Visual sensitivity to ultraviolet (UV) light is widespread in the animal kingdom. Many studies on UV vision have utilized physiological and/or anatomical methods to determine whether animals are visually sensitive to UV wavelengths. However, ultimately behavioral methods can reveal whether retinal UV sensitivity results in perceptual detection of UV stimuli. For the widely studied zebrafish (*Danio rerio*), the adult retina possesses cone photoreceptors that are sensitive to UV light. Here, we used a behavioral technique, the escape response assay, to test whether adult zebrafish can visually detect and behaviorally respond to visual cues that reflect UV. We found that adult zebrafish robustly respond to UV reflecting cues under UV light while showing no responses to the same cues under no UV light. From our results, we confirm that adult zebrafish can visually detect UV reflecting cues and show that UV perceptual sensitivity is functional in adult zebrafish. Our study highlights the utility of the fish escape response assay for UV visual behavior research.

Keywords: UV vision, visual detection, escape response, zebrafish


**Introduction**

For well over a century, we have known that animals are capable of visually sensing ultraviolet (UV) light, and since the discovery of visual UV sensitivity in ants (Lubbock, 1883), many animals, including other arthropods, birds, fish, reptiles, and some mammals, have been identified as possessing visual sensitivity into the UV portion of the light spectrum (for reviews, see Hunt, Wilkie, Bowmaker, & Poopalasundaram, 2001; Tovee, 1995). Because our eye lenses effectively cut off the transmission of UV wavelengths to our retinas, humans, on the other hand, are not capable of visual sensitivity to UV wavelengths (<400 nm). Additionally, unlike many other vertebrates, there are no photoreceptors in the human retina that are maximally sensitive to UV wavelengths. Nonetheless, the discovery of UV photoreceptors and UV opsins (SWS1) in other animals has opened up an increasingly productive area of research in the ecology and physiology of UV vision. For example, a recent study found that damselfish utilize UV visual sensitivity to detect unique UV reflecting patterns on their faces used for species and individual recognition (Siebeck, Parker, Sprenger, Mathger, & Wallis, 2010).

However, the number of photoreceptor types in an animal’s retina does not necessarily correlate with its wavelength discrimination abilities (Jacobs, 1981). While we learn more about the biology of UV vision in many animals, basic behavioral studies on UV vision are still lacking for one of the most widely studied model organisms in biology: the zebrafish (*Danio rerio*). Herein, we examine behavioral UV detection in zebrafish.

A growing body of research has focused on the ecological significance of UV vision in fish; yet, much of what is known about the physiology of UV sensitivity is derived from studies on the zebrafish visual system. Zebrafish (*Danio rerio*) have quickly become a popular model system for research on the vertebrate visual system (Fadool & Dowling, 2008). Because of their rapid and transparent development and well known genetics, zebrafish are widely used to study the development and function of the vertebrate visual system. Physiological, molecular, and morphological studies on zebrafish larvae have shown that the zebrafish retina, indeed, has UV photoreceptors that are sensitive to UV light. In fact, the UV-sensitive cones seem to be the first to be functional in larval zebrafish, exhibiting sensitivity to UV wavelengths, measured using electroretinograms (ERGs), at approximately 4 days.
post-fertilization (dpf) (Saszik, Bilotta, & Givin, 1999). UV opsin (SWS1) is first expressed in the retina by 50 hours post-fertilization (Takechi & Kawamura, 2005). Furthermore, while zebrafish have four major types of cones that are maximally sensitive ($\lambda_{\text{max}}$) to 362-nm, 420-nm, 480-nm, and 560-nm wavelengths, about 25% of the cone photoreceptors in the adult zebrafish retina are maximally sensitive to UV wavelengths (Branchek & Bremiller, 1984; Robinson, Schmitt, Harosi, Reece, & Dowling, 1993). Thus, based on retinal morphology, electrophysiology, and the presence of multiple cone types, including UV cones, we expect that adult zebrafish should exhibit some degree of visual detection of UV stimuli.

However, the number of photoreceptor types in an animal’s retina is not always directly associated with its color vision and perceptual abilities (Jacobs, 1981). Various factors, such as photic conditions and developmental plasticity, may affect how retinal signals from different photoreceptor types are processed in the brain. For example, much like the zebrafish, the goldfish retina contains four different photoreceptor cone types, yet goldfish only exhibit tetrachromatic vision under high intensity lighting, while under lower light levels, they are trichromatic (Neumeyer, 1992; Neumeyer & Arnold, 1989). While it has been known for some time that the zebrafish retina exhibits UV sensitivity ($\lambda_{\text{max}}$: 362 nm), few studies have examined visual behavior in relation to UV stimuli (see Krauss & Neumeyer, 2003; Risner, Lemerise, Vukmanic, & Moore, 2006). Given the wealth of morphological, physiological, and molecular work on the UV components of the zebrafish visual system, we set out to test the adult zebrafish’s ability to visually detect and respond to UV visual stimuli. Herein, we describe the novel application of a robust behavioral assay to assess visual detection of UV cues by adult zebrafish.

It has been suggested that for some taxa, including zebrafish, the visual system processes motion and color stimuli separately, particularly short-wavelength stimuli (Orger & Baier, 2005; Schaerer & Neumeyer, 1996). However, studies suggesting this have only evaluated wide-field visual stimuli, usually vertical square or sinusoidal gratings, using behavioral techniques such as the widely used optomotor response assay (see Orger & Baier, 2005; Schaerer & Neumeyer, 1996). Thus, to test whether adult zebrafish can detect visual cues in the UV portion of the spectrum, we utilized a behavioral assay that exploits the robust escape response (Li & Dowling, 1997) to small-field, UV cues.

**Methods**

**Escape response behavioral assay**

We used an escape response behavioral assay, adapted from Li and Dowling (1997), to test visual detection of UV cues in 20 adult zebrafish, *Danio rerio* (10 males and 10 females). We assayed each fish under two UV lighting trials: UV presence and UV absence. We used a UV light ($\lambda_{\text{max}}$: 365 nm) and a full-spectrum, white light (~400–700 nm, Duro-Test, PA, USA) to illuminate the UV transmitting test cylinder (10.5-cm diameter). We placed an opaque cylinder (3-cm diameter) in the center of the test container to prevent focal fish from moving directly from side to side of the container. For UV-absence trials, we used UV blocking filters (<10% transmission; Edmond Optics, NJ, USA) to block the UV wavelengths (~390 nm) from the same lights (see Figure 1). We measured the spectral irradiance for both UV-presence and UV-absence illuminations using a UV + VIS spectrophotometer (StellarNet) with a cosine receptor. For each trial, we tested two rotating cues presented against a white rotating drum (10 rpm). The positive cue consisted of a UV-reflective square (3 cm × 3 cm) against a non-UV-reflective background (Figure 2). The negative cue consisted of a non-UV-reflective square (3 cm × 3 cm) against a UV-reflective background (Figure 2).

For each trial, we presented the fish with the cue in both clockwise and counterclockwise directions. We counterbalanced the trial testing order (testing half of the fish under UV presence first and half of the fish under UV absence first). During each trial, after a 1-min acclimation
period, we presented the fish with the test cue in each direction for 1 min each. We used a high-resolution, micro-digital camera (Super Circuits) connected to a television monitor to observe the fish’s response. For each trial, we measured and recorded the number of times the cue approached the fish and the number of positive responses (i.e., the number of times the fish would turn and swim away from the cue). We calculated the positive response ratio by dividing the number of positive responses by the number of encounters.

Statistical analyses

We tested each fish under two UV trials for both positive and negative cues; thus, we analyzed the response data using a repeated-measures model, testing the effects of UV trial and cue. This analysis compares each response of all fish simultaneously across both UV trials and both cues, while taking into account that we measured each individual fish repeatedly. We found no evidence of a cue direction effect ($P > 0.05$), testing order effect ($P > 0.05$), nor a sex effect ($P > 0.05$). Thus, we did not include these factors in subsequent analyses. We used residuals to ensure that our data conformed to the usual assumptions of homoscedasticity and normality. All analyses were performed using PASW 17.02.

Results

All of the tested zebrafish showed a robust escape response to both the positive and negative cues when illuminated with UV light. On average, a fish encountered the cues about 11 times during each trial. During the UV-presence trials, on average, fish responded positively (i.e., swimming away from cue) about 7 times to the cue encounters. Yet, during the UV-absences trials, on average, fish responded positively only once. The difference in positive escape responses between UV presence and UV absence was statistically significant ($F = 501.6, df = 1.63, P < 0.001$), indicating that zebrafish were effectively detecting and responding to the UV cues (Figure 3). On average, fish also showed approximately 15% more escape responses to the positive UV cue than to the negative UV cue. This difference was statistically significant ($F = 9.14, df = 1.63, P = 0.004$; Figure 3). Furthermore, fish showed more positive escape responses to the UV positive cue than any other cue, yielding a significant interaction between UV trial and cue ($F = 10.35, df = 1.63, P = 0.002$).

Discussion

Here, we show that adult zebrafish (Danio rerio) can detect and behaviorally respond to UV reflecting visual cues. Zebrafish exhibit a robust escape response, immediately turning and swimming away from UV reflecting visual cues under UV light. However, when presented with the same visual cues under no UV light, zebrafish were unable to detect the UV reflecting visual cues and, thus, did not exhibit an escape response. For both negative and positive cues, the cue-to-background contrast in the UV spectrum was 86% greater than the cue-to-background contrast in the visible spectrum. Thus, the behavioral escape response to the UV cues under UV light was almost certainly mediated by inputs from the UV-sensitive photoreceptor.

![Figure 2. The spectral reflectance of the UV-reflective and non-reflective stimuli used for both cues and backgrounds in the behavioral avoidance assay drum.](image)

![Figure 3. Zebrafish mean positive escape response ratios for positive and negative cues under UV-presence and UV-absence trials. Error bars represent one standard error of the sample means.](image)
cones. These results combined with what is known about the UV components of the zebrafish visual system provide robust evidence that the UV cone photoreceptors from adult zebrafish are functional and can be utilized to visually detect UV stimuli. Our results also highlight the utility and effectiveness of the behavioral escape response assay for examining whether zebrafish, and certainly other fish, can detect and respond to UV and other spectral cues.

In some fish species, only larvae and juveniles exhibit UV visual sensitivity, presumably utilized to detect zooplankton prey (Job & Bellwood, 2007). For some salmonid fish species, UV visual sensitivity in larval fish is used for zooplankton detection (Brown, Novalesflamarique, & Hawryshyn, 1994), while in adult fish, UV sensitivity disappears and exhibits considerable plasticity in association with shifts of habitat and diet (Allison et al., 2003; Hawryshyn, Arnold, Chaisson, & Martin, 1989). Our results show that this is most likely not the case for adult zebrafish. Zebrafish clearly retain their UV photoreceptors well into adulthood (Robinson et al., 1993), and the adult zebrafish retina exhibits electroretinography (ERG) retinal responses to UV wavelengths (Saszik et al., 1999). ERG recordings reveal that adult retinal responses to UV light are considerably less robust than larval responses (Saszik et al., 1999). Yet, even though retinal responses to UV light have been shown to be reduced in adults, our study finds that adult zebrafish can still robustly detect and respond to UV visual cues.

Behavioral methods, such as startle response, optomotor, and operant conditioning assays, are widely and successfully used to assess various aspects of visual function and sensitivity in zebrafish, including screening for retinal mutations (Colwill, Raymond, Ferreira, & Escudero, 2005; Li, 2001; Risner et al., 2006). Li and Dowling (1997) first successfully utilized the escape response assay to quantify the course of dark adaptation in zebrafish and to screen for genetic retinal mutants. Adult zebrafish responded to a dark (black), presumably “threatening”, square cue by robustly swimming in the opposite direction of the cue (Li & Dowling, 1997). The escape response assay has also been successfully used to measure red cone photoreceptor sensitivity (Li et al., 2005). It has been shown that the contrast sensitivity of the escape response in zebrafish can be quite high (Ren, McCarthy, Zhang, Adolph, & Li, 2002). Ren et al. (2002) showed that the zebrafish escape response can be achieved (~70–76% positive responses) to achromatic (gray) stimuli with contrasts as low as 5% under visible light. In our experiments, less than 8% of the zebrafish tested responded positively to both cues with 3% cue-to-background contrasts in the visible spectrum. Furthermore, zebrafish in our experiment robustly exhibited the escape response toward both UV positive and negative cues only under UV light; however, zebrafish responded about 15% more to the UV positive cues (Figure 3). While it is still unknown how exactly UV stimuli are processed beyond the UV photoreceptors in the zebrafish retina, our results suggest some perceptual interaction between sensitivity to UV wavelengths in the retina and higher order processing of UV stimuli. More integrative studies are needed to better understand how exactly UV stimuli are processed within and beyond the retina. Nonetheless, the escape response assay has been shown to be a very useful tool, and our results highlight its potential for assessing visual detection ability using other spectral stimuli and other fish in future studies.

The ecological and adaptive significance of UV visual sensitivity varies greatly across the animal kingdom. Especially in fish, UV visual sensitivity is utilized in a diversity of ecological tasks, from foraging, predator and prey detection, navigation, and social communication (Tovee, 1995). However, for zebrafish, the biological significance of UV visual sensitivity is still unknown. Current studies focusing on the adaptive mechanisms and function of UV sensitivity and discrimination in zebrafish are underway. As the zebrafish continues to gain popularity as the preferred model organism for vertebrate visual system research, in order to better understand how and why the zebrafish visual system functions the way it does, future studies should aim to bridge basic visual behavioral and ecological research with the wealth of physiological, morphological, molecular, and genetic studies on the zebrafish visual system.

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