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RESEARCH ARTICLE

High complexity of aquatic irradiance may have driven the evolution of four-dimensional colour vision in shallow-water fish

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SUMMARY

Humans use three cone photoreceptor classes for colour vision, yet many birds, reptiles and shallow-water fish are tetrachromatic and use four cone classes. Screening pigments, which narrow the spectrum of photoreceptors in birds and diurnal reptiles, render visual systems with four cone classes more efficient. To date, however, the question of tetrachromacy in shallow-water fish that, like humans, lack screening pigments, is still unsolved. We raise the possibility that tetrachromacy in fish has evolved in response to higher spectral complexity of underwater light. We compared the dimensionality of colour vision in humans and fish by examining the spectral complexity of the colour signal reflected from objects into their eyes. We show that fish require four to six cone classes to reconstruct the colour signal of aquatic objects at the accuracy level achieved by humans viewing terrestrial objects. This is because environmental light, which alters the colour signals, is more complex and contains more spectral fluctuations underwater than on land. We further show that fish cones are better suited than human cones to detect these spectral fluctuations, suggesting that the capability of fish cones to detect high-frequency fluctuations in the colour signal confers an advantage. Taken together, we propose that tetrachromacy in fish has evolved to enhance the reconstruction of complex colour signals in shallow aquatic environments. Of course, shallow-water fish might possess fewer than four cone classes; however, this would come with the inevitable loss in accuracy of signal reconstruction.

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INTRODUCTION

Colour vision discriminates variation in the spectrum of light from changes in brightness, and requires the neural circuitry to compare the signals from at least two spectrally distinct cone photoreceptor classes (Jacobs, 1981). Moreover, colour vision systems seek to discriminate between objects that differ in reflectance, regardless of the lighting conditions. This is achieved by decomposing the colour signal (spectral radiance) arriving at the eye from an object into the spectral reflectance of the object and the spectral irradiance in the environment. Considering the broad absorbance spectra of cone photoreceptors and the limitations they impose on spectral resolution, it was suggested that more than three cone classes may not add enough spectral resolution to outweigh the costs of additional cone classes (Barlow, 1982; Bowmaker, 1983; Maloney, 1986; Chiao et al., 2000b). Why, then, do many reptiles, birds and shallow-water fish use four cone classes (Goldsmith et al., 1981; Goldsmith, 1990; Hawryshyn and Hárosi, 1991; Neumeyer, 1992; Hawryshyn and Hárosi, 1994; Hawryshyn et al., 2003; Sabbah et al., 2010)? Birds and diurnal reptiles often possess coloured oil droplets in their eyes. These screening pigments narrow the spectrum of photoreceptors, and thus render visual systems with four cone classes more efficient (Goldsmith, 1990; Vorobyev, 2003). To date, however, the question of high-dimensional colour vision in shallow-water fish, which lack coloured oil droplets, is still unsolved.

The numerous available cone opsin genes in teleost fish, which have resulted from multiple opsin gene duplications (Matsumoto et al., 2006; Spady et al., 2006; Ward et al., 2008), facilitate the expression of diverse complements of two to seven cone pigments. For example, zebrafish (*Danio rerio*) display seven cone pigments (Vihtelic et al., 1999; Chinen et al., 2003); guppies (*Poecilia reticulata*) (Archer and Lythgoe, 1990) and killifish (*Lucania goodei*) (Fuller et al., 2003) display five cone pigments; salmonids (Salmonidae) (Hawryshyn and Hárosi, 1994; Temple et al., 2008) display four to six cone pigments; black bream (*Acanthopagrus butcheri*) (Shand et al., 2008), goldfish (*Carassius auratus*) (Hawryshyn and McFarland, 1987; Neumeyer, 1992) and three-spine stickleback (*Gasterosteus aculeatus*) (Rowe et al., 2004) display four cone pigments; coral reef fish display two to four cone pigments (Hawryshyn et al., 2003; Losey et al., 2003); and African cichlids typically display three cone pigments (Jordan et al., 2006; Carleton et al., 2008), although several species were shown to display up to seven pigments (Parry et al., 2005). However, the presence of a large number of cone pigments in the retina may not necessarily suggest the possession of high-dimensional colour vision. First, cone pigments and the expression of cone opsin genes were shown to be distributed differently across the retina of many fishes (Denton et al., 1971; Levine et al., 1979; Allison et al., 2003; Takechi and Kawamura, 2005; Allison et al., 2006; Temple et al., 2010). Second, multiple spectral cone classes, subserved by multiple cone pigments,

may be used for tasks other than colour vision. For example, opponent interaction of the signals of two spectral classes of photoreceptors was suggested to allow elimination of the flicker generated by surface waves from the retinal image, thereby aiding in the detection of moving prey and predators (Maximov, 2000). In such cases, the dimensionality of colour vision might be lower than it appears based solely on the number of spectral cone classes present. Nonetheless, many of the species mentioned above were demonstrated to possess four cone classes that interact with one another and serve in colour vision, suggesting tetrachromatic colour vision in these species (Hawryshyn and Hárosi, 1991; Neumeyer, 1992; Hawryshyn and Hárosi, 1994; Parkyn and Hawryshyn, 2000; Hawryshyn et al., 2003; Anderson et al., 2010; Sabbah et al., 2010).

We raised the possibility that high complexity of the colour signal of aquatic objects has contributed to the emergence of four-dimensional colour vision in shallow-water fish. Such complexity accounts for variation between colour signals as well as the variation in each colour signal across wavelengths. This complexity of the colour signal, and thus the number of photoreceptors required to reconstruct it, depends on the complexity of the environmental irradiance and the reflectance of objects. The absolute number of cone classes required for reconstruction of colour signals is almost impossible to obtain. This is mostly because it is unknown how accurately animals can reconstruct colour-signal spectra, and what proportion of spectra in the environment it is biologically necessary to reconstruct. Thus, to gain insight into the dimensionality of colour vision in fish, one needs to compare the ability of fish in reconstructing colour signals with that exhibited by another animal, ideally one for which the characteristics of the visual system are largely known. Therefore, to facilitate such a comparison, in this study, we evaluated the dimensionality of colour vision of fish in comparison to that of humans and old-world primates, by examining the complexity of the colour signals of aquatic and terrestrial objects. We found that fish require four to six cone classes to reconstruct aquatic colour signals to the degree that humans reconstruct terrestrial colour signals with three cone classes. This, we show, is partly because environmental irradiance, which alters the colour signals, is more complex underwater than on land. Moreover, we found that fish cones could detect high-frequency fluctuations across wavelengths better than human cones, and that aquatic colour signals exhibit a greater proportion of these spectral fluctuations than terrestrial colour signals, suggesting that the capability of fish cones to detect high-frequency fluctuations in the colour signal confers an advantage.

MATERIALS AND METHODS

Irradiance, reflectance and colour-signal data sets

Aquatic and celestial spectral irradiance as well as spectral reflectance of aquatic and terrestrial objects were measured or adopted from previous studies (see Appendix 1 and supplementary material Fig. S1 for irradiance and reflectance spectra used). To calculate colour signals, spectral reflectance of each terrestrial object was multiplied by every single celestial irradiance spectrum, and spectral reflectance of each aquatic object was multiplied by every single aquatic irradiance spectrum. Objects were assumed to be viewed from a short distance; thus, light attenuation through air or water was neglected. All experimental and animal care procedures were approved by Queen's University Animal Care Committee under the auspices of the Canadian Council for Animal Care.

The visual system of fish

Absorbance spectra (Govardovskii et al., 2000) for cone pigments in fish were constructed based on the seven cone pigments reported in cichlid fish (λ_{max} of A_1-reconstituted visual pigments is given in parentheses): SWS1 (368 nm), SWS2b (423 nm), SWS2a (456 nm), Rh2b (484 nm), Rh2aα (519 nm), Rh2aβ (528 nm) and LWS (560 nm) (Parry et al., 2005; Spady et al., 2006). The spectral range spanned by these pigments is common to many freshwater and marine fish families (Losey et al., 2003; Bowmaker, 2008). Absorbance spectra were generated for cone pigments with either A_1 (retinal) or A_2 (3,4-dehydroretinal) chromophores, where the λ_{max} shift associated with changes in chromophore composition was accounted for. As the spectral limits of wavelength discrimination in fish are variable and largely unknown, the visible spectrum of fish was calculated as the range enclosed between the wavelengths at which the absorbance of the shortest wavelength cone (SWS1) and the longest wavelength cone (LWS) were at 1% of maximum absorbance. The visible spectrum of fish shifted and varied in width with varying chromophore composition and lens transmission. The visible spectrum (VS) was calculated for four key combinations of chromophore and lens transmission: (i) A_1 retina and a UV-transmissive lens, VS 323–685 nm, (ii) A_1 retina and a non-UV-transmissive lens, VS 357–685 nm, (iii) A_2 retina and a UV-transmissive lens, VS 323–767 nm and (iv) A_2 retina and a non-UV-transmissive lens, VS 357–767 nm. Thus, the width of the visible spectrum of fish might range between 362 and 444 nm. To correct the absorbance spectra of cone pigments for a UV-transmissive lens, we used the lens transmission of a Lake Malawi cichlid, *Metriaclima zebra*, whose wavelength at half-maximum lens transmission (T_{50}) was 350 nm (Sabbah et al., 2010). To correct the absorbance spectra of cone pigments for a non-UV-transmissive lens (T_{50}=400 nm), we used the lens transmission of another Lake Malawi cichlid, *Melanochromis auratus* (Hofmann et al., 2010). These T_{50} values are commonly found in many freshwater and marine fishes (Siebeck and Marshall, 2007; Hofmann et al., 2010). For all analyses presented, unless specified differently, the visible spectrum and spectra of cone photoreceptors in fish were calculated while accounting for a UV-transmissive lens and photoreceptors of A_1 chromophore (case i), which are commonly found in many freshwater and marine fishes (Siebeck and Marshall, 2007; Toyama et al., 2008). The shallow-water aquatic irradiance analysed in this study attained values between 10^{10} and 10^{13} photons cm^{-2} s^{-1} nm^{-1} across the visible spectrum of fish exhibiting A_1 retina. These values are well within the photopic range of many fish (DeMarco and Powers, 1991; Hawryshyn et al., 2010; Sabbah et al., 2010), and are not expected to limit the visible spectrum or the function of any of the cones reported in fish.

The visual system of humans

Absorbance spectra (Govardovskii et al., 2000) for the S-, M- and L-cone photoreceptors in humans were constructed based on absorbance templates for the A_1 chromophore using λ_{max} values of 420, 530 and 558 nm, respectively (Dartnall et al., 1983; Stockman and Sharpe, 2000). Absorbance spectra were corrected for lens (Stockman et al., 1999) and macula (Bone et al., 1992) transmission. The visible spectrum of humans was calculated as the range enclosed between the wavelengths at which the absorbance of the S-cone and the L-cone was at 1% of maximum absorbance, and ranged between 390 and 685 nm. For consistency, construction of cone absorbance spectra and estimation of the visible spectrum were performed similarly in both fish and humans; cone absorbance spectra generated for humans were quantitatively similar to the commonly used sensitivity spectra (supplementary material Fig. S2).

Characterizing spectra as linear models

To evaluate the number of independent parameters that are required for reconstructing the irradiance, reflectance and colour-signal spectra, a principal component analysis (PCA) was employed (Maloney, 1986; Chittka et al., 1994; Chiao et al., 2000b). Numerous studies have looked at the ability of animals to distinguish between different visual stimuli (Vorobyev et al., 1998; Marshall, 2000; Kelber et al., 2003; Endler et al., 2005). This was typically achieved by calculating Euclidian distances between different visual stimuli in the colour space of the animal concerned. In this study, however, we were interested primarily in investigating the complexity of colour signals rather than in assessing the ability of fish to distinguish between different stimuli; for such a task, PCA is an optimal method.

Prior to performing PCA, all spectra were normalized to be of length 1 (by normalizing each spectrum by its norm) to avoid differential weighting of different spectra in determining the choice of principal components (Maloney, 1986). To eliminate the need for subtracting the mean value (across spectra) prior to performing PCA, analysis was performed on an augmented data set that had mean zero (Maloney, 1986). This data set was generated by appending to the set of empirical spectra $S_1(\lambda),\ldots,S_n(\lambda)$ the negations of these spectra: $-S_1(\lambda),\ldots,-S_n(\lambda)$.

Spectra were expressed as the weighted sum of d fixed principal components (PCs):

$$S(\lambda) = \sum_{i=1}^{d} \sigma_i P_i(\lambda) , \quad (1)$$

where $P_i(\lambda)$ denotes PC i at wavelength λ (nm) and σ_i denotes the weight of each PC and determines the spectrum of concern, denoted $S(\lambda)$. To fit a given empirical spectrum $S_j(\lambda)$ to a given d-dimensional linear model, we used a least-squares fitting procedure, which returned the multiple correlation coefficient (R^2) as a measure of goodness of fit.

We focused on the colour signals of objects that are crucial for the survival of fish or humans – objects that are important for visual foraging and communication. For fish vision, we analysed the colour signals of algae (macroalgae covering rocks in Lake Malawi) that many fish feed in and upon, and the colour signals of the body pattern of fish (Lake Malawi cichlid fish). For human and primate vision, we analysed the colour signals of fruits and the colour signals of skin and fur of old-world primates. The colour signals of fruit are of particular interest as it was argued that humans and primates evolved trichromatic colour vision to provide acute discrimination between fruits of different degrees of ripeness and to allow for enhanced detection of fruits against the background of leaves (Osorio and Vorobyev, 1996; Regan et al., 1998; Smith et al., 2003). Celestial irradiance, and the reflectance and colour signal of terrestrial objects were analysed while accounting for the visible spectrum of humans (390–685 nm). In contrast, aquatic irradiance, and the reflectance and colour signal of aquatic objects, were analysed while accounting for the visible spectrum of fish (323–685 nm).

Characterizing spectra as band-limited functions

To identify component frequencies in spectra, the discrete Fourier transform (DFT) was applied using the fast Fourier transform (FFT) algorithm (Matlab R2009a, The Mathworks, Natick, MA, USA). DFT was calculated for each of the cone absorbance, irradiance, reflectance and colour-signal spectra examined. Many spectra had considerable levels of energy at both ends of the light spectrum. Any value difference between the two ends of a spectrum may introduce spurious, high-frequency components into the Fourier power spectrum. A Hanning window was used to attenuate these artifacts (Maloney, 1986). In order to increase the Fourier frequency resolution to 0.2 cycles μm^{-1}, cone absorbance, irradiance, reflectance and colour-signal spectra were zero-padded (Bonnardel and Maloney, 2000) (see Appendix 2 for comparison between PCA and DFT and validation of calculations).

Statistical analysis

Band-limit values for each collection of empirical spectra did not follow normal distribution (Kolmogorov–Smirnov test) and their variance differed across collections (Leven's test). Additionally, reflectance and colour-signal spectra within each collection were not independent. Therefore, to compare the mean band limit of two collections of empirical spectra, we used a randomization test, with the difference between the means of the two collections as a test statistic. The observed test statistic was compared with the null distribution estimated from 10,000 replicates, as band limits were randomly permutated while maintaining the original sample sizes (Edgington, 1995). Non-parametric percentile-based bootstrapping (10,000 replicates) was used to estimate the 95% confidence intervals around the mean band limit for each spectra collection (Efron and Tibshirani, 1994). Statistical analyses were performed using R 2.13.0 (The R Foundation for Statistical Computing, http://www.r-project.org/).

RESULTS

Complexity derived from variation between spectra

To evaluate the number of independent parameters that are required for reconstructing each collection of empirical spectra, we computed the linear model that best fits the collection using PCA. Then, we fitted each spectrum in the collection with a linear model of 1–10 principal components (PCs) to determine the goodness of fit (R^2) for each of the spectra included in the collection (see Materials and methods and supplementary material Fig. S3). It is unknown how accurately animals reconstruct spectra. Thus, the number of PCs required for signal reconstruction was defined as the number of PCs required to exceed a variance criterion of R^2=0.99. Additionally, the proportion of spectra in a collection that it is biologically necessary to reconstruct is unknown. Therefore, we calculated the number of PCs required for reconstructing, at the above criterion, 50% (median) and 95% (5th percentile) of the spectra in a collection. Note that the visual system does not seek to reconstruct spectra – it only seeks to discriminate between objects with different reflectance. However, to facilitate comparison between the dimensionality of different spectra collections, in this study, we calculated the number of PCs that are required to reconstruct spectra at a predefined variance criterion.

The exact number of PCs required for signal reconstruction depends on the variance criterion used (the acceptable reconstruction error) and the proportion of spectra in a collection that can be reconstructed. However, it is the difference in the number of PCs between fish and humans that is important. An additional PC was required for reconstructing the aquatic irradiance (N=80) *versus* celestial irradiance (N=400) (Fig. 1A; see supplementary material Fig. S4 for the first 10 PCs for celestial and aquatic irradiance). One to two additional PCs (dependent on the proportion of spectra reaching the variance criterion) were required for reconstructing the reflectance of aquatic objects (N=351) *versus* the reflectance of terrestrial objects (N=676) (Fig. 1B). Finally, one to three additional PCs were required for reconstructing the colour signal of aquatic objects (N=28,080) *versus* the colour signal of terrestrial objects (N=270,400) (Fig. 1C).

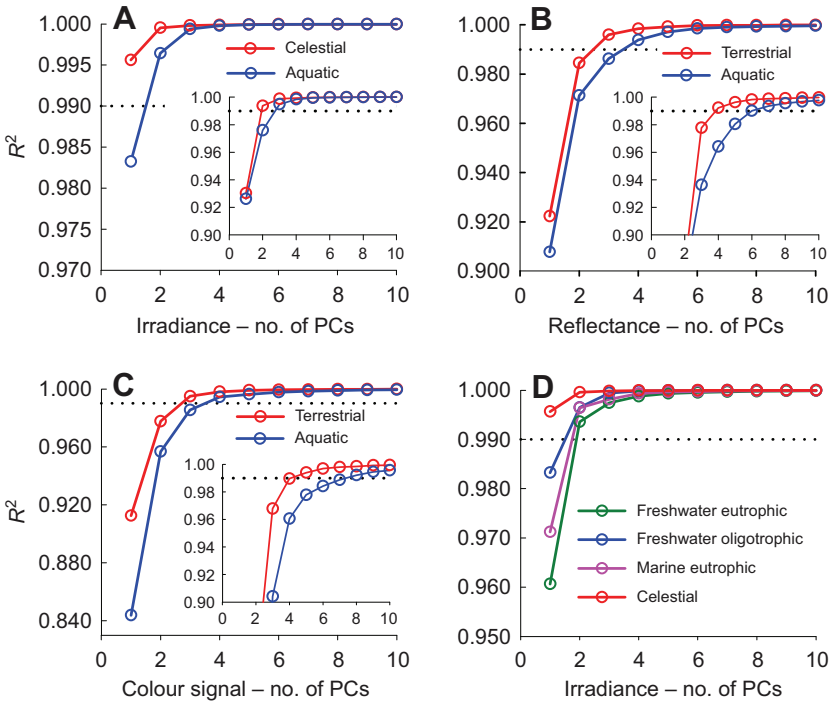

Fig. 1. Reconstruction of aquatic colour signals requires more principal components (PCs) than terrestrial colour signals. The goodness of fit, R^2, for linear models of 1–10 PCs, for irradiance, reflectance and colour-signal spectra is presented. The number of PCs required for signal reconstruction was defined as the number of PCs required to exceed a variance criterion of R^2=0.99 (horizontal dotted line). We calculated the number of PCs required for reconstructing 50% (larger plots) and 95% (insets) of the spectra in a collection. (A) Irradiance: reconstruction of 50% of spectra required one PC for celestial irradiance but two PCs for aquatic irradiance. Inset, reconstruction of 95% of spectra required two PCs for celestial irradiance but three PCs for aquatic irradiance. The number of PCs required for reconstructing the downwelling and sidewelling irradiance was similar, with two and three PCs required for reconstructing 50% and 95% of spectra in each collection, respectively (not presented). (B) Reflectance: reconstruction of 50% of spectra required three PCs for terrestrial objects but four PCs for aquatic objects. Inset, reconstruction of 95% of spectra required four PCs for terrestrial objects but six PCs for aquatic objects. (C) Colour signal: reconstruction of 50% of spectra required three PCs for terrestrial objects but four PCs for aquatic objects. Inset, reconstruction of 95% of spectra required five PCs for terrestrial objects but eight PCs for aquatic objects. (D) Aquatic irradiance for diverse water types: reconstruction of 50% of spectra required two PCs for freshwater eutrophic, freshwater oligotrophic and marine eutrophic aquatic irradiance.

To test whether the difference in the number of PCs between fish and humans depends on the visible spectrum used, aquatic irradiance and the reflectance and colour signal of aquatic objects were re-analysed while accounting for the visible spectrum of humans. The difference in the number of PCs required for reconstructing the aquatic irradiance *versus* the celestial irradiance did not change (Table 1A). However, the difference in the number of PCs required for reconstructing the reflectance and colour signal of aquatic objects *versus* terrestrial objects decreased to zero to one PC for reflectance (Table 1B) and to one to two PCs for the colour signal (Table 1C). Thus, the greater number of PCs required for reconstructing aquatic colour signals arose partly from extending the visible spectrum into the UV, but also from characteristic differences between aquatic and celestial irradiance.

The aquatic irradiance data analysed above were collected from Lake Malawi, a clear, oligotrophic lake. To test whether the reported findings hold for diverse water types, the analysis was repeated on irradiance taken from diverse ecosystems (Fig. 1D). An additional PC was required for reconstructing the aquatic irradiance in comparison to celestial irradiance, regardless of whether aquatic irradiance was collected from freshwater eutrophic (N=240), freshwater oligotrophic (N=80) or marine eutrophic (N=131) systems. Thus, the larger number of PCs required for reconstructing the colour signal of aquatic objects (higher complexity) is probably a characteristic of the aquatic environment.

Complexity derived from variation in spectra across wavelengths

Knowledge of the complexity of the colour signal arriving at the eye is indispensable; however, it is important to consider what are the constraints of the visual system that determine how the colour signal is being sampled. In fact, the broad spectra of cone photoreceptors are band-limited functions that pass little energy at frequencies beyond a given band limit or frequency (Barlow, 1982). That is, the photoreceptors cannot sample high-frequency fluctuations between wavelengths; they smooth the signal and, therefore, lose information. To study the frequency characteristics of cone photoreceptors in fish and humans, cone absorbance spectra were decomposed into their discrete Fourier components, each having a frequency expressed as cycles per wavelength [this frequency refers to the abscissa of the Fourier transform of the spectrum rather than to the frequency of light, sometimes called the comb frequency (Barlow, 1982)] (Fig. 2A–D). The band limit of a spectrum was defined as the frequency above which only 1% of the cumulative energy was found.

The band limit of cone photoreceptors decreased when moving from short (narrow bandwidth) to long (broader bandwidth) wavelength-sensitive cones. The band limits of fish cones spanned a wider range of frequencies, and the band limits of the three short-wavelength cones in fish were higher than those in humans (fish 7.23–16.02 cycles μm^{-1}, humans 8.39–14.45 cycles μm^{-1}) (Fig. 2E,F; supplementary material Table S1). Therefore, these fish cones

Table 1. R^2 coefficients for linear models of 1–10 principal components (PCs), for irradiance, reflectance and colour-signal spectra

A. Irradiance

	Median			5th percentile		
PC	Celestial (human VS)	Aquatic (fish VS)	Aquatic (human VS)	Celestial (human VS)	Aquatic (fish VS)	Aquatic (human VS)
1	**0.9956**	0.9833	0.9835	0.9304	0.9262	0.9268
2	0.9996	**0.9965**	**0.9966**	**0.9938**	0.9759	0.9761
3	0.9998	0.9994	0.9995	0.9988	**0.9948**	**0.9954**
4	0.9999	0.9998	0.9999	0.9994	0.9984	0.9991
5	0.9999	0.9999	0.9999	0.9998	0.9995	0.9996
6	1.0000	0.9999	1.0000	0.9999	0.9997	0.9998
7	1.0000	1.0000	1.0000	0.9999	0.9999	0.9999
8	1.0000	1.0000	1.0000	1.0000	0.9999	1.0000
9	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

B. Reflectance of objects

	Median			5th percentile		
PC	Terrestrial (human VS)	Aquatic (fish VS)	Aquatic (human VS)	Terrestrial (human VS)	Aquatic (fish VS)	Aquatic (human VS)
1	0.9223	0.9078	0.9312	0.7120	0.7241	0.7483
2	0.9845	0.9713	0.9855	0.8773	0.8686	0.9244
3	**0.9959**	0.9862	**0.9955**	0.9778	0.9363	0.9585
4	0.9984	**0.9939**	0.9985	**0.9922**	0.9642	0.9862
5	0.9992	0.9971	0.9992	0.9962	0.9804	**0.9942**
6	0.9997	0.9986	0.9995	0.9982	**0.9901**	0.9947
7	0.9998	0.9990	0.9997	0.9988	0.9935	0.9974
8	0.9999	0.9993	0.9998	0.9992	0.9955	0.9984
9	0.9999	0.9995	0.9998	0.9995	0.9968	0.9985
10	1.0000	0.9996	0.9999	0.9997	0.9977	0.9990

C. Colour signal of objects

	Median			5th percentile		
PC	Terrestrial (human VS)	Aquatic (fish VS)	Aquatic (human VS)	Terrestrial (human VS)	Aquatic (fish VS)	Aquatic (human VS)
1	0.9126	0.8438	0.8457	0.6372	0.4472	0.4536
2	0.9776	0.9568	0.9587	0.8487	0.6987	0.7085
3	**0.9948**	0.9855	0.9865	0.9678	0.9040	0.9088
4	0.9979	**0.9943**	**0.9952**	0.9896	0.9606	0.9635
5	0.9990	0.9963	0.9970	**0.9940**	0.9777	0.9818
6	0.9995	0.9976	0.9982	0.9969	0.9841	0.9871
7	0.9996	0.9983	0.9989	0.9979	0.9886	**0.9922**
8	0.9997	0.9990	0.9991	0.9985	**0.9921**	0.9943
9	0.9998	0.9992	0.9994	0.9991	0.9944	0.9958
10	0.9999	0.9994	0.9995	0.9993	0.9956	0.9969

The median R^2 indicates the value above which the R^2 values corresponding to 50% of spectra in a collection were formed. The 5th percentile of R^2 indicates the value above which the R^2 values corresponding to 95% of spectra in a collection were formed. The median and 5th percentile of the R^2 coefficient are given for (A) celestial (N=400) and aquatic (N=80) irradiance, (B) reflectance of terrestrial (N=676) and aquatic (N=351) objects, and (C) colour signal of terrestrial (N=270,400) and aquatic (N=28,080) objects. For aquatic irradiance, and the reflectance and colour signal of aquatic objects, spectra were fitted to linear models while accounting for the visible spectrum of fish (fish VS), and again while accounting for the visible spectrum of humans (human VS). R^2 values just exceeding the R^2=0.99 criterion are marked in bold.

(compared with all human cones) could better sample high-frequency fluctuations between wavelengths, and lost less information from the incident signal. This result is linked directly to the observation that fish typically possess a larger number of short-wavelength-sensitive cones, which can, in contrast to human cones, extend into the UV spectral range. The Nyquist sampling theorem determines the number of samples (e.g. cone photoreceptors) required to reconstruct a signal up to a certain frequency. The relationship between the band limit of a signal f (cycles μm^{-1}) and the number of significant independent samples n required to reconstruct the signal is: $n=2Wf$ where W stands for the width of the visible spectrum (μm) (Slepian and Pollak, 1961). Thus, the band limit of photoreceptors is related directly to the minimal number of photoreceptors required for reconstructing the signal up to the band limit. The number of samples required to reconstruct the band-limited colour signal that is passed by cones in fish (5.23–11.60) was higher by up to three samples (dependent on cone class) than in humans (4.96–8.53); the results for humans are consistent with past studies (Barlow, 1982; Maloney, 1986). Thus, fish would require four to six cone classes (an additional one to three cone classes in comparison to humans) to reconstruct the band-limited colour signal sampled by cones.

As shown above, given the filter properties of their photoreceptors, fish could recover higher frequencies from the

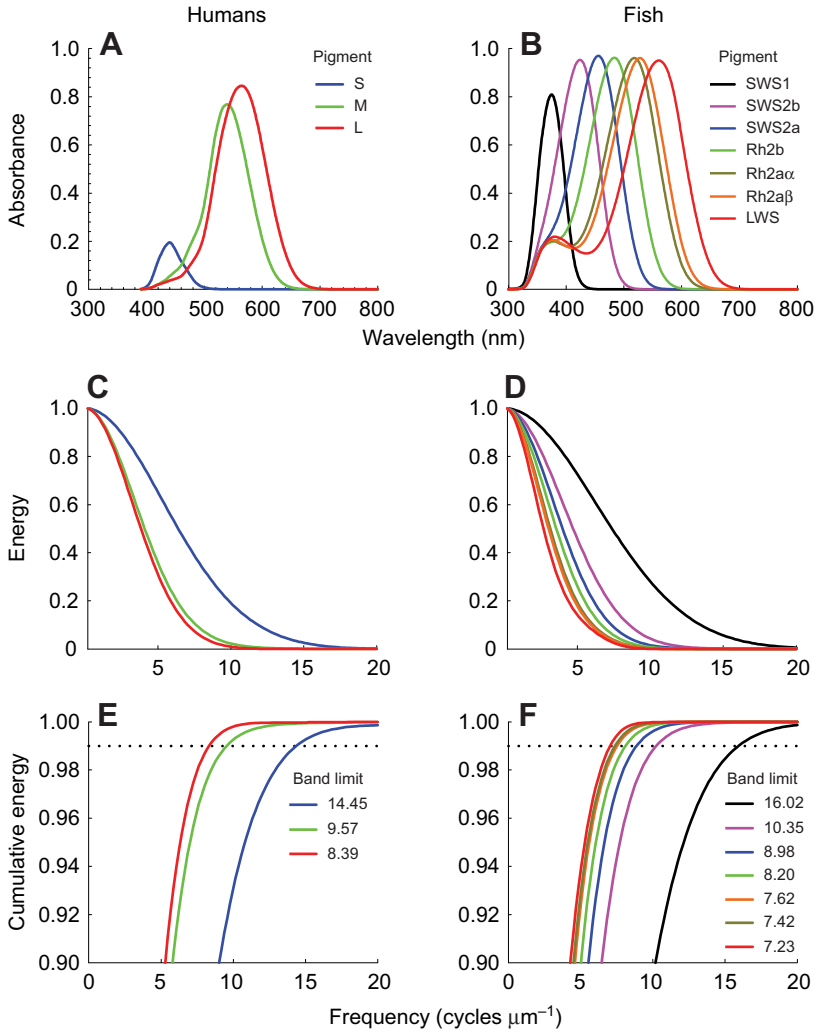

Fig. 2. Compared with human cones, fish cones recover higher frequencies from the colour signal received. (A,B) Absorbance spectra of cone photoreceptors in humans and fish. (C,D) Absorbance spectra of cone photoreceptors expressed as band-limited functions. The discrete Fourier transform (DFT) was calculated while accounting for the visible spectrum of humans and fish. (E,F) Cumulative energy of absorbance increased quickly with frequency. Band limits of the S-, M- and L-cone photoreceptors in humans equalled 14.45, 9.57 and 8.39 cycles μm^{-1}, respectively. Band limits of the SWS1 to LWS cone photoreceptors in fish ranged from 16.02 to 7.23 cycles μm^{-1}. The band limit of spectra was defined as the frequency above which only 1% of the cumulative energy was found (dotted line).

signal received (higher band limits). However, this would be advantageous only if the band limit of aquatic colour signals was equal to or higher than those of fish cones. To investigate whether this is the case, irradiance, reflectance and colour-signal spectra were expressed as band-limited functions (supplementary material Fig. S5) and their cumulative energy as a function of frequency was calculated (Fig. 3). The band limit of aquatic colour signals was significantly higher than that of terrestrial colour signals (Fig. 4A,B, left axes), indicating a correspondence between the band limits of cones and those of the colour signals. That is, the enhanced ability of fish cones to recover high-frequency fluctuations coincided with the higher proportion of high-frequency fluctuations in the colour signal of aquatic objects. Interestingly, the greater proportion of high frequencies in aquatic colour signals arose from a greater proportion of high frequencies in the aquatic irradiance (Fig. 4C) rather than in the reflectance of aquatic objects (Fig. 4D). Additionally, apart from the short-wavelength photoreceptors (S-cone in humans and SWS1 in fish), which adequately sampled the colour signal of most aquatic objects (for fish) and terrestrial objects (for humans), photoreceptors undersampled the colour signal of approximately half the relevant objects (Fig. 4A,B; left axes). That is, the band limits of photoreceptors were lower than those of the colour signals; the colour signals contain frequencies that are higher than those that can be recovered by photoreceptors, for both fish and humans. In conclusion, the capability of fish cone photoreceptors to recover high frequencies from the colour signal confers an advantage and allows enhanced reconstruction of the colour signal.

DISCUSSION

Complexity of aquatic and celestial irradiance

We found that the spectrum of aquatic irradiance in diverse freshwater and marine ecosystems is more complex than the spectrum of celestial irradiance. The greater complexity of aquatic irradiance spectra was derived from greater variation between irradiance spectra, as determined using PCA (Fig. 1D), as well as from greater variation in individual irradiance spectra across wavelengths as determined using DFT, for a freshwater oligotrophic (Lake Malawi) system (Fig. 4C), and for freshwater eutrophic [randomization test (RT), $P<0.001$, $CI_{freshwater}=9.036–9.918$, $CI_{celestial}=5.001–5.091$, $N_{freshwater}=240$, $N_{celestial}=400$] and marine eutrophic (RT, $P<0.001$, $CI_{marine}=9.192–10.055$, $CI_{celestial}=5.001–5.091$, $N_{marine}=131$, $N_{celestial}=400$) systems. The greater variation between aquatic irradiance spectra is linked directly to the stronger light attenuation in water, which results in strong dependence of the irradiance spectrum on the viewing orientation and water depth. Thus, aquatic irradiance, which is incident on objects that only slightly differ in depth or in the orientation in which they are being viewed, would be substantially different, leading to the greater complexity of aquatic irradiance. In fact, the complexity of aquatic irradiance across narrow

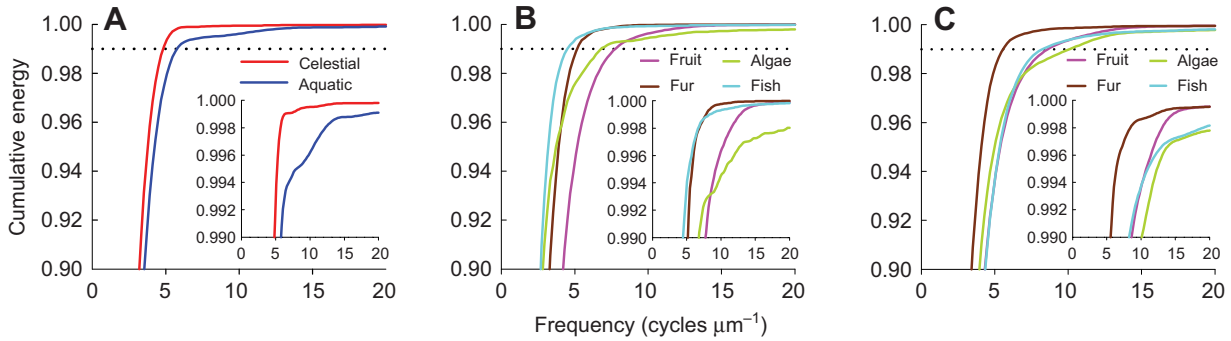

Fig. 3. Cumulative energy of irradiance (A), reflectance (B) and colour-signal spectra (C) increases quickly with frequency. To facilitate evaluation of the differences in cumulative energy between different spectra collections, insets show the same data as in the main plots but with the cumulative energy (*y*) axis magnified between 0.99 (the band-limit criterion) and 1.00.

depth ranges and at a single orientation was comparable to that of celestial irradiance. Specifically, the number of PCs required for reconstructing the celestial irradiance was similar to that of downwelling aquatic irradiance encountered across the 3–6 m depth range as well as across the 12–15 m depth range in Lake Malawi, with one and two PCs required for reconstructing 50% and 95% of spectra in each of the collections, respectively. This further illustrates the strong effect of the attenuation in water on the variation between aquatic irradiance spectra. In contrast, the greater variation in individual aquatic irradiance spectra across wavelengths might be associated with the fact that aquatic irradiance represents celestial irradiance after being refracted at the water surface and transmitted through water. Thus, any dissolved or particulate matter in the water that specifically attenuates discrete spectral ranges would render the spectrum of aquatic irradiance less 'smooth', or having greater energy at high frequencies. This may include, for example, attenuation by algae, bacteria, organic debris and minerals (Huovinen et al., 2003; Belzile et al., 2004; Morel et al., 2007). Additionally, the spectrum of aquatic irradiance can narrow considerably with depth, but still exhibit considerable energy at relatively high frequencies, leading to high complexity. Taken together, the greater complexity of aquatic irradiance arises from the stronger spectrally specific attenuation in water and the strong dependence of aquatic irradiance on depth and orientation.

Note that the celestial irradiance data set analysed in this study combined spectra from four different sources, each using different measuring equipment and protocols. Additionally, celestial irradiance was measured under a wide range of environmental and meteorological conditions, e.g. from sunrise to sunset, under variable cloud cover, fog and haze conditions, and in open fields and under vegetation. In contrast, each of the aquatic irradiance data sets was measured in a single study, in a small number of nearby sites, typically around noon, and always under clear sky. These differences in data collection procedures may suggest that the complexity differences between aquatic and celestial irradiance are even larger than estimated by our analysis, further supporting our conclusion that the spectrum of aquatic irradiance is more complex than that of celestial irradiance.

Complexity of the reflectance of aquatic and terrestrial objects

We found that the complexity of the spectral reflectance of aquatic and terrestrial objects is largely comparable, and shows a slight dependence on the spectral range examined. Using PCA, one to two additional PCs were required for reconstructing the reflectance of aquatic objects compared with the reflectance of terrestrial objects. However, this difference in the number of PCs decreased to zero to one PC when re-analysing the reflectance of aquatic objects while accounting for the visible spectrum of humans. Additionally, using DFT, the band limit of the reflectance of primate fur and skin was significantly higher than that of the body pattern of fish. However, the band limit of the reflectance of fruit and algae did not differ significantly. Thus, in general, the complexity of the reflectance of aquatic and terrestrial objects was comparable. This suggests that the greater complexity observed in the colour signal of aquatic objects, compared with terrestrial objects, probably arose from the greater complexity of aquatic irradiance.

Interestingly, the complexity of the reflectance of integumentary tissues (i.e. fish body pattern and primate fur and skin) was lower than that of fruit and algae. This difference in complexity might have arisen from differences in the mechanism of colour production between the groups. The reflectance of fruit and algae is largely determined by the pigments they contain (Gross, 1987). In contrast, the reflectance of integumentary tissues typically represents structural colours or a combination of pigments and structural colours (Fox, 1979). For example, most green colours in fish, reptiles, amphibians and birds are created by reflection of blue light coming through an over-layer of carotenoid pigments. Moreover, in many primates and other mammals, collagen arrays that constitute quasi-ordered photonic crystals are formed above arrays of microscopic holes. These tissues reflect a specific light wavelength evenly at different angles (Prum and Torres, 2004), where melanin pigments, which underlie the microscopic colour-producing structures, are crucial for the effective presentation of the structural colours (Fox, 1979; Prum and Torres, 2004). Therefore, it is possible that reflectance representing a combination of structural and pigmented colours (as in integumentary tissues) would be smoother and show less variation between wavelengths compared with reflectance produced mostly by pigments (as in fruit and algae). That is, the effect of light interference by microscopic structures may act to mask the absorbance lines of pigments, and thus also the high-frequency fluctuations between wavelengths. However, to study this phenomenon further, careful examination of the complexity of light reflected from tissues exhibiting different combinations of pigmented and structural colours should be undertaken.

Complexity of the colour signal of aquatic and terrestrial objects

Our results indicate that the complexity of aquatic colour signals is higher than that of terrestrial colour signals. Using PCA, we

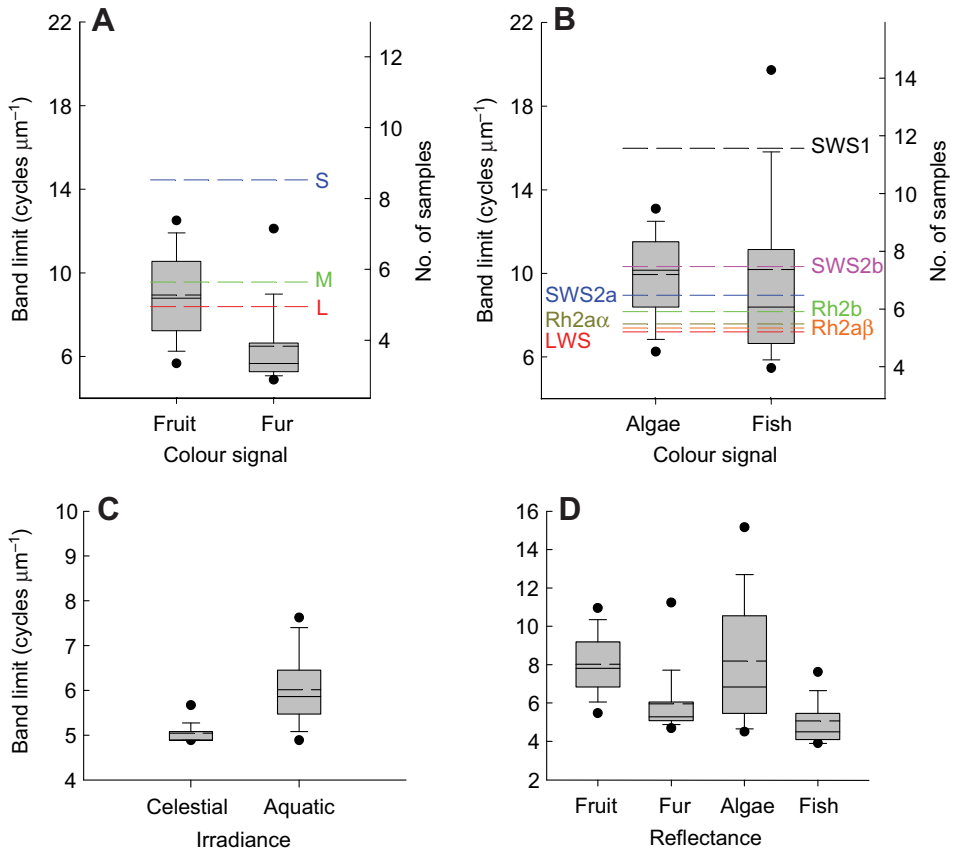

Fig. 4. Aquatic colour signals contain more high-frequency energy and require more samples to reconstruct than terrestrial colour signals. (A,B) Left axes show that the band limit of the colour signal of aquatic objects was significantly higher than that of terrestrial objects [fish–fur, randomization test (RT), P<0.001, confidence interval 2.5–97.5%, CI_{fish}=9.518–10.968, CI_{fur}=6.236–6.762, N_{fish}=24,320, N_{fur}=77,600; algae–fruit, RT, P<0.001, CI_{algae}=9.405–10.483, CI_{fruit}=8.768–9.146, N_{algae}=3760, N_{fruit}=192,800]. Photoreceptors undersampled the colour signal of objects. Humans: band limit of M- and L-cones exceeded that of the colour signal of less than 75% and 50% of fruit tissues, respectively. Fish: band limits of SWS2a to LWS exceeded that of the colour signal of less than ~50% of algae and fish tissues. Right axes show the number of samples required to reconstruct the colour signal of aquatic objects was larger than that of terrestrial objects. (C) The band limit of aquatic irradiance was significantly higher than that of celestial irradiance (RT, P<0.001, $CI_{aquatic}$=5.833–6.187, $CI_{celestial}$=5.001–5.091, $N_{aquatic}$=80, $N_{celestial}$=400). Band limits of downwelling and sidewelling irradiance did not differ significantly (RT, P=0.067, $CI_{downwelling}$=5.556–6.104, $CI_{sidewelling}$=5.939–6.401, $N_{downwelling}$=36, $N_{sidewelling}$=44) (not presented). (D) The band limits of reflectance of fruit and algae were significantly higher than those of primate fur and fish pattern (fruit–fur: RT, P<0.001, CI_{fruit}=7.885–8.178, CI_{fur}=5.711–6.236, N_{fruit}=482, N_{fur}=194; algae–fish pattern: RT, P<0.001, CI_{algae}=7.239–9.196, CI_{fish}=4.892–5.265, N_{algae}=47, N_{fish}=304); thus, the reflectance of integumentary tissues is constrained differently from that of plant and algae tissues. The band limit of reflectance of terrestrial objects was significantly higher (RT, P<0.001, CI_{fur}=5.703–6.239, CI_{fish}=4.891–5.266, N_{fur}=194, N_{fish}=304) than, or did not differ from (RT, P=0.574, CI_{fruit}=7.881–8.174, CI_{algae}=7.256–9.171, N_{fruit}=482, N_{algae}=47), that of aquatic objects; thus, the greater proportion of high frequencies in the colour signal of aquatic objects arises from the greater proportion of high frequencies in the aquatic irradiance. In A–D, the visible spectrum of fish (humans) was taken into account for the analysis of aquatic (celestial) irradiance, and the reflectance and colour signal of aquatic (terrestrial) objects. Re-analysis of aquatic irradiance, reflectance and colour-signal data with the visible spectrum of humans yielded similar results (not presented). Box: mean (dashed line), median (solid line), 25th and 75th percentiles; whiskers: 10th and 90th percentiles; points: 5th and 95th percentiles.

examined the complexity derived from variation between colour-signal spectra. We found that one to three additional PCs were required for reconstructing aquatic colour signals *versus* terrestrial colour signals. While the various PCs are orthogonal, the absorbance spectra of cone photoreceptors are not. Therefore, the number of cone photoreceptors required to reconstruct any given spectrum will be, by definition, equal to or larger than the number of PCs required to reconstruct the spectrum. Consequently, fish would require at least four to six cone classes to reconstruct aquatic colour signals to the degree that humans reconstruct terrestrial colour signals with three cone classes. Using DFT, we examined the complexity derived from variation between wavelengths across individual spectra. Aquatic colour signals included greater energy at high frequencies (higher band limits) than terrestrial colour signals. Moreover, following the Nyquist sampling theorem, we found that the number of samples required to reconstruct the band-limited colour signal that is passed by cones in fish was higher by up to three samples than in humans. Thus, also based on this approach, fish would require four to six cone classes (additional one to three cone classes in comparison to humans) to reconstruct the band-limited colour signal sampled by cones. Furthermore, we found that the shortest wavelength photoreceptors in humans and fish adequately sampled most aquatic (for fish) and terrestrial (for humans) colour signals. However, the rest of the photoreceptors undersampled approximately half of the relevant colour signals, i.e. the band limits of photoreceptors were lower than those of colour signals. This suggests that colour signals contain high-frequency fluctuations that cannot be recovered by the photoreceptors. Thus, the capability of fish cones to sample high-frequency fluctuations between wavelengths from the colour

signal might indicate an adaptation that allows enhanced reconstruction of the colour signal.

The greater number of PCs required for reconstructing aquatic colour signals as well as the greater energy of spectral fluctuations in aquatic colour signals arose partly from extending the visible spectrum into the ultraviolet, but also from characteristic differences between aquatic and celestial irradiance. This suggests that the high complexity of colour signals, and the large number of cone classes required for colour signal reconstruction, is a characteristic of the aquatic environment.

Chiao and colleagues addressed the complexity differences between the colour signals of aquatic and terrestrial objects (Chiao et al., 2000b). These authors collected colour signal (radiance) spectra of natural scenes using a multispectral imager, but referred to the data collected as reflectance rather than radiance (the authors discussed the considerable inaccuracies that might be produced by such an approximation, especially under water). Thus, to allow a meaningful comparison, we compared our colour signal results with those of Chiao and colleagues (referred to as reflectance in the original study). Linear models with four PCs were required to reconstruct (exceed the R^2=0.99 variance criterion) colour signals in forests, whereas linear models with at least five PCs were required to reconstruct aquatic colour signals. These results support our finding of the higher complexity of aquatic colour signals. Moreover, as (i) the previously reported colour signals were extracted from a small number of multispectral images (10 for forests and seven for underwater scenes) and (ii) the underwater images were only acquired at shallow depths (3–5 m), these colour signals may misrepresent the variation in the illuminant and underestimate the complexity of colour signals, especially under water.

Dimensionality of colour vision in fish

Four cone classes are found in the most basal vertebrate lineage, the lamprey (Hyperoartia) (Collin et al., 2003). All four cone opsin classes were retained by many bony fishes (Osteichthyes) that include both ray-finned fish (Actinopterygii) and lobe-finned fish (Sarcopterygii). However, opsin gene duplications that occurred early in the evolution of ray-finned fish (230 million years ago or later, after their divergence from the lobe-finned fish) had increased the number of cone opsin genes (Parry et al., 2005; Matsumoto et al., 2006; Spady et al., 2006; Shand et al., 2008; Ward et al., 2008). Thus, lobe-finned fish (represented today by the lungfishes and coelacanths), that later gave rise to the first tetrapods and to all terrestrial vertebrates (Brinkmann et al., 2004; Takezaki et al., 2004), have fewer cone opsin classes than do teleost fish, an infraclass of ray-finned fish. This suggests that the four to six cone classes, which, we found, are required for fish to reconstruct aquatic colour signals at an accuracy level comparable to that at which humans reconstruct terrestrial colour signals, are probably available in teleost fish.

Importantly, fish might possess a smaller number of cone classes but with the inevitable loss in accuracy of signal reconstruction. This idea is best illustrated by inspecting the amount of variance accounted for by linear models with 1–10 PCs (Table 1). For example, when attempting to reconstruct 95% of the colour signals in a collection with linear models that include the first two PCs, fish and humans would be able to recover only 70% and 84.9% of the variance between colour signals, respectively, whereas, when attempting to reconstruct the colour signals with linear models that include the first three PCs, fish and humans would be able to recover 90% and 96.8% of the variance between colour signals, respectively. Obviously, fish with three cone classes would do better than those with two, but humans would still be able to reconstruct the colour signals better. Many coral reef fish were suggested to display two cone classes (Losey et al., 2003) while many African cichlids were suggested to typically display three cone classes (Jordan et al., 2006; Carleton et al., 2008). Our results indicate that using two or three cone classes in these fish would lead to a reduced accuracy of colour-signal reconstruction compared with that of humans. However, definitive answers regarding the accuracy of signal reconstruction may only be derived from carefully designed behavioural studies.

Additionally, in this study, we have focused on the very first stage of colour vision, i.e. the sampling of colour signals by cone photoreceptors. This sampling stage is highly critical, as any information that is not being sampled by the photoreceptors is permanently lost, and cannot be used for later retinal processing (no matter whether it is retinal or cortical). Thus, the role of high-order processing in object discrimination under variable illumination conditions has not been addressed in the current study. Moreover, the variation between colour signals in the immediate surroundings of the animal and the visual tasks at hand may also shape the number of cone classes. For example, a hypothetical animal needs to discriminate between objects only at a specific depth, line of sight and time of day (small variation in incident irradiance). This animal would require a relatively small number of cone classes to allow discrimination between the available objects, without any need to account for changes in incident irradiance. However, greater variation in the incident irradiance would favour a visual system with a larger number of cone classes to allow accurate recovery of object reflectance and discrimination between the available objects.

Furthermore, the requirement for four to six cone classes for the reconstruction of aquatic colour signals holds only for shallow water that is characterized by irradiance of high levels and a broad spectrum. However, the gradual decrease in irradiance and narrowing of the irradiance spectrum with increasing water depth would favour colour vision systems with a gradually decreasing number of cone classes. Eventually, the dim and almost monochromatic irradiance encountered in deep water would favour one photoreceptor (either cone or rod) maximally sensitive about the wavelength of maximum transmission of irradiance. This prediction is supported by previous studies, as shallow-water fish were shown to possess a greater number of cone classes than deep-water fish (Loew and Lythgoe, 1978; Lythgoe and Partridge, 1989). The spectral location of cone pigments was also reported to vary between shallow- and deep-water fish, with the latter having cone pigments shifted toward short wavelengths, closer to the wavelength of maximum irradiance transmission (Yokoyama et al., 1999). Our prediction is also supported by the differences in the number of cone classes between aquatic mammal groups. Whales and dolphins (Cetacea) as well as seals, sea-lions and walruses (Pinnipedia), which frequently forage in deep water, show monochromatic visual systems (Peichl and Moutairou, 1998; Newman and Robinson, 2005). However, manatees (Sirenia), which live in shallow coastal lagoons, show dichromatic visual systems (Griebel and Schmid, 1996; Newman and Robinson, 2005).

Lastly, it is worth mentioning the mantis shrimp (Stomatopoda, Crustacea), which represents an extreme case of high dimensionality colour vision. Stomatopods have 16 classes of photoreceptors, with at least eight of these classes involved in colour vision through multiple dichromatic comparisons (Cronin and Marshall, 1989a; Cronin and Marshall, 1989b; Marshall et al., 1996; Chiao et al., 2000a). Stomatopods have intrarhabdomal filters, which narrow the spectral absorbance of photoreceptors (Marshall, 1988; Cronin et al., 1994). This special configuration enhances the ability of

photoreceptors to recover high-frequency energy from the arriving colour signal (higher band limit), and necessitates the use of a large number of photoreceptor classes to allow colour-signal reconstruction (Osorio et al., 1997). We suggest that the requirement for a large number of photoreceptor classes for signal reconstruction together with the relatively high complexity of aquatic irradiance may render the eight-channel colour vision system in stomatopods cost effective, representing an adaptation that may allow exquisite object discrimination capabilities.

The current study significantly advances our understanding of the evolution of four-dimensional colour-vision systems in fish. The number of cones required for reconstructing aquatic colour signals was larger than that of terrestrial colour signals, regardless of whether the unfiltered or the band-limited (passed by the cones) colour signal was considered. Therefore, our results, based on two independent approaches, show that fish would require four to six cone classes for reconstructing the colour signal of aquatic objects at the accuracy level achieved by humans viewing terrestrial objects. The need for a larger number of cone classes for colour-signal reconstruction was an attribute of the aquatic environment, and was true for diverse marine and freshwater ecosystems. This may suggest that the large diversity of cone opsin genes and four-dimensional colour vision in fish are of adaptive significance, and have probably evolved to enhance the reconstruction of the complex colour signals in aquatic environments.

APPENDIX 1

Description of irradiance and surface reflectance data sets

Aquatic spectral irradiance

Aquatic irradiance (N=80) was measured in July 2008 at three near-shore sites in Lake Malawi, near Cape Maclear on the northwestern part of the Nankumba Peninsula, Malawi (Otter Point – 14°02′21.02″S, 34°49′20.33″E; Mawlamba – 14°01′05.92″S, 34°48′59.91″E; and Mitande – 14°00′58.02″S, 34°48′33.29″E) (Sabbah et al., 2011). Lake Malawi is a clear, nutrient-low (e.g. 1–2 μg l^{-1} chlorophyll), oligotrophic lake (Guildford et al., 2007). To represent the irradiance encountered at different positions in a given habitat, spectral irradiance was measured at two orientations (downwelling and sidewelling), between 1 and 15 m depth, a depth range that Lake Malawi cichlids typically occupy (Ribbink et al., 1983; Konings, 2001). Accurate irradiance measurements closer to the wavy air–water interface could not be obtained. Irradiance was measured at 1 nm intervals using a thermoelectrically cooled spectroradiometer [effective spectral resolution 1.9 nm full width at half-maximum (FWHM) for 200–950 nm; QE65000, Ocean Optics, Dunedin, FL, USA] connected to a 30 m optical fibre (ZPK600-30-UV/VIS, Ocean Optics) that was fitted with a cosine corrector (CC-3-UV, Ocean Optics). Sampling time exceeded 2 s to average irradiance fluctuations induced by surface waves. The spectroradiometer setup was calibrated for absolute irradiance using a calibrated halogen–deuterium dual light source (200–1000 nm, DH-2000-CAL, Ocean Optics). All measurements were made at approximately noon under clear blue sky.

Two spectral irradiance data sets for eutrophic freshwater and marine systems were adopted. The first data set (N=131) included irradiance measurements taken at marine systems off the coast of the North Pacific during July–August 1991 (Saanich inlet – 48°37′N, 123°29′W and Trevor Channel – 48°50′N, 125°10′W) (Novales Flamarique and Hawryshyn, 1993). These sampling sites represent high-nutrient (e.g. 2–19 μg l^{-1} chlorophyll, depth dependent), eutrophic marine systems. The second data set (N=240) included irradiance measurements taken at Lake Cowichan, Vancouver Island, BC, Canada (48°49′N, 124°03′W) (Novales Flamarique et al., 1992). This sampling site represents a high-nutrient (e.g. 1–13 μg l^{-1} chlorophyll, depth dependent), eutrophic freshwater system. For both data sets, downwelling and sidewelling irradiance were measured at 4 nm intervals between 300 and 850 nm and down to a depth of 15 m.

Celestial spectral irradiance

Celestial (zenith) irradiance (N=18) was measured just above the water surface at each of the sampling sites in Lake Malawi using the same spectroradiometer configuration described above. Additionally, three data sets of celestial irradiance spectra were adopted. The first data set (N=66) was obtained at the Nouragues field station in French Guiana (4°05′N, 52°40′W) (Regan et al., 1998; Regan et al., 2001). The second data set (N=64) was collected at the Makerere University Biological Field Station (MUBFS) in Kibale forest, Western Uganda (Sumner and Mollon, 2000). Irradiance spectra in these two data sets were measured at 4 nm intervals from 380 to 780 nm and under diverse meteorological conditions, and were obtained from the Cambridge database of natural spectra (http://vision.psychol.cam.ac.uk/spectra/spectra.html). The third data set (N=252) was collected in Granada, Spain (37°118′N, 3°358′W) under a full range of atmospheric conditions (Hernández-Andrés et al., 1998). Irradiance spectra in this data set were measured from sunrise to sunset at 1 h intervals, at 1 nm intervals from 300 to 1100 nm, and were obtained from http://www.cns.nyu.edu/pub/ltm/Illuminants/Granada.

Choice of spectral irradiance data and biological relevance

To reliably represent the irradiance incident on terrestrial objects, zenith celestial irradiance was analysed (sidewelling celestial irradiance is typically lacking in the literature, and challenging to measure because of the inclusion of obstacles in the light path). However, light attenuation is considerably greater in water than in air (Lythgoe, 1979), rendering the effect of the path length over which light is transmitted through the medium considerably larger in the aquatic than in the terrestrial environment. Therefore, to reliably represent the irradiance incident on aquatic objects, downwelling and sidewelling aquatic irradiance that was measured at different depths was analysed.

Spectral reflectance of algae

Spectral reflectance of algae (N=47) was measured at three near-shore sites in Lake Malawi (see the ‘Aquatic spectral irradiance’ section for description of the study sites). Algae reflectance was measured using a custom-built probe that included a diving flashlight (mini Q40, Underwater Kinetics, Poway, CA, USA) and a fibre-coupled spectroradiometer (Jaz, Ocean Optics). The tip of the flashlight was fitted with an adaptor that held the optical fibre (QP600-2-UV-VIS, Ocean Optics) oriented at an angle of 45 deg to the examined surface. The far side of the adaptor included a ring of black foam that sealed the reflectance probe against the surface examined. A SCUBA diver held the reflectance probe against underwater substrates covered with algae while readings were acquired and saved on a laptop computer in a boat. The irradiance spectrum of the flashlight allowed reliable reflectance measurements between 370 and 800 nm, and the spectroradiometer configuration resulted in an effective spectral resolution of 2.06 nm (FWHM) across this range. A measurement of a Spectralon diffuse reflectance standard (WS-1-SL, Ocean Optics) was taken as 100% reflectance, and a dark measurement was taken as zero reflectance.

Spectral reflectance of the body pattern of fish

Spectral reflectance of the body pattern of fish (N=304) was measured at 1 nm intervals using a spectroradiometer (effective spectral resolution 2.06 nm FWHM for 200–950 nm; USB2000, Ocean Optics) connected to one arm of a 2 m bifurcated optical fibre (BIF600-2-UV/VIS, Ocean Optics). The other arm of the fibre was connected to a high output light source (200–1000 nm; DH-2000-BAL, Ocean Optics). The common end of the bifurcated fibre was fitted with a flat black reflectance probe that showed a 3 mm diameter tip, cut at an angle of 45 deg. A measurement of a Spectralon diffuse reflectance standard was taken as 100% reflectance, and a dark measurement was taken as zero reflectance. Fish were immersed in 500 ml water containing 2 ml of 1:10 clove oil:ethanol solution immediately after capture until the fish reached stage III anaesthesia (Jolly et al., 1972). Reflectance was measured at 16–23 different points across the submerged fish body. Spectral reflectance data were acquired from three individuals of each of five cichlid species: *Melanochromis auratus* (Boulenger 1897), *Metriaclima zebra* (Boulenger 1899), *Metriaclima aurora* (Burgess 1976), *Metriaclima callainos* (Stauffer and Hert 1992) and *Protomelas taeniolatus* (Trewavas 1935).

Spectral reflectance of fruits

A data set of fruit reflectance was adopted. This data set (N=482 from 51 plant species) was obtained from fruits collected at the MUBFS, Kibale Forest, Western Uganda. Data were also collected at other sites within Kibale Forest (which also lie within the same reserve: 0°13′ to 0°41′N and 30°19′ to 30°32′E), at Budongo Forest and in Queen Elizabeth National Park (Sumner and Mollon, 2000). Fruit reflectance spectra were measured at 4 nm intervals from 380 to 780 nm, and were obtained from the Cambridge database of natural spectra.

Spectral reflectance of integumentary tissues of primates

A data set of reflectance of fur, skin and pelts of old-world primates (Catarrhine) was adopted. This data set (N=194 from 13 species) was measured at zoos in Uganda and the UK and at the Natural History Museum, London. Reflectance spectra of integumentary tissues were measured at 4 nm intervals from 380 to 780 nm, and were obtained from the Cambridge database of natural spectra.

APPENDIX 2
Comparison between PCA and DFT and validation of calculations

Both approaches, which express spectra as linear models (PCA) or as band-limited functions (DFT), estimate the degrees of freedom of the signal. However, while PCA examines the statistics (dimensionality) of a whole collection of spectra, DFT examines the distribution of energy across comb frequencies (Barlow, 1982) in each individual spectrum. DFT is advantageous because it accounts for the filtering properties of photoreceptors, allowing estimation of the degrees of freedom of the filtered, band-limited, signal.

To verify our PCA calculations, PCA was performed on the full spectra set of 462 Nickerson–Munsell chips. Variance accounted for by the model, R^2, was in agreement with previously reported values for the 400–700 nm range (Maloney, 1986), where 0.9756, 0.9960, 0.9980, 0.9993 and 0.9996 of median variance was recovered with two to six PCs, respectively. To verify our DFT calculations, the band limits of all spectra included in the Munsell data set were calculated. Band limits were in agreement with previously reported values for the 380–770 nm range (Maloney, 1986), where 0.9459, 0.9859, 0.9990 and 0.9998 of median energy was recovered at frequencies of 3.3, 5, 10 and 15 cycles μm^{-1}, respectively.

Several of the analysed data sets included spectra measured at a resolution of 1 nm, while others included spectra measured at a resolution of 4 nm. For consistency, low-resolution spectra were spline interpolated to every 1 nm prior to analysis. To test the effect of spectral interpolation on our PCA calculations, the variance accounted for by the model, R^2, was calculated for a selected data set (Lake Malawi irradiance) measured at a spectral resolution of 1 nm, and again after this data set was sampled every 4 nm and interpolated back to 1 nm. The variance accounted for by the model did not differ between the original and interpolated data sets, where 0.9833, 0.9965, 0.9994, 0.9998, 0.9999, 0.9999 and 1.0000 of median variance was recovered with the first seven PCs. Similarly, to test the effect of spectral interpolation on our DFT calculations, the band limits of spectra included in the original and interpolated data sets were calculated and were found to be similar (randomization test, P=0.986, confidence interval 2.5–97.5%, $CI_{original}$=5.833–6.187, $CI_{interpolated}$=5.832–6.186, N=80).

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AUTHOR CONTRIBUTIONS

S.S. and N.F.T. conceived and designed the analysis. S.S. and C.W.H. designed the irradiance and reflectance measurement procedures. S.S., C.W.H. and S.M.G. performed the measurements. S.S. analyzed the data. S.S. wrote the paper. N.F.T., C.W.H. and S.M.G. commented on the paper.

COMPETING INTERESTS

No competing interests declared.

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