Opsin switch reveals function of the ultraviolet cone in fish foraging

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Although several studies have shown that ultraviolet (UV) wavelengths are important in naturally occurring, visually guided behaviours of vertebrates, the function of the UV cone in such behaviours is unknown. Here, I used thyroid hormone to transform the UV cones of young rainbow trout into blue cones, a phenomenon that occurs naturally as the animal grows, to test whether the resulting loss of UV sensitivity affected the animal’s foraging performance on *Daphnia magna*, a prey zooplankton. The distances and angles at which prey were located (variables that are known indicators of foraging performance) were significantly reduced for UV knock-out fish compared with controls. Optical measurements and photon-catch calculations revealed that the contrast of *Daphnia* was greater when perceived by the visual system of control versus that of thyroid-hormone-treated fish, demonstrating that the UV cone enhanced the foraging performance of young rainbow trout. Because most juvenile fishes have UV cones and feed on zooplankton, this finding has wide implications for understanding the visual ecology of fishes. The enhanced target contrast provided by UV cones could be used by other vertebrates in various behaviours, including foraging, mate selection and communication.

1. Introduction

Vertebrate retinas have specialized cells, termed photoreceptors, whose visual pigments capture light to begin the process of vision [1,2]. There are two types of photoreceptor in the outer retina, rods and cones, which mediate vision in the dark and light, respectively. Each visual pigment is composed of a protein (opsin) bound to a chromophore (a derivative of vitamin A) [3]. Although all rods in a given retina express up to two visual pigment opsins, cones may express up to four different classes of opsins resulting in visual pigments maximally sensitive in the ultraviolet (UV), short (blue), middle (green) or long (red) wavelength regions of the visual spectrum [2,3]. It is the combination of different spectral cone types, each expressing a predominant opsin, that mediates colour vision [1].

Until 30 years ago, when the first UV cone was discovered in the retina of a fish [4], vertebrate colour vision was thought to rely on a combination of short (S), middle (M) and long (L) wavelength-sensitive cones, as is the case in humans [1,2]. Three decades of investigations have shown, however, that the majority of non-mammalian vertebrates and small mammals that have been studied possess UV cones at some point in their lives [2,5]. In some species, these cones are present only during development and at early life stages [6–10], whereas in others, they persist throughout life [3,7,11]. Although UV cone presence has been identified in all major vertebrate taxa, from fishes to rodents to marsupials [2,3,12], no function of a UV cone in a naturally occurring, visually guided behavioural task has yet been demonstrated.

Multiple studies using fishes [13–16], amphibians [17], reptiles [18,19] and birds [20–22] have shown that UV wavelengths are important in foraging, mate selection, communication and/or orientation of these animals. Most of these works have relied on spectral filters with different light transmission properties to show that UV wavelengths are important for a given behavioural...
task. The filters, however, did not completely isolate the action of the UV cone from that of other cone types, and the absorbance of several cone types (UV, S and, sometimes, violet) straddled the cut-off wavelength (approx. 400 nm) that differentiated treatments. As such, the differences in results reported could have been due to the combined action of S (or violet) and M or L cones working as part of a chromatic discrimination pathway, without the involvement of UV cones. Furthermore, the topographical organization of spectral cone types and their visual pigments in the retinas of all but one species (the rainbow trout; [23]) used in these experiments remains unknown [5]. Because regionalization of spectral cone types occurs in vertebrate retinas and because cones can express multiple opsins [3,7,9–11,23,24], the involvement of a UV cone could further not be ascertained from such experiments. In conclusion, although there is evidence to suggest that UV cones are involved in natural visual behaviours of vertebrates, proof has remained elusive.

In this study, I tested whether the UV cone plays a role in the foraging performance of rainbow trout, a salmonid fish with UV-cone-mediated vision as a juvenile [25]. Previous research has demonstrated that salmonid fishes hatch with two types of morphological cone: singles, which are UV sensitive, and doubles, which are M/L pairs [10]. As the fish grows from a small juvenile (alevin), to a large juvenile (smolt), the single cones progressively switch opsins from SWS1 (UV) to SWS2 (blue), as part of a transformation in which the animal becomes tetrachromatic temporarily, possessing UV, S and M/L cones, and ends up as a new trichromat, possessing S and M/L cones [8–10,23,26]. The opsin switch in the single cones of alevins can be induced prematurely by exogenous treatment with thyroid hormone [27,28]. Within two weeks of such treatment, all the single cones in the alevin retina express SWS2 opsin, switching from UV to S spectral phenotype, whereas the double cones remain M/L pairs [27,28]. Thus, thyroid hormone can be used to create a functional knock-out of the SWS1 opsin while maintaining the other cone spectral phenotypes intact.

The UV cone of salmonid fishes is present only during the embryonic and early juvenile period [9,10], when the fish feeds on zooplankton [29]. The spectral transformation of single cones from UV to S parallels a progression from life in surface waters, where a full spectrum of light is present, to deeper waters, where UV light is significantly reduced or absent [25]. It is also around this time that the fish begins to switch diet from translucent zooplankton to larger, more opaque prey, like other fish [29]. These observations suggest an ecological function of UV cones in providing enhanced contrast perception of zooplankton prey, beyond that which could be achieved with other cone types. If such is the case, then the foraging performance of alevins with UV cones should be significantly enhanced compared with that of thyroid-hormone-treated equivalents, whose UV cones have been transformed to S cones [27,28].

2. Material and methods

(a) Animals and treatments

Wild stock rainbow trout (Oncorhynchus mykiss) alevins were obtained from the Lower Fraser Trout hatchery (Abbotsford, British Columbia, Canada). Fish were partitioned into two identical holding tanks with flow-through water at a temperature of 7°C, and experienced a 12:12 L:D cycle during the study. One group was exposed to L-thyroxine (T4, Sigma) for two weeks. The hormone was dissolved in 0.1 M NaOH and added to the water to a final concentration of 300 μg L⁻¹ (treatment). The other (control) group was treated identically but only the vehicle solution (0.1 M NaOH) was added to the water [27].

(b) Foraging experiments

I used silhouette video photography to film both fish groups free foraging on Daphnia magna, a natural prey zooplankton of rainbow trout [29]. The system was configured for imaging in the vertical plane, with a resolution of approximately 0.2 mm and a depth of field of 15 cm, as reported previously [13]. Each experiment consisted of filming three new fish at a time foraging for 30 min on D. magna in a 30 × 30 × 30 cm glass aquarium (filled to a depth of 15 cm and with the sides covered in black tape) following 1 h of acclimation. The fish were starved for 24 h prior to testing, and experiments were performed during the day, i.e. during the light phase of the animal’s circadian rhythm. Prey concentration in all experiments was 5 × 10⁷; and prey size was statistically the same (at α = 0.05) between experiments; the overall mean Daphnia carapace length ± s.d. was 1.3 ± 0.28 mm (n = 600). Likewise, fish size was statistically the same between experiments; the mean total length ± s.d. was 4.0 ± 0.26 cm (n = 90). During experiments, video frame acquisition was 30 s⁻¹, and the analysis of prey attack sequences was carried out frame by frame.

Like other fishes, the rainbow trout alevin searches for prey using pause–travel movements whereby the animal combines stationary periods of scanning for prey with repositioning, swimming, movements. Foraging behaviour was evaluated by measuring the prey location distance (LD) and angle associated with each attack (figure 1a). Prey LD was defined as the distance between the point at which the fish first reacted to the prey and the position of the prey itself. All attack sequences resulted in prey capture, an event characterized by fish opercular expansion. From this event, clearly identifiable on the recording, the video was backtracked to the point when the fish first spotted the prey and initiated the attack. This moment, also clearly visible on the recording, was characterized by a quick change in the fish’s head direction, increased swimming velocity and realignment of the body axis with the prey (figure 1a). The location angle (LA) was the angle between the longitudinal body axis of the fish just prior to attack initiation and the line connecting the fish’s rostrum and the position of the prey upon attack initiation. The movement parameters (distance and angle) associated with prey location are strongly correlated with the visual capabilities of the animal. If conditions improve prey visibility, then the distances and angles at which prey is located increase [13]. As such, these variables have been used as reliable indicators of fish foraging performance [13,30].

Experiments were conducted under a low intensity full light spectrum characteristic of late evening/crepuscular periods [25], or under long or short wavelength backgrounds (figure 1b). These were achieved with a 150-W Xenon lamp (Photon Technology International) whose output traversed a combination of neutral density and barrier filters (Delta Photonics) and a diffuser, to illuminate the observation aquarium quasi-uniformly from above. Irradiance measurements were acquired with a USB-2000 spectroradiometer equipped with a 600 μm diameter input, 0.22 NA, liquid light guide and a cosine collector (Ocean Optics), and without the cosine collector in the case of radiance measurements. The spectral backgrounds were chosen because: (i) salmonid foraging and the relative sensitivity of the UV cone increase in low light conditions [25], (ii) the absorbance of
both the control and treatment fish. Results were analysed
experiments were carried out for each spectral background for
mone (besides altering the absorbance of visual pigments) that
background constituted a control for any other effect of the hor-
the results and (iii) experiments under the long wavelength
allowing for a clear assessment of the UV cone contribution to
the limiting wavelength between treatments ([Delta] Photonics). This background illumination was used to
adapt the M and L cone mechanisms (i.e. the retinal pathways
associated with the M and L cones) so as to reveal any differ-
ences in UV and short wavelength sensitivity between control
and thyroid-hormone-treated fish. The stimulus channel pro-
vided a 500 ms flash stimulation of variable intensity and
wavelength, and consisted of a 150-W xenon source coupled
to a monochromator (Photon Technology International), neutral
density wheel (ThorLabs) and shutter (Vincent Associates), all
under computer control. Response amplitude versus light
intensity curves were generated for selected wavelengths
across the spectrum 360–650 nm following a 2 h adaptation
of the fish to the background light. A third degree polynomial
was fitted to the data, and a criterion voltage potential
(30 [mu]V) chosen that was in the linear part of the response–
intensity curve for all wavelengths. The sensitivity at each
wavelength was computed, as the inverse of the intensity
required to reach the criterion response. Average spectral sensi-
tivity responses were fitted with the simplex algorithm [31]
using a model equation [32] with absorbance spectra of the
visual pigments as inputs [25]. The absorbance spectra
were generated using an eighth-order polynomial template [33]
with the maximum wavelength of absorption of visual pig-
ments obtained by microspectrophotometry. Using template-
derived absorbance spectra, as opposed to those obtained
experimentally, ensures proper \beta-band representation, which
is not always the case from microspectrophotometry records.

the UV cone (none beyond [lambda] approx. 460 nm) did not straddle
the limiting wavelength between treatments ([lambda] approx. 500 nm)
allowing for a clear assessment of the UV cone contribution to
the results and (iii) experiments under the long wavelength
background constituted a control for any other effect of the hor-
more (besides altering the absorbance of visual pigments) that
could have influenced foraging performance. Five replicate
experiments were carried out for each spectral background for
both the control and treatment fish. Results were analysed
using f-tests.

(c) Optic nerve recordings
Representative fish from each group (n = 3) were used to eval-
uate spectral sensitivity at the level of the optic nerve. This was
achieved by surgically exposing the optic tectum of anaesthe-
tized fish (irrigated through the mouth with water containing
25 mg l\(^{-1}\) of buffered MS-222) and inserting a Teflon-coated
electrode with an exposed silver tip through the optic tectum
into the optic nerve to monitor extracellular compound action
potentials [25]. Recordings from this electrode were subtracted
from those of a reference electrode inserted into the nasal
fissure, pointing downward and perpendicular to the bottom
of the grid. This placement procedure ensured the analysis of
similar pieces of retina within and between treatments.
Each retina was then cut into four pieces corresponding to
dorsal, ventral, temporal and nasal sectors, and each piece
embedded in O.C.T. frozen blocks for in-situ hybridization as
per previous studies [9,23]. Sections (7 [mu]m thick) obtained
from the blocks were collected serially and deposited on slides
for double labelling with the UV and blue riboprobes, whose
specificity has been described previously [9,23]. The primers
and method used to generate riboprobes as well as the in-situ
hybridization protocol have also been published previously [9].
Digital images of sections were acquired with an E-600
Nikon microscope equipped with a DXM-100 digital camera
and DIC optics.

(d) In situ hybridization
Individual fish used for spectral sensitivity recordings were
sacrificed at the end of the experiment, in the light-adapted
state, by quick spinal bisection and decerebration. The left eyeball
was removed, the iris and lens discarded and the remaining
eyecup immersed in cryo-fixative (4% paraformaldehyde in
0.08 M phosphate buffer, pH 7.4). After 24-h fixation at 4°C,
the retina was extracted from the eyecup, flattened by making
small peripheral incisions, and laid over a grid with the optic
nerve bud at the centre of the grid, and the tip of the embryonic
fissure pointing downward and perpendicular to the bottom
of the grid. This placement procedure ensured the analysis of
similar pieces of retina within and between treatments.
Each retina was then cut into four pieces corresponding to
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hybridization protocol have also been published previously [9].
Digital images of sections were acquired with an E-600
Nikon microscope equipped with a DXM-100 digital camera
and DIC optics.

(e) Microspectrophotometry
Individual fish from each group were dark-adapted overnight.
Following this adaptation period, the fish was killed, one
eye enucleated and the retina removed under infrared illumina-
tion. Small pieces of retina were teased apart and prepared for
viewing with the dichroic microspectrophotometer (DMSP) as
per previous studies [9,34]. The DMSP is a computer-controlled,
wavelength-scanning, single-beam photometer that simulta-
nuously records average and polarized transmitted light

Figure 1. Illustration of foraging search parameters, and lighting conditions
during the experiments. (a) Schematic of a typical rainbow trout search
path and attack sequence illustrating the parameters measured to analyse
rainbow trout foraging performance. The solid dots along the line
represent stationary pauses whereas the open circle indicates a prey item.
(b) Downwelling irradiances (upper traces), and average sidewelling
(horizontal) radiances (lower traces), used in the foraging experiments, as
measured at the centre of the aquarium. Each horizontal radiance is the
average of four scans corresponding to measurements along the four sides of
the aquarium. FS, full spectrum; SW, short wavelength spectrum; LW, long
wavelength spectrum.

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fluxes through microscopic samples [34,35]. The DMSP was equipped with ultralum (Zeiss) objectives: 32/0.4 for the condenser and 100/1.20 for the objective. With the aid of reference measurements recorded through cell-free areas, individual photoreceptor outer segments were illuminated side-ways with a measuring beam of rectangular cross section of cr 2 × 0.6 μm. Absolute absorbance spectra were computed in 2 nm increments from the obtained transmittances (each spectrum consisted of an average of eight scans). The solid spectra (fits) were derived from experimental data by Fourier filtering [35].

The microspectrophotometer objective was replaced by a Zeiss ultralum 40/0.7 lens to acquire measurements through the body of live D. magna (n = 10). During recordings, the animals were held motionless, in water, between two sealed coverslips. From these measurements, the average light transmittance through the body of Daphnia was computed and used in the calculations of photon catch.

(f) Photon catch of cone photoreceptors and visual contrast estimates

Pigment absorbance spectra generated using the eighth-order polynomial template [33] were used to compute absorptance using the equation: absorptance = 1 – 1/(absorbance)+30, where S is the transverse specific density, and l is the average outer segment length of each cone type in alevin rainbow trout (S and l values were obtained from [36] and [5], respectively). The absorbance values were multiplied by the irradiance spectra (figure 1b), in radiometric units, and lens transmittance [37], and integrated to give the photon catch for each cone type under the various downwellling conditions. These values were normalized with respect to that of the cone spectral type with the lowest photon catch and used as measures of the adaptation state of each cone mechanism in each foraging experiment. Photon catches from the target (Daphnia) and background (horizontal radiance; figure 1b) were multiplied by these factors to correct for adaptation state.

The photon catch of each cone type originating from the Daphnia was calculated as the sum of photon catches from transmitted horizontal light through the Daphnia and scattered downwelling light by the Daphnia in the horizontal direction. The first component involved the product of horizontal radiance and light transmission through the body of Daphnia. The second involved the product of downwelling irradiance, and a published reflectance spectrum for D. magna obtained under a geometrical set-up relevant to this study [38]. Cone fractions did not affect photon-catch calculations as the density ratios for UV, M and L cones (in controls) and S, M and L cones (in thyroid-hormone-treated fish) were 1 : 1 : 1. Perceived contrast of the Daphnia over a given background (horizontal radiance) by alevin rainbow trout was calculated based on published interactions of cone mechanisms in cyprinid fishes [39,40]. Spectral sensitivity curves from these species indicate two common antagonistic interactions (L–M and S–M), whereas UV and S outputs were found to be additive or subtractive (i.e. UV + S or UV – S). In these works, the fishes had both UV and S cone mechanisms, being UV+S,ML tetrachromats (some fishes, such as goldfish, have single L cones in addition to M/L double cones [5]), whereas in the present study the fish were either UV+M/L (controls) or S,M/L (thyroid-hormone-treated) trichromats. Nonetheless, the spectral sensitivity functions showed some antagonistic interactions between the S and M (or UV and M) cone mechanisms, and between the L and M cone mechanisms, as featured in the literature [25,39,40]. Thus, contrasts were computed according to the following interactions: L–M, UV–M or S–M, and UV–(L–M) or S–(L–M). The latter two contrasts represent a general antagonistic interaction between the output of the single cone mechanism (UV or S) and that of the double cone mechanism (L–M). For a given experimental lighting condition, contrast was calculated as: (P_Daphnia – P_Background)/(P_Daphnia + P_Background), where P refers to photon catch according to a given interaction of cone mechanisms.

3. Results

The vast majority (over 98%) of the single cones in the retinas of control fish expressed SWS1 opsin mRNA (figure 2a), and absorbance measurements from these cones revealed a UV visual pigment with maximum wavelength of absorbance (λ_{max}) at 386 ± 5 nm (mean ± s.d., n = 18; figure 2c). By contrast, all the single cones from thyroid-hormone-treated fish expressed SWS2 opsin mRNA (figure 2b) and had a visual pigment with λ_{max} at 441 ± 6 nm (n = 21; figure 2c). There were no differences in cone densities between groups, but treatment with thyroid hormone increased the λ_{max} of the M and L cones from 498 ± 6 nm (n = 32) and 563 ± 5 nm (n = 28) in controls to 540 ± 6 nm (n = 26) and 620 ± 7 nm (n = 31) in thyroid-hormone-treated fish.

Assuming the visual pigments in control fish to be vitamin A1-based, Härösi’s [41] models can be used to compute the λ_{max} of their vitamin A2 conjugates, which are: 397 nm (UV), 521 (M) and 632 (L). Thus, a vitamin A2 shift, without a switch in the opsins, can account only for the λ_{max} of the L visual pigment in thyroid-hormone-treated fish. This is in line with the in situ hybridization results, where the single cones of thyroid-hormone-treated fish labelled solely with the SWS2 riboprobe, and with our unpublished observations of two RH2 opsins, expressed sequentially in juvenile and adult salmonids. Multiple SWS1 opsins have not been identified in salmonid fishes, and the mean λ_{max} of the UV pigment found here is close to the vitamin A2 conjugate (λ_{max} = 386 nm) predicted in another study [36]. The mean L pigment λ_{max} range found (563–620 nm) resembles that observed in other fishes and denotes a range of vitamin A1 to vitamin A2-dominated pigments [41].

Electrophysiological recordings from the optic nerve of anaesthetized live fish revealed a UV (but no S) spectral sensitivity peak in control fish and an S (but no UV) spectral sensitivity peak in thyroid-hormone-treated fish (figure 2d). Under the long wavelength (A greater than 530 nm) adapting background, the peak (α band) sensitivities of the M and L cone mechanisms were more than five times lower than those of the UV and S cone mechanisms. Thus, the sensitivity peaks in the UV and short wavelength regions were indeed driven by UV and S cone input, respectively, as the secondary (β band) sensitivities of the M and L cone mechanisms, which also absorb in the UV and short wavelengths, were approximately four times lower than those of their respective α bands. Thus, thyroid hormone treatment knocked out SWS1 opsin expression and the associated UV sensitivity.

The mean LDs and LAs were significantly greater for control compared with thyroid-hormone-treated fish under the full spectrum illumination (table 1). Likewise, the means of control and thyroid-hormone-treated fish were significantly greater under the short versus the long wavelength background. Comparison of means between control
and thyroid-hormone-treated fish under the short or the long wavelength background showed that they were not significantly different (table 1).

Light measurements acquired from different body parts of *D. magna* consistently revealed higher absorbance in the UV region of the spectrum (figure 3). Regardless of the interaction of cone mechanisms considered, the contrast of *Daphnia* under the full spectrum illumination was greater for control (UV–M: 0.47; UV–(L–M): 0.059) than for thyroid-hormone-treated fish (S–M: 0.42; S–(L–M): 0.013). In either case, the contrast difference was approximately 5 per cent. The same calculations in the case of the short wavelength background revealed a perceived contrast difference between control and treatment fish of approximately 1 per cent (for controls, UV–M: 0.026; UV–(L–M): 0.023; for thyroid-hormone-treated fish: S–M: 0.015; S–(L–M): 0.034). Under the long wavelength background, only the double cones were substantially stimulated and, based on an L–M interaction, the perceived contrast of controls was approximately 4 per cent greater than that of thyroid-hormone-treated fish.

4. Discussion

This study provides the first demonstration of a UV cone function in a naturally occurring, visually guided behaviour of a vertebrate, namely in the foraging of alevin rainbow trout. Under the full light spectrum, control fish (UV,M/L trichromats) located prey at greater distances and angles than thyroid-hormone-treated fish (S,M/L trichromats). Such enhanced foraging performance, as indicated by these variables [30], was most likely due to the presence of a UV cone in control fish, leading to greater perceived contrast of *Daphnia*.

Regardless of the interaction of cone mechanisms considered, the perceived contrast of *Daphnia* by control fish was approximately 5 per cent greater than that by thyroid-hormone-treated fish under the full spectrum illumination. In comparison, the contrast of *Daphnia* was only 1 per cent different between control and thyroid-hormone-treated fish when foraging under the short wavelength background. Such a small difference, which varied in polarity between the two fish groups depending on the interaction
of cone mechanisms considered, likely explains the statistically similar results obtained under this background, i.e. the contrast difference was too small to elicit any change in foraging behaviour. This conclusion is in line with results from several fishes where the minimum luminance contrast required for the detection of spatial gratings is 3–5% [42].

Thyroid hormone treatment also altered the absorbance of the M and L cones toward longer wavelengths such that, under the long wavelength background, the contrast difference between fish groups, based on the L–M interaction, was approximately 4 per cent. Despite this, foraging performance was not significantly different between control and thyroid-hormone-treated fish. Furthermore, under both the full spectrum and the short wavelength spectrum, contrast based on the L–M interaction was greater for thyroid-hormone-treated fish over controls, by 12 per cent (under the full spectrum) and 30 per cent (under the short wavelength spectrum). These calculations predict the opposite trends in foraging performance to those observed, indicating that the L–M interaction could not account for the differences in foraging behaviour between fish groups. When both single and double cones were stimulated under a short wavelength background, foraging performance significantly increased over that under the long wavelength background for either fish group (table 1). This result demonstrates that a trichromatic visual system involving single cone input (UV or S) was critical to enhance foraging performance.

Besides affecting opsin expression and visual pigment absorbance, thyroid hormone could have induced other physiological changes in rainbow trout alevins that may have impacted their foraging performance. If such were the case, however, control and thyroid-hormone-treated fish would have differed in their foraging performance under the long wavelength background. This was not observed, implying that any additional effects of the hormone did not alter foraging behaviour. Taken together, the foraging results and contrast calculations demonstrate that the UV cone was responsible for the enhanced foraging performance of control fish.

Like the rainbow trout, the vast majority of fishes that have been studied possess UV cones as small juveniles [6,7,9,10,41], spanning the life stages when they feed on zooplankton [13,29]. Fishes that remain small and zooplanktivorous throughout their lives (e.g. the zebrafish, giant danio, killifishes, sticklebacks) retain their UV cones [7,34,41,43], whereas those that grow large, such as the salmonid fishes, and change their diets from zooplankton to larger opaque prey (e.g. other fish) lose their UV cones as they grow [7,9,10], unless they may be used for other purposes, such as communication [15]. The present results should be applicable to other zooplanktivorous fishes and of interest in marine fish aquaculture where live zooplankton are used as larval feed [44]. Marine fish larval survival in aquaculture is poor (typically less than 10%) and a low light rearing environment containing UV/short wavelength may decrease larval mortality. More generally, the enhanced contrast provided by UV cones should apply to other vertebrates equipped with these photoreceptors and

<table>
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<tr>
<td>LD SW control</td>
<td>25.2 (9.97)</td>
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<tr>
<td>LA SW control</td>
<td>59.6 (38.3)</td>
<td>4.92</td>
<td>331</td>
<td>&lt;0.001</td>
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<tr>
<td>LA SW control</td>
<td>40.6 (32.0)</td>
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<tr>
<td>LD SW TH</td>
<td>32.9 (14.4)</td>
<td>3.12</td>
<td>246</td>
<td>0.002</td>
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<tr>
<td>LD LW TH</td>
<td>27.4 (13.6)</td>
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<tr>
<td>LA SW TH</td>
<td>62.2 (35.7)</td>
<td>4.71</td>
<td>246</td>
<td>&lt;0.001</td>
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<tr>
<td>LA LW TH</td>
<td>41.3 (33.9)</td>
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could be used in many types of behaviour including foraging, mate selection and communication.

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References


Figure 3. Absorbance of *Daphnia magna* body parts and photographic appearance under various spectral backgrounds. (a) *Daphnia magna* photographed using transmitted light from the Xenon source. Magnification bar = 0.25 mm. (b) Absorbance spectra from various regions of the *Daphnia* body illustrated in (a). (c,d) The same *D.* *magna* photographed using the filters that produced the long wavelength (LW) and short wavelength (SW) backgrounds, respectively.


31. Gaze MS, Cacheris WP. 1984 Fitting curves to data, the simplex algorithm is the answer. Byte 5, 340 – 360.


