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RESEARCH ARTICLE

Ontogeny in the visual system of Nile tilapia

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SUMMARY

Retinal neurogenesis in fish facilitates cellular rearrangement throughout ontogeny, potentially allowing for optimization of the visual system to shifts in habitat and behaviour. To test this possibility, we studied the developmental trajectory of the photopic visual process in the Nile tilapia. We examined ontogenetic changes in lens transmission, photoreceptor sensitivity and post-receptoral sensitivity, and used these to estimate changes in cone pigment frequency and retinal circuitry. We observed an ontogenetic decrease in ultraviolet (UV) photoreceptor sensitivity, which resulted from a reduction in the SWS1 cone pigment frequency, and was associated with a reduction in lens transmission at UV wavelengths. Additionally, post-receptoral sensitivity to both UV and long wavelengths decreased with age, probably reflecting changes in photoreceptor sensitivity and retinal circuitry. This novel remodelling of retinal circuitry occurred following maturation of the visual system but prior to reaching adulthood, and thus may facilitate optimization of the visual system to the changing sensory demands. Interestingly, the changes in post-receptoral sensitivity to long wavelengths could not be predicted by the changes observed in lens transmission, cone pigment frequency or photoreceptor sensitivity. This finding emphasizes the importance of considering knowledge of visual sensitivity and retinal processing when studying visual adaptations and attempting to relate visual function to the natural environment. This study advances our understanding of ontogeny in visual systems and demonstrates that the association between different elements of the visual process can be explored effectively by examining visual function throughout ontogeny.

Key words: vision, fish, photoreceptor, development, retina, cichlids.

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INTRODUCTION

Ontogeny, the developmental history of an organism throughout its lifetime, is a key programme that affects all organisms. During ontogeny, organisms may shift habitat, food source and behaviour. The teleost fish retina is an important model system for studies of ontogeny, neural plasticity and neurogenesis. In teleosts, unlike in mammals and other vertebrates (Carter-Dawson and LaVail, 1979), the eye grows throughout life, and new neurons, generated by retinal stem cells, are continuously added to the growing retina (Easter and Hitchcock, 2000; Otteson and Hitchcock, 2003). This neurogenesis in the fish retina facilitates cellular rearrangement throughout ontogeny, potentially allowing for optimization of the visual system to shifts in visual demands.

Ontogenetic changes in several elements and processes in the visual system of fish have been described. The visual process begins with light being transmitted through the ocular media to the retina. In fish, the transmission of the ocular media is typically determined by the transmission of the lens (Losey et al., 2003), which has been reported to change during ontogeny in several fish species (Losey et al., 2000; Nelson et al., 2003; Siebeck and Marshall, 2007; Thorpe et al., 1993). Within the retina, visual pigments absorb light and initiate a phototransduction cascade that leads to neural signalling and ultimately behaviour. Visual pigments are light-sensitive molecules found in the outer segments of photoreceptors and consist of a vitamin A-based chromophore bound to an opsin protein. Four cone opsin classes have been identified in vertebrates: ultraviolet sensitive (SWS1), short-wavelength sensitive (SWS2), rhodopsin-like mid-wavelength sensitive (Rh2) and long-wavelength sensitive (LWS) (Yokoyama, 2000). Cone opsin gene expression (Carleton et al., 2008; Cheng and Novales Flamarique, 2004; Shand et al., 2008; Spady et al., 2006; Veldhoen et al., 2006) and the frequency of cone photoreceptors (Allison et al., 2003; Braekevelt, 1985; Braekevelt, 1988a; Braekevelt, 1988b; Carleton et al., 2008; Evans and Fernald, 1993; Evans et al., 1993; McFarland and Loew, 1994; Shand, 1993; Shand et al., 2008; Shand et al., 1988) were reported to vary throughout the life history of several fish species. Additionally, spectral sensitivity, which is the relative efficiency of detection of light as a function of wavelength, was also reported to change during the ontogeny of some fish species (e.g. Hawryshyn et al., 1989). These alterations in spectral sensitivity were sometimes associated with a loss of certain cone classes and changes in the spatial arrangement of photoreceptors throughout the retina, i.e. cone photoreceptor mosaic (Allison et al., 2010; Allison et al., 2003; Allison et al., 2006; Shand et al., 1999).

By examining the visual system throughout ontogeny, the function of a given element/process in the visual system and its effect on other elements/processes can be explored. For example, alterations in cone opsin gene expression (measured using reverse transcription-quantitative PCR, RT-qPCR) during ontogeny were qualitatively shown to accompany alterations in the frequency of cone photoreceptors (measured using microspectrophotometry) (Allison et al., 2003; Carleton et al., 2008; Shand et al., 2008). However, most previous studies have looked at only one or two elements/processes in the visual system, making it difficult to draw

association with other elements and levels of processing. With this in mind, we studied the developmental trajectory of visual function in fry, juvenile and adult Nile tilapia through the examination of six elements: (i) lens transmission, (ii) sensitivity of photoreceptors, (iii) cone pigment frequency – estimated based on photoreceptor sensitivity, (iv) geometry of the cone mosaic, (v) post-receptoral sensitivity, and (vi) retinal circuitry – estimated based on comparison of photoreceptor and post-receptoral sensitivity. The Nile tilapia (*Oreochromis niloticus*; Cichlidae) is thought to be the riverine ancestor of the cichlid fishes of the East African Great Lakes, which represent the largest vertebrate radiation on Earth (Genner et al., 2007). It is one of the world's most heavily cultured fish species and recently has gained significance as a vertebrate model for biological research (El-Sayed, 2006).

The visual system of the Nile tilapia has been the subject of several past studies. Lens transmission in tilapia was shown to change throughout ontogeny, with the wavelength of half-maximum transmission (T_{50}) increasing with lens diameter (Thorpe and Douglas, 1993). However, it is unknown whether these changes in lens transmission are accompanied by changes in other elements of the visual system. Additionally, all four classes of cone opsin genes identified in vertebrates have been found in the Nile tilapia. However, in the tilapia, further gene duplications have resulted in numerous cone opsin genes that produce seven spectrally distinct cone pigments with differing peak sensitivities: SWS1 360 nm, SWS2b 425 nm, SWS2a 456 nm, Rh2b 472 nm, Rh2aβ 518 nm, Rh2aα 528 nm and LWS 560 nm (Spady et al., 2006). The Nile tilapia has been shown to express different complements of opsin genes throughout ontogeny, with notable changes in *SWS1* and *LWS* (Carleton et al., 2008; Spady et al., 2006). These ontogenetic changes in opsin gene expression agree qualitatively with alterations in the frequency of cone photoreceptors (Carleton et al., 2008). Spectral sensitivity, in contrast, was measured only for tilapia adults (Lisney et al., 2010); thus, the relationships between ontogenetic changes in spectral sensitivity and those in other elements of the visual system are currently unknown. Further, in tilapia, short-wavelength sensitive pigments (SWS1, SWS2b and SWS2a) reside in single cones, whereas long-wavelength sensitive pigments (Rh2b, Rh2aα, Rh2aβ and LWS) reside in double cones (Carleton et al., 2008). Adult tilapia have cone photoreceptors arranged in a square mosaic, with a single cone surrounded by four double cones (Braekevelt et al., 1998; Lisney and Hawryshyn, 2010); however, the pattern in fry and juveniles is yet to be studied. Lastly, considering the persistent retinal neurogenesis in fish, it may be that the circuitry, i.e. the synaptic and electrical coupling between different retinal cells, also changes throughout ontogeny. Such an ontogenetic change in circuitry has not been reported previously.

Here, we show that Nile tilapia undergo ontogenetic changes in lens transmission, and photoreceptor and post-receptoral sensitivity. An ontogenetic decrease in ultraviolet (UV) photoreceptor sensitivity resulted from a reduction in the frequency of the SWS1 cone pigment, and was associated with a reduction in lens transmission at UV wavelengths. We found that post-receptoral sensitivity to UV and long wavelengths decreased with age, and these changes were the result of ontogenetic changes in both photoreceptor sensitivity in the UV range and retinal circuitry in the long wavelength part of the spectrum.

MATERIALS AND METHODS

Fish care and holding conditions

Fry (N=20), juvenile (N=15) and adult (N=19) Nile tilapia, *O. niloticus* (Linnaeus 1758) (Redfish Ranch, Courtney, BC, Canada) were used. The age and body mass of fish (means ± s.d.) were 67±19 days post-fertilization (d.p.f.) and 1.08±0.55 g for fry, 119±53 d.p.f. and 8.41±1.54 g for juveniles, and 255±20 d.p.f. and 76.61±20.72 g for adults. Fish were held in aquatic facility tanks under a 12 h:12 h light–dark photoperiod at 25±1°C. Facility lighting comprised full-spectrum fluorescent lamps (UV-Blue actinic and BlueMax lamps, Full Spectrum Solutions, Jackson, MI, USA). All experimental and animal care procedures were approved by Queen`s University Animal Care Committee under the auspices of the Canadian Council for Animal Care.

Fish preparation

We recorded electroretinogram (ERG) to estimate photoreceptor and post-receptoral sensitivity. Prior to ERG recordings, fish were immersed in a solution of 125 mg l^{-1} tricaine methanesulphonate (MS-222) until they reached stage III anaesthesia (Ramsden et al., 2008). A general anaesthetic (metomidate hydrochloride, 0.3 mg g^{-1} body mass; Maranil, Syndel Laboratories, Qualicum Beach, BC, Canada) and an immobilizing agent (pancuronium bromide, 0.05 mg g^{-1} body mass; Conier Chem and Pharma, Chongqing, China) were injected subcutaneously. Test fish were placed in a holding cradle in a Faraday cage and irrigated with aerated fresh water (temperature 20±1°C, flow rate 3–10 ml s^{-1}).

ERG experimental apparatus

The optical system and recording apparatus have been described in detail elsewhere (Hawryshyn et al., 2003; Parkyn and Hawryshyn, 2000). Two background channels using 250 W halogen lamps (24 V ELC, Eiko, Kansas City, KS, USA) provided constant background illumination to light adapt the eye. A bifurcated optical fibre (fused silica, numerical aperture NA 0.22; Fiberoptic Systems, Simi Valley, CA, USA) guided light from the two background channels to the electrophysiology rig. The intensity and spectral composition of background illumination were manipulated using interference cut-off filters and neutral density filters (Corion, Franklin, MA, USA). The stimulus channel used a 150 W xenon arc lamp and monochromator (Photon Technology International, London, ON, Canada; 150 W bulb, Ushio, Cypress, CA, USA). The wavelength, intensity and duration of the stimulus were manipulated using a 0–2.7 optical density (OD) neutral density wedge (fused silica; Melles-Griot, Rochester, NY, USA), a filter wheel with nine neutral density filters of 0–4.0 OD at 0.5 OD increments, and an electronic shutter (UniBlitz D122 Shutter, Vincent Associates, Rochester, NY, USA). An optical fibre (fused silica; NA 0.55; Fiberoptic Systems) guided light from the stimulus channel to the electrophysiology rig. The background and stimulus beams were superimposed one on another to produce a beam 1.0 cm in diameter at the plane of the fish eye.

ERG recording and analysis

ERG recordings commenced at least 1 h after the onset of the light phase and were completed before the onset of the dark phase to eliminate any circadian rhythm effects (Li et al., 2005). A glass electrode (1.5 mm outer diameter, 1 mm inner diameter, borosilicate glass; World Precision Instruments, Sarasota, FL, USA) pulled to a tip diameter of 80–125 μm (P-97 Flaming/Brown Micropipette puller, Sutter Instruments, Novato, CA, USA) was loaded with saline (0.684 mol l^{-1} sodium chloride) and inserted into a saline-filled chlorided AgCl half-cell (A-M Systems, Sequim, WA, USA). The electrode tip was placed on the dorsal–nasal corneal surface of the right eye. The ground electrode was placed on the caudal fin and a chloride–silver reference electrode was placed on the head of the fish. Fish were light adapted to the background light for 30 min prior to

recordings. The duration of the light stimulus was 500 ms with an interstimulus interval of 5 s. The ERG signal was amplified and filtered using band-pass filter settings (10 Hz low pass, 100 Hz high pass) *via* an isolated bioamplifier (BMA-200 bioamplifier, CWE Incorporated, Ardmore, PA, USA). This amplified signal was then analyzed with a 16-bit A/D data acquisition system (Micro 1401, Cambridge Electronic Design, Cambridge, UK) and Signal 4.0 software. Spectral sensitivity was measured in 10 nm increments, from 340 to 700 nm, in a staggered wavelength presentation in order to prevent adaptation to specific spectral regions. At each wavelength, the ERG response to 11 stimulus intensities (irradiance levels) was determined. A third order polynomial was fitted to the response *versus* irradiance (RI) curve and the threshold irradiance that corresponded to a response criterion of 30–40 μV was interpolated (Hawryshyn et al., 2010). Sensitivity was estimated as the reciprocal of this threshold irradiance. Log relative sensitivity curves were created by normalizing the log absolute sensitivity values to the maximum sensitivity across the spectrum (Sabbah et al., 2010).

The response of the outer plexiform layer and photoreceptors

An electroretinogram accounts for the response of the outer plexiform layer of the retina, representative of the response of cone photoreceptors, horizontal cells and bipolar cells. A typical ERG waveform consists of an initial hyperpolarization phase (a-wave) representing the response of photoreceptors to the onset of light, followed by a depolarization phase (b-wave) representing the response of ON bipolar cells to the onset of light. Accordingly, the response of the outer plexiform layer (later used to estimate the post-receptoral sensitivity) was measured as the potential change between the a-wave and b-wave, whereas, the response of photoreceptors alone was measured as the amplitude of the a-wave. However, because of the strong depolarization of ON bipolar cells, the response of photoreceptors (a-wave) was typically masked. To isolate the response of photoreceptors, sodium L-aspartate (ASP) was injected into the ocular media of fish. ASP is a substrate of the glutamate transporter in photoreceptors that competitively inhibits glutamate uptake (Balcar and Johnston, 1972) and thereby increases glutamate at the synapse, reducing the light-evoked input to post-receptoral elements. ASP is routinely used to isolate photoreceptor responses in the ERG (Baron and Boynton, 1975; Bush and Sieving, 1996; Dowling and Ripps, 1972; Wakabayashi et al., 1988). To account for variation in ocular media volume, different volumes and concentrations of ASP in Ringer solution (Kraaij et al., 1998) were used for different life stages (fry, 5 μl 70 mmol l^{-1}; juvenile, 10 μl 70 mmol l^{-1}; adult, 10 μl 210 mmol l^{-1}), resulting in an estimated final ocular concentration of 6.77 mmol l^{-1}. To ensure constant effect of ASP throughout the experiments, ERG recordings commenced at least 20 min following the ASP injection and were completed within 250 min following the injection (S.S., F.E.H. and C.W.H., unpublished).

Background light conditions

Spectral sensitivity of each fish was evaluated under three background light conditions (BG1–BG3), designed to differentially activate the various cone pigment classes. Short-wavelength sensitivity was expected to be lowest under BG1, higher under BG2 and highest under BG3. The number of photons collected by the various cone pigment classes under the background conditions used was estimated using a quantum catch model:

$$Q(i) = \sum_{\lambda=300}^{800} A(i,\lambda)E(\lambda)\,, \qquad (1)$$

where $Q(i)$ denotes the quantum catch of cone pigment i, $A(i,\lambda)$ denotes the absorbance of cone pigment i at wavelength λ, and $E(\lambda)$ denotes the photon irradiance of the background light at wavelength λ. Absorbance spectra for cone pigments were constructed for the seven cone pigments reported in the Nile tilapia using the absorbance templates of Govardovskii and colleagues (Govardovskii et al., 2000) – the λ_{max} of A_1-reconstituted visual pigments is provided in parentheses: SWS1 (360 nm), SWS2b (425 nm), SWS2a (456 nm), Rh2b (472 nm), Rh2aα (518 nm), Rh2aβ (528 nm) and LWS (561 nm) (Spady et al., 2006). The spectral overlap of Rh2aα and Rh2aβ necessitated calculating a mean λ_{max} of 523 nm. The 523 nm λ_{max} absorbance spectrum was used for subsequent analysis and is hereafter referred to as Rh2a.

The irradiance provided under the various background conditions was measured at the plane of the fish eye using a spectroradiometer (QE65000, Ocean Optics, Dunedin, FL, USA) connected to a 2 m optical fibre (QP600-2-UV/VIS, Ocean Optics) that was fitted with a cosine corrector (CC-3-UV, Ocean Optics). The spectroradiometer setup was calibrated for absolute irradiance using a NIST (National Institute of Standards and Technology, Gaithersberg, MD, USA) calibrated halogen–deuterium dual light source (200–1000 nm, DH-2000-CAL, Ocean Optics).

Lens transmission measurement

The spectral transmission of the fish lens was measured following a protocol described elsewhere (Douglas and McGuigan, 1989; Lisney et al., 2010). Lenses were surgically removed from the eyes at the completion of ERG recordings and were mounted in a hole that was drilled in a black plastic block fitted inside a standard sample cuvette. Transmission measurements between 300 and 800 nm were carried out using a bench-top spectrophotometer (Cary 300, Varian, Palo Alto, CA, USA) and were normalized between 0 and 1. For each fish, 6–10 transmission measurements were acquired from both lenses and averaged.

Retinal whole-mount imaging

To study the spatial arrangement of cone photoreceptors in the retina, we viewed DAPI (4′,6-diamidino-2-phenylindole)-stained retinas of representative individuals from each age group under a laser scanning microscope. A whole-mount DAPI staining procedure was adopted from a protocol described elsewhere (Barthel and Raymond, 2000). Fish were dark adapted for 1 h before dissection and eyes were enucleated under deep red light. Neural retina was separated from other ocular tissues and fixed in 4% paraformaldehyde (PFA) buffered in phosphate-buffered saline (PBS), pH 7.4, overnight at 4°C. Retinas were washed several times in PBS and dehydrated through a graded series into methanol and stored at –20°C. Retinas were rehydrated through a graded series into PBS containing 0.1% Tween-20 (PBST) and rinsed four times in 100% PBST for 5 min each. To enhance penetration of the stain, the tissue was partially digested with 10 μg μl^{-1} of proteinase K (diluted 1/1000 in Tris-EDTA buffer) for 30 min at 37°C. The tissue was then rinsed in PBST, secondarily fixed in 4% PFA-PBS for 20 min at room temperature (RT), and rinsed with PBST (5 cycles of 5 min each) prior to staining. Retinas were incubated in 10 μl of 1:200 DAPI staining solution (10 mg ml^{-1} solution in PBS) for 1–5 min at RT and rinsed in PBS. Finally, retinas were placed on microscope slides and viewed under a laser scanning confocal microscope (LSM 710, Carl Zeiss).

Statistical model of photoreceptor spectral sensitivity

The sensitivity of retinal photoreceptors is the sum of the response of all cone pigments present in the retina. As it is not possible to

measure the sensitivity of each cone pigment separately in a single retina, an alternative approach is to infer the cone-specific sensitivity using our knowledge of how cone pigments respond and the cumulative sensitivity of photoreceptors. Cumulative sensitivity of retinal cone photoreceptors was modelled as the summed absorbance of all possible cone pigments:

$$S(\lambda) = T(\lambda)\sum_{i=1}^{n} k(i)\,A(i,\lambda)$$

$$= T(\lambda)\sum_{i=1}^{n} k(i)[aA_2(i,\lambda) + (1-a)A_1(i,\lambda)]\,, \quad (2)$$

where $T(\lambda)$ represents the lens transmission at wavelength λ, $A(i,\lambda)$ represents the absorbance of cone pigment class i, $k(i)$ denotes the contribution of cone pigment i to spectral sensitivity (cone weight), and $S(\lambda)$ is the spectral sensitivity adjusted for the measured lens transmission. To account for different proportions of A_1 (retinal) and A_2 (3,4-dehydroretinal) chromophores, the absorbance of cone pigments with A_1 and A_2 chromophores, $A_1(i,\lambda)$ and $A_2(i,\lambda)$, was taken into account, where a ($0 \leq a \leq 1$) represents the proportion of the A_2 chromophore. Cone pigment absorbance templates for the A_1 and A_2 chromophores were generated as elsewhere (Govardovskii et al., 2000) while accounting for the λ_{max} shift associated with varying A_2 proportion (Hárosi, 1994).

The statistical model is created by combining the deterministic model (Eqn 2) with the appropriate error distribution. We denote the expected spectral sensitivity by $y(\lambda)$ and the observed spectral sensitivity by $Y(\lambda)$. Thus, spectral sensitivity at a particular wavelength is distributed as $Q[y(\lambda), V(y)]$, which has a quasi-likelihood distribution with an expectation $y(\lambda)$ and variance of $V(y)$:

$$y(\lambda) = S(\lambda)\,, \quad (3)$$

$$Y(\lambda) \approx Q[y(\lambda), V(y)]\,. \quad (4)$$

As the deterministic model is non-linear, we need to pay particular attention to the error distribution, which could have a large influence on the fits. To estimate the mean–variance relationship, we calculated the mean and variance of photoreceptor sensitivity across all individual fish that were used for the measurement of sensitivity under each of the three background conditions, for each of the life stages. This analysis suggested a power relationship between the mean (y) and variance [$V(y)$] of photoreceptor sensitivity. The function has the form:

$$V(y) = dy^p\,, \quad (5)$$

which is the general form for Tweedie distributions, where d is the dispersion parameter and p determines the specific class of distribution (Jorgensen, 1987). For all groups examined, p ranged between 1.07 and 1.83, which falls within the range of compound Poisson distributions. To accommodate model fitting in a statistical software environment, we assumed p=1.5 for all groups examined. To estimate the cone weights in each individual fish, observed sensitivity was fitted to the statistical model, while the lens transmission of each respective fish was taken into account. The dispersion parameter d was estimated from the spectral sensitivity data during the fitting process. For simplicity, spectral sensitivity values that typically ranged from 10^{-13} to 10^{-16} photons cm^{-1} s^{-1} were multiplied by 10^{13} prior to analysis. Thereafter, spectral sensitivity curves were standardized. Specifically, for each individual, spectral sensitivity was divided by the maximum sensitivity across the spectrum. The resultant value was multiplied by the maximum sensitivity across all fish in the group. Analyses were performed in R 2.9.0 (R Development Core Team, 2009).

Derived cone weights could be used to estimate the frequency of cone pigments in the retina. The absolute sensitivity, time-to-peak response, and slope of the response–irradiance relationship in fish do not vary significantly between cone classes, i.e. the various cone classes have the same properties except for their spectral sensitivity (Anderson et al., 2010; Kraaij et al., 1998). Consequently, the frequency of each cone pigment in the retina F could be estimated as the weight of each cone pigment divided by the sum of weights of all cone pigments:

$$F(i) = \frac{k(i)}{\sum_{i=1}^{n} k(i)}\,. \quad (6)$$

In this regard, photoreceptor sensitivity can be used to reliably estimate the frequency of cone pigments only if photoreceptor sensitivities, and thus also the derived cone pigment frequencies, do not vary when determined under different background conditions. We will show that our results fulfil this requirement.

Statistical analysis

Prior to performing statistical analyses, normality of all data was examined using the Kolmogorov–Smirnov test, and homogeneity of variance was confirmed using Cochran's C-test (Underwood, 1997). The requirement for normality was not met; therefore, non-parametric statistical analysis was performed. To examine the variation in age and body mass across sensitivity types (photoreceptor *versus* post-receptoral), the non-parametric Mann-Whitney U-test (MW) was used. To examine the effect of background condition on cone pigment frequency (estimated based on photoreceptor sensitivity), we compared cone frequency under the three background conditions using the non-parametric ANOVA Kruskal–Wallis (KW). To examine the effect of ontogeny on cone pigment frequency, we compared cone frequency across the three life stages using KW. Following KW analysis, *post hoc* multiple comparisons were performed with P-values calculated while accounting for the number of comparisons made (Siegel and Castellan, 1988). Our results for both KW and MW are presented and interpreted without the use of conservative corrections for multiple comparisons such as the Bonferroni correction (α=0.05). While the use of Bonferroni and related procedures may reduce Type I errors, they also reduce statistical power and increase the chance of Type II error, especially in cases of small sample sizes (Nakagawa, 2004). We therefore chose to report observed effect size (i.e. η^2 values) along with exact P-values to allow the reader to evaluate biological importance. The larger the effect size, the larger the difference between treatment groups (e.g. photoreceptor *versus* post-receptoral sensitivity). The effect size for KW was calculated as $\eta^2 = H/(N-1)$, where H denotes the H statistic of KW and N denotes the total number of samples in all groups examined (Morse, 1999). The effect size for MW was calculated similarly, but with the Z-statistic of MW replacing the H-statistic. Statistical analysis was performed using Statistica software (Statsoft, Tulsa, OK, USA).

RESULTS

Lens transmission changes with ontogeny

Spectral lens transmission was measured in fry, juvenile and adult Nile tilapia. Lens transmission at all life stages increased toward long wavelengths, showing a rapid increase between 350 and 400 nm (Fig. 1A). The wavelength at half-maximum lens transmission T_{50} showed a significant positive correlation to fish age (Fig. 1B). Therefore, as the fish ages, UV wavelengths are filtered out by the lens, precluding them from reaching the retina.

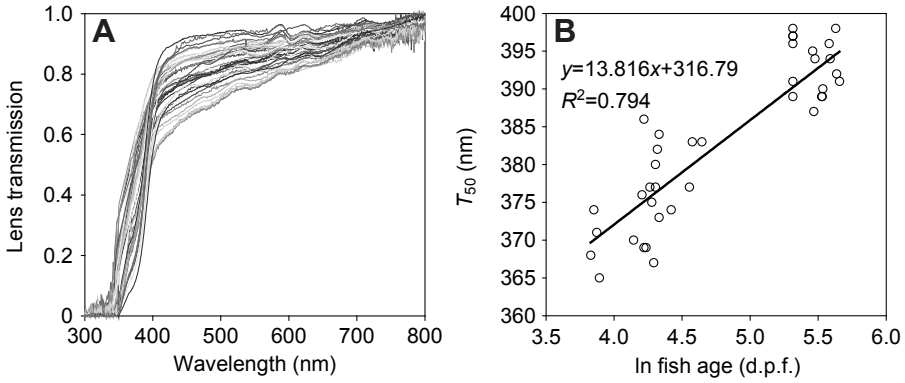

Fig. 1. Lens transmission in the Nile tilapia throughout ontogeny. (A) Spectral lens transmission (N=38) increased towards longer wavelengths. (B) The wavelength of half-maximum transmission (T_{50}) increased with the age of the fish. Age was measured in days post-fertilization (d.p.f.) and plotted on a logarithmic scale.

Photoreceptor sensitivity changes with ontogeny

Photoreceptor and post-receptoral spectral sensitivity were measured under three background light conditions for fry, juvenile and adult Nile tilapia. Photoreceptor sensitivity was estimated from the a-wave of ERGs of ASP-treated retina, whereas post-receptoral sensitivity was estimated from the b-wave of ERGs of untreated retina. See Fig. 2 for ERG waveforms recorded from untreated and ASP-treated retina, the spectral irradiance of the background conditions used, and the quantum catches of cone pigments.

Photoreceptor sensitivity between 400 and 700 nm did not vary appreciably across life stages (Fig. 3), suggesting that the contribution of cone pigments that are maximally sensitive in the 400–700 nm range (SWS2b, SWS2a, Rh2b, Rh2a and LWS) did not vary with ontogenetic stage. In contrast, photoreceptor sensitivity in the UV spectral range (340–400 nm) varied with ontogenetic stage under all background conditions, with sensitivity in juveniles and adults being lower than in fry. This reduction in sensitivity during ontogeny could arise in two possible ways: (i) a reduction in the

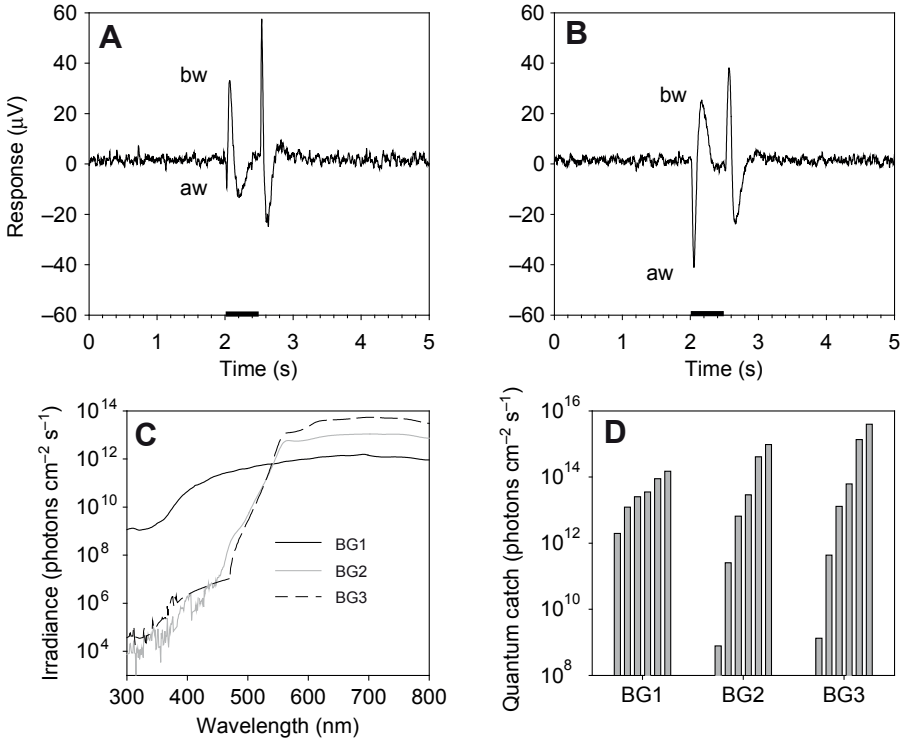

Fig. 2. Electroretinogram (ERG) waveforms recorded from untreated and sodium L-aspartate (ASP)-treated retina, the background light conditions used, and theoretical quantum catches of cone pigments. (A,B) An ERG waveform consists of an initial hyperpolarization phase (aw, a-wave) representing the response of photoreceptors to the onset of light, followed by a depolarization phase (bw, b-wave) representing the response of ON bipolar cells to the onset of light. The duration of the stimulus is indicated by the thick horizontal lines. (A) The response of the outer plexiform layer (later used to estimate the post-receptoral sensitivity) was measured as the potential change between the a-wave and b-wave from an untreated retina. (B) To isolate the response of photoreceptors, retinas were treated with ASP. The response of photoreceptors alone was measured as the amplitude of the a-wave from an ASP-treated retina. (C) Spectral sensitivity was measured under three background light conditions (BG1–BG3), each designed to emphasize the activity of different cone classes. (D) Quantum catches of cone pigments were estimated for each background condition while accounting for the lens transmission and the absorbance of visual pigments with A_2 chromophore. For each background condition, bars represent the quantum catches of (left to right) the SWS1, SWS2b, SWS2a, Rh2b, Rh2a and LWS cone pigments. Quantum catches for fry, juvenile and adult Nile tilapia were practically the same; thus, quantum catches are presented for juveniles only.

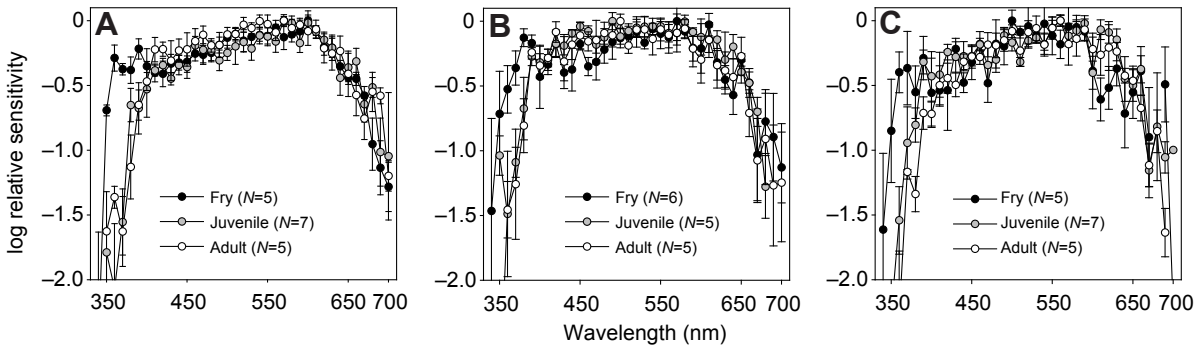

Fig. 3. Photoreceptor sensitivity across different background conditions (A, BG1; B, BG2; C, BG3) for fry, juvenile and adult Nile tilapia. Photoreceptor sensitivity in the UV spectral range (340–400 nm) varied considerably with ontogeny for all background conditions tested, with sensitivity in juveniles and adults being lower than in fry. In contrast, photoreceptor sensitivity at longer wavelengths (400–700 nm) varied only slightly with ontogeny. Note that for each life stage, photoreceptor sensitivity varied only slightly across background conditions. This suggests that the photoreceptors do not easily adapt, at least under the background conditions used. Error bars represent 1 s.e.m.

proportion of UV wavelengths reaching the retina due to a reduction in lens transmission of UV light, and (ii) a reduction in the frequency of the SWS1 cone pigment that is maximally sensitive between 360 and 373 nm (dependent on chromophore composition).

To identify the source of the reduction in photoreceptor sensitivity to UV wavelengths, we fitted a statistical model to the observed photoreceptor sensitivity to estimate the frequency of cone pigments in the retina. Photoreceptor sensitivity was modelled while accounting for the lens transmission and the absorbance of all possible cone pigments. See Fig. 4A for example fits. To examine ontogenetic changes in cone pigment frequency, we compared cone pigment frequencies across the three life stages. For each life stage, cone pigment frequency data for all fish under the three background conditions were pooled (the frequency of cone pigments did not vary significantly across background conditions; Table 1). Apart from the frequency of the SWS1 cone pigment, the frequency of all other cone pigments did not differ significantly across life stages (Fig. 4B; detailed statistics are in the figure legend). The frequency of SWS1 pigment in fry was significantly higher than in adults but not juveniles (cone pigment frequency of juveniles and adults did not differ significantly). Thus, the ontogenetic reduction of photoreceptor sensitivity to UV wavelengths was a result of a reduction in the frequency of the SWS1 cone pigment in addition to the change in lens transmission, suggesting that the frequency of the SWS1 cone pigment was higher in fry than in juveniles and adults.

Square cone mosaic does not change with ontogeny

The geometry of the square cone mosaic did not change during ontogeny, showing a single cone surrounded by four double cones (Fig. 5). Given that single cones in tilapia contain short-wavelength sensitive visual pigments, a single cone might move to contain the

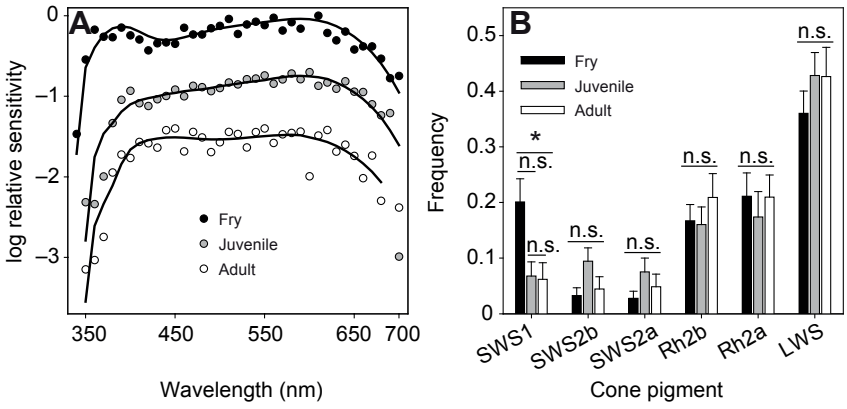

Fig. 4. Variation in photoreceptor sensitivity and cone pigment frequency with ontogeny. (A) Example fits (lines) to photoreceptor sensitivity (circles) measured in fry, juvenile and adult Nile tilapia under BG1; spectral sensitivity curves are vertically displaced for clarity. (B) Variation in cone pigment frequency with ontogeny. For each life stage, cone frequency data under the three background conditions were pooled (see Table 1). The frequency of SWS1 differed significantly with ontogeny (Kruskal–Wallis, H=9.08, d.f.=2, N=49, effect size η^2=0.189, P=0.011). *Post hoc* analysis revealed that cone pigment frequency in fry was significantly higher than in adults (P=0.029) but not juveniles (P=0.077). Cone frequency in juveniles and adults did not differ significantly (P=1.000). The frequency of all other cone pigments did not vary significantly during ontogeny (Kruskal–Wallis, d.f.=2, N=49; SWS2b: H=3.48, η^2=0.073, P=0.175; SWS2a: H=1.16, η^2=0.024, P=0.560; Rh2b: H=0.56, η^2=0.012, P=0.757; Rh2a: H=1.10, η^2=0.023, P=0.576; LWS: H=1.16, η^2=0.024, P=0.559). The proportion of the A_2 chromophore did not differ significantly across life stages (Kruskal–Wallis, H=0.39, d.f.=2, N=49, η^2=0.008, P=0.823) and equalled 0.59±0.22 (mean ± s.d.), suggesting a mixed-chromophore retina in Nile tilapia. To confirm that the UV sensitivity peak in fry reflects the contribution of the SWS1 cone pigment, we omitted the SWS1 cone pigment from the model and repeated the analysis. The reduced model was a poorer fit with a significant likelihood ratio (likelihood ratio=8.77, d.f.=1, P=0.003), suggesting that the SWS1 cone pigment is probably responsible for the UV sensitivity peak observed in fry.

Table 1. Variation in cone pigment frequency across background light conditions

	Fry			Juvenile			Adult		
Cone class	$H_{2,15}$	η^2	P	$H_{2,19}$	η^2	P	$H_{2,15}$	η^2	P
SWS1	3.29	0.235	0.192	1.16	0.064	0.559	4.57	0.326	0.101
SWS2b	0.85	0.061	0.653	6.84	0.380	**0.032**	1.04	0.074	0.594
SWS2a	1.14	0.081	0.565	2.57	0.143	0.276	2.47	0.176	0.291
Rh2b	0.01	0.001	0.993	0.63	0.035	0.728	1.83	0.131	0.399
Rh2a	5.77	0.412	0.056	0.46	0.026	0.794	3.45	0.246	0.178
LWS	1.44	0.103	0.484	1.64	0.091	0.44	4.82	0.344	0.089

To examine the variation in cone pigment frequency across background light conditions, the non-parametric ANOVA Kruskal–Wallis was used. The H statistic, degrees of freedom (d.f.) and number of samples (N) are presented as $H_{\text{d.f.},N}$. The effect size (η^2) and P-value are indicated.

The larger the effect size, the larger the difference in cone pigment frequency between fish used for measuring photoreceptor sensitivity under the three background conditions. P-values for cone pigments that showed significantly different frequency across the three background conditions ($P<0.05$) are marked in bold. Apart from the frequency of SWS2b in juveniles, the frequency of all cone pigments, for all life stages, did not vary significantly across background conditions. Therefore, cone pigment frequency data could be pooled across the three background conditions used. Additionally, this result indicates that the requirement for photoreceptor sensitivity to be used to reliably estimate the frequency of cone pigments has been fulfilled.

SWS2a/b cone pigments, or SWS1 along with SWS2a/b with changing ontogenetic stage.

Post-receptoral sensitivity changes with ontogeny

Post-receptoral sensitivity changed considerably in the UV range, with sensitivity in juveniles and adults being lower than in fry (Fig. 6). Additionally, post-receptoral sensitivity changed to a greater extent between 400 and 700 nm, with long-wavelength sensitivity (500–700 nm) in adults being lower than in fry and juveniles (similar to a sensitivity increase in the 400–500 nm range). The long-wavelength spectral range over which the sensitivity in adults was reduced might correspond to the λ_{max} range of the cone pigments Rh2a (523–560 nm) and LWS (560–625 nm), with the λ_{max} range dependent on chromophore composition. Additionally, while the sensitivity decrease over the λ_{max} range of Rh2a occurred between the fry and juvenile life stages, the sensitivity decrease over the λ_{max} range of LWS occurred mainly between the juvenile and adult life stages. The differences in post-receptoral sensitivity between the adult stage and the fry and juvenile stages could be explained by (i) comparable changes in photoreceptor sensitivity and (ii) changes in the retinal circuitry. These two mechanisms are evaluated below.

Retinal circuitry changes with ontogeny

A means to examine the extent of ontogenetic change in sensitivity is to calculate the sensitivity difference between fry and adults ('fry–adult difference'). Thus, no ontogenetic change in sensitivity would result in zero difference across the spectrum, whereas an ontogenetic change in sensitivity would result in a non-zero difference (Fig. 7A). Moreover, if ontogenetic changes in post-receptoral sensitivity arise from changes in photoreceptor sensitivity, photoreceptor and post-receptoral sensitivity are expected to correlate (Fig. 7B). To examine whether ontogenetic changes in photoreceptor sensitivity correlate to changes in post-receptoral sensitivity, we compared the fry–adult difference between photoreceptor sensitivity and post-receptoral sensitivity (Fig. 7C,D). This was possible only for the BG2 and BG3 background conditions while ensuring matching of fish age and body mass (see Table 2 for detailed statistics). In the UV spectrum (340–400 nm), differences in photoreceptor and post-receptoral sensitivity significantly correlated, indicating that ontogenetic changes in the UV range of post-receptoral sensitivity arise from changes in photoreceptor sensitivity. However, unexpectedly, at longer wavelengths (500–700 nm), differences in photoreceptor and post-receptoral sensitivity did not correlate. Additionally, differences in photoreceptor sensitivity typically did not deviate from zero difference while those in post-receptoral sensitivity did. This suggests that at long wavelengths, ontogenetic changes in post-receptoral sensitivity do not arise from changes in photoreceptor sensitivity, and probably arise from changes in the retinal circuitry. At long wavelengths, post-receptoral sensitivity in fry was higher than in adults (positive difference), indicating reduced post-receptoral sensitivity to long wavelengths in adults.

DISCUSSION

This study systematically traced ontogenetic changes across several elements and processing levels in the visual system of fish, i.e. from the transmission of the lens that controls the wavelengths reaching the retina, to the retinal circuitry that processes the signals from photoreceptors prior to being conveyed to downstream elements in the eye and visual centres in the brain. This study delivers several significant findings for the understanding of the developmental trajectory of visual function in fish. Nile tilapia was shown to

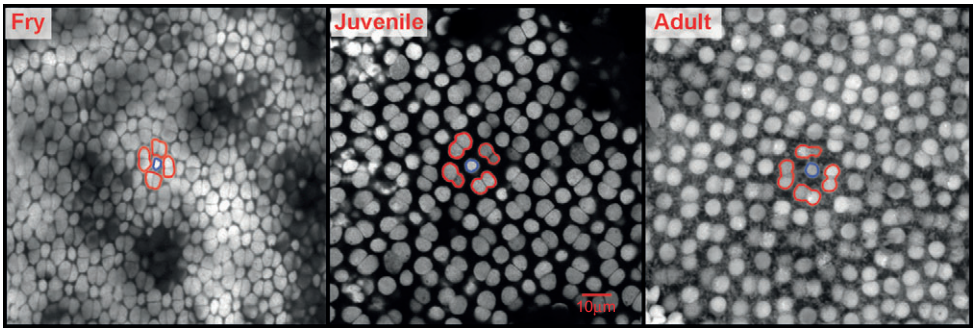

Fig. 5. Cone square mosaic in fry, juvenile and adult Nile tilapia. Spacing between cone photoreceptors in the fry was smaller than in the juvenile and adult. However, the cone square mosaic stayed fixed throughout ontogeny, exhibiting a single cone surrounded by four double cones. The age and body mass of fish were: fry, 96 d.p.f. and 1.8 g; juvenile, 138 d.p.f. and 10.2 g; adult, 273 d.p.f. and 86 g. Scale bar applies to all panels.

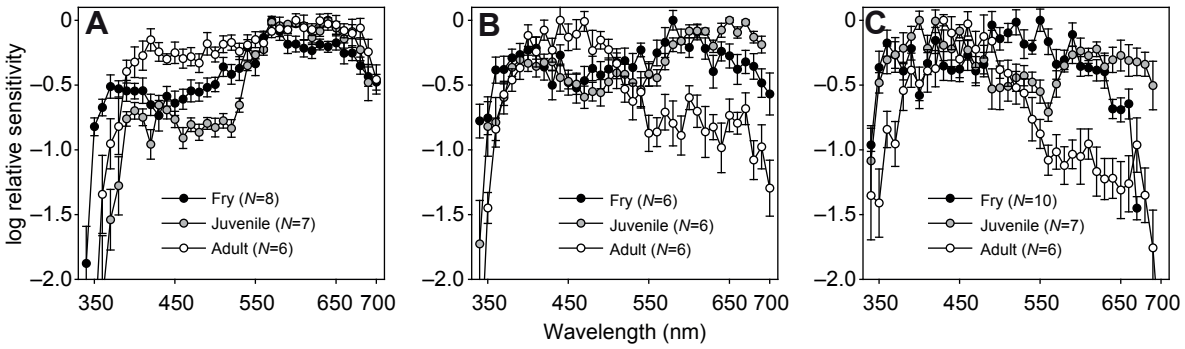

Fig. 6. Post-receptoral sensitivity across different background conditions (A, BG1; B, BG2; C, BG3) for fry, juvenile and adult Nile tilapia. In the UV range (340–400 nm), post-receptoral sensitivity in juveniles and adults was lower than in fry, whereas at long wavelengths (500–700 nm), sensitivity in adults was lower than in fry and juveniles. These trends were evident under all background light conditions. The ontogenetic sensitivity increase at short wavelengths observed under BG1 is equivalent to the ontogenetic sensitivity decrease at long wavelengths observed under BG2 and BG3 (the apparent opposite trends are merely a result of the choice of normalization method). Note that for each life stage, post-receptoral sensitivity varied considerably across background conditions. This suggests that the background conditions used differentially adapted post-receptoral element(s) in the retina. Error bars represent 1 s.e.m.

undergo ontogenetic changes in lens transmission, photoreceptor sensitivity and post-receptoral sensitivity. The decrease in photoreceptor sensitivity observed during ontogeny resulted from a reduction in the frequency of the SWS1 cone pigment, and was associated with a reduction in lens transmission at UV wavelengths. Additionally, post-receptoral sensitivity to UV and long wavelengths decreased with age. These changes in post-receptoral sensitivity were the result of (i) ontogenetic changes in photoreceptor sensitivity, mediated through a reduction in the frequency of the SWS1 pigment, in the UV spectral range, as well as (ii) ontogenetic changes in retinal circuitry leading to reduced sensitivity at longer wavelengths.

Association between different elements of the visual process

What elements/processes in the visual system can serve as predictors to the visual sensitivity of fish? The ability of elements/processes of the visual system to serve as predictors to the visual sensitivity was wavelength dependent. The reduced frequency of the SWS1 cone pigment accompanied a reduction in lens transmission to UV wavelengths, and led to a reduction in photoreceptor and post-receptoral sensitivity to UV wavelength. Thus, reduced post-receptoral sensitivity to UV wavelengths could be predicted based on the transmission of the lens, cone pigment frequency or photoreceptor sensitivity. In contrast, the observed reduction in post-receptoral sensitivity to long wavelengths could not be predicted by the lens transmission, cone pigment frequency or photoreceptor sensitivity. Therefore, any attempt to understand visual function or to relate it to the sensory world while using the lens transmission, frequency of cone pigments or photoreceptor sensitivity as predictors would probably fail. In this regard, the use of the expression of cone opsin genes or the frequency of cone photoreceptors in predicting visual sensitivity is also likely to fail because these measures are intimately associated with the frequency of cone pigments. Thus, our results emphasize the importance of considering the visual sensitivity of fish, rather than its proxies, to allow for an accurate and efficient investigation of visual functions and adaptations.

Ontogenetic changes in lens transmission

Our results show a correlation between fish age and the wavelength of half-maximum lens transmission T_{50}; that is, lens transmission of UV wavelengths declined with fish age. In tilapia, ontogenetic changes in lens transmission were reported to follow two phases (Thorpe and Douglas, 1993). In the first phase, lens diameter and rate of lenticular pigment accumulation increase, resulting in a steady increase in T_{50}. In the second phase, lenticular pigment accumulation slows down but lens diameter continues to increase, resulting in no further increase in T_{50}. We found no evidence for the levelling off of T_{50}. It is possible that the previously reported levelling off of T_{50} was found in fish older than those represented in the current study. Unfortunately, details regarding the age of the fish studied previously were not provided, therefore precluding confirmation of this possibility.

Ontogenetic changes in cone pigment frequency and photoreceptor sensitivity

We report a reduced frequency of the SWS1 cone pigment in adults compared with fry. Lisney and colleagues measured the spectral sensitivity of adult tilapia from an eye-cup (lens excluded) and found no evidence for contribution of the SWS1 pigment (Lisney et al., 2010). Additionally, gene expression studies have reported lower expression of the *SWS1* opsin gene in adults than in larvae (Carleton et al., 2008; Spady et al., 2006). Therefore, the ontogenetic decrease observed in the frequency of the SWS1 cone pigment is in agreement with previous studies.

However, our results differ from previous reports (Carleton et al., 2008; Spady et al., 2006) in the timing of the decrease in SWS1 frequency. While we detected a high frequency of the SWS1 cone pigment in fry whose age was 67±19 d.p.f., previous studies have detected only slight expression of the *SWS1* opsin gene after 60 d.p.f. Two factors may explain this discrepancy: (i) genetic differences between populations and (ii) differences in the light conditions at the fish holding facility between studies. First, the expression of cone opsin genes in cichlids (Carleton, 2009; Smith et al., 2011) and bluefin killifish (Fuller and Claricoates, 2011) was shown to differ between populations. Thus, genetic differences between the Nile tilapia populations that were used in the current and previous studies might explain the timing differences observed in the SWS1 expression. Second, the expression of cone opsin genes in the bluefin killifish (Fuller and Claricoates, 2011) as well as the frequency of cone photoreceptors (measured using microspectrophotometry) in the black bream (Shand et al., 2008) were shown to differ between fish reared under different light conditions. Additionally, in the zebrafish, a considerable variation in opsin gene expression was detected across different photoperiod regimes (Li et al., 2005). In

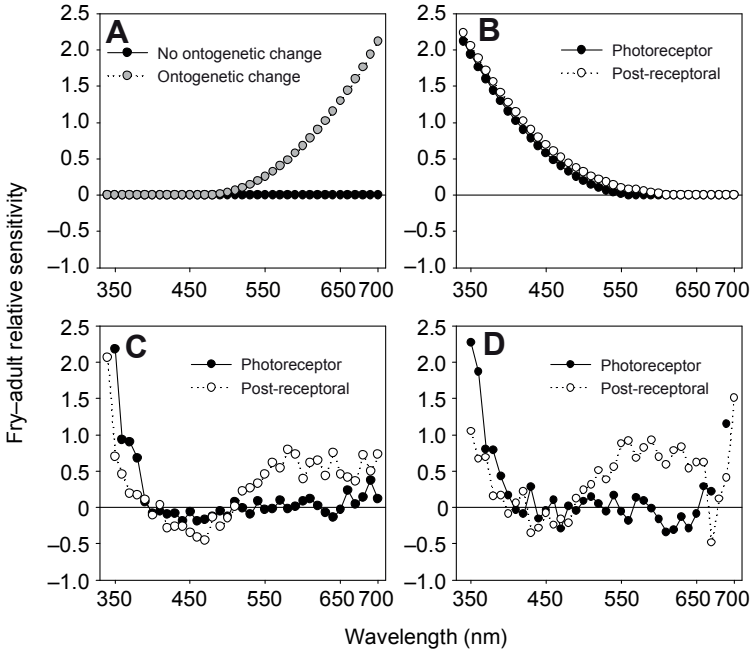

Fig. 7. Mean sensitivity difference between fry and adults. (A) A theoretical zero sensitivity difference across the spectrum that reflects no ontogenetic change in sensitivity, and a theoretical non-zero sensitivity difference that reflects an ontogenetic change in sensitivity at long wavelengths (sensitivity in fry is higher than in adults). (B) A theoretical correlation between the fry–adult difference in photoreceptor and post-receptoral sensitivity, suggesting that ontogenetic changes in post-receptoral sensitivity arise from changes in photoreceptor sensitivity. (C,D) Sensitivity difference between fry and adults was calculated for photoreceptor sensitivity as well as for post-receptoral sensitivity for background light conditions BG2 (C) and BG3 (D). Differences in photoreceptor and post-receptoral sensitivity significantly correlated in the UV spectrum (340–400 nm; BG2, Pearson r=0.9365, P=0.0059, average spectral difference 0.78 and 0.51 for photoreceptor and post-receptoral sensitivity; BG3, Pearson r=0.8858, P=0.0188, average spectral difference 1.05 and 0.44 for photoreceptor and post-receptoral sensitivity), suggesting that ontogenetic changes in the UV range of post-receptoral sensitivity arise from changes in photoreceptor sensitivity. In contrast, differences in photoreceptor and post-receptoral sensitivity did not correlate at longer wavelengths (500–700 nm; BG2, Pearson r=0.1646, P=0.4759, average spectral difference 0.04 and 0.47 for photoreceptor and post-receptoral; BG3, Pearson r=–0.3335, P=0.1629, average spectral difference 0.03 and 0.59 for photoreceptor and post-receptoral), suggesting ontogenetic changes in the retinal circuitry.

the current study, fish were held under a 12 h:12 h light–dark photoperiod with a broad light spectrum supplemented by UV blue-actinic lamps, whereas in the study by Carleton and colleagues, fish were held under a 10 h:14 h light–dark photoperiod with an unknown, and probably different, light spectrum (Carleton et al., 2008). Thus, differences in the light conditions between the current and previous studies might have resulted in the delayed reduction in the frequency of the SWS1 cone pigment. At this point, however, without controlling for light conditions and genetic variation between populations, it is difficult to evaluate these possibilities. Experiments that are designed to test the effect of holding light conditions on cone pigment frequency and spectral sensitivity in the Nile tilapia are currently underway.

Our results also show a slight, non-significant, ontogenetic increase in the frequency of the LWS cone pigment. In contrast, previous gene expression studies have reported increased expression of the *LWS* opsin gene with ontogeny (Carleton et al., 2008; Spady et al., 2006). The disagreement between the current and past studies probably arises from differences in the classification of fish to the different life stages. For example, in the current study, fish aged 67±19 d.p.f. (mean ± s.d.) were classified as the youngest group (fry), whereas in a previous study (Spady et al., 2006), fish aged 14–18 d.p.f. were classified as the youngest group (larva). It is possible that changes in the frequency of the LWS pigment occurred at an earlier age, not represented in our sample. Thus, the discrepancy between the *LWS* gene expression (past studies) and pigment frequency (current study) might be a consequence of classification differences.

Ontogenetic changes in cone mosaic

The square cone mosaic in the Nile tilapia stayed fixed throughout ontogeny, with a single cone surrounded by four double cones, as

Table 2. Variation in fish age and body mass across sensitivity types (photoreceptor *versus* post-receptoral)

	Fry				Juvenile				Adult			
	Z	*N*	η^2	*P*	*Z*	*N*	η^2	*P*	*Z*	*N*	η^2	*P*
Age												
BG1	0.00	13	0.000	1.000	3.13	14	0.241	**0.0006**	2.19	11	0.219	**0.030**
BG2	0.48	12	0.044	0.699	2.74	11	0.274	**0.004**	0.82	11	0.082	0.429
BG3	0.68	15	0.049	0.513	3.14	14	0.242	**0.0006**	1.47	11	0.147	0.177
Body mass												
BG1	0.38	13	0.032	0.724	1.61	14	0.124	0.128	2.73	11	0.273	**0.004**
BG2	0.18	12	0.016	0.937	1.65	11	0.165	0.126	0.83	11	0.083	0.429
BG3	0.00	15	0.000	1.000	1.73	14	0.133	0.087	1.29	11	0.129	0.247

To examine the variation in age (days post-fertilization, d.p.f.) and body mass (in g) across sensitivity types (photoreceptor *versus* post-receptoral), the non-parametric Mann–Whitney *U*-test (d.f.=1) was used. The *Z*-statistic, number of samples (*N*), effect size (η^2) and *P*-value are indicated.

P-values for treatments that differed significantly (P<0.05) in fish age or body mass are marked in bold. The larger the effect size, the larger the difference in age or body mass between fish used for measuring photoreceptor and post-receptoral sensitivity.

Age and body mass of fry did not differ significantly between photoreceptor and post-receptoral sensitivity for any background condition. Age, but not body mass, of juveniles differed significantly between photoreceptor and post-receptoral sensitivity for all background conditions. Age and body mass of adults differed significantly between photoreceptor and post-receptoral sensitivity for BG1 but not for BG2 or BG3. Therefore, photoreceptor sensitivity in fry could be compared with post-receptoral sensitivity across all background conditions. Photoreceptor sensitivity in juveniles could not be compared with post-receptoral sensitivity for any background condition. Photoreceptor sensitivity in adults could be compared with post-receptoral sensitivity only for BG2 and BG3.

reported for adults (Braekevelt et al., 1998; Lisney and Hawryshyn, 2010). Single cones in tilapia can contain any of the short wavelength cone pigments, SWS1, SWS2b and SWS2a (Carleton et al., 2008). Yet, single cones can also potentially contain any combination of the three short wavelength pigments. Our results show that the frequency of the SWS1 cone pigment was highest in fry and declined with ontogeny, suggesting that the SWS1 cone pigment was the primary pigment contained in single cones in fry. With ontogeny, however, the frequency of the SWS2b and/or SWS2a increased. This raises the possibility that single cones contain both the SWS2b and SWS2a cone pigments, or even all three short-wavelength cone pigments in juveniles and adults. This possibility needs to be confirmed by measuring the absorbance of individual single cones using microspectrophotometry, measuring the sensitivity of individual single cones using electrophysiological techniques, or localizing the mRNA of cone opsin genes using *in situ* hybridization. Additionally, the eccentricity of the retinal samples used for examining the spatial arrangement of the cone mosaic could not be determined. Thus, it is possible that sampling retina portions of different eccentricities would show cone mosaics of other configurations (Allison et al., 2010).

Ontogenetic changes in retinal circuitry

Our results suggest a change in the retinal circuitry when moving from fry to adults. This finding was based on a low correlation of the fry–adult difference between photoreceptor and post-receptoral sensitivity at long wavelengths. However, an alternative possibility could potentially explain the observed reduction in post-receptoral sensitivity to long wavelengths during ontogeny. As the sensitivity of fry and adults was measured at different times, a change in the spectral distribution of the background illumination throughout the course of the experiment could have generated the differences observed in post-receptoral sensitivity between fry and adults. In our study, however, the irradiance of the background channels was monitored and adjusted continuously throughout the experimentation period. Irradiance of background channels varied only slightly, with the standard deviation of relative irradiance ranging 0.005–0.116 and 0.006–0.151 log units (n=3) across the spectrum, for the two background channels. Such small variation in background irradiance cannot explain the sensitivity difference between fry and adults, which exceeded 1 log unit in some cases (Fig. 7C,D). In fact, to produce such large differences in the spectral sensitivity of adults, the spectral irradiance of background light needed to show differences of more than 4 log units (BG1–BG3; Fig. 2C and Fig. 6). Thus, this alternative explanation for the differences observed in post-receptoral sensitivity between fry and adults seems remote, supporting our suggestion of an ontogenetic change in the retinal circuitry.

Remodelling of the retinal circuitry was previously reported during early development – prior to maturation of a functional visual system and robust visual behaviours. The sequence of retinal circuitry development is conserved across vertebrate species. Ganglion and amacrine cells are the first classes to differentiate, forming the earliest functional circuits in the inner plexiform layer (Fisher, 1979). Subsequently, horizontal cells and photoreceptors differentiate and contact each other in the outer retina, forming the outer plexiform layer (Blanks et al., 1974). The networks for the vertical flow of information in the inner and outer retina are later interconnected when bipolar cells differentiate and connect to the various cells in the inner plexiform layer and outer plexiform layer (Olney, 1968). This sequence of development can reach completion in the prenatal stage, or typically no later than a few days or weeks after birth, e.g. prior to hatching in chicks, 70 h post-fertilization in zebrafish, 15 postnatal days in mice and 35 postnatal days in the ferret (Schmitt and Dowling, 1999; Wong, 1999).

Remodelling of the retinal circuitry was also reported to occur during ageing – upon reaching adulthood. This often involves photoreceptor degeneration that leads to degeneration of neuronal processes and compensatory formation of new connections (Jones et al., 2003). For example, degeneration of rod photoreceptors in mice was associated with the loss of rod bipolar cell dendrites, re-distribution of glutamate receptors, reduced complexity of the horizontal cell network (Strettoi and Pignatelli, 2000) and the transient contact of rod bipolar cells to cones (Peng et al., 2000). Ageing in mice was also associated with a reduction in the number of ganglion cells (Katz and Robison, 1986) and modification in the synaptic connections of rod bipolar cells and horizontal cells (Terzibasi et al., 2009).

In sharp contrast to remodelling of the retinal circuitry during early development and ageing, the remodelling of the retinal circuitry reported in this study occurred between the fry and adult life stages, representing processes that take place following the maturation of the visual system but prior to reaching adulthood. Thus, this study suggests remodelling of the retinal circuitry in a time window that may allow for optimization of the visual system to the changing sensory environment. However, a detailed anatomical examination would be required in order to explore the mechanisms underlying the suggested change in circuitry.

Potential adaptive significance of ontogenetic changes in post-receptoral sensitivity

Reduction in UV sensitivity with ontogeny

Tilapia adults are omnivorous, feeding mainly on macrophytes, but also on detritus, diatoms and aquatic invertebrates, including copepods, cladocerans and rotifers (Getabu, 1994; Oso et al., 2006). In contrast, tilapia fry are largely planktivorous, feeding on diatoms, amphipods insects and copepods (Beveridge and Baird, 2000; Bowen, 1982; Bruton and Boltt, 1975). Zooplankton absorb UV light (Loew and McFarland, 1990). Consequently, UV sensitivity, mediated by the SWS1 cone pigment, may enhance the contrast of zooplankton against the water background, thereby aiding in their detection (Novales Flamarique and Hawryshyn, 1994). UV sensitivity is therefore expected to be advantageous for the planktivorous tilapia fry, but not so for the omnivorous tilapia adults. Furthermore, UV sensitivity might be disadvantageous when not necessary, as presumed in tilapia adults. UV wavelengths are scattered strongly in the water media, and thus sensitivity to, and the inclusion of UV wavelengths in image-forming light may blur and degrade the retinal image (Losey et al., 1999). Thus, the ontogenetic reduction in UV sensitivity in tilapia probably has an adaptive value.

Reduction in long-wavelength sensitivity with ontogeny

Mate choice and male–male competition for territory are key processes in the life of Nile tilapia adults, and depend on visual stimulation and reliable visual assessment of the quality of conspecifics (Castro et al., 2009). Nile tilapia evolved in rivers and pools in central and eastern Africa (Lim and Webster, 2006). Like many freshwater habitats, these rivers probably exhibit turbid water that attenuates short wavelengths of light, resulting in a long-wavelength-shifted visual environment (Novales Flamarique and Hawryshyn, 1993; Seehausen et al., 2008). Thus, adult tilapia in their natural habitat are illuminated by the relatively broad light spectrum coming from above, and viewed against a long-wavelength-shifted water background. Under these conditions, a

visual system with low sensitivity to long wavelengths in the adults would be largely insensitive to the long-wavelength light from the water background. Tilapia adults, which exhibit a brightly reflecting silvery trunk (Lim and Webster, 2006), would therefore appear brighter against a dark background. Thus, low sensitivity to long wavelengths may increase the radiance (luminance) contrast and conspicuousness of conspecific adults. Unlike adult tilapia, which occupy relatively deep water (Bruton and Boltt, 1975), tilapia fry occupy very shallow water (<1 m), and would be less subject to the influence of the long-wavelength shift due to water turbidity. Reduced sensitivity to long wavelengths is therefore expected to be more advantageous for tilapia adults than for tilapia fry. Thus, the reduction in long wavelength sensitivity with ontogeny in tilapia might have an adaptive value.

Yet, a more realistic situation would depend on chromatic rather than luminance contrast. Then, signals from different cone photoreceptors would form colour-opponent channels, through which the radiance arriving from the body pattern of conspecifics and from the background would be compared. For example, the goldfish (Cyprinidae) was suggested to have a red–green opponent channel, where signals from the mid-wavelength (MWS) and long-wavelength (LWS) sensitive cones are compared (Kaneko, 1973; Kaneko and Tachibana, 1981; Neumeyer, 1984). Such a comparison may increase the conspicuousness of Nile tilapia conspecifics, whose body pattern reflections include a lower proportion of long wavelengths compared with the water background. However, evaluation of such a possibility, and its implications to the conspicuousness of the Nile tilapia, will need to wait until the configuration of colour opponency in the Nile tilapia is known.

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