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RESEARCH ARTICLE

Short-term colour vision plasticity on the reef: changes in opsin expression under varying light conditions differ between ecologically distinct fish species

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ABSTRACT

Vision mediates important behavioural tasks such as mate choice, escape from predators and foraging. In fish, photoreceptors are generally tuned to specific visual tasks and/or to their light environment, according to depth or water colour to ensure optimal performance. Evolutionary mechanisms acting on genes encoding opsin, the protein component of the photopigment, can influence the spectral sensitivity of photoreceptors. Opsin genes are known to respond to environmental conditions on a number of time scales, including short time frames due to seasonal variation, or through longer-term evolutionary tuning. There is also evidence for 'on-the-fly' adaptations in adult fish in response to rapidly changing environmental conditions; however, results are contradictory. Here, we investigated the ability of three reef fish species that belong to two ecologically distinct families, yellow-striped cardinalfish (*Ostorhinchus cyanosoma*), Ambon damselfish (*Pomacentrus amboinensis*) and lemon damselfish (*Pomacentrus moluccensis*), to alter opsin gene expression as an adaptation to short-term (weeks to months) changes of environmental light conditions, and attempted to characterize the underlying expression regulation principles. We report the ability for all species to alter opsin gene expression within months and even a few weeks, suggesting that opsin expression in adult reef fish is not static. Furthermore, we found that changes in opsin expression in single cones generally occurred more rapidly than in double cones, and identified different responses of *RH2* opsin gene expression between the ecologically distinct reef fish families. Quantum catch correlation analysis suggested different regulation mechanisms for opsin expression dependent on gene class.

KEY WORDS: Visual ecology, Diurnal/nocturnal feeders, Phenotypic variation, Adaptation, Teleost, Spectral tuning

INTRODUCTION

Detection of visual cues is often critical for behavioural tasks such as mate choice, escape from predators or foraging (Detto, 2007; Foote et al., 2004; Miyagi et al., 2012; Rick et al., 2006; Sandkam et al., 2015; Stuart-Fox et al., 2003). Therefore, tuning of photoreceptor spectral sensitivities to specific visual tasks and/or parts of the light spectrum relevant for such behaviours may be important for maintaining optimal performance (Price, 2017). This is particularly evident in fish, which have dispersed and adapted to habitats profoundly different in their light environment, including freshwater lakes and rivers, marine coastal reefs, pelagic zones and the deep sea. Considering the light conditions in these environments, fish visual systems have adapted to the overall environmental illumination of their habitat (Cronin et al., 2014; Lythgoe, 1979). However, it may be necessary for fish to adjust their visual system in adaptation to changes in seasonal light regime (Loew and McFarland, 1990; McFarland, 1990), microhabitat differences (Marshall et al., 2003), depth (Jerlov, 1977; Loew and McFarland, 1990; McFarland, 1990) or activity period (Loew and McFarland, 1990). Spectral sensitivity tuning in fish can be facilitated by various mechanisms, including structural changes, such as optical filtering of specific wavelengths (Siebeck and Marshall, 2001), or variation of photoreceptor size, number and distribution (de Busserolles et al., 2014; Taylor et al., 2015; Wagner and Kröger, 2005); or physiological changes to the properties of the light-absorbing photopigments contained in the photoreceptors (Bowmaker, 2008).

The wavelength of maximum absorbance (λ_{max}) of each photoreceptor depends primarily on two components of the visual pigment: a vitamin A-derived light-absorbing chromophore [A1 or A2 (Toyama et al., 2008; Yokoyama and Yokoyama, 1996)], and the opsin, a transmembrane protein that is covalently bound to the chromophore (Hunt and Collin, 2014). Visual opsin genes in vertebrates are classified according to their photoreceptor specificity and wavelength-dependent spectral sensitivity into one rod opsin (rhodopsin, RH1) used for dim-light vision and five classes of cone opsins used for colour vision: SWS1 (short wavelength-sensitive 1, ultraviolet), SWS2 (short wavelength-sensitive 2, violet–blue), RH2B (medium wavelength-sensitive 2B, blue–green), RH2A (medium wavelength-sensitive 2A, green), and LWS (long wavelength-sensitive, yellow–red) (Cronin et al., 2014; Yokoyama, 2008).

Various genetic mechanisms affecting the sequence structure and repertoire of opsin genes (for review see Bowmaker, 2008; Carleton et al., 2016), the type of chromophore used (A1 or A2) and differences in qualitative and/or quantitative expression of opsin genes (for a review, see Carleton, 2009) render photopigments the foundation of a versatile system for adaptation to varying environmental lighting demands (Hauser and Chang, 2017). Importantly, however, besides chromophore substitution, only qualitative or quantitative differential opsin gene expression may contribute to visual system adaptation within the same species in developing or mature fish. Such differences in expression hold the potential for highly adjustable, possibly short-term, visual system

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adaptation to changes in the prevailing light habitat (Carleton, 2009; Marshall et al., 2015).

Qualitatively differential opsin gene expression occurs most commonly between ontogenetic transitions, i.e. from larval to adult stages, and is often accompanied by migration between different light habitats or a change in diet (Archer et al., 1995; Carleton et al., 2008; Cheng and Novales Flamarique, 2004; Cortesi et al., 2015a; Cottrill et al., 2009; Loew et al., 2002; Shand et al., 2008; Temple et al., 2008). Quantitative differences in opsin expression profiles have been shown in various freshwater and marine species, including Lake Victoria cichlids (Seehausen et al., 2008) and marine damselfish (Stieb et al., 2016) inhabiting different depths, as well as killifish inhabiting spectrally distinct streams (Fuller et al., 2004), resulting in a shift of λ_{max} to match specific light conditions. Furthermore, when reared under different artificial light, plasticity in opsin expression has been shown in cichlids (Hofmann et al., 2010), black bream (Shand et al., 2008), guppies (Ehlman et al., 2015; Sakai et al., 2016) and killifish (Fuller et al., 2005). Opsin expression plasticity in adult fish has been shown in killifish (Fuller and Claricoates, 2011; Fuller et al., 2010) and African cichlids (Nandamuri et al., 2017), which alter opsin expression levels within only a few days (Fuller and Claricoates, 2011) when exposed to changed habitat light. The variable nature of these findings suggests that opsin expression plasticity may be highly species specific rather than based on a general controlling mechanism mediating opsin expression based on photoreceptor quantum catch. There are two ways in which opsin genes can mediate visual pigment performance: (1) changes in opsin expression may adjust visual sensitivities to regions of the spectrum where light is abundant (Hofmann and Carleton, 2009) [as reported for killifish (Fuller et al., 2004), black bream (Shand et al., 2008), Lake Malawi cichlids (Hofmann et al., 2010), damselfish (Stieb et al., 2016)], or (2) changes in opsin expression can lead to a decline of sensitivity in regions of the spectrum where light is abundant [reported for the blue acara (Kröger et al., 1999; Wagner and Kröger, 2000)] – a compensatory mechanism that helps maintain colour constancy (Wagner and Kröger, 2005).

The opsin repertoire in coral reef fish is less well studied, but offers excellent conditions to investigate mechanisms of visual system adaptation. With regard to species and colour richness, coral reefs are one of the most spectrally diverse ecosystems on Earth – a diversity reflected in the complexity of visual communication among reef fish (reviewed in Marshall et al., 2006; Marshall et al., 2015). Considerable variation of photoreceptor spectral sensitivity (Losey et al., 2003), ocular media transmittance (Losey et al., 2003; Siebeck and Marshall, 2001) and opsin repertoire (Cortesi et al., 2015b; Hofmann et al., 2012; Phillips et al., 2016; Stieb et al., 2017) add to the challenge in understanding this system (Marshall et al., 2015). Stieb et al. (2016) demonstrated that subtle depth-dependent differences in environmental illumination correlate with different opsin expression profiles in some damselfish species whereas others showed a stable expression profile. These findings highlight the possibility that opsin gene expression in reef fish in general may be highly species specific, perhaps because of different ecological and visual demands or phylogenetic constraints. However, it is unclear whether such changes occur during developmental stages (i.e. priming during settlement) or can also occur post-settlement in mature fish. Furthermore, it remains unknown whether changes in opsin expression under pronounced differences in environmental lighting are consistent between species, and more specifically, between ecologically distinct species.

To address this, we investigated the capacity of spectral visual system adaptation in three reef fish species, *Pomacentrus amboinensis* Bleeker 1868 (ambon damselfish), *Pomacentrus moluccensis* Bleeker 1853 (yellow damselfish) and *Ostorhinchus cyanosoma* Bleeker 1853 (yellow-striped cardinalfish). These species belong to two of the most speciose and abundant coral reef fish families, the damselfish (Pomacentridae) and cardinalfish (Apogonidae). Importantly, these families, despite sharing several ecological traits including strong association with coral, and, generally, strong site fidelity and resilience to habitat disruption (Gardiner, 2010; Marnane, 2000), feed at different times of day: damselfish are strictly diurnal whereas cardinalfish are predominantly crepuscular or nocturnal feeders (Emery, 1973; Marnane and Bellwood, 2002). With a few exceptions, damselfish and cardinalfish go through an oceanic and pelagic larval phase, after which they settle on the reef (Leis, 1991; Victor, 1991). As adults, the three species are found in small to large aggregations in clear lagoons, and coastal or seaward reefs (Randall et al., 1990). The fact that all three species co-occur in the same shallow-water coral reef zones, but are active at different times and light levels, makes them particularly interesting to test and compare mechanisms of spectral tuning.

The damselfish visual system, including opsin gene repertoire, is well understood (Hawryshyn et al., 2003; Loew and Lythgoe, 1978; Losey et al., 2003; Marshall et al., 2006; McFarland and Loew, 1994; Siebeck and Marshall, 2001; Siebeck et al., 2008; Siebeck et al., 2010; Stieb et al., 2017). *SWS* opsins appear to be expressed exclusively in single cones, whereas *RH2* and *LWS* opsins appear to be expressed exclusively in double cones (our unpublished data). However, as the cardinalfish visual system is less well known, we investigated the repertoire of expressed opsin genes in the cardinalfish *O. cyanosoma* using RNA sequencing. We then examined whether changes in spectral tuning via opsin gene expression plasticity are possible in adult reef fish and whether this is similar or different between nocturnal cardinalfish and diurnal damselfish. To do this, we exposed the two species of damselfish and the one species of cardinalfish to different lighting conditions in terms of colour and intensity for up to 6 months. We then used quantitative real-time polymerase chain reaction (qRT-PCR) experiments to quantify opsin expression. Finally, we were interested in understanding whether opsin expression follows dynamics that maximize signal strength, or functional maintenance and energy efficiency. Signal strength maximization would suggest photoreceptors shift opsin genes to increase photon catch under light conditions that provide relatively more photons at the relevant wavelengths than a reference light environment. In contrast, minimizing energy expenditure would suggest photoreceptors decrease expression of the respective opsin gene to reduce the cost of neural transmission. To address this question, we modelled the λ_{max} values of photopigments based on the identified opsin gene sequences, and used these to calculate the quantum catch of visual pigments under the experimental and natural light conditions.

MATERIALS AND METHODS

Study species

Adult specimens (*P. amboinensis*, *N*=45; *P. moluccensis*, *N*=61; *O. cyanosoma*, *N*=82) were obtained between 2015 and 2017 from an aquarium supplier (Cairns Marine Pty Ltd, Cairns, Australia), and shipped to the University of Queensland as quickly as possible (e.g. on the same day or within a few days; as per information provided by the supplier). Additionally, one adult individual of *O. cyanosoma* used for *de novo* opsin gene sequencing was collected in February 2015 on the reefs surrounding Lizard Island (14°40′S, 145°27′E), Australia, using SCUBA and hand nets, and was collected under Great Barrier Reef Marine Park Permit (G12/35005.1) and Queensland General Fisheries Permit (140763).

After undergoing light treatments, fish were anaesthetized with an overdose of clove oil (10% clove oil; 40% ethanol; 50% seawater), killed by decapitation, and retinas were dissected from the eyecup and stored in RNA-later (Ambion) for subsequent molecular analysis. For opsin studies, tissues were sampled around midday (between 11:00 h and 14:00 h), and the date and time of dissection were noted. All experimental procedures were approved by The University of Queensland Animal Ethics Committee [QBI/223/10/ARC/US AIRFORCE (NF) and QBI/192/13/ARC].

Light and control environments

At the start of the experiment, a subset of individuals for each species (*P. amboinensis*, *N*=8; *P. moluccensis*, *N*=18; and *O. cyanosoma*, *N*=18) were euthanized immediately upon arrival in our lab and used as a baseline (time point=0). Only one specimen of *P. moluccensis* was excluded from the baseline group as its eye was damaged and discoloured. All remaining individuals were kept under 12 h light:12 h dark lighting conditions in aquaria filled with 200 litres saltwater and subjected to altered light (colour) and control (intensity) environments. The three tested species were kept in the same treatment aquaria. All tanks were illuminated by broad-spectrum high intensity LED (Radion™, Ecotech Marine, Australia) and fluorescent black (FLH0T8BL/36, Toshiba, Japan) aquarium lights. Light environments (red, green and blue) were generated using spectral filter sheets (182 Light Red, 124 Dark Green, 172 Lagoon Blue; Lee Filters) (Fig. 1A).

Fish living in the three different colour habitats were euthanized after 1 month (time point=1, *O. cyanosoma*, *N*=23; *P. moluccensis*, *N*=9; *P. amboinensis*, *N*=22), 4 months (for *O. cyanosoma*, *N*=18) and 6 months (for *P. amboinensis*, *N*=15 and *P. moluccensis*, *N*=17). We decided to terminate the experiment for *O. cyanosoma* after 4 months to avoid potential health effects as previous husbandry of *O. cyanosoma* has occasionally proven difficult, reflecting the delicate nature of most cardinalfish species.

After results of colour treatments were known, more individuals of *O. cyanosoma* (*N*=23) and *P. moluccensis* (*N*=17) were placed in three additional light treatments to test the effects of light intensity. *P. amboinensis* could not be obtained because of a coral bleaching event on the Great Barrier Reef in 2016. One experimental group was exposed to unfiltered light (Fig. 1A). Two additional groups were exposed to light attenuated by neutral density filters (298, 0.15 ND; 210, 0.6 ND; Lee Filters) reducing light intensity by 60–80% (filter 298) or 20–30% (filter 210) while not completely blocking any part of the spectrum (Fig. 1A). Intensity treatments were run for 1 month (see Table S1 for a summary of specimens used), as this had been shown to be long enough to induce expression changes.

Opsin gene studies

For *P. amboinensis* and *P. moluccensis*, opsin genes and their classification have been determined previously (Hofmann et al., 2012; Stieb et al., 2016). As no such data was available for *O. cyanosoma*, and to verify the opsin genes previously identified in the damselfish, we initially sequenced retinal transcriptomes of three specimens and validated opsin gene classification using phylogenetic methods. In order to quantify opsin gene expression, we performed quantitative real-time polymerase chain reaction (qRT-PCR). All retinas were homogenized using a TissueLyser LT (Qiagen, The Netherlands) and total RNA was extracted with the RNeasy Mini Kit (Qiagen) following the manufacturer's protocol. An optional DNase digestion step was performed to eliminate traces of genomic DNA. Retinal RNA was reverse transcribed using the High Capacity RNA-to-cDNA kit (Applied Biosystems).

Opsin gene sequencing (RNA-seq) and analysis

RNA was quality checked with an Agilent 2100 BioAnalyzer 6000 NanoChip (Agilent Technologies). RNA-seq libraries were made using the TruSeq RNA Sample Preparation Kit v.2 (Illumina, San Diego, USA) and the retina-specific transcriptomes were sequenced as 125 bp paired reads on the Illumina platform (HiSeq2000 v4) by the sequencing facility within the Queensland Brain Institute at the University of Queensland, Australia. Samples were multiplexed at 12 samples per lane obtaining 20–30 million sequenced reads per sample.

Data were processed using the online Bioinformatics platform Galaxy (Research Computing Centre, The University of Queensland, Australia) (Afgan et al., 2015). Reads were quality checked using FastQC, and high copy sequences, such as primers and library indices were removed using Trimmomatic. Furthermore, regions with quality scores below 20 were removed by cropping at the start and end of a read, as well as by using a sliding window quality crop. Finally, reads with lengths less than 80 bp were dropped from the analysis. Trinity was used for *de novo* assembly of transcripts, with a group pair distance of 250 bp, and minimum inchworm kmer coverage of 2.

Further bioinformatics analyses were performed using Geneious software (version 9.0.4). For *P. amboinensis* and *P. moluccensis*, assembled transcripts were mapped to species-specific known and publicly available opsin genes (*P. amboinensis*: *SWS1*, GenBank accession no. HQ286506; *SWS2B*, HQ286516; *RH2B*, HQ286526; *RH2A*, HQ286536; *LWS*, HQ286546; *RH1*, HQ286556; *P. moluccensis*: *SWS1*, KU745428; *SWS2B*, KU745427; *RH2B*, KU745429; *RH2A*, KU745430; *LWS*, KU745432; *RH1*, KU745431). To identify *SWS2A* opsin genes of *O. cyanosoma*, the assembled transcripts were also mapped to reference opsin gene sequences from the dusky dottyback (*Pseudochromis fuscus*: KP004335.1). For each species and all opsin genes identified, we further followed the methods described in de Busserolles et al. (2017) to manually check for gene duplications. Briefly, after identification of candidate gene coding sequences, unassembled reads were mapped to the opsin gene repertoire of the species using medium sensitivity settings (70% identity threshold). Deviating reads were then extracted by working from single polynucleotide polymorphism (SNP) to SNP by exploiting paired-end matching to cover gaps, and their consensus sequence was used as species-specific reference for repeated high-specificity (100% identity) mapping of unassembled reads until maximum obtainable sequence length was reached.

To confirm the assignment of the newly identified opsin genes of *O. cyanosoma* to the known opsin classes, we aligned their amino acid sequences with the opsin genes of the zebrafish (*Danio rerio*), the Japanese ricefish (*Oryzias latipes*), the bluefin killifish (*Lucania goodie*), the Lake Malawi cichlid (*Metriaclima zebra*) and the Nile tilapia (*Oreochromis niloticus*) (see Fig. 2 for GenBank accession numbers). We then estimated maximum likelihood phylogenies for each gene based on the amino acid sequences using RAxML 8.2.10 (Stamatakis, 2014) on the web-based platform CIPRES (Miller et al., 2010), followed by a rapid bootstrap analysis with 1000 replicates. The highest scoring tree was selected as the best tree.

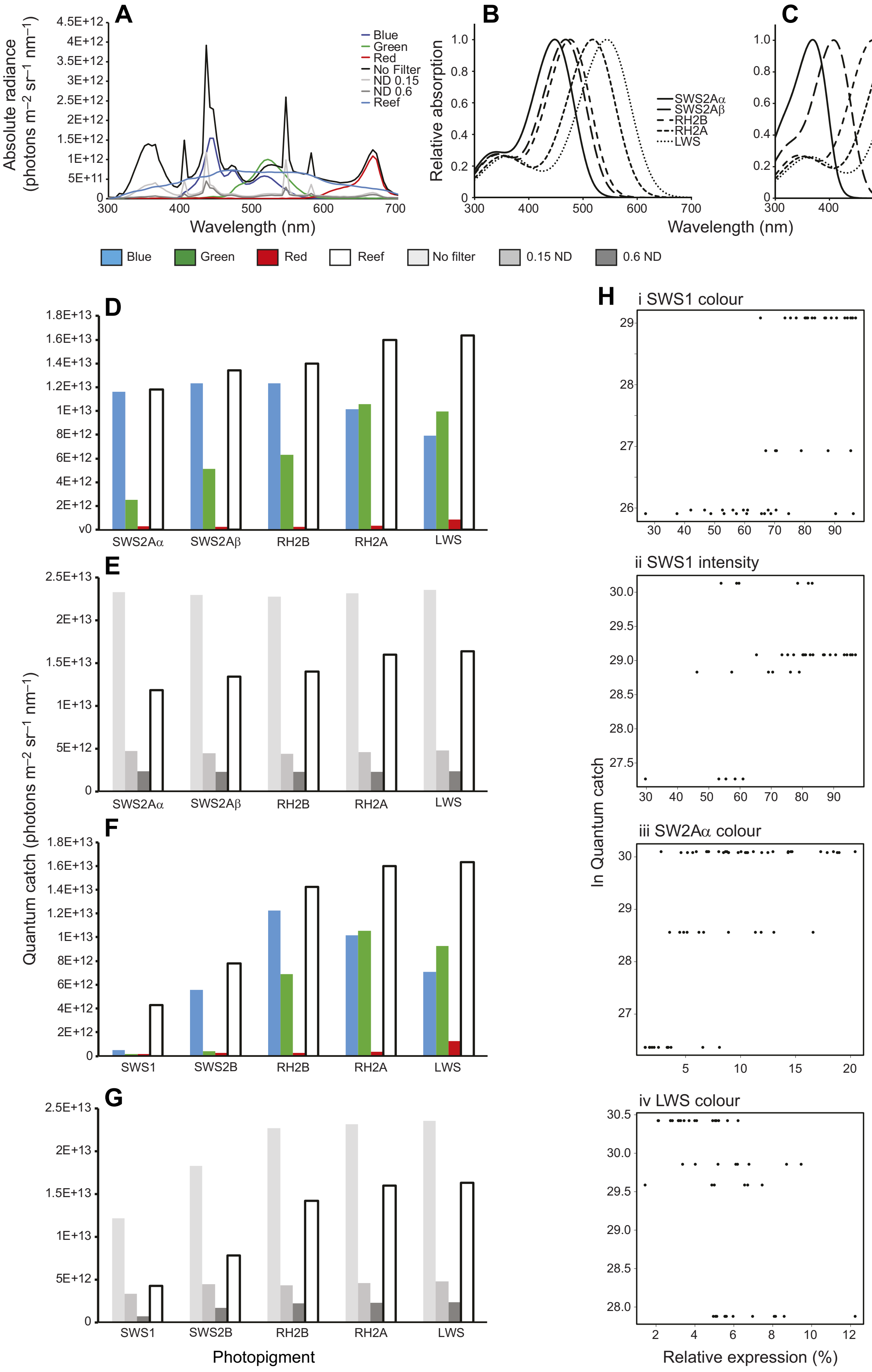

Fig. 1. See next page for legend.

Fig. 1. Radiance of light treatments used in the experiments, and absorption and quantum catch of opsins in reef fishes. (A) Absolute radiance on a shallow water reef (reef), unfiltered laboratory light (no filter), colour (blue, green, red) and intensity (0.15 ND, 0.6 ND) treatments. (B,C) Spectral absorbance curves of photopigments based on modelled λ_{max} values of photopigments categorized by identified opsin classes in *Ostorhinchus cyanosoma* (B) and in *Pomacentrus moluccensis* and *Pomacentrus amboinensis* (C). (D–G) Absolute quantum catch of modelled photopigments containing one of the identified cone opsins each under reef illumination compared with colour and intensity treatments: *O. cyanosoma*, colour (D) and intensity (E) treatments; *P. moluccensis*, colour (F) and intensity (G) treatments. (H) Quantum catch plotted against relative expression of opsins showing plastic expression changes: SWS1 (i) and LWS (iv) quantum catch under colour, and SWS1 (ii) under intensity treatments in *P. moluccensis*; SWS2Aα (iii) quantum catch under colour treatments in *O. cyanosoma*.

Opsin gene expression using quantitative real-time polymerase chain reaction (qRT-PCR)

We quantified relative opsin gene expression using qRT-PCR [SYBR Green master (Rox) dye (Roche)] on a StepOnePlus Real-Time PCR System (Applied Biosystems, USA). Following previously described methods (Carleton and Kocher, 2001; Stieb et al., 2016, 2017), relative opsin gene expression was calculated from the efficiency and critical cycle number (C_t), as relative single (*SWS* opsin genes) and double (*RH2* and *LWS* genes) cone opsin expression as a fraction of the total of single, or double cone opsin genes expressed and relative rod opsin gene expression as the fraction of all opsin genes expressed. The distinction of single and double cone opsin was chosen as *in situ* hybridization assays on several damselfish and other reef fish species suggest this expression is specific to particular cell types (unpublished data), and because it prevents the masking of expression changes in the less abundant single cone opsins by highly abundant double cone opsins. All percentage values are reported as median and interquartile range.

For qPCR reactions targeting opsin genes of *P. amboinensis* and *P. moluccensis*, we used publicly available and already validated primers (Table S2) from Stieb et al. (2016). For *O. cyanosoma*, we followed the methods as per Stieb et al. (2016, 2017) and designed unique primers (Table S2) for each opsin gene with either the forward or the reverse primer spanning an exon–exon boundary (except for the intronless *RH1*) to ensure only cDNA would be amplified with a product length of 60–100 bp. Primer efficiencies (Table S2) were tested using a five orders of magnitude dilution

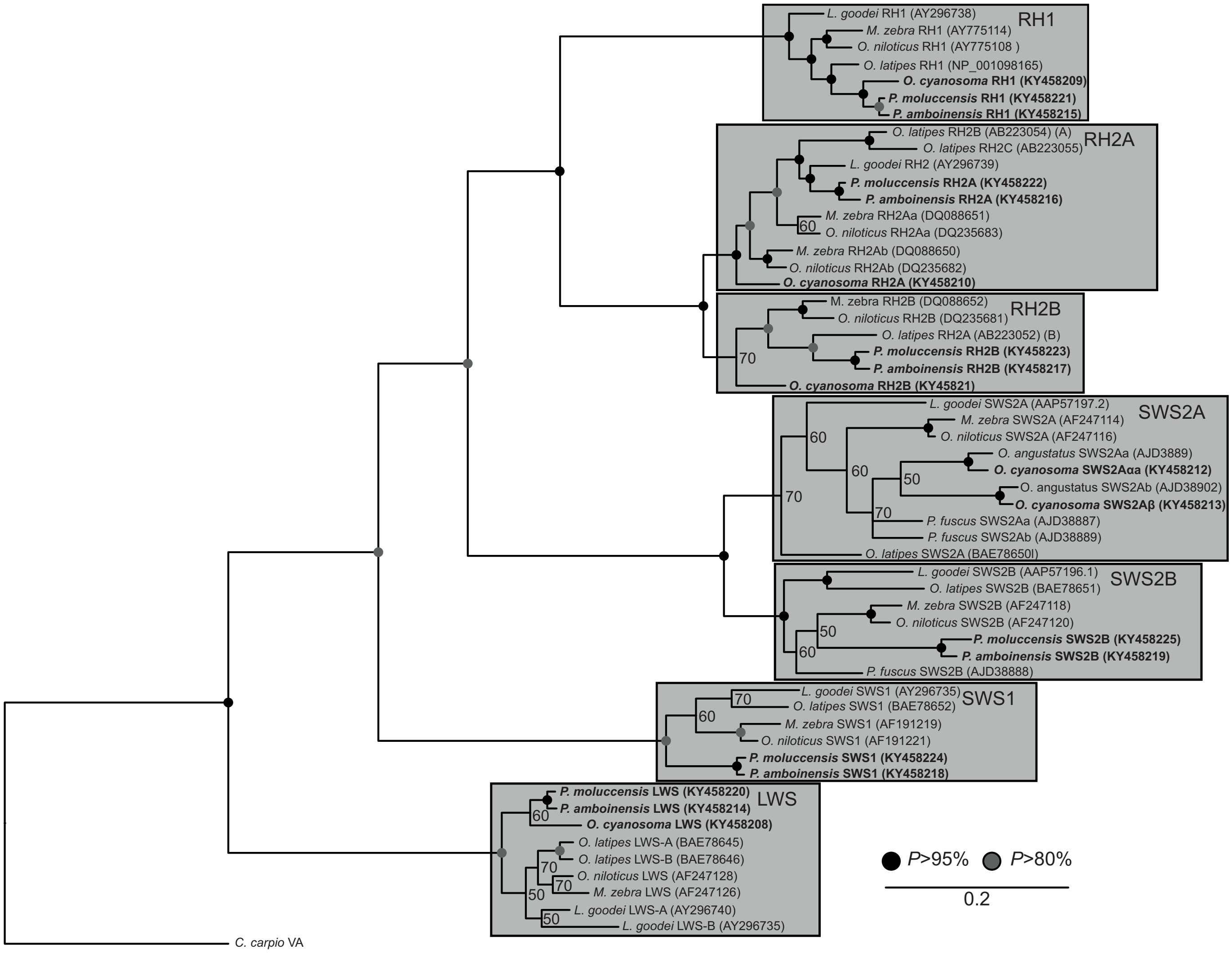

Fig. 2. Maximum likelihood reconstruction of the phylogenetic relatedness of opsin gene sequences identified in *Ostorhinchus cyanosoma*, *Pomacentrus moluccensis* and *Pomacentrus amboinensis*. Fish species examined in this study are shown in bold. GenBank accession numbers of opsin genes are depicted.

series of a species-specific opsin pool. The opsin pool contained equal ratios of fragments of each opsin gene (molarity of fragments was measured using an Agilent 2100 BioAnalyzer High Sensitivity DNA Chip, Agilent Technologies) that were amplified from cDNA using pool primers (see Table S2) specifically designed for each opsin gene identified in the *O. cyanosoma* transcriptome. Products were separated via gel electrophoresis, then cut out from the gel and purified using the QIAquick PCR Purification Kit (Qiagen). The *RH1* amplicon was additionally Sanger sequenced for sequence verification. All experiments were carried out with three technical replicates, and samples originating from the different light and control experiments were randomly assigned to each qPCR plate.

Relationship of opsin expression with light and control treatments

To test whether opsin gene expression changed as a consequence of exposure to different light and control treatments, we used the beta regression method based on the R package BETAREG (Cribari-Neto and Zeileis, 2009). This regression allows handling of non-transformed data to model percentages and proportions. The beta distribution can be of variable shape, and is, therefore, suitable for the analysis of relative opsin gene expression as our dependent variable. To identify relationships between relative opsin expression and different time points, each time point was used as a categorical factor. To test for differences between different light and control treatments, categorical factors were set for each treatment. Repeated hypothesis testing was accounted for by using a Bonferroni correction considering six tested hypotheses for comparisons between treatments and baseline, and five tested hypotheses for comparisons between treatments and no filter treatment (one per treatment group). *P*-values were thus calculated according to $P=\alpha/m$, with $m=6$ or $m=5$, respectively. Analyses were performed in R (https://www.r-project.org/) using the interface RSTUDIO (version 0.98.1062).

Spectral sensitivity modelling

The spectral sensitivities of the different cone and rod opsin classes identified in *O. cyanosoma*, *P. amboinensis* and *P. moluccensis* were modelled using the translated amino acid sequences. Opsin amino acid sequences were aligned with bovine rhodopsin (GenBank accession no.: NP_001014890.1). This allowed us to infer the loci of transmembrane regions and the identification of known retinal chromophore binding pocket sites, as well as previously discovered tuning sites, based on the protein structure (summarized in Hunt et al., 2001; Yokoyama, 2008).

Furthermore, amino acid comparisons of the identified opsin genes to those of other fish species with known opsin λ_{max}, was used to infer tuning effects where possible. Our opsin gene sequences were aligned with those of the cichlids *Metriaclima zebra* and *Oreochromis niloticus*, the killifish (*Lucania goodei*), the Japanese ricefish (*Oryzias latipes*), as well as the dusky dottyback (*Pseudochromis fuscus*). The opsin genes found in these species have been studied extensively, including *in vitro* opsin protein expression studies to assess pure protein spectral absorbance and microspectrophotometry (MSP; Carleton, 2009; Cortesi et al., 2015a). Damselfish photoreceptor absorbance had previously been measured using MSP (summary in Stieb et al., 2016).

We focused on variable amino acid residues that occurred either in areas corresponding to the retinal binding pocket and were substitutions that resulted in a change in polarity, or at known tuning sites (Dungan et al., 2016; Takahashi and Ebrey, 2003; Yokoyama, 2008). Final calculations were based on pure opsin spectral absorbance of the photopigments of *Oreochromis niloticus* (Spady et al., 2006), *Lucania goodei* (Yokoyama et al., 2007) or *Oryzias latipes* (Matsumoto et al., 2006; RH1). Tuning effects of the amino acid residues present at the sites in question were inferred directly at sites of known tuning effects. Tuning effects of other variable amino acid residues between the species were inferred based on the λ_{max} differences of the known sequences and the variable sites present (see Table S5 for a summary of bovine rhodopsin aligned site effects considered). Errors in inferred spectral sensitivities will have small effects on quantum catch calculations.

It is notable that we did not test chromophore composition at any stage in our experiment as it is unlikely to occur in our test species. Evidence suggests a limitation to the A1 chromophore in marine fish, on which we based our λ_{max} calculations. Shifts from one chromophore to another are thought to occur only in fish that undergo extreme habitat changes that bring about a drastic change in environmental conditions, such as eels or salmon (Beatty, 1966, 1975, 1984; Wood et al., 1992). Our test species, however, are confined to a marine environment throughout their adult life history.

Light measurements and quantum catch calculation

The light spectrum in each treatment tank, and, for comparison, on a shallow reef (2 m) off Lizard Island around midday in March 2015, were determined by measuring 45 deg downwelling radiance using a UV/VIS 100 µm optic fibre (Ocean Optics) connected to a USB2000 spectrophotometer (Ocean Optics), and the software Spectrasuite (Ocean Optics). For absolute radiance measurements, the fibre and spectrophotometer were calibrated using a Xenon Arc calibration light source (Ocean Optics).

Visual system efficiency, as determined by the total quantum catch (*Q*) of each photopigment under the experimental light conditions, was estimated using the equation:

$$Q = \int R_a(\lambda) R(\lambda) d\lambda,$$

where $R_a(\lambda)$ is the absolute radiance spectrum, and $R(\lambda)$ is the photoreceptor absorption calculated using the equations for the bovine rhodopsin template, as explained in detail by Govardovskii et al. (2000).

The λ_{max} values, as determined by amino acid-based modelling (SWS2B, SWS2A, RH2B, RH2A, LWS) and MSP (SWS1), were used to generate photoreceptor absorbance curves and to calculate the quantum catch of hypothetical photopigments, one for each identified cone opsin class and under each lighting condition. As an additive sites model for site effects in *SWS1* genes is not well supported, we conclude that SWS1 λ_{max} gained from MSP measurements undertaken in *P. amboinensis* (Losey et al., 2003) are more robust than any estimate. As *SWS1* is only expressed in damselfish and *SWS1* sequences of both test species are identical in tuning sites, we therefore used those SWS1 λ_{max} values for both *P. amboinensis* and *P. moluccensis*. We are also aware that increasing evidence suggests opsin gene co-expression in cones (Dalton et al., 2014, 2015, 2017; Torres-Dowdall et al., 2017); however, as this information is unavailable for our study species, we believe that for the overall conclusion of our study, our estimated quantum catches will provide a good enough first estimate.

To test for correlation between relative opsin expression and opsin specific photopigment quantum catch, we used Kendall's τb correlation using the Kendall package in R (2005). Quantum catch

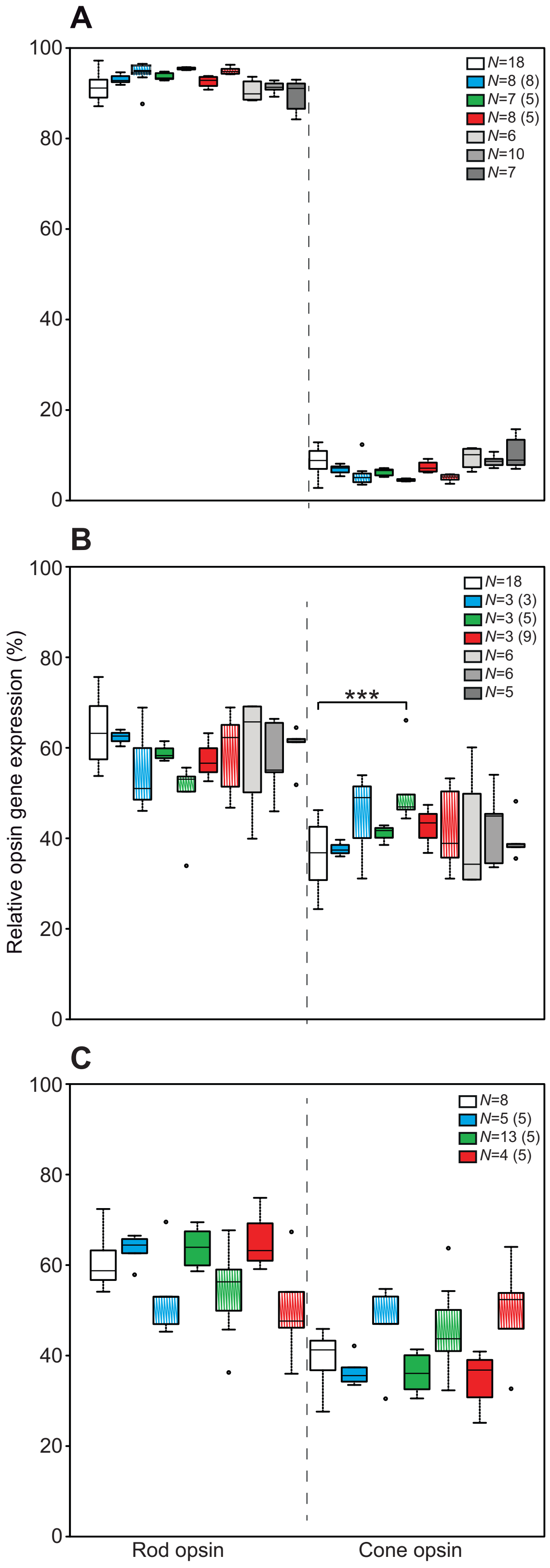

Fig. 3. Relative expression of cone and rod opsin genes as a fraction of total opsin after light treatments. (A) *Ostorhinchus cyanosoma*, (B) *Pomacentrus moluccensis* and (C) *Pomacentrus amboinensis*. White, baseline; blue, blue treatment; green, green treatment; red, red treatment; light grey, no filter treatment; medium grey, 0.15 ND treatment; dark grey, 0.6 ND treatment. Solid boxes show results after 1 month (time point 1); hatched boxes after 4 or 6 months (time point 2). Note that for *P. amboinensis*, no intensity treatments are available. *N* indicates number of specimens used; numbers in parentheses are *N* for time point 2. Boxes show 25th and 75th percentiles with median; whiskers are 5th and 95th percentiles; dots are outliers. Significant difference in beta regression analysis is shown, ***$P\leq 0.00017$.

data was ln transformed for visualization purposes only. Kendall's τb allows the correlation of non-normally distributed data by assigning and correlating the ranked data.

RESULTS

Opsin gene sequences using RNA-seq

RNA sequencing and *de novo* transcript assembly reconstructed complete coding sequences of five different opsin gene classes in *O. cyanosoma*. Their identity was confirmed by amino acid-based maximum-likelihood phylogenetic inference that grouped the newly discovered genes with those of previously well-studied fish species (Fig. 2): four cone opsins (*SWS2A*, *RH2B*, *RH2A*, *LWS*) and one rod opsin (*RH1*). Two sister copies of the *SWS2A* gene (*SWS2Aα*, *SWS2Aβ*), and one sister gene each of the *RH2B* (*RH2B2*) and *RH2A* (*RH2A2*) genes were identified. However, as both *RH2* duplicates were expressed at low levels and could not be assembled to full coding sequence length, these two genes were omitted from further analyses.

RNA sequencing and *de novo* assembly of the retinal transcriptome of *P. moluccensis* and *P. amboinensis* confirmed previous reports of six opsin genes (encoding five cone opsins: *SWS1*, *SWS2B*, *RH2B*, *RH2A*, *LWS*; and one rod opsin: *RH1*) found in damselfish (Hofmann et al., 2012; Stieb et al., 2016), and furthermore allowed complete reconstruction of their coding sequence (Fig. 2).

Light-dependent opsin expression

Opsin expression values (for a summary see Table S3) are presented in relative percentage, were normalized within cone types (single cones, double cones), and are presented as median percentage and interquartile range. At time 0 (baseline), the yellow-striped cardinalfish expressed *SWS2Aα*, *SWS2Aβ*, *RH2B*, *RH2A*, *LWS* and *RH1*. The total retinal opsin repertoire was dominated by RH1 opsin, making up 91.2% (3.9) of all expressed retinal opsin (Fig. 3A). In single cones, *O. cyanosoma* almost exclusively expressed *SWS2Aβ* (90.6%, 7.5). In double cones, *RH2A* was expressed strongest (82.5%, 8.6), *RH2B* at lower levels (17.1%, 8.5) and *LWS* expression was very low (0.4%, 0.9). Variability in expression was greatest among *RH2* (i.e. *RH2B*: 7.1–43.6%) and small among *SWS2A* genes.

At time 0 (baseline), both damselfish species expressed *SWS1*, *SWS2B*, *RH2B*, *RH2A*, *LWS* and *RH1*. Rod opsin made up only 63.2% (11.0) (*P. moluccensis*) and 58.7% (6.5) (*P. amboinensis*) of total opsin expressed (Fig. 3B,C). Among single cone opsins, both species expressed predominantly *SWS1* (*P. amboinensis*: 86.6%, 9.6; *P. moluccensis*: 85.0%, 12.5). It is notable that levels of SWS genes showed large variability, particularly in *P. amboinensis*. In double cones, both species expressed comparable amounts of *RH2B* and *RH2A* (*P. amboinensis*: *RH2B*=45.0%, 4.0; *RH2A*=54.5%, 3.9; *P. moluccensis*: *RH2B*=43.4%, 3.1; *RH2A*=52.2%, 2.7), but differed in *LWS* expression (*P. amboinensis*: *LWS*=0.4%, 0.4; *P. moluccensis*: *LWS*=3.6%, 2.1, $P<0.00017$).

Under altered illumination we observed major shifts in opsin expression in the three investigated reef fish species. We observed effects following altered light spectrum, light intensity and treatment duration, as well as differences in effects between opsin gene classes and fish family. A summary of the beta-regression statistics for the tested comparisons of baseline and treatment expression levels is provided in Table S4. The ratio of single cone opsins in damselfish, namely *SWS1* and *SWS2B*, showed the largest changes (Figs 4–7). In colour treatments low in short wavelength radiation, rapid shifts from *SWS1* (370 nm) to *SWS2B* (408 nm) (see Table 1) expression in damselfish were observed (Figs 4–7, panels C–F). Generally, these shifts occurred rapidly after only 1 month, and were even stronger after 6 months. *P. moluccensis* showed a shift in *SWS1* to *SWS2B* expression ratio similar in nature to that observed in *P. amboinensis*, but this was not statistically significant, perhaps due to the low number of replicates. Rapid shifts towards *SWS2B* were also observed in the intensity treatments, but the extent of the shift was less when more UV radiation was available (Fig. 7D–F). In *O. cyanosoma*, we also observed rapid shifts among single cone opsins (Figs 4–7). Here, the ratio of *SWS2Aα* (448 nm) to *SWS2Aβ* (468 nm) (see Table 1) shifted to the longer-tuned photopigment after 1 month of red treatment (Fig. 6A). Under the two brightest intensity treatments (Fig. 7A,B; no filter and 0.15 ND), the ratio of *SWS2Aα* to *SWS2Aβ* expression shifted to the shorter-tuned photopigment. Under blue, green and 0.6 ND treatment, single cone opsin expression remained unchanged.

Expression levels among double cone opsins were generally more rigid, showing, where present, only delayed changes in opsin expression under the different treatment conditions. There were also differences in affected genes between damselfish and cardinalfish. In *O. cyanosoma*, the ratio of *RH2B* to *RH2A* opsin expression (476 nm vs 518 nm, see Table 1) shifted towards *RH2A* in the colour treatments (Figs 4–6, panels A,B); however, this effect only showed in the 6 month treatment groups. The no filter and 0.6 ND group showed a shift from *RH2A* to *RH2B* after 1 month; in the 0.15 ND group double cone opsin expression was unchanged (Fig. 7A,B). *LWS* (544 nm, see Table 1) expression was unaffected by changed light conditions. In damselfish, double cone opsin expression remained comparably stable following changes in lighting conditions. Observed changes that were statistically significant were generally small, or reverted back to baseline levels – and are thus likely to be mostly attributable to natural variability. However, *LWS* (554 nm, see Table 1) expression in *P. moluccensis* saw a small increase after 6 months of blue light, 1 and 6 months of green light, 6 months of red light, and 1 month of unfiltered aquarium light. *RH2* opsin gene expression in both species was largely unaffected by colour treatments or reverted back to pre-exposure levels (i.e. Fig. 5E,F), except for reduced *RH2A* (518 nm, see Table 1) expression in *P. moluccensis* in the no filter and the 0.15 ND groups.

Spectral sensitivity modelling

An overview of the considered amino acid positions in each investigated gene, as well as the known or estimated substitution effects, are given in Table S5. On the whole, there were few variable tuning sites compared to other species with known $\lambda_{\max}$, suggesting our predictions are reasonable approximations. In addition, because of the tuning site similarities for both *Pomacentrus* species, their gene predictions are identical for all opsins.

A summary of our calculated $\lambda_{\max}$ values and any reported MSP measurements from the same or related species are given in Table 1. We calculated the resulting photopigments in damselfish to be maximally sensitive to 370 nm (SWS1), 408 nm (SWS2B), 480 nm (RH2B), 518 nm (RH2A) and 554 nm (LWS). These calculations fit well with values previously obtained using MSP. In *O. cyanosoma*, we calculated the photopigments to be maximally sensitive to 448 nm (SWS2Aα), 468 nm (SWS2Aβ), 476 nm (RH2B), 518 nm (RH2A) and 544 nm (LWS). To our knowledge, MSP measurements in Apogonidae have been reported only on *Pristiapogon kallotperus* (Losey et al., 2003); thus we list these for reference.

Light measurements and quantum catches

An overview of light environment spectra used (Fig. 1A), visual pigment absorbance curves generated from $\lambda_{\max}$ calculations for *O. cyanosoma* (Fig. 1B), *P. amboinensis* and *P. moluccensis* (Fig. 1C), as well as calculated quantum catches (Fig. 1D–G) and correlation plots with opsin gene expression (Fig. 1H) are shown. A summary of all calculated quantum catches is provided in Table 2. Calculated quantum catches in treatments were of similar magnitudes to those calculated for a natural reef setting, except for the red, ND 0.15 and ND 0.6 treatments in which quantum catch was up to 20-fold lower. Overall, under reef illumination, quantum catch was dominant for double cones, slightly less for single cones in *O. cyanosoma*, and markedly less in *P. moluccensis* and *P. amboinensis*.

Under blue treatment illumination, quantum catch was short-wavelength shifted. As a result, single cone opsins collected relatively more photons than double cone opsins in *O. cyanosoma*. However, in *P. moluccensis*, single cone opsin quantum catch was very low for SWS1 pigment, but remained comparably high for SWS2B pigment. In both species among double cone opsins, RH2B collected the most photons. Under green illumination, quantum catch in both species was long-wavelength shifted, such that double cone quantum catch dominated single cone quantum catch. In these conditions, RH2A was best tuned to the available light spectrum in both species. The strong light-attenuating properties of the red filter led to conditions such that in both species quantum catch for all photopigments except the LWS-based one was up to 20-times lower than under a reef spectrum. Owing to UV filter properties of the colour filters used, neither of the three colour treatments strongly stimulated the SWS1-based pigment.

All three light intensity treatment spectra resulted in similar quantum catch ratios between the opsin-based pigments. Differences in total caught quanta matched the expectation based on the light intensity. In *O. cyanosoma*, quantum catch of the five pigments was almost equal, suggesting a relative increase in single cone opsin expression compared with double cone opsin, and among single cone opsin, quantum catch was similar for SWS2Aα and SWS2Aβ. Total quantum catch, however, was between two times greater (no filter) and five times (0.6 ND) lower than under a reef spectrum. In *P. moluccensis*, relative quantum catch between the five opsin-based pigments was similar under the different conditions, albeit at different total numbers of absorbed quanta.

Test results for whether shifts in opsin expression are proportionate to the quantum catch resulting from the different light treatments using Kendall τ correlation coefficients are given in Table 3. Owing to the small extent of changes in *RH2* opsin genes in *P. moluccensis* and lack of changes in *LWS* expression in *O. cyanosoma*, we emphasize the relationship of single cone opsin, *RH2* opsins in *O. cyanosoma* and *LWS* in *P. moluccensis*. Overall, in *P. moluccensis* and *O. cyanosoma*, single cone opsin expression strongly correlated with quantum catch (Fig. 1Hi–iii). *SWS1* expression correlated positively with quantum catch under colour and intensity treatments (Fig. 1Hi,ii), whereas *SWS2Aα* expression correlated positively with quantum catch under colour treatments only. In *O. cyanosoma*, neither of the *RH2* opsin genes showed correlation with quantum catch across all treatments (intensity+colour). However, analysis of intensity and colour separately revealed that under colour treatments, decreased *RH2B* expression

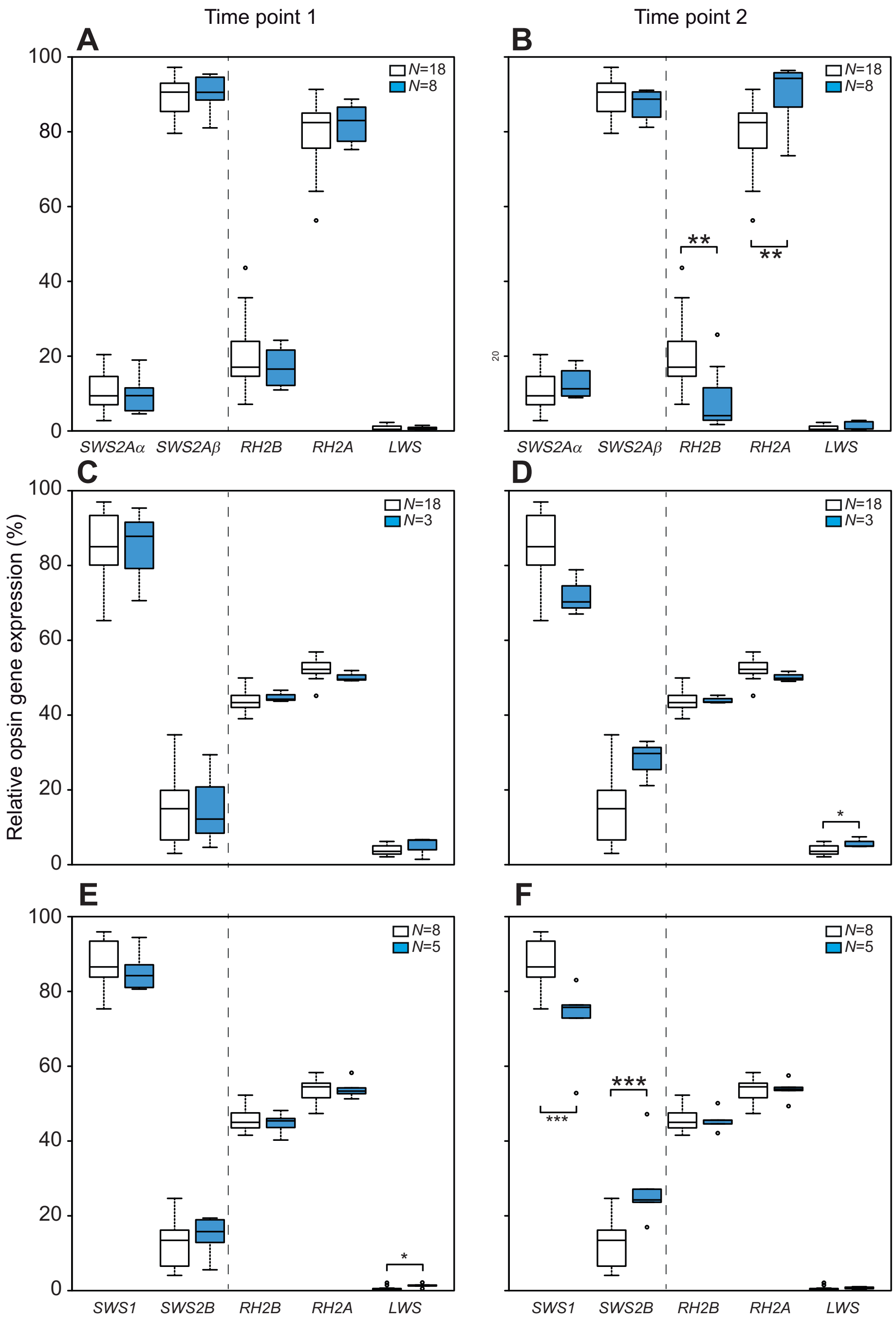

Fig. 4. Relative cone opsin gene expression after exposure to blue aquarium illumination. Gene expression in *O. cyanosoma* (A,B), *P. moluccensis* (C,D) and *P. amboinensis* (E,F) at the start of the experiment (baseline; white boxes) and after exposure to blue aquarium illumination for 1 month (time point 1; A,C,E) and 4 months (time point 2; B, *O. cyanosoma*) or 6 months (time point 2; D,F, *P. moluccensis*, *P. amboinensis*) (blue boxes). Expression values are shown as fraction of total single cone opsin genes [*SWS2Aα*, *SWS2Aβ* (A,B); *SWS1*, *SWS2B* (C–F)], or as fraction of total double cone opsin (*RH2B*, *RH2A*, *LWS*); dashed line marks separation. *N* indicates number of specimens used. Boxes show 25th and 75th percentiles with median; whiskers are 5th and 95th percentiles; dots are outliers. Significant differences in beta regression analysis are indicated by *$P\leq0.0083$, **$P\leq0.0017$ and ***$P\leq0.00017$.

coincided with decreased RH2B quantum catch, and increased *RH2A* expression coincided with increased RH2A quantum catch. In intensity treatments, on the other hand, quantum catch did not correlate with opsin expression, thus masking these effects in the combined analysis. In *P. moluccensis*, *LWS* opsin gene expression correlated with quantum catch in colour treatments but not in intensity treatments (Fig. 1Hi,ii,iv).

DISCUSSION

In this study, we investigated the possibility that phenotypic plasticity in opsin expression affects photoreceptor spectral sensitivity in adult coral reef fish by comparing species with a diurnal versus nocturnal feeding activity. We first identified the previously unknown opsin complement in cardinalfish (Fig. 2). We then exposed damselfish and cardinalfish to altered light conditions in the lab (Fig. 1A) and measured the levels of expressed opsin genes in the retinal tissue. We found that in the cardinalfish retina, the total opsin pool is dominated by rhodopsin (*RH1* ~90%) and thus different from damselfish in which rhodopsin only makes up ~60% of total opsin (Fig. 3). This is consistent with their different ecologies, as nocturnal cardinalfish expressed more of the low light-sensitive rhodopsin compared with diurnal damselfish. Furthermore, we found that the damselfish cone opsin repertoire is tuned to shorter wavelengths than the cardinalfish opsin repertoire, by means of UV-sensitive SWS1 and SWS2B opsins (Fig. 1B,C). Our experiments further demonstrate that opsin gene expression can vary within several months or weeks, but that this plasticity differs between damselfish and cardinalfish (Figs 4–7). Our data further indicate that environmental light can induce opsin

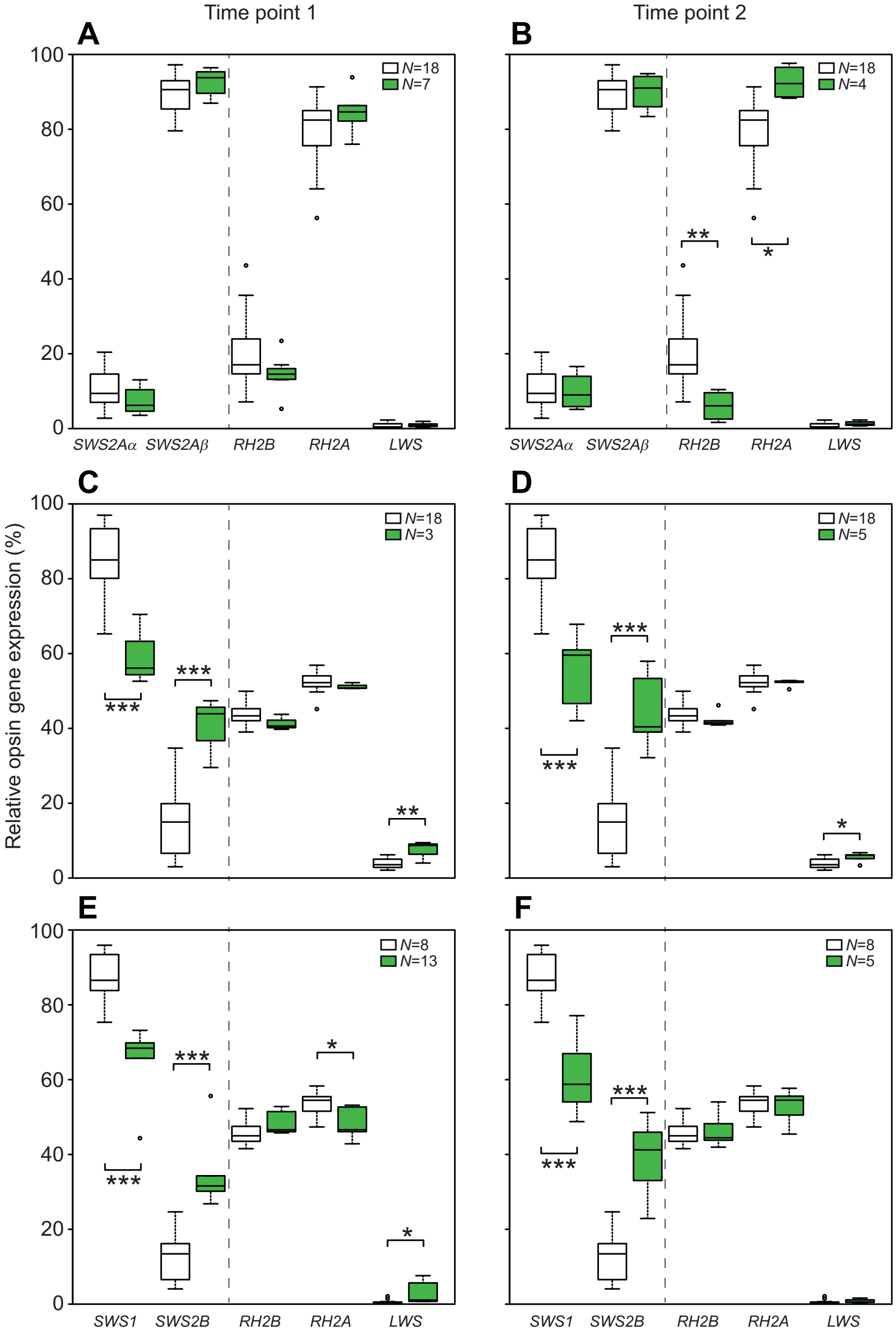

Fig. 5. Relative cone opsin gene expression after exposure to green aquarium illumination. Gene expression in *O. cyanosoma* (A,B), *P. moluccensis* (C,D) and *P. amboinensis* (E,F) at the start of the experiment (baseline; white boxes) and after exposure to blue aquarium illumination for 1 month (time point 1; A,C,E) and 4 months (time point 2; B, *O. cyanosoma*) or 6 months (time point 2; D,F, *P. moluccensis*, *P. amboinensis*) (green boxes). Expression values are shown as fraction of total single cone opsin genes [*SWS2Aα*, *SWS2Aβ* (A,B); *SWS1*, *SWS2B* (C–F)], or as fraction of total double cone opsin (*RH2B*, *RH2A*, *LWS*); dashed line marks separation. *N* indicates number of specimens used. Boxes show 25th and 75th percentiles with median; whiskers are 5th and 95th percentiles; dots are outliers. Significant differences in beta regression analysis are indicated by $^*P\leq0.0083$, $^{**}P\leq0.0017$ and $^{***}P\leq0.00017$.

expression changes via variation of intensity and spectrum; however, this is true only in cone opsin genes as rhodopsin levels remain unaffected. We found that single cone opsin gene expression demonstrated a more rapid change under most conditions compared with double cone opsin gene expression. Furthermore, correlation analysis indicates that quantitative opsin expression does correlate with quantum catch; however, there are differences between specific opsin gene classes (Fig. 1D,H; Tables 2 and 3).

Opsin repertoire and spectral sensitivities in damselfish and cardinalfish

RNA sequencing confirmed the damselfish opsin repertoire previously described (Hofmann et al., 2012; Stieb et al., 2016, 2017). Five cone opsins (*SWS1*, *SWS2B*, *RH2B*, *RH2A*, *LWS*), as well as one rod opsin (*RH1*), are expressed in the damselfish retina. The retinal opsin gene repertoire we identified in the yellow striped cardinalfish, *O. cyanosoma*, comprises five cone opsins (*SWS2Aα*, *SWS2Aβ*, *RH2B*, *RH2A*, *LWS*) and one rod opsin (*RH1*).

Whereas our calculation of damselfish visual sensitivities match well with values previously obtained using MSP, those calculated for the cardinalfish only match in part (Table 1). For cardinalfish, there is little data on photoreceptor spectral sensitivity (Cronin et al., 2014; Losey et al., 2003). In the iridescent cardinalfish, only one type of single cone and two spectrally distinct double cone types were identified using MSP (Losey et al., 2003). The deviations of sensitivity from our calculations may be explained by opsin co-

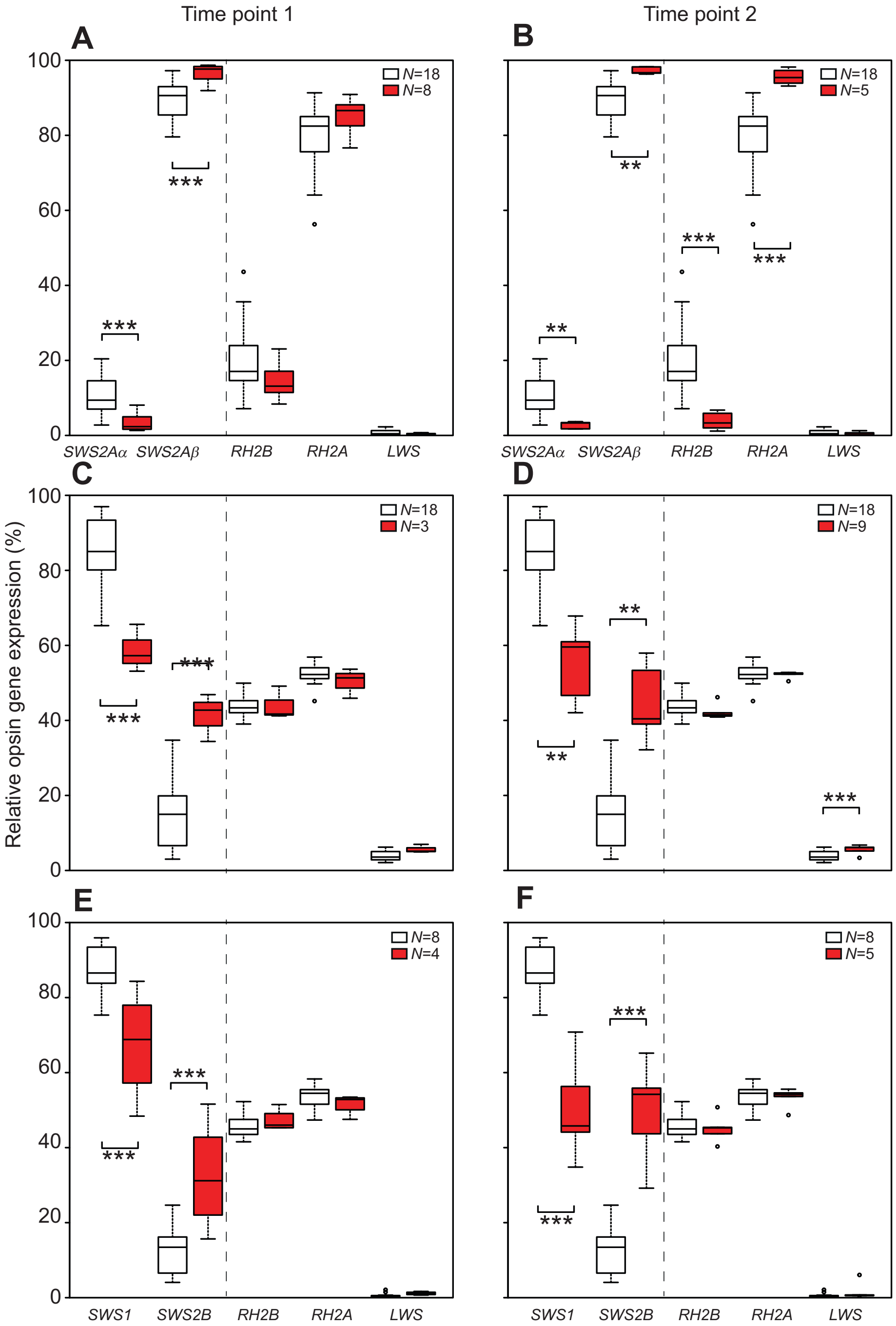

Fig. 6. Relative cone opsin gene expression after exposure to red aquarium illumination. Gene expression in *O. cyanosoma* (A,B), *P. moluccensis* (C,D) and *P. amboinensis* (E,F) at the start of the experiment (baseline; white boxes) and after exposure to blue aquarium illumination for 1 month (time point 1; A,C,E) and 4 months (time point 2; B, *O. cyanosoma*) or 6 months (time point 2; D,F, *P. moluccensis*, *P. amboinensis*) (red boxes). Expression values are shown as fraction of total single cone opsin genes [*SWS2Aα*, *SWS2Aβ* (A,B); *SWS1*, *SWS2B* (C–F)], or as fraction of total double cone opsin (*RH2B*, *RH2A*, *LWS*); dashed line marks separation. *N* indicates number of specimens used. Boxes show 25th and 75th percentiles with median; whiskers are 5th and 95th percentiles; dots are outliers. Significant differences in beta regression analysis are indicated by $^{**}P\leq0.0017$ and $^{***}P\leq0.00017$.

expression, which is not accounted for in our modelling, or if genes expressed at very low levels are not functional (*LWS*, 1% of double cone opsin). However, MSP measurements of single cones in the dusky dottyback (Cortesi et al., 2015a) concur mostly with our SWS2A calculations.

Considering the opsin repertoire of *O. cyanosoma* – high *SWS2Aβ* and *RH2A*, low *RH2B* and *SWS2Aα*, and very low *LWS* – it is possible that their colour vision system operates using a dichromatic opponency mechanism under photopic light conditions. However, without further insight into how and where in the retina these opsins are expressed, this remains unclear. Dichromacy has been suggested to be highly efficient in environments that offer two main colours, such as algae and corals, or in a restricted light environment (Chiao et al., 2000; Lythgoe, 1979; Marshall et al., 2003). Cardinalfish are nocturnal, spending the night primarily feeding on benthic or planktonic invertebrates (Barnett et al., 2006; Marnane and Bellwood, 2002). With a rod opsin to cone opsin ratio of ~9:1, the cardinalfish visual system shows typical adaptations to life in dimly lit environments (de Busserolles and Marshall, 2017; Hunt et al., 2001; Wikler and Rakic, 1990). This increased rhodopsin (*RH1*) ratio suggests that colour vision in cardinalfish may be restricted in its functionality, and adapted to subserve mostly specific colour tasks during the day, such as social interaction (Kuwamura, 1985).

The expression patterns we found in damselfish, high *SWS1*, *RH2B* and *RH2A*, and low *LWS* and *SWS2B*, confirmed those previously reported in wild specimens (Stieb et al., 2016, 2017), and are used to discriminate between colours (Siebeck et al., 2008). The

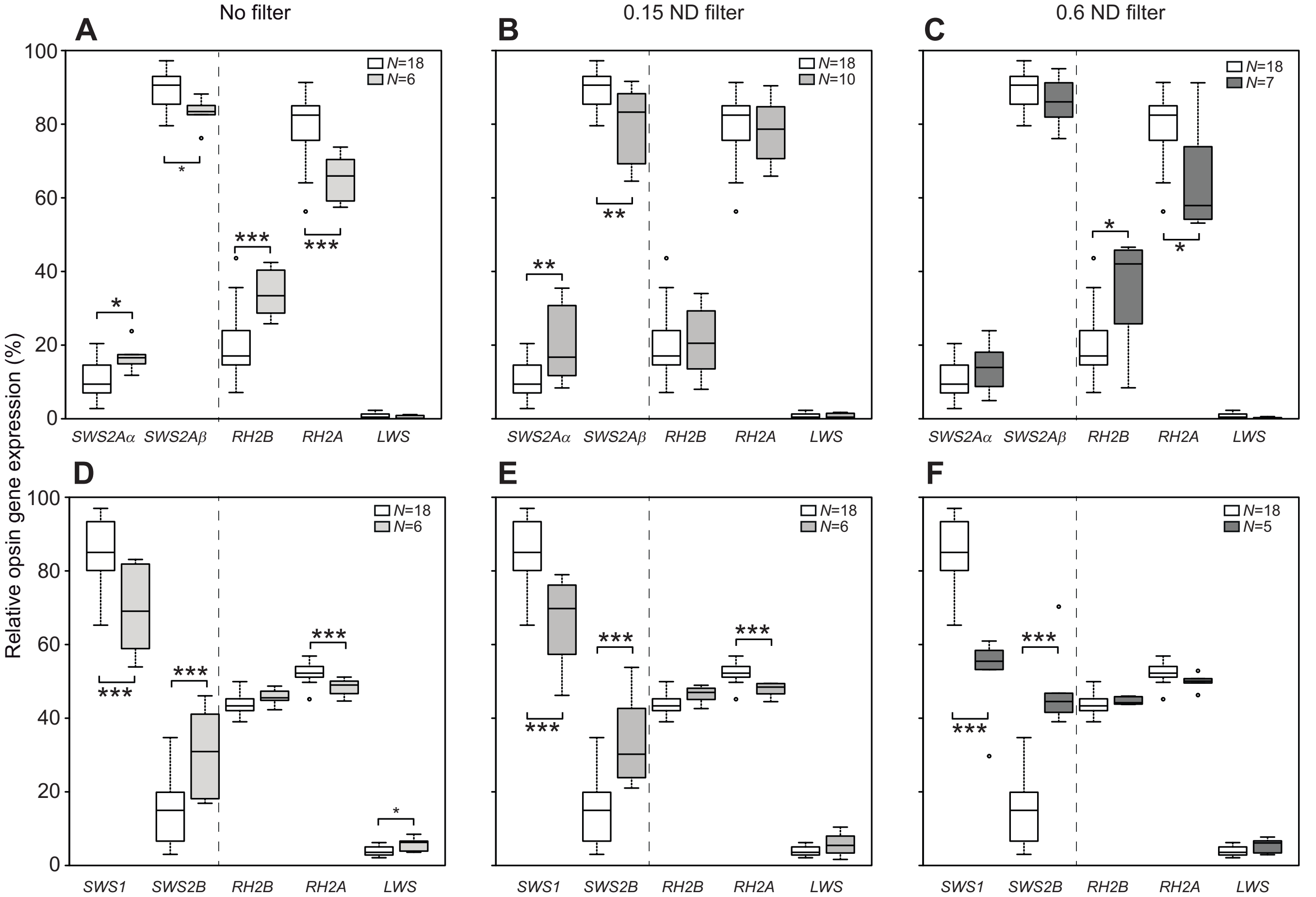

Fig. 7. Relative cone opsin gene expression after exposure to unfiltered and 0.15 ND or 0.6 ND filtered aquarium light. Gene expression in *O. cyanosoma* (A–C) and *P. moluccensis* (D–F) at the start of the experiment (baseline; white boxes) and after 1 month exposure to unfiltered aquarium light (A,D, light grey boxes), 0.15 ND filtered aquarium light (B,E, medium grey boxes) and 0.6 ND filtered aquarium light (C,F, dark grey boxes). Expression values are shown as fraction of total single cone opsin genes [*SWS2Aα*, *SWS2Aβ* (A,B); *SWS1*, *SWS2B* (C–F)], or as fraction of total double cone opsin (*RH2B*, *RH2A*, *LWS*); dashed line marks separation. *N* indicates number of specimens used. Boxes show 25th and 75th percentiles with median; whiskers are 5th and 95th percentiles; dots are outliers. Significant differences in beta regression analysis are indicated by $^*P\leq0.0083$, $^{**}P\leq0.0017$ and $^{***}P\leq0.00017$.

difference between opsin repertoires in both families with the greatest functional impact is probably the presence of transcripts for UV-sensitive SWS1 and violet-sensitive SWS2B opsin in damselfish in place of blue-sensitive SWS2Aα and SWS2Aβ in cardinalfish, shifting spectral sensitivity in damselfish into ultraviolet wavelengths. UV-reflecting body patterns are common in both families (Marshall, 2000; Stieb et al., 2017), supporting hypotheses that small reef fish may benefit from a covert short-range communication channel invisible for larger, UV-blind predatory fish (Siebeck et al., 2010). All damselfish investigated to date express an *SWS1* opsin gene (Stieb et al., 2017), forming visual pigments sensitive to UV light (Hawryshyn et al., 2003; Loew and Lythgoe, 1978; Losey et al., 2003; Marshall et al., 2006; McFarland and Loew, 1994) and possess ocular media transmitting UV light

Table 1. Summary of λ_{max} values for photopigments in reef fishes determined by our modelling along with MSP values for reference

		λ_{max} (nm)		
Species	Opsin	Modelling	MSP in same species	MSP in related species
P. moluccensis	SWS1		370 (*P. amboinensis*)[1]	347–376 (Pomacentridae)[1–4]
	SWS2B	408		404 (*Chromis ovalis*)[2], 410 (*Chromis verater*)[2]
	RH2B	480	480 (*P. amboinensis*)[1]	475–486 (Pomacentridae)[1–5]
	RH2A	518	523 (*P. amboinensis*)[1]	519 (Pomacentridae)[1–4]
	LWS	554		560 (*P. melanochir*)[5]
O. cyanosoma	SWS2Aα	448		448 (*P. fuscus*)[6], 441 (*P. kallopterus*)[2]
	SWS2Aβ	468		457 (*P. fuscus*)[6]
	RH2B	476		494 (*P. kallopterus*)[2]
	RH2A	518		516 (*P. kallopterus*)[2]
	LWS	544		

[1]Siebeck et al., 2010; [2]Losey et al., 2003; [3]Hawryshyn et al., 2003; [4]McFarland and Loew, 1994; [5]Loew and Lythgoe, 1978; [6]Cortesi et al., 2015a.

Table 2. Summary of calculated quantum catch by hypothetical photopigments under all investigated light conditions in *P. moluccensis* and *O. cyanosoma*

		Quantum catch (absolute quanta captured in photons m^{-2} sr^{-1} nm^{-1})						
Species	Treatment	SWS1	SWS2B	SWS2Aa	SWS2Ab	RH2B	RH2A	LWS
P. moluccensis	Reef	4.27E+12	7.82E+12			1.42E+13	1.6E+13	1.63E+13
	Blue	4.96E+11	5.57E+12			1.23E+13	1.02E+13	7.07E+12
	Green	1.89E+11	4.19E+11			6.91E+12	1.06E+13	9.26E+12
	Red	1.79E+11	2.42E+11			2.53E+11	3.61E+11	1.28E+12
	No filter	1.22E+13	1.83E+13			2.27E+13	2.32E+13	2.36E+13
	0.15 ND	3.32E+12	4.44E+12			4.34E+12	4.59E+12	4.8E+12
	0.6 ND	6.94E+11	1.66E+12			2.23E+12	2.25E+12	2.32E+12
O. cyanosoma	Reef			1.18E+13	1.34E+13	1.4E+13	1.6E+13	1.64E+13
	Blue			1.16E+13	1.23E+13	1.23E+13	1.02E+13	7.91E+12
	Green			2.53E+12	5.12E+12	6.32E+12	1.06E+13	9.98E+12
	Red			2.82E+11	2.64E+11	2.56E+11	3.61E+11	8.8E+11
	No filter			2.33E+13	2.29E+13	2.27E+13	2.32E+13	2.36E+13
	0.15 ND			4.74E+12	4.44E+12	4.36E+12	4.59E+12	4.8E+12
	0.6 ND			2.34E+12	2.28E+12	2.24E+12	2.25E+12	2.32E+12

(Siebeck and Marshall, 2001). *P. amboinensis* has been shown to use these features to identify both conspecifics and heterospecifics based on UV-reflecting facial markings (Siebeck et al., 2010).

Phenotypic plasticity in opsin expression induced by changed lighting conditions

Our results provide evidence for the presence of phenotypic opsin expression plasticity in coral reef fish from two families. In diurnal damselfish, UV-sensitive (*SWS1*) and violet-sensitive (*SWS2B*) single cone opsins appeared most susceptible to changes in light conditions. Similarly, but to a lesser extent, blue-sensitive single cone opsins (*SWS2Aα*, *SWS2Aβ*) in nocturnal cardinalfish responded to changes in light conditions, suggesting a high degree of plasticity and adaptability in these opsins. Double cone opsins were less plastic, showing large shifts only in *RH2* genes in the nocturnal cardinalfish. It is important to highlight that expression changes were also observed in fish exposed to the unfiltered (no filter) aquarium light treatment when compared with our baseline. In *P. moluccensis*, these changes were not significantly different to those observed under the other treatments, thus clouding the distinction between effects of light environment and potential other confounding factors for this species, e.g. stress, season or dietary changes (Table S4). This is further complicated since the no filter spectrum can hardly be considered a negative control as it also differs from the conditions present on the reef. Additionally, holding times may have affected opsin gene expression. For example, time spent in the supplier's facility reportedly did not exceed a few days; however, as they were provided by an external supplier, we cannot be certain that this was consistent for all individuals.

Table 3. Summary of Kendall's τb coefficients for correlation analysis of quantum catch and opsin gene expression under all treatments combined, and colour and intensity treatments analysed separately

Treatment	Species	Gene	Kendall's τb	*P* (τb)
Colour and intensity	*O. cyanosoma*	*SWS2Aα*	0.32	**<0.0003*****
		SWS2Aβ	−0.266	**<0.003****
		RH2B	0.177	0.029
		RH2A	−0.172	0.033
		LWS	0.14	0.085
	P. moluccensis	*SWS1*	0.323	**<0.003****
		SWS2B	−0.378	**<0.0003*****
		RH2B	0.136	0.149
		RH2A	0.124	0.189
		LWS	−0.236	**<0.017***
Colour	*O. cyanosoma*	*SWS2Aα*	0.443	**<0.0003*****
		SWS2Aβ	−0.443	**<0.0003*****
		RH2B	0.297	**<0.003****
		RH2A	−0.288	**<0.017***
		LWS	0.082	0.419
	P. moluccensis	*SWS1*	0.49	**<0.0003*****
		SWS2B	−0.49	**<0.0003*****
		RH2B	0.231	0.048
		RH2A	0.269	0.022
		LWS	−0.435	**<0.0003*****
Intensity	*O. cyanosoma*	*SWS2Aα*	−0.023	0.86
		SWS2Aβ	0.023	0.86
		RH2B	−0.017	0.9
		RH2A	0.017	0.9
		LWS	0.202	0.1
	P. moluccensis	*SWS1*	0.319	**<0.017***
		SWS2B	−0.319	**<0.017***
		RH2B	0.07	0.61
		RH2A	0.077	0.568
		LWS	−0.004	0.99

After Bonferroni correction for three tested hypotheses ($P=\alpha/m$, with $m=3$), *$P\leq0.017$, **$P\leq0.003$ and ***$P\leq0.0003$ were considered significant (shown in bold).

Changes in opsin expression could serve as a rapid mechanism to shift photoreceptor spectral sensitivity in order to adapt to altered optical conditions of the environment, thus maintaining optimal visual perception of vital cues in their immediate surrounding. Altered conditions of the light environment can be associated with seasonal change, a phenomenon particularly common in freshwater systems, but also known to occur in marine habitats, particularly in coastal regions and tropical coral reefs due to increased terrestrial organic matter run-off as a result of wet-season rainfall (Lythgoe, 1979; McClanahan, 1988; Munz and McFarland, 1977). Such effects are commonly associated with algal blooms, or increased amounts of other particulate matter in the water column, and a result of prolonged daylight duration, increased water temperature, increased rainfall and increased land run-off, variable solar radiation intensity or a combination of these factors (Lythgoe, 1979; Munz and McFarland, 1977; McFarland and Munz, 1975). Increased particulate organic matter, or increased phytoplankton, generally leads to a long-wavelength shift of the available light spectrum due to light absorption, suggesting that the visual system response observed in this study under altered lighting conditions could be found in nature. In fact, there are several accounts reporting seasonal periodicity in fish visual system characteristics, such as altered spectral sensitivity in the three-spined stickleback (*Gasterosteus aculeatus*) (Cronly-Dillon et al., 1968), altered opsin gene expression in the damselfish (*Pomacentrus nagasakiensis*) (Stieb et al., 2016) and the Japanese ricefish (*Oryzias latipes*) (Shimmura et al., 2017). Whether the expression changes observed in *P. nagasakiensis* by Stieb et al. (2016) were in fact due seasonal change remained unclear. Thus, further investigation in this direction is needed to clarify the effects of season on reef fish opsin expression.

Differences in ambient light at different depths is also important to consider. Both damselfish species and *O. cyanosoma* reportedly occur at up to 40 m depth (Allen et al., 2003; Randall et al., 1990). Stieb et al. (2016) showed that damselfish collected from different habitat depths did differ in opsin expression as a result of adaptation to changed lighting conditions. However, it remained unclear whether the observed changes resulted from plasticity during juvenile settlement, or whether the animals retained expression plasticity in adulthood. As our data suggests, the tested damselfish may have developed those expression changes after they reached maturity. Nevertheless, damselfish and cardinalfish generally display high site fidelity (Gardiner and Jones, 2005; Marnane, 2000; Petersen, 1995), making change of habitat depth or geographical relocation unlikely candidates to demand visual system adaptation in adults of these species.

According to the offset hypothesis, a dichromatic visual system is best tuned to an environment if one of its sensitivities is matched to the overall environmental backlight and the other is offset from this background in order to allow contrast detection (Lythgoe, 1979). This principle may explain the differences observed between single and double cone opsin gene expression, and therefore possibly, the differences observed between damselfish and cardinalfish. In the above scenario, the overall environmental backlight is likely to be the least-variable parameter. Overall, environmental backlight on coral reefs peaks around 500 nm (Marshall et al., 2003; Matz et al., 2006), a wavelength to which reef fish double cones are well matched (Losey et al., 2003). Wavelengths at either end of the spectrum, by contrast, are attenuated rapidly with increasing depth or distance (Lythgoe, 1979) while 500 nm light intensity remains comparably constant. Under these circumstances, constructing a visual system with set double cone but comparably plastic single cone spectral sensitivities, would supply the most feasible adaptive system.

Rhodopsin expression remained largely unaffected by changes in light in all tested species, regardless of shifts in spectral distribution or light intensity. For a monochromatic visual system, any change in light condition effectively only changes perceived intensity because different spectral channels for comparison are not available (Gegenfurtner and Kiper, 2003). Our study species and other investigated shallow water reef fish express only one RH1 opsin, and to date, more than one rhodopsin has been found among fish only in several deep-sea species (de Busserolles et al., 2015; Partridge et al., 1992; Pointer et al., 2007), carp (Lim et al., 1997) and the zebrafish (Morrow et al., 2017). Hence, if changed light conditions had had an effect, we would have expected to see this in all treatment groups differing in light intensity. In fact, from other systems, rhodopsin expression is known to fluctuate following a circadian rhythm (Korenbrot and Fernald, 1989); however, it seems that this is not affected in the timeframes we investigated here.

How to improve visual sensitivity with changing light conditions: direction of expression change in cone opsins differs between opsin class

To investigate whether changes in the spectral composition of the light environment favour upregulation or downregulation of opsin gene expression by means of visual pigment performance, we calculated quantum catches of each photopigment modelled from the peak spectral absorbance calculated using its amino acid sequence. Our results suggest that both strategies are employed and that it depends on the nature of the environmental change, as well as on the affected pigment, which strategy will drive opsin expression change. For instance, *SWS1* opsin gene expression in Pomacentrids correlated positively with increased quantum catch of the SWS1 photopigment. Consequently, *SWS1* expression in comparison to *SWS2B* expression dropped in a proportionate manner upon exposure to gradually decreasing levels of UV radiation. This agrees with reports of long-wavelength shifted spectral sensitivities in black bream reared in short wavelength reduced conditions (Shand et al., 2008), but is seemingly in contrast to a reported reduction of blue-sensitive single cones in blue acara, when reared under monochromatic blue light (Kröger et al., 1999, 2003; Wagner and Kröger, 2000; reviewed in Wagner and Kröger, 2005). The non-compensatory principle apparently controlling SWS gene expression in damselfish may greatly influence visual system capabilities in an ecological context such as their ability to use UV signals for conspecific and heterospecific identification (Siebeck et al., 2010). As known habitat depth extends to 40 m (Randall et al., 1990), a significant difference in available UV illumination from high in surface waters to almost nothing at depth on the reef is a real consideration here (Cronin et al., 2014).

In *O. cyanosoma*, *RH2B* expression positively correlated with quantum catch in colour treatments, suggesting the adaptive response is driven by *RH2B* expression. *LWS* expression in *P. moluccensis*, in comparison, correlated negatively with quantum catch, further illustrating that the effect of changed light conditions is dependent on the nature of the change and the available opsin repertoire.

Potential mechanisms facilitating differential cone opsin gene expression

Differential opsin gene expression could be facilitated by various mechanisms. The number of photoreceptor cells or outer segment size may change and thus provide more or less room for the opsin. In cichlids reared under chromatically deprived light conditions, both mechanisms were observed (Kröger et al., 1999; Wagner and Kröger, 2000, 2005). Increasing evidence suggests that gene co-expression can also shift spectral sensitivity via differential opsin gene expression. Opsin co-expression in individual photoreceptors is known to occur in fish (Dalton et al., 2014, 2015, 2017; Takechi and Kawamura, 2005; Torres-Dowdall et al., 2017) and allows rapid adjustments of the opsin complement without making structural changes to the retinal anatomy. By yielding photoreceptors with peak spectral sensitivities intermediate between those of pure opsin-based photoreceptors, co-expression may furthermore be an effective means to achieve optimal backlight matching.

Lastly, species differences in total amounts of retinal cone opsin and the relative proportion of photoreceptors subserving colour and luminance vision, respectively, may facilitate different adaptive responses in opsin gene expression changes. If one assumes two visual systems with similar proportion of photoreceptors tasked with colour and luminance vision, but with different total cone opsin volumes, the one with less cone opsin available (i.e. cardinalfish) should show greater sensitivity to expression changes, since smaller increases or decreases of gene expression have a larger effect on relative differential gene expression. As a consequence, even if the net change in gene expression in both systems were similar, relative expression changes would be greater in the system with less cone opsin overall. If the colour task in this species were background matching, changes in lighting conditions would have a greater relative effect. Such effects could be affected by differential topographic distribution of cone opsins, a phenomenon known at the molecular level, among others, from work on cichlids and zebrafish (Dalton et al., 2015; Takechi and Kawamura, 2005). On a morphological level differential distribution of different photoreceptor classes is common in many animals, including many reef fish (Collin and Shand, 2003). Such differences are thought to relate directly to visual system demands and may be related to tasks specific for certain cone classes (e.g. background matching; Temple, 2011). Such a spatial differentiation of tasks may then result in differential distribution of opsins across the retina (as in the human fovea, which contains fewer blue-sensitive cones; Curcio et al., 1991; Roorda and Williams, 1999) and as a consequence, make these different retinal areas respond differently to altered light conditions.

Conclusion

Increasing evidence suggests that gene expression plasticity seems to be crucial for sensory system adaptation to environmental conditions. Our study shows that retinal opsin expression in adult reef fish is plastic, and that it can be modulated by spectral and intensity changes in environmental light. Such expression adjustments may allow rapid adaptation to changing light conditions in the wild because of changed habitat depth or seasonal variability. Our results largely concur with previous reports on opsin gene expression plasticity in fish; however, the effects observed here differ between species and opsin gene class, suggesting that interactions of this nature need to be assessed at the species level.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.L., S.M.S., K. L. Cheney, N.J.M.; Methodology: M.L., S.M.S., K. L. Cheney, N.J.M.; Formal analysis: M.L., S.M.S., K. L. Carleton; Investigation: M.L., S.M.S., A.P.; Resources: M.L., S.M.S.; Data curation: M.L., S.M.S.; Writing - original draft: M.L., S.M.S.; Writing - review & editing: M.L., S.M.S., K. L. Carleton, K. L. Cheney, N.J.M.; Visualization: M.L.; Supervision: K. L. Cheney, N.J.M.; Project administration: M.L., S.M.S., K. L. Cheney, N.J.M.; Funding acquisition: K. L. Cheney, N.J.M.

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Data availability

New opsin gene sequences have been deposited in the GenBank database, and all accession numbers are given in Fig. 2. Primer sequences are available in Table S2.

Supplementary information

Supplementary information available online at http://jeb.biologists.org/lookup/doi/10.1242/jeb.175281.supplemental

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Table S1: Overview of numbers of specimens used per light treatment and per sampling timepoint. Timepoint 0 = Start of experiment; Timepoint 1 = 1 month; Timepoint 2 = 4 months (*O. cyanosoma*) / 6 months (*P. moluccensis, P. amboinensis*).

Species	Treatment												
	Baseline	Blue		Green		Red		No Filter		ND0.15		ND0.6	
	0	1	2	1	2	1	2	1	2	1	2	1	2
O. cyanosoma	18	8	8	7	5	8	5	6	n/a	10	n/a	7	n/a
P. moluccensis	18	3	3	3	5	3	9	6	n/a	6	n/a	5	n/a
P. amboinensis	8	5	5	13	5	4	5	n/a	n/a	n/a	n/a	n/a	n/a

Table S2: Primer names and sequences used for PCR and sequencing of the pool of opsins for *O. cyanosoma*; and summary of qPCR primer combinations and efficiencies for each species. Primer names and sequences for *P. amboinensis* and *P. moluccensis* were obtained from Stieb et al. (2016).

opsin	**species**	**primer pool**	**sequence**
SWS2Aα	*O. cyanosoma*	pSWS2AA_F1	5’-GCCATGGCTAACCTCATTGT-3’
		pSWS2AA_R4	5’-TTTGGAGACTTCAGTTACTGATGCT -3’
SWS2Aβ	*O. cyanosoma*	pSWS2AB_F3	5’-AACTTGGCCTTTTCCAACCT -3’
		pSWS2AB_R5	5’-ACTTCAGTCACCGACTGG -3’
RH2B	*O. cyanosoma*	pRH2B_F3	5’-CTCCGGCAACCTCTGAACTT -3’
		pRH2B_R3	5’-GTATGGGGTCCAAGCAACAA-3’
RH2A	*O. cyanosoma*	pSWS2AB_R5	5’-GGCAACCGCTGAACTACATC-3’
		pRH2A_R1	5’-AGCCAAAGACCATCAAGACG-3’
LWS	*O. cyanosoma*	pLWS_F1	5’-TCAGCGTATGCAACCAGTTC-3’
		pLWS_R1	5’-GGCATATCCAGGGTTAGCAG-3’
RH1	*O. cyanosoma*	pRH1_F2	5’-GCGTTGTCCGGAGTCCTTAT-3’
		pRH1_R2	5’-TCCACATGAGCACTGCATTC-3’
opsin	**species with primer efficiencies (%)**	**primer qPCR**	**sequence**
SWS1	*P. amboinensis* (90), *P. moluccensis* (94)	SWS1_forward_1	5’-CTCCAAGAGCTCCTGCGTCT-3’
		SWS1_reverse_1	5’-TGATGCAGGCGTTGAACTGTTTG-3’
SWS2B	*P. amboinensis* (91), *P. moluccensis* (91)	SWS2B_forward_1	5’-GGTGAAAGCGGTAGCAAAGG-3’
		SWS2B_reverse_1	5’-CCATCTTGGTCACCTCCCGCTC-3’
SWS2Aα	*O. cyanosoma* (103)	SWS2AA_F3	5’- ATAAACAGTTCCGTGGGTGCATGAT -3’
		SWS2AA_R3	5’- TTGGAGACTTCAGTTACTGATGCTG -3’
SWS2Aβ	*O. cyanosoma* (100)	SWS2AB_F1	5’- TAACGCTTGGTGGGATGGTGAG -3’
		SWS2AB_R1	5’- GCTAAAGCGTGGTCAGGTTTGAAC -3’
RH2B	*P. amboinensis*	RH2B_forward_1	5’-GGTGGGCTATTTCTCCTTGGG-3’
	P. moluccensis	RH2B_forward_2	5’-GATGGGCTATTTCTCCTTGGGG-3’
	P. amboinensis (93), *P. moluccensis* (96)	RH2B_reverse_1	5’-CACAGAGACACTTGACCTCCG-3’
	O. cyanosoma (95)	RH2B_F1	5’- CTGCTTTGGCTTCACCATCACC -3’
		RH2B_R5	5’-ACTTGACCTCCCAGTGTAGCCATG-3’
RH2A	*P. amboinensis* (91), *P. moluccensis* (94)	RH2A_forward_1	5’-CATTCTTGGACCCACTTTCTGCG-3’
		RH2A_reverse_1	5’-CCAGAGAGCAACTTCACCTCCA-3’
	O. cyanosoma (91)	RH2A_F2	5’-ATGCAGGAGCTGGAGTTGCTTTC-3’
		RH2A_R2	5’-GGTACCTGGACCAGCCACC-3’
LWS	*P. moluccensis*	LWS_forward_1	5’-ACACCAATCACACCAAAGATCCC-3’
	P. amboinensis	LWS_forward_3	5’-CCAATTACACCAAAGATCCC-3’
	P. amboinensis (96), *P. moluccensis* (95)	LWS_reverse_2	5’-GACAAACATCCAGGCTGTGGC-3’
	O. cyanosoma (96)	LWS_F2	5’-TTCGGATGGAGCAGGTACTGG-3’
		LWS_R2	5’-ATCATGTACGACTGGACTCCAGG-3’
RH1	*P. amboinensis* (91), *P. moluccensis* (90)	RH1_forward_1	5’-CCACTGCATGATCACCACCT-3’
		RH1_reverse_1	5’-GATGCTCCCTCCTCTTCTTCG-3’
	O. cyanosoma (81)	RH1_F3	5’-CCATCAGCAACTTCCGCTTTGG-3’
		RH1_R3	5’-GGGGTACGGAGCAAGCAGC-3’

Table S3: Summary of total relative opsin expression under different colour treatments and after different treatment durations in the three investigated reef fish species. Values are given as median fraction of total single cone opsin (%) and interquartile range for each of the SWS cone opsins present in each respective species (*O. cyanosoma*: SWS2Aα, SWS2Aβ; *P. amboinensis*/*P. moluccensis*: SWS1/SWS2B), as median fraction of total double cone opsin (RH2B, RH2A, LWS) and interquartile range, and as median fraction of cone and rod opsin of total opsin (%) and interquartile range.

Species	time spent in tank [months]	treatment	n	Opsin gene								
				SWS1	SWS2B	SWS2Aα	SWS2Aβ	RH2B	RH2A	LWS	Total Cone	Total RH1
P. amboinensis	0	baseline	8	86.6, 9.6	13.4, 9.6	-	-	45.0, 4.0	54.5, 3.9	0.4, 0.4	58.7, 6.5	41.3, 6.5
	1	blue	5	84.2, 6.1	15.8, 6.1	-	-	45.4, 2.4	53.4, 1.6	1.3, 0.2	64.4, 3.1	35.6, 3.1
		green	13	68.4, 4.1	31.6, 4.1	-	-	46.6, 5.4	46.6, 6.6	1.1, 4.9	63.9, 7.5	36.1, 7.5
		red	4	68.8, 13.2	31.2, 13.2	-	-	46.0, 2.6	52.8, 1.8	1.1, 0.4	63.2, 4.5	36.8, 4.5
	6	blue	5	75.7, 3.5	24.3, 3.5	-	-	45.6, 1.0	53.9, 0.9	0.5, 0.4	52.9, 6.1	47.1, 6.1
		green	5	58.8, 12.9	41.2, 12.9	-	-	44.5, 4.4	54.5, 5.0	0.6, 0.6	56.3, 9.1	43.7, 9.1
		red	5	45.8, 12.2	54.2, 12.2	-	-	45.3, 1.7	54.2, 1.0	0.6, 0.2	47.6, 7.9	52.4, 7.9
P. moluccensis	0	baseline	18	85.0, 12.5	15.0, 12.5	-	-	43.4, 3.1	52.2, 2.7	3.6, 2.1	63.2, 11.0	46.8, 11.0
	1	blue	3	87.8, 12.4	12.2, 12.4	-	-	44.3, 1.5	49.6, 1.4	6.6, 2.6	62.6, 1.8	37.4, 1.8
		green	3	56.1, 8.9	43.9, 8.9	-	-	40.6, 2.0	50.8, 0.8	8.7, 2.7	58.3, 2.2	41.7, 2.2
		red	3	57.3, 6.2	42.7, 6.2	-	-	41.7, 4.0	51.3, 3.9	5.1, 1.0	56.6, 5.3	33.4, 5.3
		no filter	6	69.1, 21.9	30.9, 21.9	-	-	45.5, 2.1	49.0, 2.6	6.3, 2.2	65.7, 15.5	34.3, 15.5
		0.15 ND	6	69.8, 14.4	30.2, 14.4	-	-	47.0, 2.4	48.4, 2.6	5.5, 4.0	55.1, 8.2	44.9, 8.2
		0.6 ND	5	55.5, 5.2	44.5, 5.2	-	-	44.2, 2.0	50.1, 1.2	6.1, 3.3	61.3, 0.7	38.7, 0.7
	6	blue	3	70.3, 5.9	29.7, 5.9	-	-	43.5, 1.0	49.8, 1.3	5.0, 1.3	51.0, 11.4	49.0, 11.4
		green	5	59.6, 14.3	40.4, 14.3	-	-	41.5, 0.9	52.4, 0.3	6.1, 1.0	53.1, 3.3	46.9, 3.3
		red	9	66.6, 25.9	33.4, 25.9	-	-	41.5, 3.6	49.9, 4.4	6.0, 2.6	62.3, 12.1	37.7, 12.1
O. cyanosoma	0	baseline	18	-	-	9.4, 7.5	90.6, 7.5	17.1, 8.5	82.5, 8.6	0.4, 0.9	91.2, 3.9	8.8, 3.9
	1	blue	8	-	-	9.4, 5.5	90.6, 5.5	16.5, 8.9	83.0, 8.5	0.7, 0.5	92.8, 0.8	7.2, 0.8
		green	7	-	-	6.2, 5.7	93.8, 5.7	14.5, 2.9	84.6, 4.1	0.8, 0.7	93.4, 1.3	6.6, 1.3
		red	8	-	-	2.3, 2.4	97.7, 2.4	13.1, 5.1	86.6, 5.1	0.4, 0.2	92.9, 1.6	7.1, 1.6
		no filter	6	-	-	16.6, 2.1	83.4, 2.1	33.4, 10.0	65.9, 9.4	0.5, 0.6	89.9, 3.3	10.1, 3.3
		0.15 ND	10	-	-	16.7, 17.9	83.3, 17.9	20.5, 12.5	78.6, 11.3	0.4, 1.0	91.3, 1.3	8.7, 1.3
		0.6 ND	7	-	-	13.9, 9.3	86.1, 9.3	42.0, 20.0	57.9, 19.7	0.1, 0.3	91.1, 5.6	8.9, 5.6
	4	blue	8	-	-	11.3, 5.6	88.7, 5.6	4.1, 5.6	94.3, 6.6	1.5, 1.7	94.9, 1.5	5.1, 1.5
		green	5	-	-	9.0, 6.4	91.0, 6.4	6.1, 6.2	92.2, 7.2	1.2, 0.5	95.5, 0.3	3.5, 0.3
		red	5	-	-	3.3, 1.7	96.7, 1.7	3.3, 3.9	95.4, 3.4	0.6, 0.5	94.8, 1.1	5.2, 1.1

Table S4: Summary of beta regression models showing results for baseline datasets tested against light treatments (blue, green, red, no filter, ND0.15, ND0.6) after different time points, and results for no filter datasets tested against light treatments (blue, green, red, ND0.15, ND0.6). After Bonferroni-Correction for six (baseline dataset) respectively five (no filter dataset) tested treatment hypotheses (p = α/m, with m=6 resp. 5), p-values less than or equal to 0.0083, 0.0017, and 0.00017 resp. p-values less than or equal to 0.01, 0.002, and 0.0002 were considered significant and are marked with *, **, or ***, respectively. Statistically significant P-values are shown in bold.

species	**time spent in tank [months]**	**light & control treatment**	**opsin gene**							
			SWS1	**SWS2B**	**SWS2Aα**	**SWS2Aβ**	**RH2B**	**RH2A**	**LWS**	**RH1**
			baseline	**baseline**	**baseline**	**baseline**	**baseline**	**baseline**	**baseline**	**baseline**
P. amboinensis	1	blue	0.385	0.385	n/a	n/a	0.508	0.865	**0.002***	0.337
		green	**2.77E-08*****	**2.77E-08*****	n/a	n/a	0.09	**0.004***	**0.007***	0.277
		red	**7.02E-05*****	**7.02E-05*****	n/a	n/a	0.404	0.245	0.009	0.194
	6	blue	**1.05E-04*****	**1.05E-04*****	n/a	n/a	0.922	0.982	0.169	0.071
		green	**1.01E-14*****	**1.01E-14*****	n/a	n/a	0.78	0.676	0.046	0.091
		red	**2.67E-13*****	**2.67E-13*****	n/a	n/a	0.697	0.823	0.999	0.015
P. moluccensis	1	blue	0.947	0.947	n/a	n/a	0.465	0.16	0.715	0.683
		green	**1.85E-05*****	**1.85E-05*****	n/a	n/a	0.108	0.452	**2.23E-04****	0.222
		red	**5.83E-06*****	**5.83E-06*****	n/a	n/a	0.902	0.22	0.009	0.126
		no filter	**7.65E-04****	**7.65E-04****	n/a	n/a	0.09	**7.68E-04****	**0.002***	0.349
		0.15 ND	**4.00E-05*****	**4.00E-05*****	n/a	n/a	0.019	**6.13E-04****	0.13	0.044
		0.6 ND	**3.5E-10*****	**3.5E-10*****	n/a	n/a	0.414	0.051	0.05	0.257
	6	blue	0.01	0.01	n/a	n/a	0.863	0.151	**0.008***	0.076
		green	**4.96E-09*****	**4.96E-09*****	n/a	n/a	0.235	0.849	**0.006***	**7.38E-05*****
		red	**3.64E-04****	**3.64E-04****	n/a	n/a	0.14	0.12	**8.25E-08*****	0.058
O. cyanosoma	1	blue	n/a	n/a	0.521	0.521	0.63	0.602	0.357	0.204
		green	n/a	n/a	0.109	0.109	0.204	0.213	0.145	0.076
		red	n/a	n/a	**1.74E-05*****	**1.74E-05*****	0.184	0.134	0.527	0.416
		no filter	n/a	n/a	**0.003***	**0.003***	**1.01E-04*****	**9.68E-05*****	0.75	0.417
		0.15 ND	n/a	n/a	**0.002***	**0.002***	0.627	0.609	0.834	0.707
		0.6 ND	n/a	n/a	0.343	0.343	**0.003***	**0.003***	**0.001***	0.156
	4	blue	n/a	n/a	0.227	0.227	**2.41E-04****	**7.62E-04****	0.029	0.015
		green	n/a	n/a	0.736	0.736	**0.002***	**0.002***	0.029	0.011
		red	n/a	n/a	**2.01E-04****	**2.01E-04****	**3.43E-05*****	**2.73E-05*****	0.836	0.013
			no filter	**no filter**	**no filter**	**no filter**	**no filter**	**no filter**	**no filter**	**no filter**
P. moluccensis	1	blue	0.046	0.046	n/a	n/a	0.514	0.18	0.306	
		green	0.183	0.183	n/a	n/a	**0.002***	0.03	0.349	
		red	0.121	0.121	n/a	n/a	0.365	0.304	0.995	
		0.15 ND	0.606	0.606	n/a	n/a	0.435	0.528	0.477	
		0.6 ND	0.011	0.011	n/a	n/a	0.323	0.259	0.591	
O. cyanosoma	1	blue	n/a	n/a	**5.44E-04****	**5.44E-04****	**1.25E-08*****	**9.86E-09*****	0.147	
		green	n/a	n/a	**3.34E-06*****	**3.34E-06*****	**6.49E-09*****	**8.03E-09*****	0.061	
		red	n/a	n/a	**2.97E-14*****	**2.97E-14*****	**4.52E-12*****	**7.63E-13*****	0.748	
		0.15 ND	n/a	n/a	**0.004***	**0.004***	**5.7E-04****	**3.44E-04****	0.895	
		0.6 ND	n/a	n/a	0.178	0.178	0.782	0.751	0.037	

Table S5: Overview of sites and site effects considered for λ_{max}-calculations for *Pomacentrus moluccensis*, *P. amboinensis*, and *Ostorhinchus cyanosoma*. Bottom site numbers indicate amino acid residue position of Bovine rhodopsin, top site numbers indicate amino acid (AA) residue position in the target genes. All site effects and overall sensitivities are given in nm. AA changes in sites that our λ_{max}-calculations are based on are highlighted in blue, and are either known tuning sites (SWS2B W265Y, SWS2Aβ S94A, LWS S164A, LWS Y261F: Lin et al., 1998; Takahashi and Ebrey, 2003; Yokoyama, 2008; Dungan et al., 2016) or in close vicinity (RH2B C157V: Chan et al., 1992; Wilkie et al., 2000), are estimated on MSP mesaurements (SWS2Aα L216F; RH2B I49C, RH2B S109G, LWS: Cortesi et al., 2015; Spady et al., 2006; Siebeck et al., 2010; Losey et al., 2003; Stieb et al., 2016), or are based on a change in polarity (RH2B C98A, RH2B S124A, RH2A F158/166L, RH2A F158/166A).

SWS2B

Species	Variable site							Estimated tuning effect (nm)						Estimate (nm)	Actual lmax (nm)	MSP
	49	52	124	169	174	209	271	49	52	124	169	174	271			
***P. moluccensis* SWS2B**	F	F	T	F	A	Y	Y	0	0	0	0		-15	408		(404 - 410 in other damselfish)
***P. amboinensis* SWS2B**	F	F	T	F	A	Y	Y	0	0	0	0		-15	408		
P. fuscus SWS2B	F	F	T	C	A	Y	W	0	0	0	0		0	423		
M. zebra SWS2B	F	F	T	C	A	Y	W	0	0	0	0		0	423	423	
O. niloticus SWS2B	F	F	T	C	A	Y	W	0	0	0	0		0	423	423	
O. latipes SWS2B	L	V	A	C	S	F	Y	-3	6	-10	0	4	-15	405	405	
L. goodei SWS2B	L	F	G	F	T	Y	Y	-3	0	-10	0	2	-15	397	397	
Bovine RH1	Y	L	T	M	A	F	W									
	43	46	118	163	168	203	265	43	46	118	163	168	265			

SWS2A

Species	Variable site											Estimated tuning effect (nm)										Estimate (nm)	Actual lmax (nm)
	47	49	88	100	115	169	174	222	275	298	305	47	49	100	115	169	174	222	275	298	305		
***O. cyanosoma* SWS2Aα**	A	F	A	A	A	F	A	F	A	S	A	0	0	0	0	0	0	-8	0	0	0	448	
P. fuscus SWS2Aα	A	F	A	A	A	F	S	F	A	S	A	0	0	0	0	0	0	-8	0	0	0	448	448
M. zebra SWS2A	A	F	A	A	A	F	S	L	A	S	A	0	0	0	0	0	0	0	0	0	0	456	
O. niloticus SWS2A	A	F	A	A	A	F	S	L	A	S	A	0	0	0	0	0	0	0	0	0	0	456	456
L. goodei SWS2A	A	Y	A	A	A	F	S	L	A	A	T	0	0	0	0	0	0	0	0	8	2	460	448
O. latipes SWS2A	A	F	A	A	A	C	A	F	A	S	T	0	0	0	0	0	0	-8	0	0	2	450	439
***O. cyanosoma* SWS2Aβ**	S	Y	S	T	V	I	S	L	A	S	A	0	0	14	-2	0	0	0	0	0	0	468	
P. fuscus SWS2Aβ	A	F	A	A	A	F	S	L	T	S	A	0	0	0	0	0	0	0	6	0	2	464	457
Bovine RH1	A	Y	A	L	A	A	A																
	41	43	82	94	109	163	168	216	269	292	299	41	43	94	109	163	168	216	269	292	299		

RH2B

Species	Variable site										Estimated tuning effect (nm)										Estimate (nm)	Actual lmax (nm)
	45	50	95	96	99	110	125	158	204	293	45	50	95	96	99	110	125	158	204	293		
O. cyanosoma RH2B_1	M	C	T	F	A	G	A	V	Y	A	0		0	0	-2	8	-2	-2	0	0	474	
P. amboinensis RH2B	M	C	T	I	C	G	S	C	Y	A	0		0	0	0	8	0	0	0	0	480	480
P. moluccensis RH2B	M	C	T	I	C	G	S	C	Y	A	0		0	0	0	8	0	0	0	0	480	
O. niloticus RH2B	M	I	T	I	C	S	S	C	Y	A											472	472
M. zebra RH2B	I	I	T	I	C	S	S	C	Y	A	0	0	0	0	0	0	0	0	0	0		484
O. latipes RH2A	M	L	C	I	C	G	S	C	F	S	0	0	-10	0	0	0	0	0	-1	-8	453	452
Bovine RH1	M	M	T	L	S	G	A	V	F	A												
	44	49	94	95	98	109	124	157	203	292	44	49	94	95	98	109	124	157	203	292		

RH2A

Species	Variable site			Estimated tuning effect (nm)			Estimate (nm)	Actual lmax (nm)
	117	166	298	117	166	298		
***O. cyanosoma* RH2A_1**	A	A	I	0	0	0	518	
***P_amboinensis* RH2A**	F	I	L	0	0	0	518	
***P. moluccensis* RH2A**	F	I	L	0	0	0	518	
M. zebra RH2Aa	F	F	L	0	10	0		528
O. niloticus RH2Aa	F	F	L	0	10	0		528
M. zebra RH2Ab	F	L	I	0	0			518
O. niloticus RH2Ab	F	L	I	0	0			518
Bovine RH1	G	A	I					
	109	158	290	109	158	290		

LWS

Species	Variable Site		Tuning effect (nm)		Estimate (nm)	Actual lmax (nm)
	177	274	177	274		
***O. cyanosoma* LWS**	A	F	-7	-10	544	
***P. amboinensis* LWS**	A	Y	-7	0	554	
***P. moluccensis* LWS**	A	Y	-7	0	554	
O. niloticus LWS	S	Y	0	0	561	561
L. goodei LWS	S	Y	0	0	561	573
L. goodei LWS	S	Y				
O. latipes LWS	S	Y	0	0	561	
O. latipes LWS	S	Y	0	0	561	
Bovine RH1	A	F				
	164	261	164	261		

RH1

Species	Variable site				Estimated tuning effect (nm)			Estimate (nm)	Actual lmax (nm)
	83	168	298	299	83	298	299		
***O. cyanosoma* RH1**	N	S	S	A	-6	0	-2	494	
***P. amboinensis* RH1**	N	A	S	S	-6	0	0	496	
***P moluccensis* RH1**	N	A	S	S	-6	0	0	496	
M. zebra RH1	D	A	A	A	0	8	-2	508	
O. niloticus RH1	D	A	S	S	0	0	0	502	
L. goodei RH1	D	A	A	S	0	8	0	510	
O. latipes RH1	D	A	S	S	0	0	0		502
Bovine RH1	D	A	S	A					
	83	168	298	299	83	298	299		

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