The Journal of Experimental Biology 216, 3110-3122
© 2013. Published by The Company of Biologists Ltd
doi:10.1242/jeb.081331

RESEARCH ARTICLE

Modulation of environmental light alters reception and production of visual signals in Nile tilapia

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SUMMARY

Signal reception and production form the basis of animal visual communication, and are largely constrained by environmental light. However, the role of environmental light in producing variation in either signal reception or production has not been fully investigated. To chart the effect of environmental light on visual sensitivity and body colouration throughout ontogeny, we measured spectral sensitivity, lens transmission and body pattern reflectance from juvenile and adult Nile tilapia held under two environmental light treatments. Spectral sensitivity in juveniles reared under a broad-spectrum light treatment and a red-shifted light treatment differed mostly at short wavelengths, where the irradiance of the two light treatments differed the most. In contrast, adults held under the same two light treatments did not differ in spectral sensitivity. Lens transmission in both juveniles and adults did not differ significantly between environmental light treatments, indicating that differences in spectral sensitivity of juveniles originated in the retina. Juveniles and adults held under the two environmental light treatments differed in spectral reflectance, and adults transferred to a third, white light treatment differed in spectral reflectance from their counterparts held under the two original treatments. These results demonstrate that environmental light plays a crucial role in shaping signal reception in juveniles and signal production throughout ontogeny, reinforcing the notion that environmental light has the capacity to influence animal communication, and suggesting that the characteristics of environmental light should be considered in models of ecological speciation.

Key words: ontogenetic variation, visual sensitivity, body colouration, sensory drive, animal communication.

Received 10 October 2012; Accepted 23 April 2013

INTRODUCTION

Communication allows individuals to exchange information that induces changes from the cellular to the organismal level. However, communication is constrained by a suite of environmental biophysical factors (reviewed in Endler, 1992; Endler, 1993). Visual signals, those that are received and processed by the eye, are largely constrained by an interaction of natural and sexual selection, and the prevailing environmental light (Endler, 1991). Two key processes govern the successful transfer of visual signals: reception and production. Signal reception is defined as the process whereby individuals make decisions based on behavioural displays or some other sensory information that has been received (Endler, 1993). The successful transfer of visual signals is dependent ultimately upon the receiver's signal reception capabilities (Guilford and Dawkins, 1991). However, signal production, defined as changes to the environment caused by one or more individuals, also plays a large role in animal communication (Endler, 1993). Thus, variation in signal production (e.g. variation in body colouration) works in tandem with variation in signal reception (e.g. variation in visual pigment complement). This ultimately provides many facets for natural and sexual selection to act upon, creating a greater probability for divergence between populations (Ryan, 1990).

Ecological speciation has recently drawn increasing attention as its framework allows the evolution of reproductive isolation without the requirement of physical barriers to gene flow (e.g. Rundle and Nosil, 2005; Cadillo-Quiroz et al., 2012). One such model is sensory drive (Endler, 1992), which predicts that differential selection acting on sensory and signalling structures would produce premating isolation between populations, eventually leading to reproductive isolation and speciation. Perhaps one of the best examples of sensory drive promoting speciation is the adaptive radiation of Lake Victoria cichlids (Seehausen et al., 2008). There, two closely related species (*Pundamilia pundamilia* and *P. nyererei*) that occupy separate depths in the water column, were shown to diverge in the opsin gene sequence of the long-wavelength-sensitive (LWS) visual pigment, and this was associated with light and water-depth gradients, and variation in body colouration (Seehausen et al., 2008).

The spectrum and intensity of environmental light place constraints on the visual system (Lythgoe, 1968; Lythgoe, 1979; Sabbah et al., 2013a), and influence the success of visual tasks such as mate choice (Sundin et al., 2010) and foraging (Maddocks et al., 2001). Nonetheless, while there is some evidence that environmental light can drive variation in signal reception (Shand et al., 2008; Fuller and Claricoates, 2011; Fuller et al., 2010; Smith et al., 2012), evidence for the ability of environmental light to drive variation in signal production is scant (but see Imanpoor and Abdollahi, 2011; Kelley et al., 2012).

Our objective was to evaluate whether environmental light has the capacity to drive variation in signal reception and signal production, and whether this effect varies throughout ontogeny. We

measured spectral sensitivity and spectral reflectance in juvenile and adult Nile tilapia *Oreochromis niloticus* (L.) that were reared and held under different environmental light treatments. Nile tilapia form an ancestral outgroup to the lacustrine African cichlids (Kocher, 2004), and are a growing presence in laboratory research (El-Sayed, 2006). Cichlid fish show variation in signal reception, as visual pigment complements show a great range of natural variation (Carleton and Kocher, 2001; Parry et al., 2005; Sabbah et al., 2010; O'Quin et al., 2010). Additionally, cichlids are notorious for their variation in signal production, showing myriad behaviours (Barlow, 2000) and body colourations (Kornfield and Smith, 2000), many of which are species specific (Hofmann et al., 2009). When these sources of variation are coupled with changes in the spectral quality of available environmental light, which, for example, occur as water depth varies (e.g. Sabbah et al., 2011), the basic requirements for sensory drive are met. However, the role of environmental light in these sources of variation, particularly signal production, remains somewhat elusive.

Nile tilapia have been shown to differentially express seven visual pigments throughout ontogeny: SWS1 (360 nm), SWS2b (425 nm), SWS2a (456 nm), Rh2b (472 nm), Rh2aβ (518 nm), Rh2aα (528 nm) and LWS (561 nm) (Spady et al., 2006). Juveniles predominantly express SWS2b, SWS2a, Rh2a (a combination of Rh2aβ and Rh2aα, as their maximum absorbance wavelengths, λ_{max}, substantially overlap) and LWS (Carleton et al., 2008). Adults predominantly express SWS2a, Rh2a and LWS (Carleton et al., 2008), although evidence for a fourth, violet-sensitive (VS) visual pigment (380–420 nm) has also been reported (Lisney et al., 2010). Additionally, Nile tilapia possess dermal chromatophores, thought to be composed of opsin-based light-sensitive pigments (Ban et al., 2005), suggesting that Nile tilapia may have the ability to alter body colouration independently of the visual system.

We implemented two environmental light treatments for juveniles and adults: one that provided a broad spectrum, and one that provided a reduced short-wavelength (400–470 nm), or red-shifted, spectrum. We later implemented a third, white environmental light treatment, where a sample of fish from each original treatment was held. We recorded corneal electroretinograms (ERGs) and measured the spectral reflectance of the fish body pattern to characterize the effect of environmental light on visual sensitivity and body colouration, respectively. ERG was recorded from a whole-fish preparation. Thus, we also measured the transmission of light through the ocular media, largely determined by lens transmission (Douglas and McGuigan, 1989), in order to tease apart retinal *versus* non-retinal effects of environmental light on spectral sensitivity. We hypothesized that if environmental light altered the visual world of Nile tilapia by shaping signal production and signal reception, then (i) fish reared under different environmental light treatments would show differences in spectral sensitivity, (ii) fish reared under different environmental light treatments would show differences in spectral reflectance, and (iii) fish transferred from their original light treatments and held under a white light treatment would show similar spectral sensitivity and spectral reflectance.

We found that environmental light had an age-dependent effect on spectral sensitivity, an age-independent effect on spectral reflectance, and no effect on lens transmission in Nile tilapia. Our results demonstrate that environmental light plays a key role in shaping signal reception in juvenile fish as well as in shaping signal production throughout ontogeny.

MATERIALS AND METHODS

Fish care and holding conditions

Nile tilapia larvae, *O. niloticus* (Northern Tilapia, Lindsay, ON, Canada), were held in our aquatic facility under a 12 h:12 h light:dark photoperiod at a temperature of 25±2°C and fed pellets (Martin Mills, Elmira, ON, Canada) once daily. 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.

Experimental design

Two 80 l plastic tanks were placed under an array of broad-spectrum blue fluorescent lamps (UV-Blue Actinic lamps, Full Spectrum Solutions, Jackson, MI, USA). Black Coroplast (Coroplast, Cornwall, ON, Canada) was used to cover the walls of the tanks to minimize sidewelling light from contaminating either treatment tank. Tanks were fitted with UV-transmissible Plexiglas lids (Acrilyte, Evonik Industries, NJ, USA). A yellow-coloured film (Rosco, Markham, ON, Canada) was fixed to one of the lids, which effectively reduced the amount of short-wavelength light entering the tank. The other tank was left with only the Plexiglas lid, providing a broad spectrum of environmental light. Thus, we created two environmental light treatments: (i) a broad spectrum that may simulate an open, clear water body (T1), and (ii) a reduced short-wavelength (red-shifted) spectrum that may simulate a turbid water body (T2). Spectral irradiance was measured from the centre–bottom of each tank using a spectroradiometer (QE65000; Ocean Optics, Dunedin, FL, USA) connected to a 2 m optical fibre (QP600-2-UV/VIS; Ocean Optics) 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). Fig. 1A shows irradiance spectra for tanks T1 and T2.

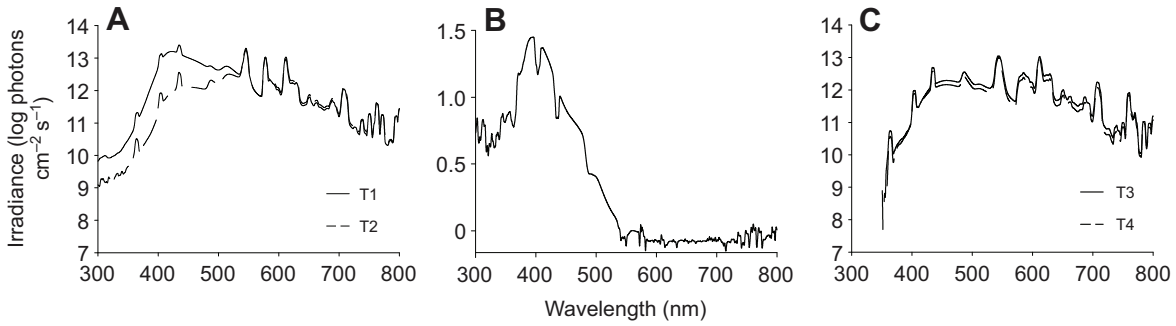

Fig. 1. Spectral irradiance in experimental tanks. (A) Irradiance in tanks 1 and 2 (T1 and T2) differed at short wavelengths (300–470 nm). (B) The irradiance difference curve (T1–T2) shows that the peak irradiance difference falls between 400 and 440 nm. (C) Irradiance in tanks 3 and 4 (T3 and T4) was similar. In these tanks, irradiance at wavelengths shorter than 350 nm was too low to be considered reliable, and thus is not presented.

Eighty larvae (12 days post-fertilization, d.p.f.) from a stock tank were randomly divided between the two light treatments (N_{T1}=40, N_{T2}=40). Fish were exposed to their respective light treatment for 60 days, after which they were considered juveniles (72 d.p.f., body mass 2.05±0.95 g), and measurements of spectral sensitivity, lens transmission and reflectance commenced. Upon completion of a 30 day measurement session from juveniles, two additional experimental tanks were implemented to study whether the effects of environmental light on spectral sensitivity, lens transmission and reflectance were reversible. These two new tanks were placed under an array of white fluorescent lamps (BlueMax lamps, Full Spectrum Solutions) and were not isolated from each other (hereafter 'white light treatment'). Fig. 1C shows irradiance spectra for tanks T3 and T4. Ten fish were randomly sampled from T1 and placed into T3, and 10 fish were randomly sampled from T2 and placed into T4. All fish (103 d.p.f.), from all four tanks (T1–T4), were then exposed to their respective light treatment for an additional 60 days, thus reaching sexual maturity (163 d.p.f., body mass 58.4±19.63 g) (El-Sayed, 2006). A measurement session from adults then began. Thus, juveniles' age at the time of measurement ranged between 72 and 102 d.p.f., and adults' age at the time of measurement ranged between 163 and 193 d.p.f. See Fig. 2 for a schematic timeline detailing the experimental progression. For all spectral sensitivity, lens transmission and reflectance measurements, fish were randomly sampled from the different tanks that corresponded to the different light treatments and life stages. Spectral sensitivity could be measured from only one fish per day. Consequently, for each life stage, measurements of spectral sensitivity and lens transmission (performed immediately following spectral sensitivity measurements) were carried out throughout 1 month. Spectral reflectance was measured roughly during the middle of this 1 month period.

Spectral sensitivity

Fish handling

To estimate spectral sensitivity, we recorded corneal ERGs. Prior to ERGs, 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 inside a Faraday cage and irrigated with aerated fresh water (temperature 20±1°C, flow rate 0.2 l min^{-1} for juveniles, 0.35 l min^{-1} for adults).

ERG experimental apparatus

The optical system and recording apparatus have been described in detail elsewhere (Hawryshyn et al., 2003; Sabbah et al., 2010). Two background channels using 250 W halogen lamps provided constant 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 background channels to the electrophysiology rig. The intensity and spectral composition of background illumination were manipulated using neutral density and interference cut-off filters (Corion, Franklin, MA, USA). The stimulus channel used a 300 W xenon arc lamp and monochromator (Newport Oriel, Irvine, CA, USA). The stimulus' intensity and duration were manipulated using a 0–3 optical density (OD) neutral density wedge (fused silica; Melles-Griot, Rochester, NY, USA) 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. Background and stimulus optical fibres were fitted to a beam splitter, producing a stimulus beam (diameter 0.5 cm at the plane of the fish eye) contained within the background beam (diameter 1 cm).

ERG electrode configuration

A borosilicate glass electrode (1 and 1.5 mm inner and outer diameter, respectively; World Precision Instruments, Sarasota, FL, USA) was pulled to a tip diameter of 80–125 μm (P-97

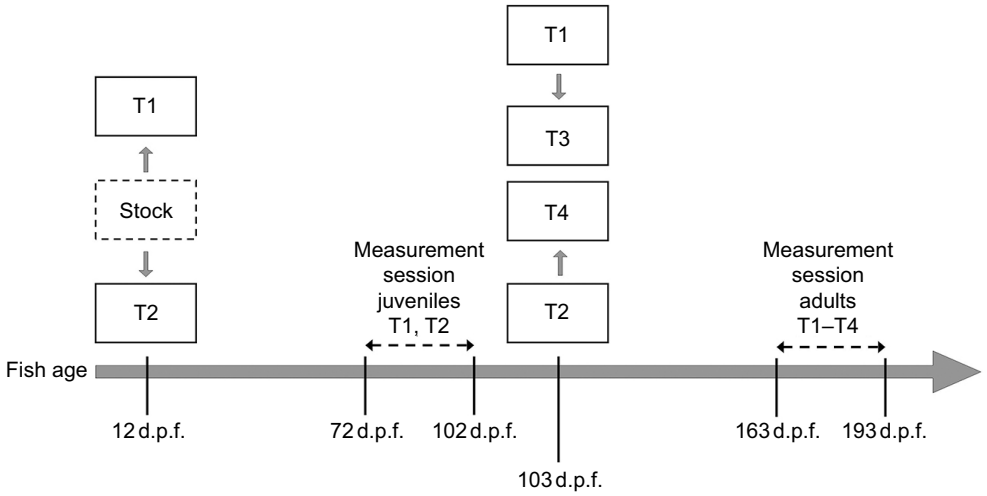

Fig. 2. Experimental time line. Eighty larvae (12 days post-fertilization, d.p.f.) from a stock tank were randomly divided between two tanks that represented two distinct light treatments – a broad-spectrum light treatment (T1) and a reduced short-wavelength (red-shifted) light treatment (T2). Fish were exposed to their respective light treatment for 60 days, after which they were considered juveniles (72 d.p.f.), and measurement of spectral sensitivity, lens transmission and reflectance commenced. Upon completion of a 30 day measurement session of juveniles, two additional experimental tanks were created and represented a third, white light treatment. Ten fish were randomly sampled from T1 and placed into T3, and 10 fish were randomly sampled from T2 and placed into T4. All fish (103 d.p.f.), from all four tanks (T1–T4), were then exposed to their respective light treatment for an additional 60 days, thus reaching sexual maturity (163 d.p.f.). Then, a 30 day measurement session of adults began.

Flaming/Brown Micropipette puller; Sutter Instruments, Novato, CA, USA), loaded with saline (0.68 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 cornea of the right eye. A ground electrode was placed on the caudal fin; a Teflon-coated chlorided silver reference electrode (0.5 mm, A-M Systems) was placed on the head of the fish.

ERG recording procedure

The duration of the light stimulus was 500 ms, with an interstimulus interval of 5 s. The recorded signal was amplified and filtered (10 Hz low pass, 100 Hz high pass; BMA-200, CWE Incorporated, Ardmore, PA, USA), and 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 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 curve and the threshold irradiance, corresponding to a response criterion of 15 μV was interpolated (Sabbah et al., 2013b). Sensitivity was estimated as the reciprocal of this threshold. 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 amplitude of the b-wave of the ERG waveform, mainly representing the response of ON-bipolar cells to the onset of light (Slaughter and Miller, 1981), was measured under a short-wavelength isolating background condition. This condition was designed to chromatically adapt the LWS cone mechanism, accentuating the response of the short-wavelength-sensitive cone mechanisms. The background illumination light adapted the fish eye for 30 min prior to the start of, and during, the experiment. The number of photons collected by the various cone pigments under the background condition and environmental light treatments was estimated using a quantum catch model:

$$Q_i = \sum_{\lambda=300}^{800} A_i(\lambda) E(\lambda), \quad (1)$$

where Q_i denotes the quantum catch of cone pigment i summated over the 300–800 nm wavelength range, $A_i(\lambda)$ represents the spectral absorbance of cone pigment i, and $E(\lambda)$ represents the spectral photon irradiance of the background/environmental light field. Spectral irradiance of the background illumination was measured at the plane of the fish eye using the spectroradiometer configuration described above. Absorbance spectra were generated for the seven cone pigments reported in Nile tilapia using visual pigment absorbance templates (Govardovskii et al., 2000). The wavelength of absorbance maxima (λ_{max}) of visual pigments with an A_1 chromophore was: SWS1, 360 nm; SWS2b, 425 nm; SWS2a, 456 nm; Rh2b, 472 nm; Rh2aβ, 518 nm; Rh2aα, 528 nm; and LWS, 561 nm (Spady et al., 2006). Because of substantial spectral overlap between Rh2aα and Rh2aβ, the absorbance spectra of these two visual pigments were combined (hereafter referred to as Rh2a), and the mean λ_{max} (523 nm) was used for subsequent analyses. Background illumination quantum catch monotonically increased from UVS to LWS: SWS1, 1.56×10^4 photons cm^{-2} s^{-1}; SWS2b, 2.44×10^6 photons cm^{-2} s^{-1}; SWS2a, 7.11×10^7 photons cm^{-2} s^{-1}; Rh2b, 5.08×10^8 photons cm^{-2} s^{-1}; Rh2a, 1.08×10^{11} photons cm^{-2} s^{-1}; and LWS, 4.47×10^{11} photons cm^{-2} s^{-1}.

Visual pigment template fitting

To relate spectral sensitivity to cone visual pigments, normalized spectral sensitivity curves were fitted with visual pigment templates (Hawryshyn et al., 2010). Considering that (i) the ERG represents the summed response of all cones and neurons in the outer retina, and (ii) opponent and non-opponent neural interactions between cones may remodel spectral sensitivity, the process of fitting visual pigment templates to spectral sensitivity might be challenging and prone to errors. Despite these limitations, fitting visual pigment templates to spectral sensitivity curves may still provide insight into the effect of environmental light on the contribution of the various visual pigments to spectral sensitivity. As a first-order approximation, fish were assumed to receive input from visual pigments whose opsin gene expression levels surpassed a threshold of 5%. To determine which opsin genes exceeded the 5% threshold, we followed Carleton et al. (Carleton et al., 2008), which perhaps provides the most comprehensive report of variation of opsin gene expression throughout ontogeny in Nile tilapia. In accordance with this report, juveniles were assumed to receive input from four visual pigments (SWS2b, SWS2a, Rh2a and LWS) and adults from three (SWS2a, Rh2a and LWS). See fig. 3 in Carleton et al. (Carleton et al., 2008) for detailed gene expression results for juveniles (50 d.p.f.) and adults (~155 and 195 d.p.f.); these ages of fish represent the best match to the ages of fish used in the current study.

Visual pigments are composed of a protein moiety, opsin, that is bound to a chromophore, a light-activated derivative of vitamin A. In fish and amphibians, the chromophore can exist in two states, A_1 (11-*cis* retinal) and A_2 (3,4-didehydroretinal) (Dartnall et al., 1961; Bowmaker, 1995). The λ_{max} of each visual pigment exhibits a defined wavelength shift as the A_2 proportion changes (Hárosi, 1994). This shift and the transmission of the lens were accounted for when generating the absorbance spectra of visual pigments for varying A_2 proportions. We combined absorbance spectra for visual pigments with an A_1 and A_2 chromophore. The proportion of the A_2 state was presented using a fraction parameter, a($0\leq a\leq1$), and therefore the absorbance spectrum of a given visual pigment exhibiting an A_2 proportion of a was calculated as:

$$A(a) = A_1 (1 - a) + A_2\, a\,. \quad (2)$$

Because visual sensitivity in Nile tilapia is dominated by LWS (Carleton et al., 2008), we determined a by least-squares fitting the LWS template to the spectral sensitivity curve. Thereafter, we used a to fit the remaining templates, while a parameter representing the contribution of each visual pigment to spectral sensitivity, k, was left unrestricted.

Lens transmission

The spectral transmission of the fish lens was measured following a protocol described elsewhere (Lisney et al., 2010; Sabbah et al., 2012). Lenses were surgically removed following the completion of ERG recordings and mounted inside a cuvette. Lens transmission was measured between 300 and 800 nm using a bench-top spectrophotometer (Cary 300; Varian, Palo Alto, CA, USA), and was normalized between 0 and 1 (maximum transmission was typically observed at the longest measured wavelength, 800 nm). For each fish, three transmission measurements were acquired from both lenses and averaged.

Spectral reflectance

Fish handling

Spectral reflectance (an approximation of the biologically relevant body colouration) was measured as described elsewhere (Gray et

al., 2011). Fish were immersed in water and anaesthetized with MS-222. Upon reaching stage III anaesthesia, fish were transferred into an open, black, water-filled chamber for reflectance measurements. Upon completion of measurements, fish were immersed in aerated water for recovery, and returned to their respective tanks. Note that the use of anaesthetics affects the spectral reflectance of the body pattern of fish, with MS-222 being no exception (Gray et al., 2011). However, the reflectance of all fish from all treatments was measured following the same protocol; thus, the effect of environmental light on body pattern reflectance could be compared across treatments.

Reflectance measurement apparatus and procedure

Spectral reflectance measurements were taken using a USB2000 spectroradiometer (Ocean Optics) connected to a 2 m bifurcated fibre optic cable (BIF600-2- UV/Vis, Ocean Optics) and a Deuterium–Halogen balanced light source (Ocean Optics). The spectroradiometer exhibited an effective spectral resolution of 2.06 nm (full width at half maximum, FWHM) between 200 and 950 nm (Sabbah et al., 2010). The reflectance probe exhibited a 3 mm diameter tip cut at an angle of 45 deg; black felt was placed over the tip to block extraneous light. This ensured that all measurements were taken at the same distance and angle, increasing the likelihood of measuring diffuse rather than specular reflectance. Unlike specular reflection, diffuse reflection does not vary with angle because all measured surfaces are equally likely to reflect light at any angle (Fleishman et al., 2006). Fish skin was assumed to act as a diffuse reflector. A measurement of a Spectralon diffuse reflectance standard (WS-1-SL, Ocean Optics) was taken as 100% reflectance; a dark measurement was taken as zero reflectance.

All reflectance recordings were performed under the same conditions, and fish were always kept on their left side, with the ventral surface facing the experimenter. The small size of juveniles allowed the measurement of no more than four distinct focal reflectance patches (the operculum, above the pectoral fin, above the anal fin and the caudal peduncle). For consistency, the number of focal patches remained the same in adults. We chose not to measure fin reflectance because of the increased probability that light transmitted through the transparent fin may 'contaminate' the reflectance measurement (Fleishman et al., 2006). We systematically measured reflectance from the darker pigmented bars on the fish trunk. However, considering (i) the tip diameter of the reflectance probe (3 mm), and (ii) the relatively large proximity of bright and dark bars on the trunk of juvenile fish, we cannot exclude the possibility that reflectance measurements in juveniles represent a combination of reflectance of bright and dark bars. Additionally, stress may potentially induce changes in the spectral reflectance of fish. Although great care was taken to minimize stress, it is possible that handling fish before and during reflectance measurements increased stress level. However, as all fish were handled similarly, experimenter-induced stress in all fish was likely similar. Stress level might have also varied between different light treatments. Indeed, variation in environmental light was reported to be associated with slight differences in cortisol levels in Nile tilapia (Volpato and Barreto, 2001). These differences, however, were all within the reported basal levels of cortisol (see Auperin et al., 1997). Thus, the different light treatments used in this study likely did not induce substantial differences in stress level between fish from different light treatments.

Statistical analysis

Prior to statistical analyses, all data were assessed for normality using the Shapiro–Wilk test, and for homogeneity of variance using an *F*-test (Quinn and Keough, 2002); thereafter, the appropriate tests were chosen. Reflectance spectra correlated with each other, and each spectrum comprised a large number of data points. Therefore, we used principal component analysis (PCA) to reduce the redundancy in the reflectance dataset (Smith, 2002). For each life stage, PCA was performed on the whole spectral reflectance dataset that included all fish from all light treatments, and the loadings of the various principal components (PCs) were obtained. Thereafter, the number of substantially contributing PCs was determined using a threshold criterion that compared the standard deviation of all PCs with the standard deviation of the first PC (Tong and Crowe, 1995). The loadings for the substantially contributing PCs were divided according to their respective treatments, and were used to test for differences between treatments. To test the effect of environmental light modulation on spectral reflectance, randomization tests were performed on the PCA loadings (Edgington, 1995; Adams and Anthony, 1996), with the difference between the means of any two treatments as the test statistic. The observed test statistic was compared with a null distribution estimated from 10,000 replicates, where PCA loadings were randomly permuted while maintaining the original sample sizes. Two-tailed 95% confidence intervals were also computed by bootstrapping the data (10,000 replicates).

To test whether modulation of environmental light affected lens transmission, we calculated the wavelength of half-maximum lens transmission (T_{50}), used for estimating the degree of short-wavelength light reaching the retina (Douglas and McGuigan, 1989), and compared T_{50} data between treatment groups using two-sample *t*-tests. In addition, to test whether lens transmission differed across the entire spectrum in response to environmental light modulation, we used PCA, randomization tests and bootstrap procedures as described above. In short, for each life stage, PCA was performed on the whole spectral lens transmission dataset that included all fish from all light treatments, and the loadings of the substantially contributing PCs were determined. Thereafter, randomization tests were performed on the PCA loadings to test the effect of environmental light modulation on the spectral lens transmission, and two-tailed 95% confidence intervals of the PCA loadings were computed using bootstrapping. All statistics were performed in R version 2.13.2 (R Development Core Team, 2009) with the significance level taken as $\alpha=0.05$.

RESULTS

Spectral sensitivity, lens transmission and spectral reflectance were measured in juvenile and adult Nile tilapia. Juveniles and adults were held under two different environmental light treatments, a broad spectrum and a reduced short-wavelength (red-shifted) spectrum. A third, white environmental light treatment was created for a sample of adults to test the possibility of short-term plasticity in spectral sensitivity, lens transmission and spectral reflectance. Neither juveniles nor adults from the different treatment groups differed significantly in body mass (Table 1), indicating that comparisons between treatments could justifiably be made.

How does environmental light affect spectral sensitivity throughout ontogeny?

To investigate how environmental light affects spectral sensitivity, and how this effect varies throughout ontogeny, we measured spectral sensitivity from juveniles and adults held under different environmental light treatments. Spectral sensitivity was measured under a short-wavelength isolating background that adapted the LWS cone mechanism.

Table 1. Body mass of juvenile and adult Nile tilapia

A. Variation in body mass

Experimental tank	Juveniles (g)	Adults (g)
T1	2.2±0.96	51.0±22.6
T2	1.9±0.94	50.6±18.6
T3		64.0±11.5
T4		68.0±25.8

B. Mann–Whitney test results

Experimental tank	Life stage	*U*	d.f.	*P*
T1 *vs* T2	Juvenile	8.50	13	0.314
T1 *vs* T2	Adult	1.50	8	0.841
T3 *vs* T4	Adult	0.50	8	1.00
T1,T2 *vs* T3,T4	Adult	19.0	18	0.166

(A) Body mass in the experimental tanks, shown as mean ± s.d. (B) To study the variation in body mass between experimental tanks, the non-parametric Mann–Whitney test was used. The *U* statistic, degrees of freedom (d.f.) and *P*-value are presented.

Spectral sensitivity in juveniles from a broad spectrum (T1, N_{T1}=5) and a red-shifted spectrum (T2, N_{T2}=5) differed slightly at long wavelengths (690–700 nm), but considerably between 420 and 440 nm (Fig. 3A). The latter difference in sensitivity agreed well with the peak difference in spectral irradiance between environmental light treatments (Fig. 1A,B). To estimate whether the contribution of visual pigments to spectral sensitivity varied under the two light treatments, spectral sensitivity was fitted with visual pigment templates for the four visual pigments reported in Nile tilapia juveniles – SWS2b (425 nm), SWS2a (456 nm), Rh2a (523 nm) and LWS (561 nm) (Carleton et al., 2008). See Materials and methods for details of the assumptions underlying the fitting procedure. The absorbance spectra of the four visual pigments could explain reasonably well the observed spectral sensitivity in juveniles. However, an additional, unexplained sensitivity peak was detected around 370 nm; this peak may reflect the sensitivity of the UV-sensitive SWS1 visual pigment (see Discussion). The contribution of the Rh2a and LWS visual pigments to spectral sensitivity did not vary appreciably between light treatments (Table 2 and Fig. 3B,C). Moreover, differences in the contribution of the various pigments to spectral sensitivity between juveniles from the two environmental light treatments correlated qualitatively to differences in the quantum catch (QC) of the pigments under the two light treatments. That is, the larger the difference in the contribution of a given pigment to spectral sensitivity between light treatments, the larger is the QC difference of that pigment under the two light treatments (Fig. 4A,B).

Spectral sensitivity in adults held under the broad spectrum (T1, N_{T1}=5) and the red-shifted spectrum (T2, N_{T2}=5) light treatment was similar (Fig. 5A). Thus, spectral sensitivity differences apparent between T1 and T2 as juveniles disappeared in the adult stage. Additionally, as expected, spectral sensitivity in adults held under the white light treatment (T3 and T4; N_{T3}=5, N_{T4}=5) was similar, but differed slightly at short wavelengths from that in adults from T1 and T2. Spectral sensitivity was fitted with visual pigment templates for the three visual pigments reported in Nile tilapia adults – SWS2a (456 nm), Rh2a (523 nm) and LWS (561 nm) (Carleton et al., 2008). The absorbance spectra of these three visual pigments could explain reasonably well the observed spectral sensitivity in adults from T3 and T4. However, in T1 and T2, an additional, unexplained sensitivity peak was detected around 400 nm; this peak may reflect the sensitivity of the SWS2b visual pigment or of an as yet undefined VS pigment (see Discussion) (Table 2 and Fig. 5B–E). The contribution of the various visual pigments to spectral sensitivity was similar for adults from T1 and T2, and did not correlate with QC differences of these pigments between the two light treatments (Fig. 4C,D). No appreciable differences were detected in either the contribution of visual pigments to the spectral sensitivity of adults from T3 and T4 or differences in the QC of the pigments under the two light treatments (Fig. 4E,F).

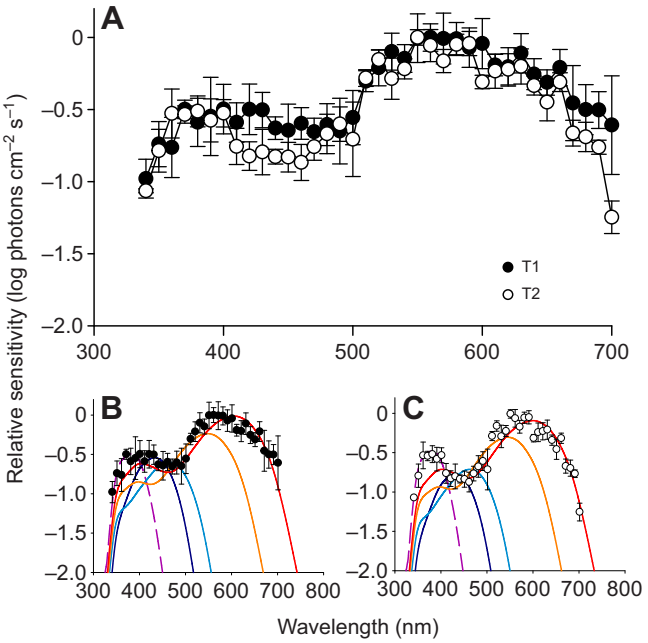

Fig. 3. Spectral sensitivity and visual pigment template fits for juvenile Nile tilapia. (A) Spectral sensitivity differed between juveniles from T1 (N_{T1}=5) and T2 (N_{T2}=5). Note the prominent short wavelength (410–440 nm) sensitivity difference between the two groups. (B,C) Visual pigment templates fitted to spectral sensitivity of juveniles from T1 (B) and T2 (C). Coloured lines represent the four visual pigments reported in juvenile Nile tilapia: SWS2b (dark blue), SWS2a (blue), Rh2a (orange) and LWS (red). A sensitivity peak around 370 nm could not be accounted for by the visual pigments reported. This sensitivity peak could be explained by the templates of the SWS1 visual pigment (dashed purple line). Error bars denote ±1 s.e.m.

For both juveniles and adults, the best fits of visual pigment templates to spectral sensitivity were attained with visual pigments of mixed A_1–A_2 chromophore. A_2 proportion varied throughout ontogeny, ranging between 0.55 and 0.62 in juveniles and between 0.99 and 1.00 in adults (Table 2).

How does environmental light affect lens transmission throughout ontogeny?

To investigate how environmental light affects the transmission of the fish lens, and how this effect changes throughout ontogeny, we compared the wavelength of half-maximum lens transmission (T_{50}) between environmental light treatments for juvenile and adult Nile tilapia. In addition, we employed PCA to test for differences across the entire spectrum between environmental light treatments, for juveniles and adults. We tested the effect of environmental light treatment on the loadings of each of the PCs that collectively accounted for >99% of the variation (tolerance criterion=0.1) (Hubert et al., 2005); however, for each treatment, we stopped our analysis once non-significant results were obtained.

Lens transmission in juveniles did not differ significantly between environmental light treatments (Fig. 6A), either in respect to T_{50}

Table 2. Results of fitting visual pigment templates to spectral sensitivity curves

Life stage	Experimental tank	Visual pigment	Fitted spectral range (nm)	Estimated λ_{max} (nm)	R^2	k	k'*	A_2 proportion
Juvenile	T1	SWS1†	340–400	382	0.561	0.492	0.514	0.62
		SWS2b	410–450	434	0.177	0.542	0.464	
		SWS2a	450–490	465	n.a.	0.600	0.405	
		Rh2a	470–540	548	0.741	0.236	0.773	
		LWS	540–690	606	0.532	0.012	1.000	
	T2	SWS1†	340–400	382	0.911	0.480	0.573	0.55
		SWS2b	410–450	433	n.a.	0.776	0.247	
		SWS2a	450–490	464	n.a.	0.712	0.316	
		Rh2a	470–540	545	0.673	0.304	0.767	
		LWS	540–690	600	0.716	0.093	1.000	
Adult	T1	SWS2a	370–480	467	0.474	0.517	0.526	0.99
		Rh2a	490–560	563	0.598	0.402	0.651	
		LWS	540–640	633	0.875	0.081	1.000	
	T2	SWS2a	370–480	467	0.251	0.442	0.610	1.00
		Rh2a	490–560	563	0.685	0.362	0.694	
		LWS	540–640	633	0.826	0.080	1.000	
	T3	SWS2a	370–480	467	0.682	0.805	0.231	0.99
		Rh2a	490–560	563	0.911	0.520	0.569	
		LWS	540–640	633	0.831	0.157	1.000	
	T4	SWS2a	370–480	467	0.582	0.850	0.197	0.99
		Rh2a	490–560	563	0.390	0.550	0.590	
		LWS	540–640	633	0.625	0.237	1.000	

λ_{max}, wavelength of maximum absorbance. A_2 is the 3,4-didehydroretinal chromatophore.

*k represents the automatically selected shift of a given visual pigment template along the sensitivity (ordinate) axis to allow for the best fit between empirical spectral sensitivity and template. Accordingly, k=0 represents the maximum possible contribution of the visual pigment under consideration to the observed sensitivity; and the larger the k, the smaller the contribution of that visual pigment to spectral sensitivity. For simplicity, we subtracted all k values from 1 and normalized the resulting value to the maximum value in each treatment group, such that k'=1 indicates the largest possible contribution of a given visual pigment. The contribution (k') of the SWS2a pigment to spectral sensitivity in juveniles from T1 and T2, as well as the contribution of the SWS2b pigment to spectral sensitivity in juveniles from T2 was relatively low and was associated with poor fits (n.a. indicates negative R^2 values).

†Spectral sensitivity in juveniles showed an additional peak around 370 nm that could not be accounted for by the visual pigments reported in juvenile tilapia. This sensitivity peak could be explained by the SWS1 visual pigment.

values (t-test, t=–0.253, d.f.=8, P=0.816) or across the entire spectrum [randomization test, PC1: P=0.880, confidence interval (CI) 2.5–97.5% – CI_{T1}=0.32–0.34, CI_{T2}=0.32–0.34, N_{T1}=4, N_{T2}=5). Similarly, lens transmission in adults from T1 and T2, as well as in adults from T3 and T4 did not differ significantly (Fig. 6B), either in respect to T_{50} values (t-$test_{T1:T2}$, t=–0.412, d.f.=6, P=0.720; t-$test_{T3:T4}$, t=0.967, d.f.=6, P=0.405) or across the entire spectrum (randomization $test_{T1:T2}$, PC1: P=0.060, CI_{T1}=0.38–0.41, CI_{T2}=0.35–0.37, N_{T1}=4, N_{T2}=3; randomization $test_{T3:T4}$, PC1: P=0.122, CI_{T3}=0.38–0.39, CI_{T4}=0.36–0.38, N_{T3}=3, N_{T4}=4).

How does environmental light affect spectral reflectance throughout ontogeny?

To investigate how environmental light affects spectral reflectance of fish, and how this effect changes throughout ontogeny, we measured the spectral reflectance from four focal patches on the bodies of juvenile and adult fish, held under different environmental light treatments. Fig. 7 shows photographs of representative adults from all experimental light treatments, and the location of the four focal patches. We used PCA to break down the variation into smaller vectors representing the loadings of the PCs that are responsible for most variation in the data. Sample sizes indicate the number of reflection measurements used in analyses.

Spectral reflectance in juveniles differed significantly between environmental light treatments, i.e. T1 (N_{T1}=3) *versus* T2 (N_{T2}=5) (randomization test, PC1: P<0.0001, CI_{T1}=0.21–0.29, CI_{T2}=0.03–0.10, N_{T1}=12, N_{T2}=20; PC2: P=0.0002, CI_{T1}=–0.04–0.15, CI_{T2}=–0.19 to −0.11, N_{T1}=12, N_{T2}=20; PC3: P=0.222, CI_{T1}=–0.98–0.04, CI_{T2}=–0.18 to −0.02, N_{T1}=12, N_{T2}=20). Juvenile fish from a red-shifted light treatment typically showed lower reflectance between 470 and 800 nm (Fig. 8A–D). However, an exception to this trend was the reflectance measured above the pectoral fin, which was lower across the spectrum under the red-shifted light treatment.

Spectral reflectance in adults differed significantly between environmental light treatments, i.e. T1 (N_{T1}=5) *versus* T2 (N_{T2}=5) (randomization test, PC1: P<0.0001, CI_{T1}=0.02–0.04, CI_{T2}=0.21–0.24, N_{T1}=19, N_{T2}=19; PC2: P=0.182, CI_{T1}=–0.12 to −0.03, CI_{T2}=–0.09–0.07, N_{T1}=19, N_{T2}=19). Spectral reflectance varied considerably between adults. Nonetheless, fish held under the two light treatments showed clear differences in reflectance between 390 and 440 nm as well as between 470 and 800 nm (Fig. 8E–H).

To test whether the effect of environmental light on spectral reflectance depended on the life stage of the fish, adults from T1 and T2 were compared against their juvenile counterparts. Adults and juveniles from T1 differed significantly in spectral reflectance, as did adults and juveniles from T2 (randomization test, PC1: P<0.0001 for T1 and T2). Adults from T3 (N_{T3}=5) and T4 (N_{T4}=5) did not differ significantly in spectral reflectance (randomization test, PC1: P=0.351, CI_{T3}=0.14–0.18, CI_{T4}=0.13–0.16, N_{T3}=20, N_{T4}=20; Fig. 8I–L). Additionally, adults from T1 and T3 as well as adults from T2 and T4 differed significantly in spectral reflectance (randomization test, PC1: P<0.0001 for both pairs).

DISCUSSION

This study provides evidence for an important role of environmental light in shaping signal reception and production throughout ontogeny. Our results show that environmental light can influence

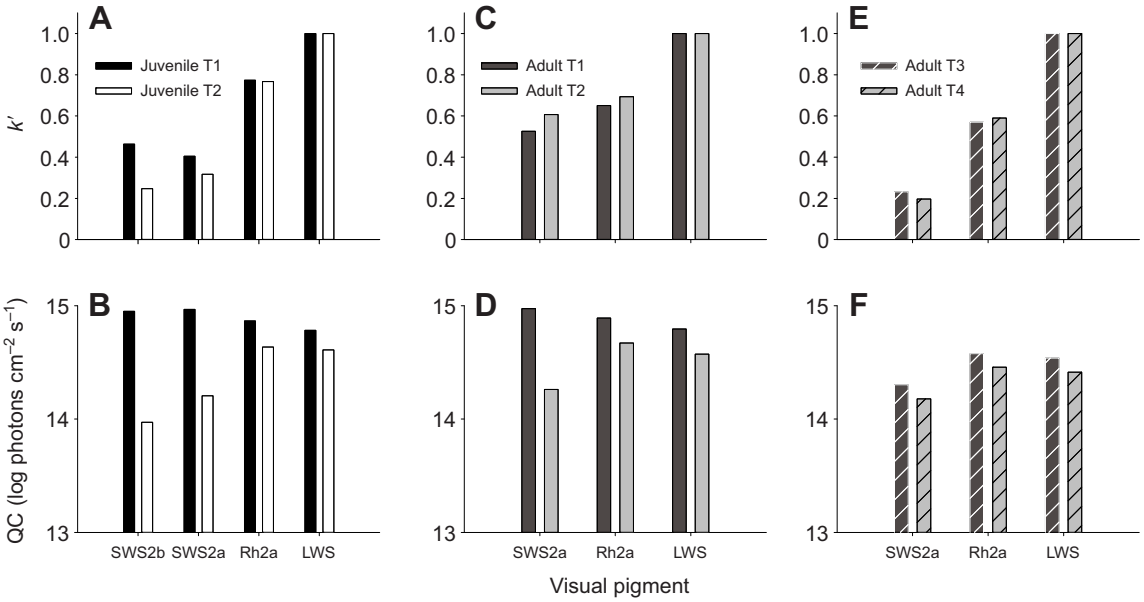

Fig. 4. Normalized contribution of visual pigments to spectral sensitivity (*k′)* and quantum catch (QC) of pigments in juvenile and adult Nile tilapia. The contribution of visual pigments to spectral sensitivity was estimated for each of the environmental light treatments examined. (A) Normalized *k* (*k′)* for the SWS2b visual pigment in juveniles reared under the broad-spectrum light treatment (T1) was nearly twice as large as the *k′* in juveniles reared under the red-shifted spectrum treatment (T2). (B) The latter difference in *k′* of the SWS2b pigment coincided with a larger QC of that pigment in T1 juveniles compared with T2 juveniles. (C) *k′* in adults from T1 and T2 was similar, and did not correlate with differences in the QC of pigments between light treatments (D). (E) Neither *k′* of visual pigments in adults held under the white light treatment (T3 and T4) nor the QC of these pigments differed appreciably (F). Note, however, that *k′* of the SWS2a in adults from T1 and T2 differed slightly from that in adults from T3 and T4 (D,F).

spectral sensitivity, but only during an age-dependent time window. In addition, environmental light appears to influence spectral reflectance (an approximation of the biologically relevant body colouration) regardless of age. These results highlight the important role of modulation of environmental light in body colouration plasticity.

Effect of environmental light on signal reception throughout ontogeny

We hypothesized that if modulation of environmental light altered the visual world of Nile tilapia by shaping signal reception, then fish reared under a short-wavelength-deprived (red-shifted) light spectrum would show a different spectral sensitivity from that of fish reared under a broad light spectrum. We also hypothesized that if the effect of modulation of environmental light on spectral sensitivity is reversible, then fish reared under the two original environmental light treatments and then transferred to a third, white light treatment would show similar spectral sensitivity.

We found that juveniles reared under a red-shifted light spectrum were less sensitive to short-wavelength light (mostly between 420 and 440 nm) compared with juveniles reared under a broad spectrum. Interestingly, the spectral location of this difference in sensitivity matched that of the peak difference in spectral irradiance between environmental light treatments (Fig. 1B). Furthermore, we found that environmental light had no influence on lens transmission regardless of life stage, indicating that the changes in the spectral sensitivity of juveniles observed under the two environmental light treatments were likely induced by changes occurring in the retina. This suggests that the reduction in short wavelength sensitivity among juveniles was likely driven by a localized effect on the contribution of short-wavelength-sensitive pigments to spectral sensitivity. Indeed, our results from fitting visual pigment templates to spectral sensitivity showed that the normalized contribution of the SWS2b and SWS2a visual pigments to spectral sensitivity in juveniles reared under the red-shifted light treatment was lower than in fish reared under the broad-spectrum light treatment. However, the relatively low contribution of the SWS2a pigment to spectral sensitivity as well as the associated poor fits of spectral sensitivity to the SWS2a pigment absorbance template may suggest that the differences observed in short wavelength sensitivity were mediated mainly through variation in the contribution of the SWS2b pigment. Interestingly, spectral sensitivity in juveniles reared under both light treatments showed an additional peak around 370 nm, a sensitivity peak that could not be accounted for by the visual pigments reported in juvenile tilapia. This peak may reflect the sensitivity of the UV-sensitive SWS1 visual pigment that is highly expressed in larvae and fry tilapia (Carleton et al., 2008). Thus, considering the strong effect of environmental light on the contribution of visual pigments to spectral sensitivity, it is possible that the light treatments examined in this study acted to extend the expression of the *SWS1* opsin gene and the pigment it encodes into the juvenile life stage. Indeed, our results from fitting visual pigment templates to spectral sensitivity suggest that the normalized contribution of the SWS1 visual pigment to spectral sensitivity in juveniles was substantial (Table 2).

Our findings are consistent with the idea that the expression level of an opsin gene is proportional to the irradiance level at the spectral range that maximally excites the pigment it encodes. For example, Hofmann and colleagues (Hofmann et al., 2010) found that three out of four Lake Malawi cichlids reared in the lab under a UV-deprived light treatment showed decreased expression of the opsin gene that encodes a UV-sensitive visual pigment (*SWS1*) compared with wild-caught fish (exposed to natural levels of UV light). Our results add to this previous report, and demonstrate that modulation of environmental light not only affects the expression level of cone opsin genes but also affects the contribution of visual pigments to

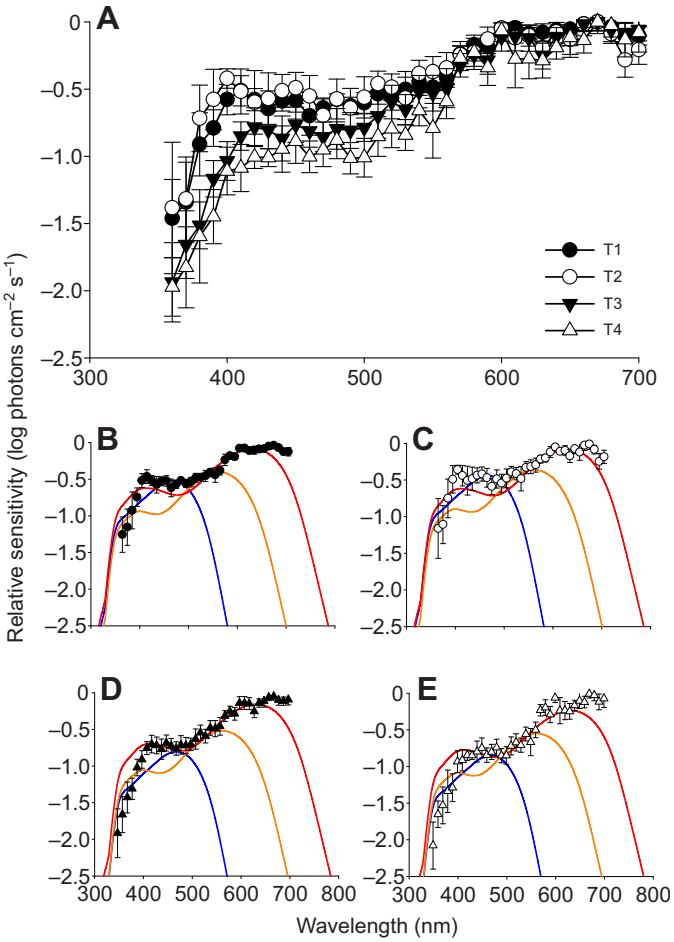

Fig. 5. Spectral sensitivity and visual pigment template fits for adult Nile tilapia. (A) Spectral sensitivity did not differ between adults from T1 (N_{T1}=5) and T2 (N_{T2}=5), or between adults from T3 (N_{T3}=5) and T4 (N_{T3}=5); however, short wavelength spectral sensitivity did differ slightly between fish from T1 and T2 and fish from T3 and T4. (B–E) Visual pigment templates fitted to spectral sensitivity of adults from T1 (B), T2 (C), T3 (D) and T4 (E). Coloured lines represent the three visual pigments found in adult Nile tilapia: SWS2a (blue), Rh2a (orange) and LWS (red). Note that the LWS visual pigment template could describe well the entire sensitivity spectrum for adults reared in all tanks (B–E). Error bars denote ±1 s.e.m.

spectral sensitivity. This implies that the visual system is able to respond to the spectral quality and availability of light and tune itself accordingly.

In contrast to juveniles, adults held under the broad-spectrum and red-shifted treatments showed similar spectral sensitivity. Additionally, spectral sensitivity in adults that were transferred to the white light treatment (in two separate tanks, T3 and T4) was also similar. Adults were previously reported not to possess the SWS2b visual pigment (Carleton et al., 2008), supporting the observed lack of response of spectral sensitivity in adults from T1 and T2 to modulation of environmental light. It is important to test whether other environmental light treatments, differing in the irradiance level at spectral ranges that maximally excite visual pigments found in adults, would alter spectral sensitivity in adult fish. Interestingly, spectral sensitivity of adults from T1 and T2 differed slightly at short wavelengths from that of adults transferred to T3 and T4. This difference in short wavelength sensitivity was probably a result of variation in the contribution of the SWS2a pigment to spectral sensitivity (Table 2 and Fig. 4C,E); however, the mechanism underlying this modulation of spectral sensitivity between the two pairs of tanks is currently unknown. Note that, in addition to fitting the absorbance templates of the three visual pigments reported in adult Nile tilapia, we also attempted to fit the VS visual pigment identified by Lisney et al. (Lisney et al., 2010); however, the resultant fit was very poor (not presented). This may indicate limitations of the fitting procedure rather than the lack of the VS visual pigment, as spectral sensitivity in T1 and T2 did show some short-wavelength sensitivity unaccounted for by the absorbance templates (Fig. 5). Additionally, the discrepancy between our study that of Lisney et al. (Lisney et al., 2010) might be a consequence of differences in background illumination used in ERG recordings, or the method used for the estimation of sensitivity. It is possible that these methodological differences obscured the sensitivity peak of the VS visual pigment concerned in the current study.

Interestingly, A_2 proportion was found to change between juveniles and adults, ranging between 0.55 and 0.62 in juveniles and between 0.99 and 1.00 in adults. Such ontogenetic variation in A_2 proportion was previously reported in salmonids and was suggested to prepare the fish for migration from the freshwater to the marine environment (Temple et al., 2006). Shifts in A_2 proportion were also reported to correlate with seasonal changes in temperature (Allen and McFarland, 1973; Tsin and Beatty, 1977) and with changes in photoperiod and light spectrum (Allen, 1971). However, the functional significance of plasticity in A_2 proportion of Nile tilapia is unclear at present. Moreover, spectral sensitivity in adults was dominated by the contribution of the LWS visual pigment. This was most evident from the results of the visual pigment template fitting, where the LWS visual pigment template gave a close approximation of the entire sensitivity spectrum in adults. Such an outcome might be expected as the expression of the *LWS* opsin gene was reported to account for 80% of total opsin gene expression in adults (Carleton et al., 2008). Additionally, despite the lack of effect of environmental light observed on spectral sensitivity in adults, the capacity of environmental light to shape signal reception in such a way that would influence adults and sexual selection does exist (see Fuller and Noa, 2010), and thus merits continued investigation.

Effect of environmental light on signal production throughout ontogeny

If environmental light shaped signal production, we hypothesized that fish reared under different light treatments would differ in spectral reflectance. Additionally, if environmental light has the capacity to induce a reversible effect on signal production, we hypothesized that fish transferred from their original light treatments and reared under a third, white light treatment would show similar spectral reflectance. Our results show that juveniles reared under a red-shifted light spectrum produced different spectral reflectance compared with juveniles reared under a broad-spectrum light treatment, tending to show reduced reflectance between 470 and 800 nm. Moreover, adults reared under a red-shifted light spectrum produced different spectral reflectance compared with adults reared under a broad-spectrum light, tending to show greater reflectance between 390 and 440 nm and lower reflectance between 470 and 800 nm. Adults also differed in spectral reflectance compared with their juvenile counterparts. Finally, fish from the original light treatments that were transferred to the white light treatment did not show differences in spectral reflectance, but were different from the fish that remained under the two original light treatments. Together, these results suggest that spectral reflectance is progressively and continuously shaped by environmental light, highlighting its role in body colour plasticity.

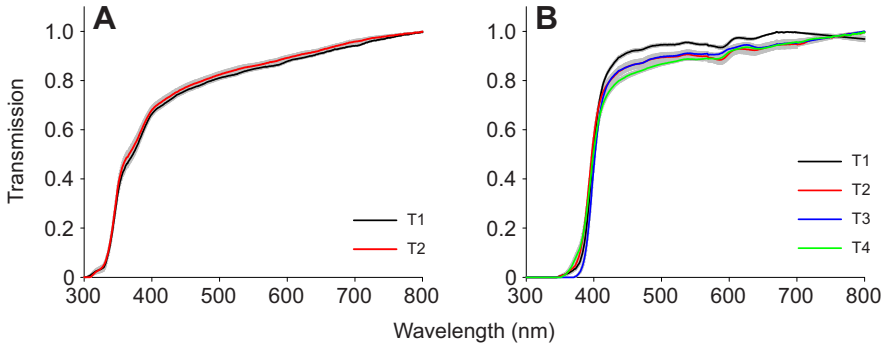

Fig. 6. Lens transmission of juvenile and adult Nile tilapia. Spectral lens transmission did not differ considerably between environmental light treatments for (A) juveniles (N_{T1}=4, N_{T2}=5) or (B) adults (N_{T1}=4, N_{T2}=3, N_{T3}=3, N_{T4}=4). However, lens transmission differed substantially throughout ontogeny, both in shape and in the wavelength of half-maximum lens transmission (T_{50}). The increase in lens transmission towards longer wavelengths was gradual in juveniles, but rather abrupt in adults, with the largest slope around 400 nm. Additionally, T_{50} in adults was significantly larger than in juveniles (randomization test: P<0.0001, $CI_{juveniles}$=357–376, CI_{adults}=399–405, $N_{juveniles}$=10, N_{adults}=18). That is, the lens of adult Nile tilapia was less transmissive of UV/short wavelengths. The barely noticeable grey shading (±1 s.e.m.) around the transmission curves suggests that lens transmission varied only slightly within treatment groups (maximum s.e.m. across the spectrum: juveniles, T1=±0.017, T2=±0.025; adults, T1=±0.020, T2=±0.020, T3=±0.024, T4=±0.024).

We found that juveniles and adults reared under a red-shifted light spectrum typically showed lower reflectance across the middle- and long-wavelength spectral regions (470–800 nm) compared with their counterparts reared under broad-spectrum light. Adults reared under a red-shifted light spectrum also tended to show higher reflectance across the UV and short-wavelength spectral regions (330–450 nm) (Fig. 8); this strong effect of environmental light on the spectral reflectance of adults stands in marked contrast to the absence of an effect of environmental light on spectral sensitivity. These findings are in agreement with the contrast hypothesis (Lythgoe, 1968). In environments where short-wavelength light is reduced (as in our red-shifted spectrum light treatment), it may be beneficial for conspecific recognition if body colour is offset relative to the prevailing background light (showing increased reflectance at short wavelengths), as this may increase the contrast of the fish against the water background without the need for a shift in spectral sensitivity. This prediction is consistent with our findings. Additionally, Sabbah and colleagues have shown that adult Nile tilapia exhibit lower sensitivity to long wavelengths compared with fry (Sabbah et al., 2012). They argued that lower long-wavelength sensitivity might facilitate conspecific recognition by increasing contrast against a red-shifted light environment. Thus, an observer with low long-wavelength sensitivity coupled with individuals showing high UV and short-wavelength spectral reflectance would result in very high contrast, and would likely facilitate conspecific recognition and mate choice.

We also found that spectral reflectance varied throughout ontogeny. It is well known that body colouration is highly variable among cichlids, with the vast array of colour morphs likely a result of strong sexual selection (Salzburger, 2009). However, less is known about ontogenetic body colour variation. The African cichlid *Haplochromis burtoni* was reported to show ontogenetic colour changes; the timing of which depended on social status (Fernald and Hirata, 1979). In the Central American firemouth cichlid *Thorichthys meeki*, 16 different colour morphs were observed as fish matured, possibly a result of a suite of age-dependent selective forces and developmental constraints (Beeching and Pike, 2010). The adaptive value of ontogenetic body colour variation was also explored in the tropical python *Morelia viridis*. Wilson and colleagues found that ontogenetic body colour differences in pythons reflect a changing foraging paradigm between juveniles and adults, suggesting an adaptive mechanism that camouflages both life stages from avian predators (Wilson et al., 2007). Our results demonstrate that environmental light shapes body colouration throughout ontogeny; however, the extent to which this may represent an adaptive response is currently unknown and demands further investigation.

Finally, spectral reflectance in adults reared under the two original light treatments as juveniles but transferred to a white environmental light treatment showed a reversible effect of environmental light. That is, spectral reflectance in adults held under the white light treatment differed markedly from that of their counterparts reared under the two original light treatments, and became almost indistinguishable. These adults showed little variation in reflectance from each other (Fig. 8I–L), and were more silver in colour than adults from either of the other treatments (Fig. 7). This

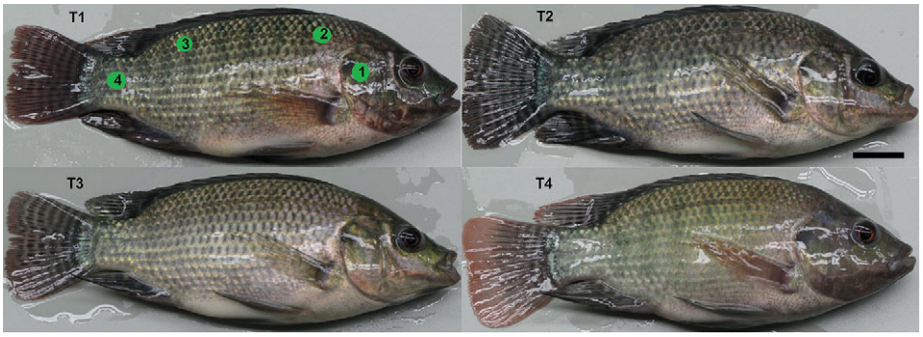

Fig. 7. Photographs of representative adults from all experimental light treatments. Green circles in the top left photograph indicate the approximate location of reflection focal patches used across all fish, for juveniles and adults. Visual inspection of fish photographs reveals that fish from T1 generally showed more yellows, while those from T2 generally showed more blues. Fish from T3 and T4 were generally more silver coloured. Scale bar in the top right photograph indicates 2 cm and applies to all photographs.

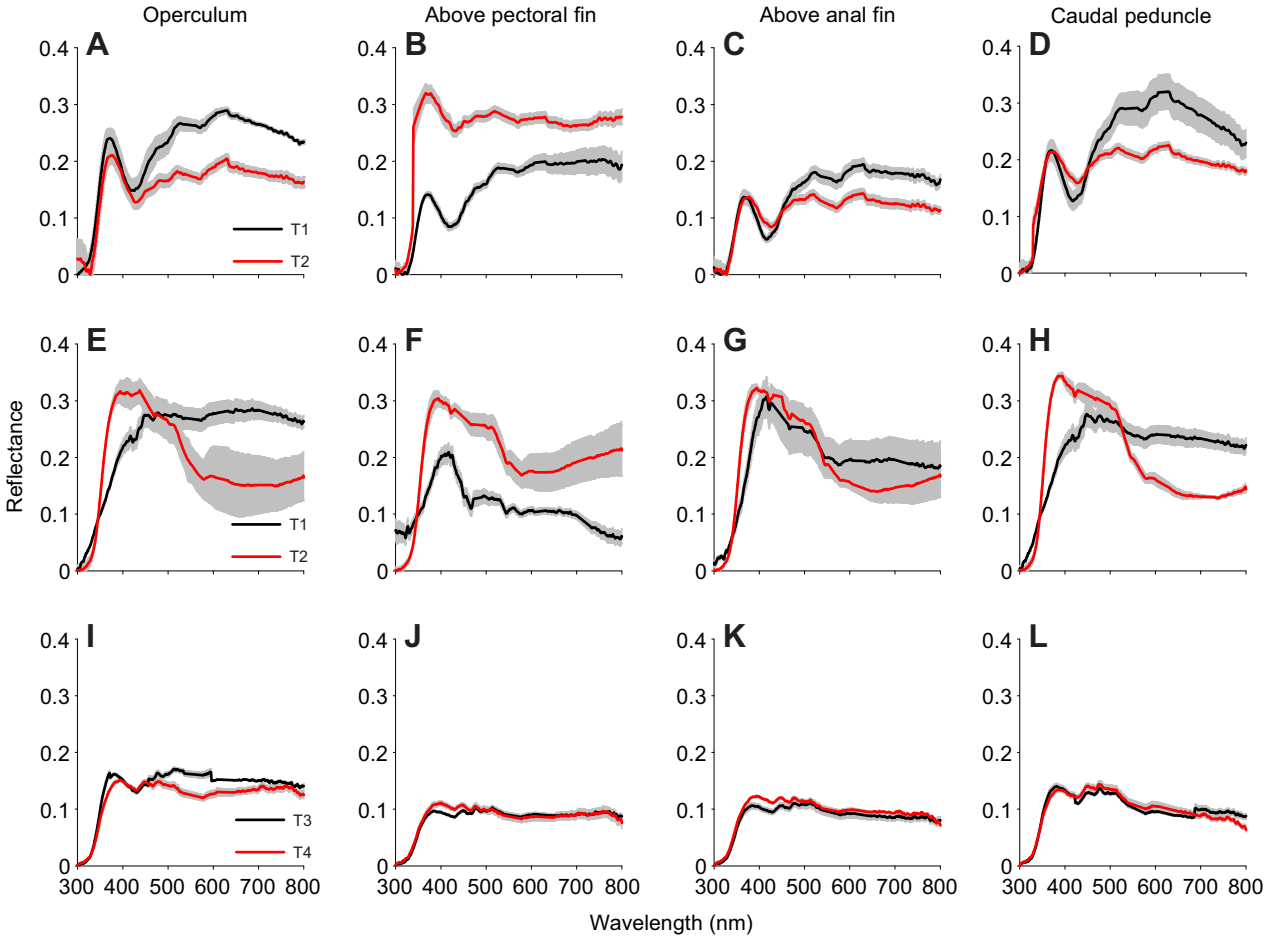

Fig. 8. Averaged raw reflection spectra for juvenile and adult Nile tilapia. (A–D) Juveniles from T1 (N_{T1}=3) and T2 (N_{T2}=5) differed significantly in spectral reflectance. (E–H) Adults from T1 (N_{T1}=5) and T2 (N_{T2}=5) differed significantly in spectral reflectance, both between treatments and between their juvenile counterparts. (I–L) Adults from T3 (N_{T3}=5) and T4 (N_{T4}=5) did not differ significantly in spectral sensitivity, but fish tested against the treatment they came from did differ significantly. See Results for detailed statistics. Grey shading denotes ±1 s.e.m. in all panels.

result demonstrates the continuous influence of environmental light on body colouration.

Note that sexually mature Nile tilapia males develop subtle, but detectable, changes in body colouration relative to females. Similar differences in spectral reflectance are also typically observed between dominant and subordinate males. The effect of sex or dominance hierarchy on the spectral reflectance of adult fish was not considered in the current study. However, we opted not to measure reflectance from any fins that may show large reflectance differences between females and males as well as between males that differ in dominance hierarchy. This, we believe, allows for the examination of the effect of environmental light on the reflectance of fish, while removing the potentially confounding effect of sex differences and variation in dominance hierarchy.

Nile tilapia are thought to possess dermal chromatophores with opsin-based light-sensitive pigments (Ban et al., 2005). Unlike visual cells that respond to light through changes in membrane potential (hyperpolarization among vertebrates, depolarization among invertebrates), tilapia dermal chromatophores do not require such changes to initiate a photoresponse. Instead, chromatophore response is likely driven by changes in cAMP levels, through a G-protein photocascade event (Ban et al., 2005). This suggests that tilapia chromatophores can respond to light independently of the visual system, unlike the well-known cuttlefish *Sepia officinalis*, which receives information about its surroundings visually and aggregates or disperses the pigment granules in its chromatophores accordingly (Barbosa et al., 2007; Mäthger et al., 2008). The spectral composition of the prevailing environmental light changes dramatically with water depth (McFarland and Munz, 1975; Sabbah et al., 2011), and the spectrum of underwater light is typically red-shifted in turbid water bodies (Utne-Palm, 2002), resembling our red-shifted environmental light treatment. Thus, it is possible that modulation of environmental light, associated with variation in water depth and clarity, plays a role in determining body colouration, at least in fish with dermal chromatophores. However, additional work that considers these factors should be carried out, especially in systems where dermal chromatophores are thought to be able to detect and respond to light independently of the visual system.

CONCLUSIONS

This study shows that environmental light influences spectral sensitivity in an age-dependent manner and body colouration in an age-independent manner, but does not influence lens transmission. We provide experimental evidence that plasticity in signal reception, likely through a decreased contribution of the SWS2b visual pigment to spectral sensitivity, can be induced by modulation of environmental light at the juvenile life stage. However, plasticity in signal production (body colouration) could be induced and

progressively moulded throughout ontogeny. This is especially interesting, as Nile tilapia have been shown to rely upon visual signals for tasks such as conspecific recognition and mate choice (Castro et al., 2009), suggesting that environmental light may play a key role in tilapiine communication and mating. These results reinforce previous reports (see Endler, 1993) that environmental light has the capacity to be a large driving force in models of animal communication, and possibly also in models of ecological speciation.

ACKNOWLEDGEMENTS

We thank Zahra Dargaei, Kathleen Allen, Michael Sutton, Maheen Habib-Nayany, Tomas Money and Changhai Zhu for valuable input and assistance with data collection. In addition, we wish to thank two anonymous reviewers for their helpful comments and constructive criticism on a previous version of the manuscript.

AUTHOR CONTRIBUTIONS

M.A.W.H. and S.S. conceived and designed the experiments. M.A.W.H. performed the measurements. M.A.W.H. and S.S. analyzed the data. M.A.W.H. and S.S. wrote the paper. R.M.R. and C.W.H. commented on the paper.

COMPETING INTERESTS

No competing interests declared.

FUNDING

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant [106102-07]; NSERC Research Tools and Instrumentation Grant [359714-2008]; Canada Foundation for Innovation, Ontario Innovation Trust [202821]; and the Canada Research Chair Program to C.W.H. S.S. was supported by a Vanier Canada Graduate Scholarship from NSERC.

REFERENCES

Adams, D. C. and Anthony, C. D. (1996). Using randomization techniques to analyse behavioural data. *Anim. Behav.* **51**, 733-738.
Allen, D. M. (1971). Photic control of the proportions of two visual pigments in a fish. *Vision Res.* **11**, 1077-1112.
Allen, D. M. and McFarland, W. N. (1973). The effect of temperature on rhodopsin-porphyropsin ratios in a fish. *Vision Res.* **13**, 1303-1309.
Auperin, B., Baroiller, J. F., Ricordel, M. J., Fostier, A. and Prunet, P. (1997). Effect of confinement stress on circulating levels of growth hormone and two prolactins in freshwater-adapted tilapia (*Oreochromis niloticus*). *Gen. Comp. Endocrinol.* **108**, 35-44.
Ban, E., Kasai, A., Sato, M., Yokozeki, A., Hisatomi, O. and Oshima, N. (2005). The signaling pathway in photoresponses that may be mediated by visual pigments in erythrophores of Nile tilapia. *Pigment Cell Res.* **18**, 360-369.
Barbosa, A., Mäthger, L. M., Chubb, C., Florio, C., Chiao, C.-C. and Hanlon, R. T. (2007). Disruptive coloration in cuttlefish: a visual perception mechanism that regulates ontogenetic adjustment of skin patterning. *J. Exp. Biol.* **210**, 1139-1147.
Barlow, G. W. (2000). *The Cichlid Fishes: Nature's Grand Experiment in Evolution*. Cambridge: Perseus Publishing.
Beeching, S. C. and Pike, R. E. (2010). Ontogenetic colour change in the firemouth cichlid, *Thorichthys meeki*. *Copeia* **2010**, 189-195.
Bowmaker, J. K. (1995). The visual pigments of fish. *Prog. Retin. Eye Res.* **15**, 1-31.
Cadillo-Quiroz, H., Didelot, X., Held, N. L., Herrera, A., Darling, A., Reno, M. L., Krause, D. J. and Whitaker, R. J. (2012). Patterns of gene flow define species of thermophilic Archaea. *PLoS Biol.* **10**, e1001265.
Carleton, K. L. and Kocher, T. D. (2001). Cone opsin genes of African cichlid fishes: tuning spectral sensitivity by differential gene expression. *Mol. Biol. Evol.* **18**, 1540-1550.
Carleton, K. L., Spady, T. C., Streelman, J. T., Kidd, M. R., McFarland, W. N. and Loew, E. R. (2008). Visual sensitivities tuned by heterochronic shifts in opsin gene expression. *BMC Biol.* **6**, 22.
Castro, A. L. S., Gonçalves-de-Freitas, E., Volpato, G. L. and Oliveira, C. (2009). Visual communication stimulates reproduction in Nile tilapia, *Oreochromis niloticus* (L.). *Braz. J. Med. Biol. Res.* **42**, 368-374.
Dartnall, H. J. A., Lander, M. R. and Munz, F. W. (1961). Periodic changes in the visual pigment of a fish. In *Progress in Photobiology* (ed. B. C. Christensen and B. Buchman), pp. 203-213. Amsterdam: Elsevier.
Douglas, R. H. and McGuigan, C. M. (1989). The spectral transmission of freshwater teleost ocular media – an interspecific comparison and a guide to potential ultraviolet sensitivity. *Vision Res.* **29**, 871-879.
Edgington, E. S. (1995). *Randomization Tests*. New York, NY: Marcel Dekker.
El Sayed, A. F. M. (2006). *Tilapia Culture*. Wallingford: CABI.
Endler, J. A. (1991). Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Res.* **31**, 587-608.
Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *Am. Nat.* **139**, S125-S153.
Endler, J. A. (1993). Some general comments on the evolution and design of animal communication systems. *Philos. Trans. R. Soc. B* **340**, 215-225.
Fernald, R. D. and Hirata, N. R. (1979). The ontogeny of social behavior and body coloration in the African cichlid fish *Haplochromis burtoni*. *Z. Tierpsychol.* **50**, 180-187.
Fleishman, L. J., Leal, M. and Sheehan, J. (2006). Illumination geometry, detector position and the objective determination of animal signal colours in natural light. *Anim. Behav.* **71**, 463-474.
Fuller, R. C. and Claricoates, K. M. (2011). Rapid light-induced shifts in opsin expression: finding new opsins, discerning mechanisms of change, and implications for visual sensitivity. *Mol. Ecol.* **20**, 3321-3335.
Fuller, R. C. and Noa, L. A. (2010). Female mating preferences, lighting environment, and a test of the sensory bias hypothesis in the bluefin killifish. *Anim. Behav.* **80**, 23-35.
Fuller, R. C., Noa, L. A. and Strellner, R. S. (2010). Teasing apart the many effects of lighting environment on opsin expression and foraging preference in bluefin killifish. *Am. Nat.* **176**, 1-13.
Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G. and Donner, K. (2000). In search of the visual pigment template. *Vis. Neurosci.* **17**, 509-528.
Gray, S. M., Hart, F. L., Tremblay, M. E. M., Lisney, T. J. and Hawryshyn, C. W. (2011). The effects of handling time, ambient light, and anaesthetic method, on the standardized measurement of fish colouration. *Can. J. Fish. Aquat. Sci.* **68**, 330-342.
Guilford, T. and Dawkins, M. S. (1991). Receiver psychology and evolution of animal signals. *Anim. Behav.* **42**, 1-14.
Hárosi, F. I. (1994). An analysis of two spectral properties of vertebrate visual pigments. *Vision Res.* **34**, 1359-1367.
Hawryshyn, C. W., Moyer, H. D., Allison, W. T., Haimberger, T. J. and McFarland, W. N. (2003). Multidimensional polarization sensitivity in damselfishes. *J. Comp. Physiol. A* **189**, 213-220.
Hawryshyn, C. W., Ramsden, S. D., Betke, K. M. and Sabbah, S. (2010). Spectral and polarization sensitivity of juvenile Atlantic salmon (*Salmo salar*): phylogenetic considerations. *J. Exp. Biol.* **213**, 3187-3197.
Hofmann, C. M., O'Quin, K. E., Marshall, N. J., Cronin, T. W., Seehausen, O. and Carleton, K. L. (2009). The eyes have it: regulatory and structural changes both underlie cichlid visual pigment diversity. *PLoS Biol.* **7**, e1000266.
Hofmann, C. M., O'Quin, K. E., Smith, A. R. and Carleton, K. L. (2010). Plasticity of opsin gene expression in cichlids from Lake Malawi. *Mol. Ecol.* **19**, 2064-2074.
Hubert, M., Rousseeuw, P. J. and Branden, K. V. (2005). ROBPCA: A new approach to robust principal component analysis. *Technometrics* **47**, 64-79.
Imanpoor, M. R. and Abdollahi, M. (2011). Effects of tank color on growth, stress response and skin color of juvenile Caspian kutum *Rtilus frisii* Kutum. *Global Veterinaria* **6**, 118-125.
Kelley, J. L., Phillips, B., Cummins, G. H. and Shand, J. (2012). Changes in the visual environment effect colour signal brightness and shoaling behaviour in a freshwater fish. *Anim. Behav.* **83**, 783-791.
Kocher, T. D. (2004). Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* **5**, 288-298.
Kornfield, I. and Smith, P. F. (2000). African cichlid fishes: model systems for evolutionary biology. *Annu. Rev. Ecol. Evol. Syst.* **31**, 163-196.
Lisney, T. J., Studd, E. and Hawryshyn, C. W. (2010). Electrophysiological assessment of spectral sensitivity in adult Nile tilapia *Oreochromis niloticus*: evidence for violet sensitivity. *J. Exp. Biol.* **213**, 1453-1463.
Lythgoe, J. N. (1968). Visual pigments and visual range underwater. *Vision Res.* **8**, 997-1012.
Lythgoe, J. N. (1979). *The Ecology of Vision*. Oxford: Clarendon Press.
Maddocks, S. A., Church, S. C. and Cuthill, I. C. (2001). The effects of the light environment on prey choice by zebra finches. *J. Exp. Biol.* **204**, 2509-2515.
Mäthger, L. M., Chiao, C.-C., Barbosa, A. and Hanlon, R. T. (2008). Color matching on natural substrates in cuttlefish, *Sepia officinalis*. *J. Comp. Physiol. A* **194**, 577-585.
McFarland, W. N. and Munz, F. W. (1975). Part II: the photic environment of clear tropical seas during the day. *Vision Res.* **15**, 1063-1070.
O'Quin, K. E., Hofmann, C. M., Hofmann, H. A. and Carleton, K. L. (2010). Parallel evolution of opsin gene expression in African cichlid fishes. *Mol. Biol. Evol.* **27**, 2839-2854.
Parry, J. W. L., Carleton, K. L., Spady, T., Carboo, A., Hunt, D. M. and Bowmaker, J. K. (2005). Mix and match color vision: tuning spectral sensitivity by differential opsin gene expression in Lake Malawi cichlids. *Curr. Biol.* **15**, 1734-1739.
Quinn, G. P. and Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press.
R Development Core Team (2009). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Available at: http://www.R-project.org/.
Ramsden, S. D., Anderson, L., Mussi, M., Kamermans, M. and Hawryshyn, C. W. (2008). Retinal processing and opponent mechanisms mediating ultraviolet polarization sensitivity in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **211**, 1376-1385.
Rundle, H. D. and Nosil, P. (2005). Ecological speciation. *Ecol. Lett.* **8**, 336-352.
Ryan, M. J. (1990). Sexual selection, sensory systems, and sensory exploitation. *Oxf. Surv. Evol. Biol.* **7**, 157-195.
Sabbah, S., Laria, R. L., Gray, S. M. and Hawryshyn, C. W. (2010). Functional diversity in the color vision of cichlid fishes. *BMC Biol.* **8**, 133.
Sabbah, S., Gray, S. M., Boss, E. S., Fraser, J. M., Zatha, R. and Hawryshyn, C. W. (2011). The underwater photic environment of Cape Maclear, Lake Malawi: comparison between rock- and sand-bottom habitats and implications for cichlid fish vision. *J. Exp. Biol.* **214**, 487-500.
Sabbah, S., Hui, J., Hauser, F. E., Nelson, W. A. and Hawryshyn, C. W. (2012). Ontogeny in the visual system of Nile tilapia. *J. Exp. Biol.* **215**, 2684-2695.
Sabbah, S., Troje, N. F., Gray, S. M. and Hawryshyn, C. W. (2013a). High complexity of aquatic irradiance may have driven the evolution of four-dimensional colour vision in shallow-water fish. *J. Exp. Biol.* **216**, 1670-1682.

Sabbah, S., Habib-Nayany, M. F., Dargaei, Z., Hauser, F. E., Kamermans, M. and Hawryshyn, C. W. (2013b). Retinal region of polarization sensitivity switches during ontogeny of rainbow trout. *J. Neurosci.* **33**, 7428-7438.
Salzburger, W. (2009). The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Mol. Ecol.* **18**, 169-185.
Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D. J., Miyagi, R., van der Sluijs, I., Schneider, M. V., Maan, M. E., Tachida, H. et al. (2008). Speciation through sensory drive in cichlid fish. *Nature* **455**, 620-626.
Shand, J., Davies, W. L., Thomas, N., Balmer, L., Cowing, J. A., Pointer, M., Carvalho, L. S., Trezise, A. E. O., Collin, S. P., Beazley, L. D. et al. (2008). The influence of ontogeny and light environment on the expression of visual pigment opsins in the retina of the black bream, *Acanthopagrus butcheri*. *J. Exp. Biol.* **211**, 1495-1503.
Slaughter, M. M. and Miller, R. F. (1981). 2-amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. *Science* **211**, 182-185.
Smith, L. I. (2002). *A Tutorial on Principal Components Analysis*. Available at: http://www.sccg.sk/~haladova/principal_components.pdf. Accessed May 20, 2012.
Smith, A. R., Ma, K., Soares, D. and Carleton, K. L. (2012). Relative LWS cone opsin expression determines optomotor thresholds in Malawi cichlid fish. *Genes Brain Behav.* **11**, 185-192.
Spady, T. C., Parry, J. W. L., Robinson, P. R., Hunt, D. M., Bowmaker, J. K. and Carleton, K. L. (2006). Evolution of the cichlid visual palette through ontogenetic subfunctionalization of the opsin gene arrays. *Mol. Biol. Evol.* **23**, 1538-1547.
Sundin, J., Berglund, A. and Rosenqvist, G. (2010). Turbidity hampers mate choice in a pipefish. *Ethology* **116**, 1-9.
Temple, S. E., Plate, E. M., Ramsden, S., Haimberger, T., Roth, W. M. and Hawryshyn, C. W. (2006). Seasonal cycle in vitamin A(1)/A(2)-based visual pigment composition during the life history of coho salmon (*Oncorhynchus kisutch*). *J. Comp. Physiol. A* **192**, 301-313.
Tong, H. and Crowe, C. M. (1995). Detection of gross errors in in data reconciliation by principal component analysis. *Process Syst. Eng.* **41**, 1712-1722.
Tsin, A. T. C. and Beatty, D. D. (1977). Visual pigment changes in rainbow trout in response to temperature. *Science* **195**, 1358-1360.
Utne-Palm, A. C. (2002). Visual feeding of fish in a turbid environment: physical and behavioural aspects. *Mar. Freshwat. Behav. Physiol.* **35**, 111-128.
Volpato, G. L. and Barreto, R. E. (2001). Environmental blue light prevents stress in the fish Nile tilapia. *Braz. J. Med. Biol. Res.* **34**, 1041-1045.
Wilson, D., Heinsohn, R. and Endler, J. A. (2007). The adaptive significance of ontogenetic colour change in a tropical python. *Biol. Lett.* **3**, 40-43.