Diminished foraging performance of a mutant zebrafish with reduced population of ultraviolet cones

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Ultraviolet (UV) cones are photoreceptors that sense light in the range 300–450 nm and are found in the retinas of non-mammalian vertebrates and small mammals. Despite their widespread presence across taxa, the functions that these cones exert in the lives of animals remain largely unknown. In this study, I used the zebrafish lor (lots of rods) mutant, characterized by a diminished UV cone population compared to that of wild-type zebrafish, to test whether its foraging performance differed from that of the wild-type (control). The mean location distance and angle (variables that are reliable indicators of foraging performance) at which control fish detected zooplankton prey were, on average, 24 and 90% greater than corresponding measures for lor fish. Such inferior foraging performance of the mutant could be explained by reduced contrast perception of the prey, resulting from the diminished population of UV cones and associated sensitivity. Thus, UV cones enhance the foraging performance of zebrafish, a crucial ecological function that may explain why small zooplanktivorous fishes retain UV cones throughout their lives.

1. Introduction

The retinas of many vertebrates, including fishes, amphibians, reptiles, birds, monotremes, marsupials and mammals possess a cone photoreceptor that is maximally sensitive to ultraviolet (UV) light, in the range 360–390 nm [1]. This sensitivity is determined by the combination of a UV-sensitive visual pigment protein (SWS1 opsin) and a vitamin A-derived chromophore which, together, form the light-capturing visual pigment expressed in UV cones. Since the discovery of a UV cone in the retina of a cyprinid fish [2], many studies have demonstrated the use of UV light in vertebrate behaviours such as foraging, orientation, mate selection and communication [3–12]. The role of the UV cone in these visually mediated behaviours has, however, remained unknown as previous studies did not determine its action from that of other cone types, and alternative combinations of cone mechanisms (i.e. cone types and their associated circuitries that drive the visual response) can account for UV sensitivity [13,14]. Thus, the functional roles of UV cones have remained largely conjecture in the scientific literature.

It is only recently that an ecological function was demonstrated for the UV cone in the retina of a fish, the rainbow trout [15]. The juveniles of this species hatch with a trichromatic visual system based on cones that are UV, green or M (expressing RH2 opsin) and red or L (expressing LWS opsin) [16,17]. As the juvenile grows, a physiological transformation involving thyroid hormone changes the UV cones into blue (S) cones by switching opsin production from SWS1 to SWS2. Prior to this natural transformation, the juvenile can be treated exogenously with thyroid hormone to induce, prematurely, the opsin switch [18,19]. Using thyroid hormone treatment and the appropriate controls, the UV cone was shown to improve the foraging performance of small zooplanktivorous rainbow trout [15]. Whether such a result holds for species that retain UV cones throughout their lives remains unknown.
Among fishes, there appears to be two major life-history-dependent strategies with respect to the presence of UV cones in the retina. Fishes that are small and zooplanktivorous, like the zebrafish, giant Danio, killifishes and threespine stickleback, retain UV cones throughout life [19–22]. Fishes that grow from small juveniles into much larger adults, like the salmonid fishes and flatfishes, progressively lose their UV cones [17,23]. This progressive loss parallels a change in lifestyle from residence in surface waters (where a full light spectrum with UV wavelengths penetrates) to deeper waters (where UV light is absent or much reduced), and a change in diet from translucent zooplankton to opaque prey. Because the translucent zooplankton that juvenile fish prey upon can absorb or scatter more UV light with respect to the background [15,24,25], thereby enhancing their contrast to a UV-sensitive predator, it has been hypothesized that zooplanktivory is the major determinant driving the retention of UV cones in small fishes [15,26–28].

The zebrafish is a typical, small zooplanktivorous fish that retains UV cones as part of a tetrachromatic visual system based, additionally, on S, M and L cones [19,23,29–31]. Mutation screens carried out in the Fadool laboratory identified the lor (lots of rods) mutant characterized by greatly diminished numbers of UV cones, their place in the photoreceptor mosaic occupied, instead, by rods [32]. Thus, the lor mutant provides a unique opportunity to test whether UV cones improve the foraging performance of zebrafish. If fish with normal numbers of UV cones (controls) are able to detect zooplankton from greater distances and angles compared with lor mutants, then we can conclude that the presence of UV cones is important for the detection of zooplankton, probably owing to contrast enhancement. Here, this hypothesis was tested using behavioural experiments of foraging performance based on a physiological characterization of the lor visual system.

Materials and methods can be found in the electronic supplementary material.

2. Results

(a) Functional characterization of the lor retina

Because lor zebrafish photoreceptors have only been characterized by molecular and immunohistochemical means [32], and there is no knowledge of the absorbance of the visual pigments or the functional output of the retina, I first characterized the photoreceptor visual pigments and spectral sensitivity of this mutant. This knowledge was essential to assess the visual capabilities of the animal. Using microspectrophotometry, I found identical visual pigments in lor versus control fish, with the exception of the UV visual pigment in lor fish which I could not record from since the associated cone type was scarce in the retina. The wavelengths of maximum absorbance ($\lambda_{\text{max}}$) ± s.d. of the visual pigments in the lor retina were: 420 ± 7 nm (S), 478 ± 6 nm (M), 564 ± 6 nm (L) and 498 ± 5 nm (rod) (figure 1b). These values are statistically the same as those previously published for wild-type zebrafish [19,29]. In zebrafish, there are four RH2 isoforms with corresponding $\lambda_{\text{max}}$ of vitamin A1 reconstituted visual pigments ranging from 467 nm (RH2-1) to 505 nm (RH2-4) [33]. Expression of RH2-1 and, especially, RH2-2 ($\lambda_{\text{max}}$: 476 nm) predominate throughout the central retina, whereas RH2–3 ($\lambda_{\text{max}}$: 488 nm) and RH2–4 are confined to small patches in the ventral and peripheral retina [34].

![Figure 1.](http://rspb.royalsocietypublishing.org/) Light backgrounds, and absorbance of visual pigments in lor zebrafish. (a) Spectral backgrounds (downward irradiance and horizontal radiance) used in the foraging experiments. (b) Absorbance of visual pigments in the retina of lor zebrafish (mean of n = 12 each, from three fish). Cone visual pigment abbreviations: S, short wavelength; M, middle wavelength; L, long wavelength. (Online version in colour.)

Table 1. Statistics of foraging experiments (n = 100 per fish group). (ANOVA statistics were $F = 12.15$, $p < 0.0001$ for location distance and $F = 21.54$, $p < 0.0001$ for location angle. lor, lots of rods; FS, full spectrum; 450 LP, 450 nm long pass.)

<table>
<thead>
<tr>
<th>fish group</th>
<th>light background</th>
<th>location distance ± s.d. (mm)</th>
<th>location angle ± s.d. ($^\circ$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>FS</td>
<td>32.4 ± 14.7</td>
<td>73.5 ± 40.4</td>
</tr>
<tr>
<td>lor</td>
<td>FS</td>
<td>26.2 ± 11.0</td>
<td>38.7 ± 36.2</td>
</tr>
<tr>
<td>control</td>
<td>450 LP</td>
<td>22.8 ± 10.2</td>
<td>40.7 ± 27.9</td>
</tr>
<tr>
<td>lor</td>
<td>450 LP</td>
<td>26.5 ± 12.5</td>
<td>45.0 ± 34.4</td>
</tr>
</tbody>
</table>

*Group significantly different from others following ANOVA with SNK and Tukey’s HSD grouping tests.

centro-temporal retina, which mediated the majority of prey attacks (table 1), the predominant opsin transcript is RH2-2 [34], corresponding to the M cone measured by microspectrophotometry in this and other studies [19,29]. Together, these results illustrate the overall dominant expression of the RH2-2 opsin isoform in the zebrafish retina. UV cones were only present sparsely in the central retina (figure 2a,b), though their numbers could increase towards the peripheral retina of some fish (figure 2c,d). There was, however, no regular pattern...
to the presence of UV cones in lor fish. By comparison, control fish had the typical row mosaic arrangement with alternating UV and S cones throughout the retina (figure 2e,f) [19,29]. Cone density measurements from three retinas revealed mean UV : S : M : L cone fractions of 0.08 : 0.9 : 2 : 2 for the lor retina and 1 : 1 : 2 : 2 for the control retina.

When the fish were adapted to the full spectrum background used in behavioural experiments (figure 1a), electroretinogram (ERG) recordings showed responses to the onset (ON) and termination (OFF) of a 500 ms light stimulus that varied with wavelength (figure 3a). The amplitude of the ON response to UV wavelengths was significantly greater for controls compared with lor fish (figure 3a). This translated into a mean spectral sensitivity function which peaked at 360 nm (corresponding to the \( \lambda_{\text{max}} \) of the UV visual pigment) [19,29] for control fish, and lower UV sensitivity for lor fish (figure 3b). By contrast, the OFF response was smaller in the UV region of the spectrum regardless of fish type examined (figure 3a,b), indicating that the UV cone mechanism contributed little to the OFF response under this adapting background.

(b) Foraging performance
I used two measures of prey localization, the location distance and location angle at which fish first detected prey upon launching an attack [15], to assess foraging performance of the two fish groups. Foraging theory predicts that if a given condition improves the foraging performance of an animal (e.g. more UV cones in the retina), then this advantage should translate into overall greater distances and angles at which prey is detected [35]. Free-foraging zebrafish controls detected *Daphnia magna*, a natural prey zooplankton, at significantly greater distances and angles than did their lor counterparts under the full spectrum background (table 1). The mean difference was 6.2 mm for location distance and 34.8° for location angle, corresponding to increases of approximately 24% and 90%, respectively, over the lor means.

Because these results could have arisen from a difference in overall photon catch by cones between the two fish groups (as may be expected from the replacement of UV cones by rods in the lor retina) [32], additional experiments were carried out under a 450 long pass (450 LP) background (figure 1a). The intensity of the 450 LP background was 1.15 times that of the full spectrum background, resulting in an estimated total photon catch of lor fish that was 1.02 times that of control fish under the full spectrum background. Since sensitivity of the retina is positively correlated to stimulus intensity, and hence photon catch, the lor mutants were expected to perform better than controls in the visually driven task of foraging. Yet the mean location distance and angle of lor fish under the 450 LP background remained significantly smaller than equivalent measures for control fish under the full spectrum background, but similar to those of control fish under the 450 LP background (table 1). Therefore, differences in total photon catch could not account for the enhanced prey location capabilities of control fish under the full spectrum background. Perceived contrast of *D. magna*, as estimated from physiological, anatomical, and optical measures provided an explanation for the results.

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Figure 2. Single cones in the retina of control and lor zebrafish. (a,b) Tangential (a) and radial (b) micrographs of single cones in the central retina of lor zebrafish show a minute presence of UV cones (green fluorescence) and overwhelming presence of S cones (red fluorescence). The inner segment of S cones expressed mCherry, whereas the outer segment of UV cones was labelled with a cy 3 fluorophore conjugated to a UV opsin antibody (zfUV). (c,d) Tangential (c) and radial (d) micrographs from the peripheral retina of lor zebrafish illustrating higher incidence of UV cones. (e,f) Tangential micrographs of control zebrafish retina labelled with the zfUV antibody against the SWS1 opsin (e) and with the zfblue antibody against the SWS2 opsin (f). Labelled UV and S cone outer segments are indicated with green and red arrows, respectively; UV and S cone inner segments (unlabelled) are likewise indicated with green and red arrowheads. Magnification bar in (b), 10 \( \mu \)m, applies to all panels. (Online version in colour.)
The greatest contrast of *D. magna* was found for the UV-(L-M) interaction of cone mechanisms under the full spectrum illumination (table 2). Under this background, interactions involving the UV cone resulted in greater perceived contrast for control compared with *lor* fish, in line with the enhanced foraging performance of control fish under this background. Interactions lacking the UV cone resulted in contrasts that were the same (or similar, less than 3% difference) between fish groups under either spectral background and greater under the 450 LP background. Such cone interactions could therefore not account for the foraging trends observed. In addition, the high contrasts found for interactions involving the UV cone under the 450 LP background (which were greater than those found for the UV-M interaction under the full spectrum background) indicate that UV cone input was critical to enhance foraging performance, as supported by the ERG sensitivity results.

### Table 2. Relative contrast of *Daphnia magna* based on known interactions of different cone mechanisms in the retina of zebrafish. (Abbreviations as per table 1.)

<table>
<thead>
<tr>
<th>cone interaction</th>
<th>fish group (light background)</th>
<th>control (FS)</th>
<th><em>lor</em> (FS)</th>
<th>control (450 LP)</th>
<th><em>lor</em> (450 LP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-M</td>
<td></td>
<td>0.60</td>
<td>0.60</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>UV-M</td>
<td></td>
<td>0.27</td>
<td>0.24</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>S-M</td>
<td></td>
<td>0.32</td>
<td>0.32</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>UV-(L-M)</td>
<td></td>
<td>0.99</td>
<td>0.82</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>S-(L-M)</td>
<td></td>
<td>0.14</td>
<td>0.12</td>
<td>0.19</td>
<td>0.17</td>
</tr>
</tbody>
</table>

3. Discussion

Although UV cones have been found in the retinas of most vertebrates that have been studied, their functions have remained elusive. This is because optical isolation of UV cone function during behavioural experiments has proved challenging since all other cone types absorb in the UV region of the visual spectrum (310–400 nm) and because their combined action can mimic that of the UV cone [14]. Previous investigations [5–12,25,36,37] used methods to differentiate between UV-encompassing and UV-excluding stimuli that cut off at wavelengths around 400 nm (often using filters with up to 20% transmission at 380 nm); such methods are inadequate to isolate the action of the UV cone, which absorbs wavelengths above 400 nm. Furthermore, none of these studies measured the spectral sensitivity of the animal and, as such, the UV cone contribution to the results could never be ascertained as the strength of the corresponding retinal signal (i.e. the output of the UV cone mechanism) varies both as a function of spectrum and intensity of background illumination [14,38]. In addition, some of these studies could not account for differences in intensity between treatments [36,37], used intensities that were more than $10^5$ times than that found at crepuscular periods [36] (and such intensities are known to depress the UV cone mechanism [38]), or did not know whether the animals tested had UV cones [9,25]. From such investigations, it is only possible to conclude, given a positive outcome (i.e. if UV wavelengths improve some aspect of behaviour), that UV cones may be involved in the process. The shortcomings in methodology and the absence of spectral sensitivity data do not permit any relevant conclusion as to UV cone involvement or lack thereof in the case of negative results (i.e. if UV light does not make any difference to the behaviour examined). Given the persistent challenges in achieving UV cone isolation and proving its level of activity during behavioural experiments, genetic or environmentally modified animals whose UV cone-driven sensitivity has been altered from the wild-type condition have become crucial to unravel UV cone function.

In this study, I used the *lor* mutant zebrafish to demonstrate that UV cones enhance the performance of zebrafish foraging on *D. magna*, a naturally occurring translucent zooplankton prey. As opposed to control (wild-type) zebrafish, which had UV cones throughout the retina and showed prominent UV cone-mediated sensitivity, the *lor* retina had few UV cones and their contribution to the spectral sensitivity function was minimal or inexistent from ERG recordings. This is because, for the *lor* fish, sensitivity in the UV range was always similar or lower than in the rest of the spectrum and so such could be attributed to absorbance by other cone types [14,38]. If the additional UV
cone-driven sensitivity of control zebrafish was responsible for their superior foraging performance compared to that of lor mutants, then compensation for any reduced photon catch by the mutant (owing to its diminished UV cone numbers) would be inconsequential to the results. This is indeed what was found under the 450 LP background as lor fish consistently exhibited smaller location distances and angles compared with controls. In addition, when controls foraged under the 450 LP background (rendering the UV cones inactive, but stimulating the longer wavelength absorbance of S cones and the α band absorbances of M and L cones), their foraging performance was similar to that of lor fish, further attesting to the importance of the UV cone mechanism in prey detection.

To explain this difference in foraging performance, the visual contrast of D. magus as would appear to a control versus a lor fish was estimated under the various light backgrounds. These computations indicated that UV cones mediated higher perceived contrast of the prey, whereas interactions involving S, M and L cones alone could not account for the foraging results. The lack of, or minor S cone mechanism influence on the foraging results appeared to be supported by the low spectral sensitivity found at 410 nm, regardless of fish type examined. Previous studies using zebrafish have shown lower sensitivity of the S cone mechanism with respect to the UV cone mechanism under light backgrounds that favour isolation of both mechanisms [30,31]. The S cone mechanism was similarly depressed under the spectral backgrounds used in this study. The same trend has been found in juvenile rainbow trout [38], whereby the UV cone mechanism becomes progressively dominant over the S cone mechanism as the light background approaches late evening/crepuscular conditions in nature, as was the case in this study. Also as in rainbow trout [14,38], the UV cone mechanism of zebrafish primarily contributed to the ON response, with comparatively much smaller potential contribution to the OFF response. This suggests that reflections from the prey (as opposed to light absorption by the prey) constitute the principal signal that drives fish detection of zooplankton, especially when viewed against a lower intensity background as was the case in this study.

UV cones may exert other functions in fishes including mate selection and communication [7,11,39]. In addition, a recent study involving mathematical modelling of cell pattern formations suggests that UV cones are necessary to establish the cone mosaic of zebrafish [40]. Although such a developmental role is consistent with the earliest morphological appearance of this cone type and its regular position in the cone mosaic of some fishes [16,41] the expression of SWS1 opsin (as opposed to other opsin types) has not been explained by developmental function. Furthermore, multiple fishes do not exhibit chromatically regular cone mosaics during development, yet still express SWS1 opsin in a subset of localized cones [23]. The observation that UV cones improve the foraging performance of fishes on translucent zooplankton provides an ecological reason for the expression of SWS1 opsin at early life stages [3,15,23,27]. It also explains why many fishes that feed on zooplankton throughout life tend to retain their UV cones, whereas others which grow large, changing diets to opaque prey, and move from life in surface to deeper waters, tend to lose them [17,23,42].

Lens absorption in fishes appears to change in accordance with the UV cone fate in that small zooplanktivorous fishes with UV-violet visual pigments have full spectrum transmitting lenses, whereas the lenses of those species that grow large and lose UV cones become progressively opaque to the UV spectrum [43–46]. One study suggests that adult lifestyle, more than zooplanktivory, may shape the opacity of lenses in reef fish larvae [47], though even in this study the larvae of zooplanktivorous species had lenses with greater UV transmission than those of non zooplanktivorous species. Additionally, in species that are constantly exposed to full sunlight during the larval stages, lens transmittance may have evolved as a balance between the advantages of UV vision and the risk of tissue damage by UV radiation [28,45,46]. Thus, within the potential constraints of deleterious short-wavelength UV radiation exposure, both optical and retinal components appear to have evolved to optimize foraging on zooplankton during the early juvenile life stages of fishes, a crucial period when fast growth is the primary determinant of survival [48].

Ethics. All animal use was approved by the Animal Care committees of Simon Fraser University (protocol no. 1126B-10) and the University of Victoria (protocol no. 2013-005), which abide by regulations set by the Canadian Council for Animal Care.

Data accessibility. Supporting data have been included in the electronic supplementary material.

Competing interests. I have no competing interests.

Funding. This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant 238889.

Acknowledgements. I thank Dr Rachel Wong for the lor zebrafish, Dr David Hyde for the opsin antibodies and Lisa Grebinsky for logistical help with the foraging experiments.

References


