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Adult plasticity in African Cichlids: Rapid changes in opsin expression in response to environmental light differences

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Abstract

Phenotypic plasticity allows organisms to adapt quickly to local environmental conditions and could facilitate adaptive radiations. Cichlids have recently undergone an adaptive radiation in Lake Malawi where they inhabit diverse light environments and tune their visual sensitivity through differences in cone opsin expression. While cichlid opsin expression is known to be plastic over development, whether adults remain plastic is unknown. Adult plasticity in visual tuning could play a role in cichlid radiations by enabling survival in changing environments and facilitating invasion into novel environments. Here we examine the existence of and temporal changes in adult visual plasticity of two closely related species. In complementary experiments, wild adult *Metriacroma mbenji* from Lake Malawi were moved to the lab under UV-deficient fluorescent lighting; while lab raised *M. benetos* were placed under UV-rich lighting designed to mimic light conditions in the wild. Surprisingly, adult cichlids in both experiments showed significant changes in the expression of the UV-sensitive single cone opsin, *SWS1*, in only three days. Modeling quantum catches in the light environments revealed a possible link between the light available to the *SWS1* visual pigment and *SWS1* expression. We conclude that adult cichlids can undergo rapid and significant changes in opsin expression in response to environmental light shifts that are relevant to their habitat and evolutionary history in Lake Malawi. This could have contributed to the rapid divergence characteristic of these fantastic fishes.

Keywords

opsin; gene expression; plasticity; cichlid; vision

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DATA ACCESSIBILITY

Opsin expression data for the Natural to Narrow and Narrow to Broad experiments (including normalization to total opsin within cone type and *GNAT2*) as well as relative *SWS1* cone opsin expression data for all species in Figure 6 have been deposited in Dryad (doi: 10.5061/dryad.v20gh).

AUTHOR CONTRIBUTIONS

S.P.N designed the Natural to Narrow experiment, and M.R.Y designed the Narrow to Broad experiment. Both S.P.N and M.R.Y performed analyses and drafted the manuscript. K.L.C conceptualized the study and critically reviewed the article. All authors declare no conflicts of interest.

INTRODUCTION

Phenotypic plasticity is the ability of an individual genotype to produce multiple distinctive phenotypes in response to varied environmental conditions (Price *et al.* 2003; Travis 1994; West-Eberhard 2003). The environment presents certain triggers, whether biotic, such as social interactions between potential mates or predators, or abiotic, such as seasonal changes in temperature or the current light environment. Additionally, these triggers are not fixed, but in a constant state of flux, fluctuating variably throughout the lifetime of the organism. Responding appropriately to these heterogeneous environmental cues is linked to organismal fitness and is crucial for executing fundamental tasks such as foraging, mate-choice and predator avoidance. Thus, phenotypic plasticity can increase the persistence of a species in variable environments and can also facilitate the ability to adapt to novel niches (Pigliucci 2001; West-Eberhard 2005).

Historically, phenotypic plasticity has been overlooked as being important to adaptive evolution because it could possibly shelter the genotype from the effects of selection (Grant 1977; Stebbins 1950). However, recent theoretical modeling and experimental research has revealed that under the right conditions, environmentally induced plasticity can promote diversification and speciation events, such as in adaptive radiations (Ehrenreich & Pfennig 2016; Ghalambor *et al.* 2007; Pfennig & McGee 2010; Wund *et al.* 2008; Schneider & Meyer 2017). This could be accomplished in several ways, some of which include allowing the immediate exploitation of different niches in a novel environment, producing a rapid response to selection pressures in one generation, and enabling the repeated evolution of traits suited to different environments (Pfennig *et al.* 2010; Yeh & Price 2004). Phenotypic plasticity is ubiquitous in nature and has been observed for several ecologically relevant traits in phenotypically diverse lineages such as social behavior in Hymenopterans (Cahan *et al.* 2004), courtship behavior in sticklebacks (Shaw *et al.* 2007), life history traits in nematodes (Gutteling *et al.* 2007), color morphs in spiders (Brewer *et al.* 2015) and limb length in Anoles (Losos *et al.* 2000). In the present study, we concentrated on visual system plasticity, mainly as changes in the opsin gene expression in response to environmental lighting that could enhance an animal's visual function and ultimately its fitness.

Photoreceptors lead the visual response, being specially harnessed to capture light. The absorption of photons by the photoreceptors starts a signal transduction cascade resulting in neural output (Wald 1935). As such, the ability of an organism to detect and respond to light will depend on the absorption properties of the visual pigment residing within the photoreceptors (Bowmaker 1995; Hunt *et al.* 2006). Indeed, it has been extensively shown that subsets of vertebrate photoreceptors have spectral sensitivities that closely match the most abundant wavelengths in the surrounding environment (Levine & Macnichol 1979; Loew & Lythgoe 1978; Lythgoe 1984). This spectral "tuning" of the photoreceptors to ambient light is ecologically relevant and serves to enhance contrast detection in visually driven tasks (Osorio & Vorobyev 2008). Additionally, variation in the properties of the photoreceptors due to differences in the spectral range of environmental light has been known to foster population differentiation and speciation (Endler 1991; Seehausen *et al.* 2008).

Visual sensitivities of animals can be adapted to their environmental light via genetic modifications and/or phenotypic plasticity. Genetic changes such as structural replacements in specific amino acids within the opsin protein moiety shape the sensitivity of the visual pigments (Hunt *et al.* 2007; Yokoyama 2008). These genetic modifications usually take place slowly, accumulating gradually over evolutionary time (Lamb *et al.* 2007; Bloch *et al.* 2015). On the other hand, plasticity induced changes fine tune visual acuity much more rapidly, within the lifetime of the organism (Beaudet & Hawryshyn 1999; Collin & Shand 2003). Some of these physiological mechanisms include seasonal reversals in chromophore usage in connection with migration behavior (Flamarique 2005) or alterations in the dietary availability of carotenoids (Sandkam *et al.* 2016; Toomey & McGraw 2011).

One other molecular mechanism for altering spectral sensitivity that can be both genetically determined and environmentally induced, thereby having the potential to act across varied time scales, is changes in opsin gene expression (Secondi *et al.* 2017; Macias-Munoz *et al.* 2015; Carleton *et al.* 2010; Carleton *et al.* 2008; Fuller *et al.* 2005). Across species, opsin expression variation in African cichlids causes significant differences in spectral sensitivities between closely related taxa (Carleton 2009; Parry *et al.* 2005). This differential opsin expression results in closely related species with huge differences in their visual capabilities and aids cichlid diversification across all major axes such as habitat, foraging and mating preference (Dijkstra *et al.* 2005; Dobberfuhl *et al.* 2005; Jordan *et al.* 2004; Kidd *et al.* 2006). Within a single species, opsin expression changes can be hardwired ontogenic events, often correlated with changes in habitats or foraging behaviors (Carleton *et al.* 2008). For example, cone opsins in riverine Tilapia convert their expression from shorter to longer wavelengths as the fish grow and reach adulthood. This kind of developmental progression has also been observed in other fish species such as salmon (Temple *et al.* 2008), goatfish (Shand 1993), eel (Cottrill *et al.* 2009), trout (Cheng & Flamarique 2007), flounder (Hoke *et al.* 2006) and black bream (Shand *et al.* 2002).

In addition to genetic mechanisms described above, several studies have examined whether there can be phenotypic plasticity in opsin expression in response to changes in environmental light conditions. However, results from these studies are often conflicting; with some organisms showing plastic changes while others have little/no plasticity whatsoever. When reared under different light regimens, developmental plasticity in opsin expression was observed in guppies (Ehlman *et al.* 2015) and black bream (Shand *et al.* 2008). Contrary to these results, rearing conditions had no effect on opsin expression in cave-dwelling mollies (Tobler *et al.* 2010) and South American cichlid *Aequidens pulcher* (Wagner & Kroger 2000). Although not tested during development, adult sticklebacks showed minor plasticity in opsin expression (Flamarique *et al.* 2013). So far, the only known system that shows both developmental and adult plasticity in opsin expression is the bluefin killifish (*Lucania goodei*) that occurs in a wide-range of lighting conditions, from tannin swamps to clear freshwater (Fuller *et al.* 2005; Fuller *et al.* 2004). Two separate experiments have documented that killifish show pronounced developmental and adult plasticity in opsin expression (Fuller & Claricoates 2011; Fuller *et al.* 2010). Larval killifish reared in tea-stained water have higher *RH2* and *LWS* expression, while fish from the same brood reared under clear water have increased expression of *SWS1* and *SWS2B* opsins. Similar changes

were also observed in swamp and clear spring adult fish when placed in reciprocal water conditions. Importantly, these plastic changes have been observed in as little as three days.

The cichlids of East African rift lakes are one of the fastest speciating vertebrates with over 500 species from Lake Malawi having evolved from a common ancestor less than a million years ago (Danley & Kocher 2001; Genner *et al.* 2007; Kocher 2004). Due to their recent evolutionary history, all cichlid species have nearly identical sequences for each of their seven corresponding cone opsin genes (*SWS1*, *SWS2B*, *SWS2A*, *RH2B*, *RH2A β* , *RH2A α* and *LWS*) (Spady *et al.* 2005). Large differences in visual sensitivities among cichlid species are achieved by changing the complement of expressed opsin genes (Carleton 2009; Carleton & Kocher 2001). Adult cichlids from Lake Malawi express a subset of the seven cone opsin genes in their retinas, leading to sizeable differences in the spectral properties of their photoreceptors.

In cichlids, modification of spectral sensitivity due to changes in opsin expression are coordinated both by genetic and environmental factors. Multiple hybrid crosses between cichlid species with differing opsin expression patterns have revealed that the largest opsin expression differences are controlled by a few loci (Carleton *et al.* 2010; Nandamuri *et al.* 2017; O'Quin *et al.* 2012). Additionally, environmentally induced plasticity plays an important role in tuning visual sensitivity during development. Previously, Lake Malawi cichlids reared under fluorescent light conditions in the lab expressed small but significantly different opsin expression profiles than their wild counterparts (Hofmann *et al.* 2010b). Further, differences in opsin expression were observed when individual broods were split and raised under different lighting conditions (Smith *et al.* 2012). Also, developmental plasticity was observed in cichlids with increased *LWS* expression in dorsal retinas when fish were raised under upwelling fluorescent light, (Dalton *et al.* 2015). Although these earlier studies were successful in establishing that the visual system in cichlids is developmentally plastic, two crucial questions remain unanswered: **1) Can plastic changes in opsin expression occur in adulthood or are they restricted to early development? 2) What are the temporal dynamics of the plastic change in opsin expression? In other words, how quickly can adult individuals modify their opsin expression in response to an altered environmental cue?**

The current study has three main goals. First, we want to examine if adult cichlids exhibit plastic changes in opsin expression due to differences in lighting conditions. If visual tuning by plasticity is only restricted to development, it could limit the ability of cichlid species to adapt to changing light environments at later life history stages. Second, we want to monitor the tempo of changes in opsin expression to determine how swiftly plastic changes are observed. If the plastic response to an altered light regime takes too long to come into effect, it might adversely affect the optimum functioning of the visual system in the new light environment. Third, we want to compare opsin expression relative to a cone-specific housekeeping gene with opsin expression normalized to the total opsin expression. Many of our previous studies have compared opsin expression relative to total pool of opsins within cone type (Hofmann *et al.* 2010b; Carleton *et al.* 2010). While this enables comparisons of opsin ratios, it obscures which opsin is actually changing in expression due to differences in illumination. To study plasticity, we set up two complementary experiments: 1) we took

adult wild-caught cichlids from a single population and housed them in narrow spectrum lighting devoid of UV wavelengths (Natural to Narrow); and 2) we placed lab-reared adult cichlids in high intensity UV-rich broadband illumination designed to mimic light in the natural lake environment (Narrow to Broad). Results from this study give us the first insights that cichlids with short wavelength sensitivities can indeed respond on a rapid time scale to light changes.

MATERIALS AND METHODS

Species selection

Two species from a single genus were chosen for the experiments. *Metriaclima mbenji* were used for the Natural to Narrow study and *Metriaclima benetos* were used in the Narrow to Broad experiments. These sister species are thought to be less than 100,000 years divergent to each other and to *Metriaclima zebra*, which is the representative cichlid species from Lake Malawi (Brawand *et al.* 2014; Meyer 1993). Both species occupy similar ecological niches in the lakes with identical foraging, mating and breeding behaviors. Most importantly, adult wild-caught individuals of both species have the same opsin expression profiles in their retinas - *SWS1* and minor levels of *SWS2B* expression in the single cones; and *RH2B* and *RH2A* expression in the double cones (Carleton 2009). When raised under different lighting conditions, a plastic response in opsin expression has been previously demonstrated in *M. mbenji* (Hofmann *et al.* 2010b). Since *M. benetos* is a sister species to *M. mbenji*, it was presumed that they would also be plastic. Additionally, developmental plasticity has been observed in *M. benetos* when reared in the lab (Carleton *et al.* 2008). For the Natural to Narrow study, adult *M. mbenji* individuals from a single population (n=49) were collected in 2015 from Mbenji Island (13° 26' 23. 50" S 34° 29' 35" E) and housed in the lab for the duration of the experiment. Adult *M. benetos* (n=47) used for the Narrow to Broad study were multiple generation progeny produced from wild-caught stocks collected from Mazinzi reef in 2008 (14 ° 5' 33" S 35 ° 1' 94" E).

Light environments

The light environments were selected to address several questions. First, did moving fish under altered environments change their gene expression? For this we chose to move fish from the natural light conditions in the lake to the simplest light conditions of the fluorescent lights typical in labs (Natural to Narrow). Second, what aspect of the lab lighting affects the change in opsin expression (Narrow to Broad)? Is it the intensity of light or spectral content or both? We therefore chose to compare lights of the same spectra varying in intensity (dim (DIM) vs bright fluorescent illumination (BFLR)) and then varied spectral content (fluorescent vs metal-halide illumination, UV-rich).

Natural to Narrow—The natural light environment was estimated from measurements taken at Thumbi Island (14°1'8"S 34°48'53"E) (Dalton *et al.* 2010). *M. mbenji* are normally found in the top 20m of the lake, so side-welling irradiance measurements at 3m and 10m were used for comparison to the narrow spectrum lab environment (Ribbink *et al.* 1983). The irradiance spectra for the lab fluorescent environment was measured from the underwater side-welling light in the tanks using a cosine collector attached to the Ocean

Optics USB4000 spectrometer (Ocean Optics, Dunedin, FL, USA) with a 1 micron diameter, 2m UV transmissive fiber. The tanks were housed under Phillips 32W (Philips, Somerset, NJ, USA) fluorescent bulbs.

Narrow to Broad—Three light environments were chosen for this study- dim fluorescent (DIM), bright fluorescent (BFLR), and metal halide (UV). Similar to the Natural to Narrow study, the irradiance spectra were measured underwater for side-welling light. The DIM and BFLR treatment tanks were under Phillips 32W fluorescent bulbs and the UV treatment tanks were under broad-spectrum metal halide XM 10,000 K bulbs (XM lighting, Springfield, OH, USA). DIM tanks had an intensity of less than 0.2 klux as measured by a HI 97500 Portable Luxmeter (Hanna Instruments, Woonsocket, RI, USA). Intensity in BFLR and UV tanks was ~2.5 klux. The halide UV treatment was intended to simulate water conditions in the lake. It should be noted that the UV treatment was broadband illumination but still less intense compared to light incident on the Lake Malawi water surface and equivalent to intensity at a depth of 20m in the lake. All the light environments operated under a peak 12–12 day/night photoperiod.

Sample collection

Natural to Narrow—Adult *M. mbenji* from a single population were shipped to the University of Maryland after being held for several weeks in an outdoor greenhouse in Malawi, being kept in the dark during shipment. Immediately on arrival, 20 individuals were euthanized and phenotyped for opsin expression (DAY0). The remaining fish (n= 29) were placed in a single 50-gallon tank under fluorescent light conditions. The overall intensity of light was several orders of magnitude lower than that in the lake and importantly was UV deficient. 10 fish each were then sampled at 3 days (DAY3), 3 weeks (DAY21) and 5 weeks (DAY35) (the DAY35 sample had 9 individuals), to look for changes in opsin expression (Fig. 1a). The light conditions were kept constant for the entire duration of the experiment. To reduce time of day effects, all samples were collected between 3–5 PM. Individuals were sampled randomly with respect to sex since no correlation between sex and opsin expression has been found in wild-caught or lab-reared Lake Malawi cichlids (data not shown). Specimens were euthanized using an overdose of MS222 and retinas were dissected and stored at –20 °C in RNAlater.

Narrow to Broad—Lab-raised *M. benetos* were F2 progeny of wild-caught fish that were more than 3 years old and resided in dim fluorescent tanks. Fish from 3 unrelated families with at least 2 individuals from each family were included in the baseline dim fluorescent (DIM) group. Specimens were either collected from DIM tanks or moved to bright fluorescent (BFLR) or metal halide (UV) tanks for 3 or 7 days prior to sampling (Fig. 1b). Samples were collected using the same methods as Natural to Narrow with either 9 or 10 individuals collected for each treatment.

Quantification of opsin expression

Opsin expression was quantified using formerly described methods (Carleton 2011). Briefly, RNA was extracted from dissected retinas using the QIAshredder and RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and transcribed into cDNA (Superscript III, Invitrogen,

Carlsbad, CA, USA). cDNA from each sample was quantified by real-time, quantitative PCR (RT-PCR) using Taqman primers (Life Tech, Carlsbad, CA, USA) and probes specific for each opsin on a Roche Lightcycler 480 (Roche, Bavaria, Germany). As in previous work, *RH2Aα* and *RH2Aβ* genes that are more than 99% identical were combined to become the 'RH2A' measurement. Plates were run with a mixture of experimental groups to reduce plate effects and each reaction was performed in duplicate. PCR efficiencies were determined by amplifying an opsin construct containing a single copy of each of the six opsins (Spady *et al.* 2006). Expression of each opsin relative to single and double cones was calculated as a percent of the total opsin within that cell type. For example, the fraction, f , of *SWS1* expression in the single cones is calculated as:

$$f_{SWS1,SC} = T_{SWS1} / (T_{SWS1} + T_{SWS2B} + T_{SWS2A})$$

where T is the transcript level for each of the single cone opsin genes. Similarly, the fraction of *LWS* expression in double cones is:

$$f_{LWS,DC} = T_{LWS} / (T_{RH2A} + T_{RH2B} + T_{LWS})$$

To eliminate the effects of normalization to the opsin pool within a cone type, opsin expression was also normalized to another gene, *GNAT2* (Dalton *et al.* 2015). *GNAT2* is the cone-specific alpha subunit of transducin, which is part of the photoreceptor signal transduction cascade and is therefore likely constantly and highly expressed in the cone cells of the retina. PCR efficiencies were calculated from RT-PCR measurements of serial dilutions of a mixture of *GNAT2* PCR product and the opsin construct. Using the PCR efficiencies (E_i) and critical numbers (C_{ti}) for each opsin, the normalized cone opsin expression to *GNAT2* was calculated (in percentage) as:

$$T_i / T_{GNAT2} = (1 + E_{GNAT2})^{C_{tGNAT2}} / (1 + E_i)^{C_{ti}}$$

where 'i' is the cone opsin of interest, E_{GNAT2} and C_{tGNAT2} are the PCR efficiency and critical number for *GNAT2* respectively.

Quantum catch calculations

Absolute quantum catch was estimated for each visual pigment (opsin protein bound to the A1 chromophore) to approximate how many incident photons each pigment would absorb in the different light environments (Kelber *et al.* 2003). Quantum catch thus helps measure how each visual pigment is stimulated in the wild and the different lab treatment conditions. Quantum catch is estimated using:

$$Q_i = \int I(\lambda) L(\lambda) R(\lambda) d\lambda$$

where $I(\lambda)$ represents the normalized irradiance spectra of the environment, $L(\lambda)$ is the lens transmission, and $R(\lambda)$ represents the photoreceptor absorption values at each wavelength (Govardovskii *et al.* 2000). Since opsin gene sequences and visual pigment peak sensitivities

are similar for different *Metriaclima* species, the $\lambda_{\max}$ of *Metriaclima zebra* visual pigments were used. For visual pigments not expressed in *M. zebra*, an average of sensitivities from different Lake Malawi species was used to calculate quantum catches (Carleton 2009). Lens transmittance was measured from four lenses of adult lab-reared *M. benetos* (Escobar-Camacho *et al.* 2017). Since *M. mbenji* occur in the top 20m of the water column all quantum calculations for Natural to Narrow experiment were performed at a depth of 3m and 10m.

Statistical tests

To test for significance of the change in opsin expression between each experimental light environment, post hoc Tukey's multiple comparisons of means with 95% family-wise confidence level was used. Statistical tests were performed in R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria) and were corrected for multiple comparisons using the Bonferroni adjustment.

RESULTS

Changes in opsin expression

Overall, opsin expression varied as a function of both time and treatment. Significant differences in opsin expression were observed in both experiments. Results from post-hoc Tukey's multiple comparisons for all the treatment and time points are provided in Table 1.

Natural to Narrow—When normalized to total opsins in single cones, *SWS2B* increased significantly within three days (Fig. 2c). While *SWS1* decreased within the same period, the change was not significant (Fig. 2a). While no significant changes in *SWS2B* were observed when normalized to *GNAT2*, expression of *SWS1* significantly decreased within three days (Fig. 2d), indicating that *SWS1* was downregulated. Following the three-day time period, *SWS1* and *SWS2B* remained unchanged for the duration of the experiment. No changes in *SWS2A* were observed, irrespective of the normalization (Fig. S1a, S1b).

When normalized to total opsin in double cones, *RH2B* decreased significantly after three days in the lab environment (Fig. S1c). Post three days, *RH2B* remains unchanged. When normalized to *GNAT2*, a significant decrease in expression was observed between DAY0 and both DAY3 as well as DAY21, denoting that *RH2B* was downregulated as well (Fig. S1d). *RH2A* showed drastically different patterns for each normalization procedure. When normalized to total opsin within double cones, *RH2A* showed no changes between arrival and the later time points (Fig. S1e). However, when normalized to *GNAT2*, a significant decrease was observed between arrival and both DAY3 (Fig. S1f). Regardless of normalization, no changes in *LWS* were observed in the double cones (Fig. S1g, S1h).

Narrow to Broad—Adult fish show significant changes after treatment for only three days (Fig. 3). Only some opsins showed differences in expression in the different light environments. Longer duration treatments also seemed to increase the strength of differences.

In the single cones, *SWS1* increased in the UV environment. Both time points in the UV (UV3 and UV7) were significantly higher than DIM when normalized to opsin within single cones (Fig. 3a). With *GNAT2* normalization, UV7 had significantly higher *SWS1* than DIM treatment indicating that *SWS1* opsin was upregulated and that this upregulation increased with more time (Fig. 3b). When normalized to total opsin in single cones, *SWS2B* showed the opposite trend from *SWS1*, with expression in both UV3 and UV7 being significantly less than in DIM (Fig. 3c). However, no significant changes were observed in *SWS2B* when normalized to *GNAT2* (Fig. 3d). In *M. benetos*, *SWS2A* is only detected at very low levels in the retina. When normalized to opsin expression within single cones, expression in the BFLR7 was significantly higher than both the UV3 and UV7 (Fig. S2a). The *GNAT2* normalization showed a significant decrease in *SWS2A* in UV3 relative to DIM but the values are extremely small and likely not biologically significant (Fig. S2b).

When normalized to total opsin in double cones, *LWS* expression in the BFLR7 was significantly higher than both UV3 and UV7 (Fig. 3e). Compared to the baseline DIM, *LWS* opsin showed a significant increase in the bright fluorescent environment but only for the 7-day treatment (BFLR7) suggesting that upregulation of *LWS* in the BFLR tanks is intensified with longer duration in the treatment (Fig. 3f). Neither *RH2B* nor *RH2A* showed consistent differences with treatment. Compared to the baseline DIM, when normalized to total opsin within double cones, both *RH2B* and *RH2A* remained unchanged in the UV treatment (Fig. S2c, S2e). While no changes were observed in *RH2A* expression with *GNAT2* normalization, *RH2B* decreased in BFLR7 and both UV3 and UV7 when compared to BFLR3 (Fig. S2d, S2f).

Quantum catches

The calculated quantum catch values suggest that visual pigments are stimulated differently in each light environment. The absolute quantum catch values of all treatment conditions for both the experiments are presented in Table 2.

Natural to Narrow—Although there is variation, in the wild, all visual pigments performed relatively well at both depths. Since light transmission decreases with depth, quantum catches at 3m were approximately three times higher for all opsins than those for 10m. Consistent with the higher irradiance levels towards the longer wavelength end of the spectra (Fig. 4a), quantum catches for double cone pigments (*RH2B*, *RH2A β* , *RH2A α* , *LWS*) were about 4 times higher than those for single cones (*SWS1*, *SWS2B*, *SWS2A*) (Fig. 4b). Owing to their red-shifted spectra, the difference in quantum catches doubled to about eight times between the two cone types under fluorescent lights (Fig. 4a). Except for *SWS1*, quantum catch for all other opsins was ~1.2 orders of magnitude lower in the lab environment than in the wild. It is important to note that the quantum catch for *SWS1* falls to zero in the UV deficient lab environment (Table 2A). This means that in individuals shifted from the lake to the lab, the *SWS1* photoreceptors in the retina received no stimulation.

Narrow to Broad—The metal halide (UV) environment was relatively effective in simulating the environmental conditions in the wild. Although the intensity of the UV

treatment (~ 2.5 klux) was at least 40 times lower than the shallow water conditions in the lake (~ 100 klux), the treatment was successful in providing a wider spectrum that stimulated all visual pigments (Fig. 5a). As expected, the fluorescent spectra for both the BFLR and DIM treatments were shifted heavily to longer wavelengths (Fig. 5a). Consequently, the quantum catches of the double cone pigments were higher than the single cone pigments (Fig. 5b).

Similar to *SWS1* in the Natural to Narrow experiment, there is zero quantum catch for *SWS1* in the DIM treatment (Fig. 5b, Table 2B). The quantum catch for *SWS2B* was lowest in the DIM, but nearly the same in the BFLR and UV treatments. For all other visual pigments, quantum catches were highest in the BFLR treatment (Fig. 5b). Of note, the quantum catch for *LWS* in BFLR was three times higher than in the UV treatment, and nearly nine times higher than DIM. Also, *SWS1* is the only visual pigment with quantum catch values higher in UV compared to all other treatments (Table 2B).

DISCUSSION

Plasticity in opsin expression in adult cichlids

Using quantitative PCR, we have shown that adult cichlids from Lake Malawi exhibit plastic changes in opsin expression mediated by differences in spectral irradiance. Even more striking is that these plastic changes occurred relatively fast, with some changes recorded three days after being placed in the new environment. This suggests that retinal sensitivities can change on a rapid timescale, enabling adult fish to adjust to short-term changes in lighting conditions. This might be helpful for fish swimming to different depths to find new habitats or from changes in local water clarity from runoff during seasonal rains or algal blooms.

As mentioned earlier, most of our previous studies have measured opsin expression relative to the total pool of opsins. The reasoning for this is that color vision is contingent on the differences in the inputs received by the cone cells in the retina (Kelber *et al.* 2003). Differences in the expression of each cone type relative to total opsin either echo differences in the relative abundance of specific cone cells in the retina or more likely the packing of the opsin pigment in the outer segments of cones, which could change color perception. The downside of this normalization is that it obfuscates the mechanism by which the differences in visual tuning through opsin expression arise. Since expression changes of each opsin are no longer independent, it becomes impossible to predict which opsin is being up/down regulated. To circumvent this problem, we have normalized opsin expression to the expression of a cone specific gene, *GNAT2*, similar to what has been done previously (Fuller & Claricoates 2011). *GNAT2*, as part of the phototransduction cascade, is constantly expressed at high levels in the cone cells of the retina and should be expressed in conjunction with the cone opsins thus varying minimally between samples and between treatments. Indeed, results from 27 cichlid retinal transcriptomes confirm that *GNAT2* is relatively constant in expression (data not shown). It has a coefficient of variation comparable to that of beta-actin (and better than several other genes often used as housekeeping genes including *EF1a*, *PAX6*, and *UBE2z*). In the Natural to Narrow experiment, although normalization to total single cone opsins indicates that *SWS2B*

increases significantly after just three days under fluorescent lights (Fig. 2), *GNAT2* normalization suggests that adult animals only decrease *SWS1* while keeping *SWS2B* constant.

Plastic changes in opsin expression could be explained by several mechanisms. It is possible that the outer segments of the photoreceptors could increase in size. Such changes in photoreceptor length have been previously observed following long-term exposure to highly intense monochromatic light (Wagner & Kroger 2000). This is unlikely within the time frame observed within our study (three days). Another option is that the expression differences arise from changes in the relative numbers of cone types, with additional cone cells being added to the retina. Again, this too is highly improbable, as it has been demonstrated that complete differentiation of cone cells in gold fish takes approximately three weeks (Wu *et al.* 2001). A third possibility is an increase in the density of the visual pigment in the cone photoreceptors. Since optimal opsin protein packing in the photoreceptor cilia is critical for proper functionality of the phototransduction cascade and kinetics of light response, this seems unlikely as well (Wen *et al.* 2009). We hypothesize that the plastic differences in expression arise from the expression of multiple opsins in the same photoreceptor, i.e. co-expression (Dalton *et al.* 2014). Earlier studies in the lab have shown that *M. zebra* coexpressed opsins in their cone cells and that this level of coexpression increased with altered environmental background spectra (Dalton *et al.* 2015). Since *M. benetos* and *M. mbenji* are from the same genus, it seems likely that plastic changes could cause an increase in coexpression of opsins in these individuals as well. Future studies with in-situ hybridization or electrophysiology from individual cone photoreceptors might throw some light on the cellular modifications underlying plasticity.

In this study, it is clear from the quantum catches (Table 2, Fig. 4 & Fig. 5) that the largest difference between the two experiments arises from the absence/presence of ultraviolet illumination. As expected, major plastic changes are observed in the single cones, principally along the *SWS1-SWS2B* axis (ignoring the very low levels of *SWS2A* expression that are not likely to be physiologically relevant). Although quantum catches of the double cone opsins vary with light treatments, the magnitude of change is much smaller. Further, this problem is likely compounded by the fact that the two *RH2A* opsins have been merged in our study. Future experiments with more severe shifts in the spectral space bounded by the appropriate wavelengths of the double cones might lead to more pronounced plasticity in the double cones.

Both intensity and spectral composition of the incident light seem to play a role in mediating plastic changes, with longer duration times generally enhancing the differences in opsin expression. Previous studies have speculated that the spectral content of light rather than intensity played a bigger role in plasticity (Hofmann *et al.* 2010b). In the current study, we showed that at least for some opsins, namely *LWS*, intensity of light might also play a part (fish in the BFLR7 treatment have high *LWS* expression compared to the DIM) (Fig. 3f). Additional plasticity studies on adult species are underway to help further delineate the role of spectral makeup and intensity in stimulating expression plasticity.

Lastly, while the light changes dramatically between Lake Malawi and the lab, there are several other factors that differ between those two environments including temperature, salinity, circadian period, diet, and presence or absence of predators. Although unlikely, there is a possibility that any of these factors could have an effect on opsin expression in the Natural to Narrow experiment. Given that complementary changes in opsin expression were seen in the Narrow to Broad experiment where all the lab conditions were the same and only the light composition was changed, we postulate that changes in opsin expression in Natural to Narrow were most likely due to the light environment differences.

Genetic inferences of plasticity

From this study, it is evident that *SWS2A* is never turned on in *Metriaclima* species in spite of the further improvements it would make to the quantum catch efficiency. This result is not novel and has been formerly observed when broods from other rock-dwelling cichlids were raised under fluorescent lights (Hofmann *et al.* 2010b). Also, this is consistent with the developmental program in *Metriaclima* that entails an ontogenetic progression from *SWS1* in larvae to *SWS1-SWS2B* as adults in the single cones but never to *SWS2A* expression (Carleton *et al.* 2008). These findings suggest that in Lake Malawi cichlid species with short wavelength sensitivities, *SWS2A* is controlled by an independent set of factors that are different from the genetic factors that co-ordinate both *SWS1* and *SWS2B*. In fact, this hypothesis is supported by data from a recent hybrid cross in the lab between two genera that express *SWS1* and *SWS2B* (Nandamuri et al. 2017). Since *SWS1* and *SWS2B* are expressed in the same cone cell, it makes sense that they would share some, if not all, genetic factors.

Furthermore, plasticity experiments in killifish have illustrated that the extent of plastic opsin expression varies among families proposing that plasticity is under genetic control (Fuller *et al.* 2005). Though traditionally debated, it is now accepted that plasticity loci do exist and studies examining the genetic basis of plasticity have become common (Bergland *et al.* 2008; Gutteling *et al.* 2007; Parsons *et al.* 2016). At present, we have a hybrid cross between a plastic species *M. mbenji* and a non-plastic species *A. baenschii* (Nandamuri et al. 2017). Splitting the F2 broods of this cross and raising them under UV-rich and UV-deficient lighting will help map genetic factors contributing to the plasticity of *SWS1/SWS2B* expression.

Comparing developmental and adult plasticity

A summary of what we currently know about developmental and adult plasticity is shown in Figure 6. The results from this current study are compared against previously collected developmental progression in the lab and wild adult data in the context of *SWS1* expression (Carleton *et al.* 2008; Hofmann *et al.* 2010a; Hofmann *et al.* 2010b; Smith *et al.* 2011). A comparison of the Natural to Narrow results to the *M. benetos* lab developmental series can be informative about how plastic changes in opsin expression are mediated during adulthood versus during development (Fig. 6 left) Lab-raised *M. benetos* larvae (10–20 days) show the highest levels of *SWS1* (~95%, *M. benetos* lab development in Fig. 6). As the larvae grow older in the lab, the levels of *SWS1* steadily decrease with adults (150–180 days) having an average *SWS1* closer to 50% (similar to *SWS1* in lab-raised *M. mbenji* adults, Fig. 6).

Currently, no wild developmental data is available for comparison, but wild caught adult *Metriaclima* species have an average *SWSI* of around 85% (wild Thumbi, wild Mazinzi and wild Mbenji adults in Fig. 6). Since wild-caught adult *Metriaclima* species have high *SWSI* as adults, it is probable that they retain a predominance of *SWSI* throughout development and into adulthood.

It is therefore likely that the *M. benetos* lab developmental series shows the effects of plasticity in opsin expression during development. This is equivalent to the Natural to Narrow experiment in the current study that shows plasticity in opsin expression as adults. A closer look at these two parallel studies yields some interesting preliminary trends. The temporal dynamics of developmental plasticity seem to be slower than that of adult plasticity. One possible explanation for this is that during development, the plastic response is occurring in opposition to a preprogrammed genetic program, resulting in slower change. In adults, no such opposition exists, resulting in a quicker change in *SWSI* in the absence of the required UV signal. More work on the genetic basis and gene regulatory networks of opsin expression and plasticity might provide further insights.

In our Narrow to Broad experiment, *SWSI* increased in adult lab-raised fish to 58% (BFLR) and 67% (UV) in seven days (Fig 6 right). Additional work would establish whether lab-raised adults can ever achieve the wild expression levels (~85%) with increased light intensity. In the current study, the UV environment only emulated Lake Malawi quantum catches at a depth of 20m, so it is possible that providing a stronger source of illumination that offers improved quantum catches for *SWSI* might produce a stronger plastic response.

Ecological context of plasticity

In the present study, we have shown that changes in the light environment leads to plasticity, which suggests some mechanism by which light is sensed and integrated into signals that both promote and inhibit opsin gene expression. This mechanism seems to be in place during both development and adulthood, allowing cichlids to be responsive to the environment throughout their lives and not just during a narrow sensitive developmental window. On short ecological time scales, such as one lifetime, opsin expression plasticity could be important for fish experiencing seasonal or man-made shifts in water quality that drastically change the light environment (Ehlman *et al.* 2015; Vonlanthen *et al.* 2012). Such seasonal changes in water quality and light attenuation in Lake Malawi have been previously reported (Guildford *et al.* 2007). Whether the seasonal effects on the light spectrum in Lake Malawi actually cause shifts in opsin expression in cichlids needs to be studied in more detail. Nonetheless, analogous findings in both the lab and Lake Victoria have shown that the spectral transmittance of water plays a major role in the interpretation of color signals in cichlids, a key element in mate choice and recognition (Seehausen & van Alphen 1998; Seehausen *et al.* 1997).

Additionally, the depth of Lake Malawi also provides opportunity for shifts in light environment within species ranges. *Metriaclima* species typically inhabit the top 20m of the lake, which shows ever-decreasing amounts of ultraviolet light and therefore less efficiency of the *SWSI* photoreceptor to perceive light with depth (Dalton *et al.* 2010). A fish moving down to inhabit a different part of the habitat range could be enough to induce plasticity

similar to what we have demonstrated in this study. Wild *Metriaclima* species in the lake typically express miniscule amounts of *SWS2B* and large amounts of *SWS1* (Hofmann et al. 2010b). A fish moving to a habitat niche that opens up around 15–20 meters in the clear waters of Lake Malawi would likely have very little ultraviolet light. This would necessitate a speedy shift in expression from *SWS1* to *SWS2B* in the single cones, similar to what we observed in the Natural to Narrow experiment. Interestingly, Zimbabwe rock is one location in Lake Malawi where adult *M. zebra* were found to have only 55–60% *SWS1* in their retinas (Smith et al. 2011). This pinnacle is very steep and extends to some of the deeper *M. zebra* habitats in the lake, supporting that depth could induce shifts from *SWS1* to *SWS2B*.

Evolutionary implications of plasticity

Cichlids are famous for their incredibly accelerated adaptive radiation in the African Rift Lakes (Kocher 2004; Seehausen 2006). Along with opsin expression as depicted in the present study, plasticity acts across a broad range of cichlid phenotypes. These include trophic morphology (Muschick et al. 2011), body shape (Wimberger 1992), gill and brain size in response to hypoxia (Crispo & Chapman 2010), foraging (Parsons et al. 2016) and social behavior (Maruska & Fernald 2010). Plasticity allows an organism to quickly respond to an environmental shift instead of changing a phenotype through purely genetic means (mutation in the coding or regulatory sequences), which would simply be too slow. Over time, selection can then act on the range of phenotypes produced by plasticity to canalize them by genetic assimilation or allow them to remain plastic (Lande 2009; Pigliucci & Murren 2003; Pigliucci et al. 2006; Waddington 1953).

Ancestral state reconstruction of the African cichlid tree reveals that the ancestral riverine species that seeded Lake Malawi likely possessed the long opsin combination (*SWS2A*, *RH2B*, *LWS*) (O'Quin et al. 2010). The Nile tilapia is largely considered to be an extant representative of this riverine ancestor. Despite having the long opsin combination in adulthood, Nile Tilapia expresses all seven opsins at some point in its lifetime (Carleton et al. 2008). Additionally, Tilapia seems to exhibit developmental plasticity, but has lost adult plasticity in opsin expression (Hornsby et al. 2013). It is likely that the developmental plasticity possessed by this riverine ancestor allowed canalization of the array of potential opsin expression phenotypes into the different opsin combinations seen in Lake Malawi today. For example, *Metriaclima* species typically express the short combination as adults (*SWS1*, *RH2B*, *RH2A*), which is the same as larval expression observed in Nile tilapia (Carleton et al. 2008). Perhaps *Metriaclima* have genetically solidified the ancestral neotenic expression pattern for use in adults. Neoteny may also explain the mechanism of how *Metriaclima* could have maintained plasticity in adulthood.

Retaining plasticity is likely costly (Callahan et al. 2008; Relyea 2002) so why preserve the ability to change opsin expression into adulthood? Moreover, if not utilized, plasticity is likely to be lost through drift (Pigliucci et al. 2006). Selection to maintain plasticity has to overcome these prohibitive costs or suffer random loss. The geological history of Lake Malawi provides some context for the possible selective force to sustain plasticity. In the last 1.3 million years lake levels have continuously changed; at times dropping 600m to confine the lake to one 100m deep pool, and at other times filling to 200m above current levels

(Lyons *et al.* 2015). Further characterization has shown dry periods of “green lake” conditions with low lake levels and high eutrophication punctuated by about 100,000 year intervals of the deeper “blue lake” conditions much like the lake of today (Ivory *et al.* 2016). With the extreme shifts in light environment that would occur during these alternating blue and green lake periods, it is not hard to speculate that the ability to switch visual sensitivity through plasticity would be important. Theory predicts that stable environments will favor genetic assimilation while plasticity is more likely to be maintained in unstable environments (Callahan *et al.* 2008; Pigliucci *et al.* 2006). It seems likely then that Lake Malawi would be a good candidate for retention of plasticity in different cichlid lineages.

CONCLUSION

Light-induced plasticity seems to be a powerful fine-tuning mechanism that allows cichlids to visually evaluate their immediate surroundings more efficiently. Since vision is critical for cichlid fitness, this work brings attention to the connection between plasticity and rapid diversification. Lastly, studying phenotypic change in response to an environmental shift in the context of adaptive radiations goes a long way into consolidating plasticity into modern evolutionary biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Scheme for the experimental light conditions and sampling time points. a) Natural to Narrow- wild *M. mbenji* adults were collected from Lake Malawi and brought into the lab where they were sampled upon arrival (Day 0) and Days 3, 21 and 35. b) Narrow to Broad-lab-raised *M. benetos* adults residing in dim tanks (DIM) were either sampled for baseline or moved under bright fluorescent tanks (BFLR) or under metal halide lamps (UV). Samples were taken on Days 3 and 7.

Fig. 2. Natural to Narrow opsin expression for different normalization schemes: a) *SWS1* and c) *SWS2B* relative to total single cone expression, b) *SWS1* and d) *SWS2B* relative to *GNAT2* expression. Significant Tukey's multiple comparisons after Bonferroni corrections are indicated with an asterisk.

Fig. 3.

Narrow to Broad opsin expression for different normalization schemes: a) *SWS1* c) *SWS2B* and e) *LWS* relative to total double cone expression, as compared to b) *SWS1* d) *SWS2B* and f) *LWS* relative to *GNAT2* expression. Significant Tukey's multiple comparisons after Bonferroni corrections are indicated with an asterisk. BFLR3/7- Bright fluorescent environment Day 3/Day7, UV3/7 – Metal halide UV-rich environment Day 3/Day 7.

Fig. 4.

Natural to Narrow light environment spectra and quantum catch. The spectrum of Lake Malawi is much broader and more intense than the lab environment (a). Lake Malawi spectra are measured on the secondary axis. Side-welling irradiance is provided in photons/cm²/msec. Light environment spectra were used to calculate absolute quantum catch for each visual pigment (b). The lab fluorescent environment is greatly diminished in quantum catch for every visual pigment compared to Lake Malawi. Inset shows the close-up of the *SWS1* quantum catch. Quantum catch data for Natural to Narrow experiment can be found in Table 2A.

Fig. 5.

Narrow to Broad light environment spectra and quantum catch. The spectra of the UV environment is much broader, but less intense than BFLR (a). BFLR and DIM environment spectra are measured on the secondary axis. Side-welling irradiance is provided in photons/cm²/msec. Light environment spectra were used to calculate absolute quantum catch for each visual pigment (b) BFLR had the highest quantum catch for each visual pigment except for *SWS1* where the UV environment had the highest. Inset shows the close-up of the *SWS1* quantum catch. Quantum catch data for Narrow to Broad experiment can be found in Table 2B.

Fig. 6.

A summary of current knowledge about Lake Malawi *SWS1* opsin expression in the context of development and plasticity. Open squares represent findings from the present Natural to Narrow and Narrow to Broad studies (right of dashed line) compared to wild adults, lab adults, and lab developmental series (left of dashed line). Wild adults include *M. mbenji* sampled from Mbenji Island, *M. zebra* sampled from Thumbi Island, and *M. barlowi* sampled from Mazinzi Reef. We do not have wild caught *M. benetos* for comparison. The lab developmental series for *M. benetos* is similar to that of other *Metriaclicma* species. Data used for this figure can be found in the data sheet under Data Accessibility.

TABLE 1

Post-hoc Tukey’s comparisons for all the opsins for different treatments and time points. Shown are the adjusted p-values for each opsin and each normalization procedure. All significant p-values (< 0.05) are bolded, and significant p-values after Bonferroni correction are denoted with an asterisk. For each opsin, the p-values are provided for both normalization procedures (total opsin within cell type and *GNAT2*).

IA: Natural to Narrow Single cone opsins:							
	<i>SWS1</i> _{totSC}	<i>SWS1</i> _{<i>GNAT2</i>}	<i>SWS2B</i> _{totSC}	<i>SWS2B</i> _{<i>GNAT2</i>}	<i>SWS2A</i> _{totSC}	<i>SWS2A</i> _{<i>GNAT2</i>}	
DAY3 – DAY0	0.0088	0.0002*	0.0079*	0.9485	0.7643	0.5786	
DAY21 – DAY0	0.0583	0.0019*	0.0514	0.9518	0.5309	0.314	
DAY35 – DAY0	0.0187	0.0091	0.017	0.5947	0.7694	0.6596	
DAY21 – DAY3	0.9176	0.9136	0.9227	0.9999	0.9871	0.9783	
DAY35 – DAY3	0.9989	0.7571	0.9989	0.9178	0.9999	0.9998	
DAY35 – DAY21	0.9628	0.9849	0.9657	0.9141	0.9903	0.9668	

Double cone opsins:							
	<i>RH2B</i> _{totDC}	<i>RH2B</i> _{<i>GNAT2</i>}	<i>RH2A</i> _{totDC}	<i>RH2A</i> _{<i>GNAT2</i>}	<i>LWS</i> _{totDC}	<i>LWS</i> _{<i>GNAT2</i>}	
DAY3 – DAY0	0.0049*	< 0.0001*	0.2139	0.0009*	0.5264	0.996	
DAY21 – DAY0	0.8184	0.0026*	0.603	0.0107	0.8444	0.3641	
DAY35 – DAY0	0.0594	0.0095	0.1561	0.1343	0.9999	0.6941	
DAY21 – DAY3	0.11	0.6888	0.9217	0.8942	0.2449	0.6101	
DAY35 – DAY3	0.8874	0.5334	0.9968	0.4413	0.6233	0.8679	
DAY35 – DAY21	0.4291	0.9922	0.8439	0.8443	0.9209	0.973	

IB: Narrow to Broad Single cone opsins:							
	<i>SWS1</i> _{totSC}	<i>SWS1</i> _{<i>GNAT2</i>}	<i>SWS2B</i> _{totSC}	<i>SWS2B</i> _{<i>GNAT2</i>}	<i>SWS2A</i> _{totSC}	<i>SWS2A</i> _{<i>GNAT2</i>}	
BFLR3 – DIM	0.8095	0.621	0.8298	0.9163	0.7961	0.9425	
BFLR7 – DIM	0.0717	0.0277	0.0497	0.3117	0.148	0.5934	
UV3 – DIM	0.0018*	0.6364	0.002*	0.0085	0.3738	0.0073*	
UV7 – DIM	0.0009*	0.0003*	0.001*	0.0429	0.5647	0.0183	
BFLR7 – BFLR3	0.5294	0.486	0.4124	0.8189	0.0128	0.9577	

IB: Narrow to Broad Single cone opsins:						
	<i>SWS1</i> _{totSC}	<i>SWS1</i> _{GNAT2}	<i>SWS2B</i> _{totSC}	<i>SWS2B</i> _{GNAT2}	<i>SWS2A</i> _{totSC}	<i>SWS2A</i> _{GNAT2}
UV3 – BFLR3	0.0444	0.9999	0.0437	0.0841	0.9579	0.0608
UV7 – BFLR3	0.0296	0.0278	0.0277	0.2859	0.9967	0.1322
UV3 – BFLR7	0.6697	0.4714	0.7787	0.5371	0.0018*	0.2491
UV7 – BFLR7	0.593	0.6225	0.6997	0.8976	0.0038*	0.4426
UV7 – UV3	0.9999	0.0261	0.9999	0.9562	0.9963	0.9928

Double cone opsins:						
	<i>RH2B</i> _{totDC}	<i>RH2B</i> _{GNAT2}	<i>RH2A</i> _{totDC}	<i>RH2A</i> _{GNAT2}	<i>LWS</i> _{totDC}	<i>LWS</i> _{GNAT2}
BFLR3 – DIM	0.2133	0.0422	0.3249	0.1312	0.9999	0.5949
BFLR7 – DIM	0.0173	0.3479	0.9973	0.5904	0.0108	0.0071*
UV3 – DIM	0.6019	0.8161	0.1609	0.9781	0.5229	0.8749
UV7 – DIM	0.1481	0.5442	0.0139	0.1667	0.2992	0.8259
BFLR7 – BFLR3	<0.0001*	0.0002*	0.2036	0.8851	0.02	0.2451
UV3 – BFLR3	0.0089	0.003*	0.0015*	0.3905	0.4601	0.1502
UV7 – BFLR3	0.0006*	0.0006*	<0.0001*	0.9997	0.256	0.1126
UV3 – BFLR7	0.399	0.9371	0.3163	0.9071	0.0001*	0.0006*
UV7 – BFLR7	0.8631	0.9956	0.0409	0.9381	<0.0001*	0.0003*
UV7 – UV3	0.9145	0.9934	0.8758	0.4694	0.9969	0.9999

Table 2

Quantum catches of the opsins for different lighting environments. Quantum catches are provided in photons/cm²/msec.

2A: Natural to Narrow			
Opsin	Lake Malawi @ 3m	Lake Malawi @10m	Lab environment
<i>SWS1</i>	6.36E+11	1.78E+11	0
<i>SWS2B</i>	3.24E+12	9.39E+11	5.39E+10
<i>SWS2A</i>	5.41E+12	1.71E+12	1.02E+11
<i>RH2B</i>	9.94E+12	3.25E+12	2.13E+11
<i>RH2Aβ</i> *	1.2E+13	3.85E+12	3.49E+11
<i>RH2Aα</i> *	1.22E+13	3.88E+12	3.84E+11
<i>LWS</i>	1.2E+13	3.45E+12	4.99E+11

2B: Narrow to Broad			
Opsin	DIM	BFLR	UV
<i>SWS1</i>	0	1.04E+10	2.37E+10
<i>SWS2B</i>	3.83E+09	1.5E+11	1.65E+11
<i>SWS2A</i>	1.34E+10	2.6E+11	2.06E+11
<i>RH2B</i>	4.32E+10	5.47E+11	3.3E+11
<i>RH2Aβ</i> *	9.79E+10	9.18E+11	4.02E+11
<i>RH2Aα</i> *	1.13E+11	1.03E+12	4.26E+11
<i>LWS</i>	1.68E+11	1.45E+12	5.28E+11

* Although quantum catches are calculated separately for *RH2Aα* and *RH2Aβ* opsins, the opsins were combined in the RT-PCR expression assay.