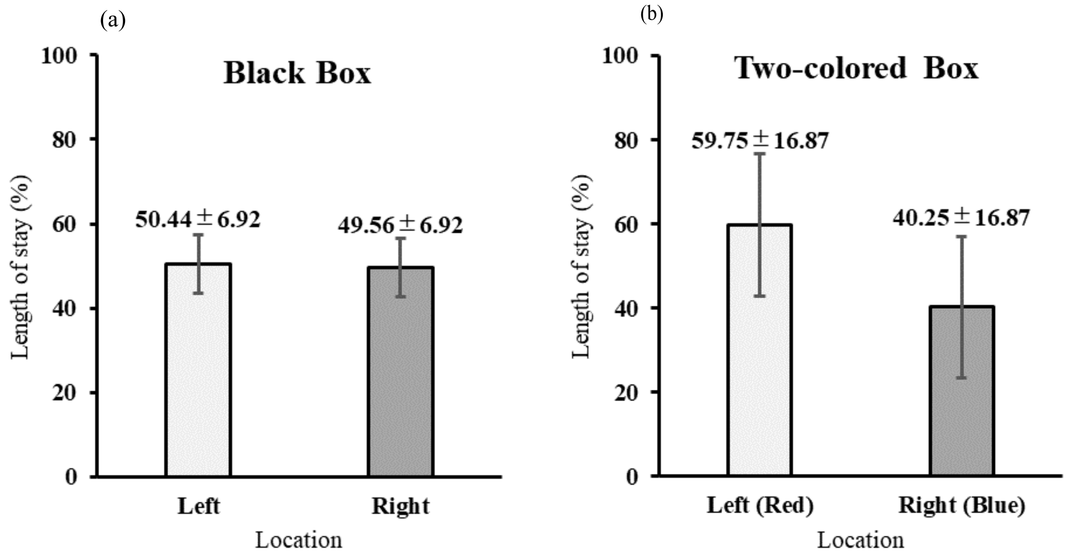

Figure 1 (a) the percentage of time that salamanders spent in the left and right area of the Black Box and (b) the percentage of time they spent in the red and blue area of the Two-colored Box. The mean ± SEM

The length of time spent in the four distinctive areas (up-left, down-left, up-right, and down-right) was also compared. Figure 2 presents the percentages of the average duration that the Tokyo salamanders spent in each of these four areas. These findings provide evidence that the salamanders were actively moving within the confines of the experimental boxes.

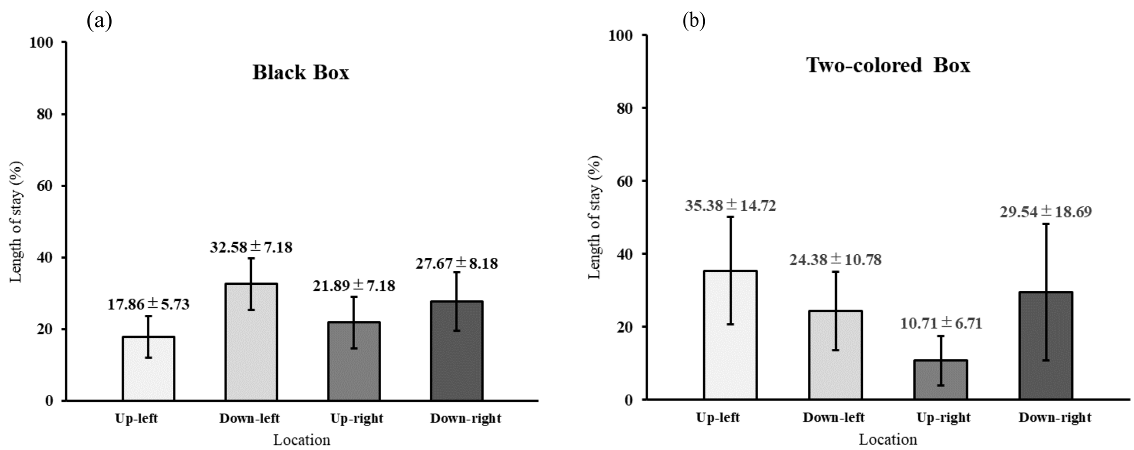

Figure 2 (a) the percentage of time salamanders spent in 4 equal size areas (up-left, down-left, up-right, down-left) of the Black Box and (b) Two-colored Box. The mean ± SEM

DISCUSSION

One of the primary goals of this project was to identify whether Tokyo salamanders possess color vision. The current study suggests a potential for color vision in this species, as they altered behavior in response to red and blue stimuli as compared to their behavior in the black box. This finding is consistent with that of Przyrembel (1995) that indicated trichromatic color vision in fire salamanders.

The color vision and cognitive abilities of salamanders have not been known until now. Therefore, it is hoped that this kind of research will advance our understanding and that conservation activities tailored to their characteristics will flourish. For example, by knowing their color vision, it will be possible to install breeding facilities and lighting that take into consideration the colors they can see.

However, given the small sample size, these results should be interpreted with caution, as the findings may not represent the color vision and behavior of the entire Tokyo salamander population. Another area of uncertainty is related to the brightness of colors utilized in the experiments. Since I did not quantitatively measure the color brightness, it remains uncertain whether the salamanders were affected by brightness rather than the actual color wavelength.

These findings carry significant implications for furthering research in color vision, especially given the paucity of studies in this area and the potential evidence of color vision in Tokyo salamanders. Such insights contribute to the refinement of experimental designs aimed at investigating color vision more comprehensively. Future research should consider implementing experimental methods that utilize the three primary colors of light to determine the range of light wavelengths perceivable by salamanders while ensuring an ample sample size.

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