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Are Corals Colorful?

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ABSTRACT

Using *in situ* spectrometry data and visual system modeling, we investigate whether the colors conferred to the reef-building corals by GFP-like proteins would look colorful not only to humans, but also to fish occupying different ecological niches on the reef. Some GFP-like proteins, most notably fluorescent greens and nonfluorescent chromoproteins, indeed generate intense color signals. An unexpected finding was that fluorescent proteins might also make corals appear less colorful to fish, counterbalancing the effect of absorption by the photosynthetic pigments of the endosymbiotic algae, which might be a form of protection against herbivores. We conclude that GFP-determined coloration of corals may be an important factor in visual ecology of the reef fishes.

INTRODUCTION

GFP (green fluorescent protein)-like fluorescent proteins attain a remarkable diversity of colors in reef-building corals (1–3). These proteins are responsible for the majority of coral coloration superimposed upon the brownish background provided by the endosymbiotic algae (4). Currently known coral GFP-like proteins can be subdivided into several color types: cyan, shortwave green, longwave green, yellow, red and nonfluorescent purple–blue (5). The exact function of this diversity remains unknown. The only fluorescent color for which functionality has been experimentally demonstrated is fluorescent green, which may provide photoprotection for algal endosymbionts when present in high concentrations (6,7). The function of other colors, as well as the role of green fluorescent proteins in more typical low concentrations, is still debated (8,9). Among different putative functions, recently reviewed in (10), there is a possibility that the coral coloration evolved to produce a visual signal—in other words, it may be beneficial for corals to appear colorful to other inhabitants of the reef (11). It may even be possible that the coral color diversity, whatever might have been the reason for its appearance, served as a trigger to the evolution of colorfulness in other reef organisms through adaptation of their visual systems to the coral environment (10). The critical question remains, however: Are the eyes of the present-day reef organisms suited to see the coral colors? GFP-determined color contrasts in corals tend to appear quite strong to humans (12), but this is not necessarily so for the reef inhabitants, whose visual systems and color perception may be very different (13–15). Here we investigate whether the spectral differences between differently colored (according to human perception) coral parts or morphs are sufficient to be discriminated by divergent color vision systems of three species of reef fish, to determine whether coral coloration may in principle be relevant for their visual ecology.

MATERIALS AND METHODS

In situ spectrometry. *In situ* spectra were measured by SCUBA divers with the use of a USB2000 spectrometer (Ocean Optics, Dunedin, FL) controlled by a handheld computer. The setup was battery powered and enclosed in a waterproof casing (Wills Housings, Sydney) with an attached measuring optical fiber and controls allowing manipulation of the handheld's buttons. Modified Palm-Spec software (Ocean Optics, Dunedin, FL) was used that allowed control of the system with the handheld's buttons only. Data collected for each coral subject involved measurements of the ambient light spectrum from the 100% reflective standard placed near the coral, radiance from the colored coral surface(s) under ambient light and radiance from the same surface when illuminated by a blue flashlight BlueStar (NightSea, Andover, MA) to estimate positions of the fluorescence peaks. The raw spectra have been corrected for the sensitivity of the spectrometer's detector with the use of a calibration curve obtained with the use of a standard LS-1 light source (Ocean Optics, Dunedin, FL).

Coral samples. We measured several species of coral that appeared colorful to us at 7–15 m depth at the Heron Island Research Station on the Great Barrier Reef. These species included *Lobophyllia hemprichii* (Fig. 1A, red mouth versus green tentacles), *Favites abdita* (Fig. 1D, green oral disk versus pale red theca) and also the contrast between two uniformly colored species, pink *Stylophora pistillata* and blue *Porites sp.*, Fig. 1N, which were growing near each other. In the Caribbean, we measured red, green and blue color morphs of the great star coral *Montastrea cavernosa* at 20 m depth off Long Key, FL (Fig. 1G–I).

Fish visual systems. We modeled the visual responses of three species: damselfish (*Chromis ovalis*), butterflyfish (*Forcipiger flavissimus*), and barracuda (*Sphyrena helleri*). We selected these species as representatives of three different ways of life on the reef and because their visual systems were clearly different. Damselfish live within a small territory comprising just one or two coral colonies, and usually remain very close to the coral surface. One may expect that they would not have much use for perception of coral color, unless at the recruitment stage. Butterflyfish have larger territory and swim higher in the water column, viewing the reef from greater distance. Butterflyfish are corallivorous and therefore may need to discriminate between different corals better than damselfish. Barracuda have the largest territory, perceive the reef from the greatest distance, and

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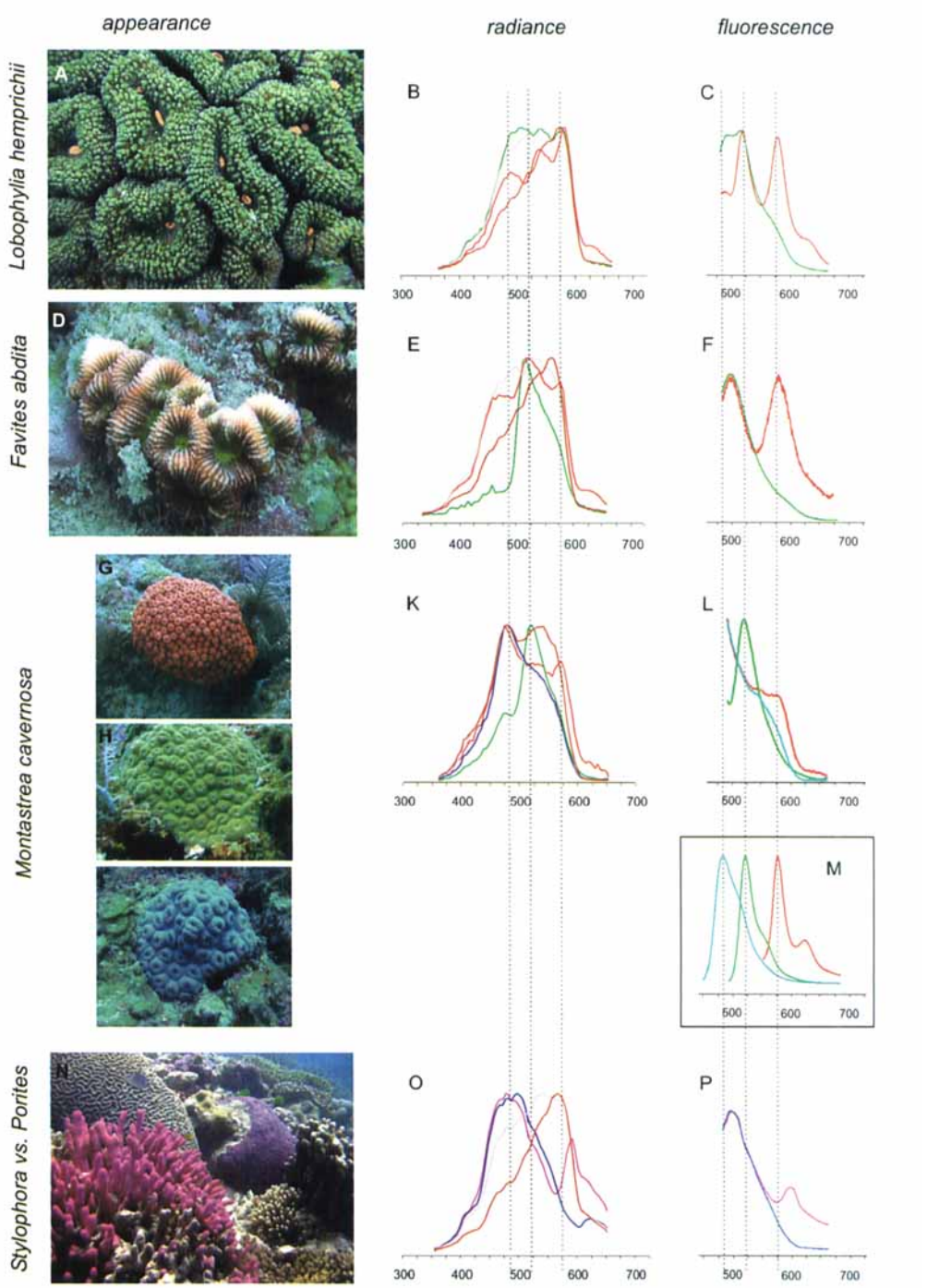

Figure 1. Appearances (A, D, G, H, I and N), normalized radiances (B, E, K and O) and normalized fluorescence spectra (C, F, L and P) of the coral subjects. The boxed fluorescence panel (M) shows fluorescence spectra of the three major types of GFP-like proteins found in *Montastrea cavernosa*—cyan, green, and red, each shown as a curve of corresponding color. Three vertical dotted lines trace the positions of the maxima from panel M across the plots to provide reference. On panels B, E, K and O (radiances) the gray curve is the luminant (radiance of a white standard), and brown is modeled radiance of a coral lacking GFP-like proteins. The color of the remaining curves corresponds to the apparent color of the measured surfaces. In *Lobophyllia hemprichii* (A), we measured the red mouth area and the green theca; in *Favites abdita* (D)—green oral disk and the pale red theca. In *Montastrea cavernosa* the color of the morphs shown on panels G, H and I was studied. Finally, pink *Stylophora pistillata* and the blue nearby-growing blue *Porites sp.* were measured (N). On all the graphs, horizontal axis is wavelength in nanometers; the vertical axis is radiance or fluorescence intensity.

are piscivorous. It may be reasoned that, other than wanting to detect fish against corals, barracuda would not want to distinguish different coral types.

Color vision in the fishes was modeled on the basis of the absorption spectra of individual photoreceptors determined previously with the use of microspectrophotometry of isolated retinas (15,16). In addition to rod-opsins, which are involved in luminosity detection and were not included in color vision calculations, fishes possess two (barracuda) or three (damselfish and butterflyfish) types of visual pigments with different absorption properties (Fig. 2). Typically, one of these pigments absorbs in the UV-blue region and is specific to a particular type of cone photoreceptor cells. Due to the transmission characteristics of the cornea, the UV sensitivity of these photoreceptors at shorter wavelength is not realized, which was incorporated into the calculations. Of the three fishes compared, the damselfish has the most extended UV vision (Fig. 2). In damselfish and butterflyfish, the other two pigments are contained within so-called double cones (two closely juxtaposed cone cells possessing different pigments). The mechanism of double-cone function is not yet understood. Most importantly, it is unclear whether the two component cones may act as independent photoreceptors, in which case the damselfish and butterflyfish would possess trichromatic vision (17). Alternatively, a double cone may act as a single photoreceptor integrating over the responses of its components, leading to dichromatic vision in all fishes examined (Fig. 2). In this article, we modeled both possibilities. We also modeled human vision responses as a comparison.

Calculations of discrimination capability. To analyze the perception of coral colors, we used the previously developed model by (18), which assumes that receptor noise limits the color discrimination (19). An important feature of this model, the predicted perceptual distance between signals, does not depend on intensity; that is, this model estimates the

differences only in chromaticities of colors. The model predicts behavioral spectral sensitivity in a number of species, including human beings (18–20). The quantum catches, Q_i in photoreceptor of a spectral type i were calculated as

$$Q_i = \int_\lambda R_i(\lambda)S(\lambda)\,d\lambda, \quad (1)$$

where $R_i(\lambda)$ denotes the spectral sensitivity of a receptor i, and $S(\lambda)$ is the spectrum of a fluorescent coral. The contrast between two spectra is calculated as the logarithm of the quotient of quantum catches from Spectrum 1 and Spectrum 2. The result of this calculation is the contrast Δf for each receptor type i:

$$\Delta f_i = \ln(Q_i[\text{spec1}]) - \ln(Q_i[\text{spec2}]) = \ln(Q_i[\text{spec1}]/Q_i[\text{spec2}]) \quad (2)$$

To quantify discrimination with the use of all receptor types in a given visual system, each receptor class is first assigned a noise value ω based on its individual Weber fraction (v) and on the receptor proportion (n); see also (18):

$$\omega_i = v_i/\sqrt{n_i} \quad (3)$$

The actual value of a Weber fraction for the long-wavelength sensitivity mechanism (21) is not known for reef fish. In the studied vertebrates, it ranges from 0.02 (humans [22]) to 0.1 (some birds [23]). In all our calculations here, we assumed a Weber fraction of 0.05, which is close to the median value and therefore represents as good a guess as we can make at the moment. The Weber fraction for humans was also set to 0.05 to facilitate comparison, because in this article we are analyzing the adaptation of visual pigments rather than finesse of visual processing. The proportions of individual cones in fish used in calculations were based on morphological studies of fish retina (Marshall, unpublished): for trichromatic models, shortwave cones to midwave cones to longwave cones (S:M:L) = 1:1:1; for dichromatic models, S:L = 1:2. We also did calculations for human visual system using the cone proportions for a typical human observer (0.05, 0.35 and 0.6 for S,M,L cones, respectively) (22).

Then, we calculate the distance ΔS between two spectra for dichromatic and trichromatic visual systems.

$$\text{Dichromat: } (\Delta S)^2 = (\Delta f_1 - \Delta f_2)^2/(\omega_1^2 + \omega_2^2) \quad (4)$$

$$\text{Trichromat: } (\Delta S)^2 = [\omega_1^2(\Delta f_3 - \Delta f_2)^2 + \omega_2^2(\Delta f_3 - \Delta f_1)^2 + \omega_3^2(\Delta f_1 - \Delta f_2)^2]/[(\omega_1\omega_2)^2 + (\omega_1\omega_3)^2 + (\omega_2\omega_3)^2] \quad (5)$$

The difference in perception of the two colors $\Delta S = 1$ corresponds to just noticeable difference (jnd), because the signal corresponds to one standard deviation of the receptor noise. Colors with $\Delta S < 1$ would not be possible to distinguish, because the difference will be masked by the receptor noise even under favorable viewing conditions. For the values of ΔS increasingly higher than 1, the two colors become more and more distinguishable, providing increasingly rapid discrimination under difficult conditions.

Note that the color distance is inversely proportional to the Weber fraction. Should fish Weber fraction be twice higher or twice lower than our guess of 0.05 (which would cover virtually the whole possible range, from 0.025 to 0.1) the calculated distances will simply increase or decrease by the factor of 2. Although this may bring some of the poorly distinguishable color contrasts above or below the threshold of 1, the general conclusions concerning which color is more or less contrasting will not be affected.

RESULTS AND DISCUSSION

Coral colors

Three of the model coral species—*Lobophyllia hemprichii*, *Favites abdita* and *Montastrea cavernosa*—belong to the *Faviina* suborder of stony corals and share the same paralogous groups of GFP-like proteins (24, Alieva and Matz, unpublished). The colors produced by these paralogs include fluorescent cyan, green, and red (Fig. 1M) and have been most thoroughly studied in *M. cavernosa* (3). It has been demonstrated that the natural color polymorphism in this species (such as represented on Fig. 1G–I) is due to variation in relative expression levels of several genes coding for proteins of different fluorescent color (3). The three morphs considered here are no exception: the blue morph contains only cyan fluorescent protein; green morph, only green fluorescent protein; and red morph contains a mixture of cyan and red fluorescent proteins (Fig. 1L). From the position and shape of the radiance and fluorescence maxima observed in *Lobophyllia* and *Favites* (Fig. 1B, C, E and F)

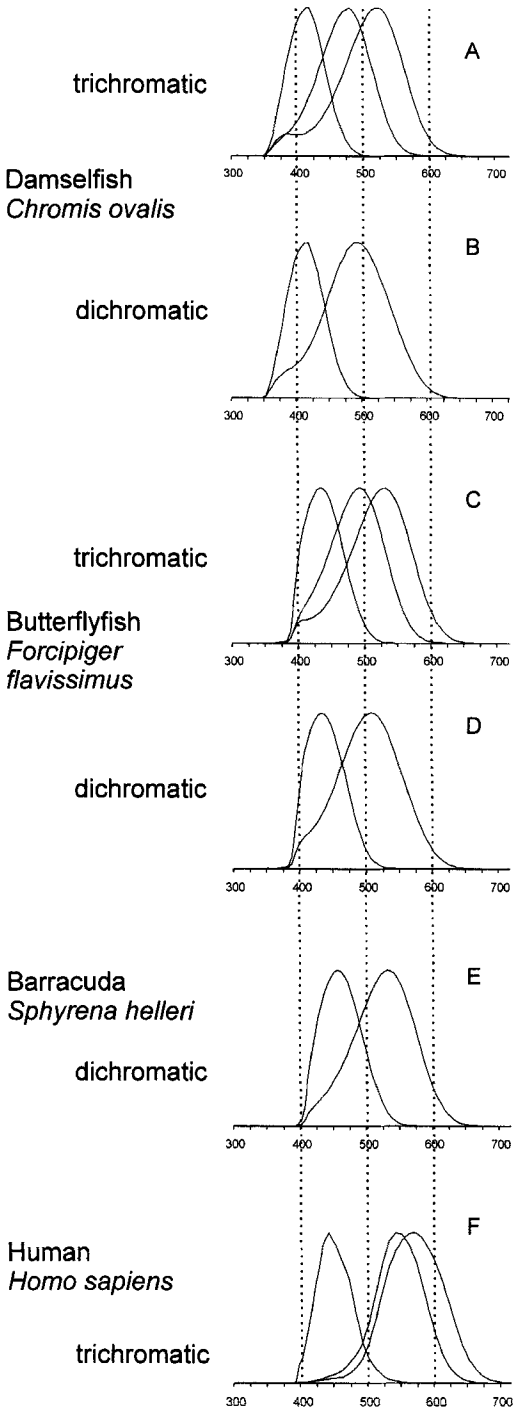

Figure 2. Vision models used in this study. The graphs show the absorption spectra of individual visual pigments in different species, truncated on the UV side by absorption of the cornea. The dichromatic models for damselfish and butterflyfish combine absorption of two pigments found in the double cones into one type of sensitivity. Horizontal axis—wavelength in nanometers, vertical axis—absorption intensity.

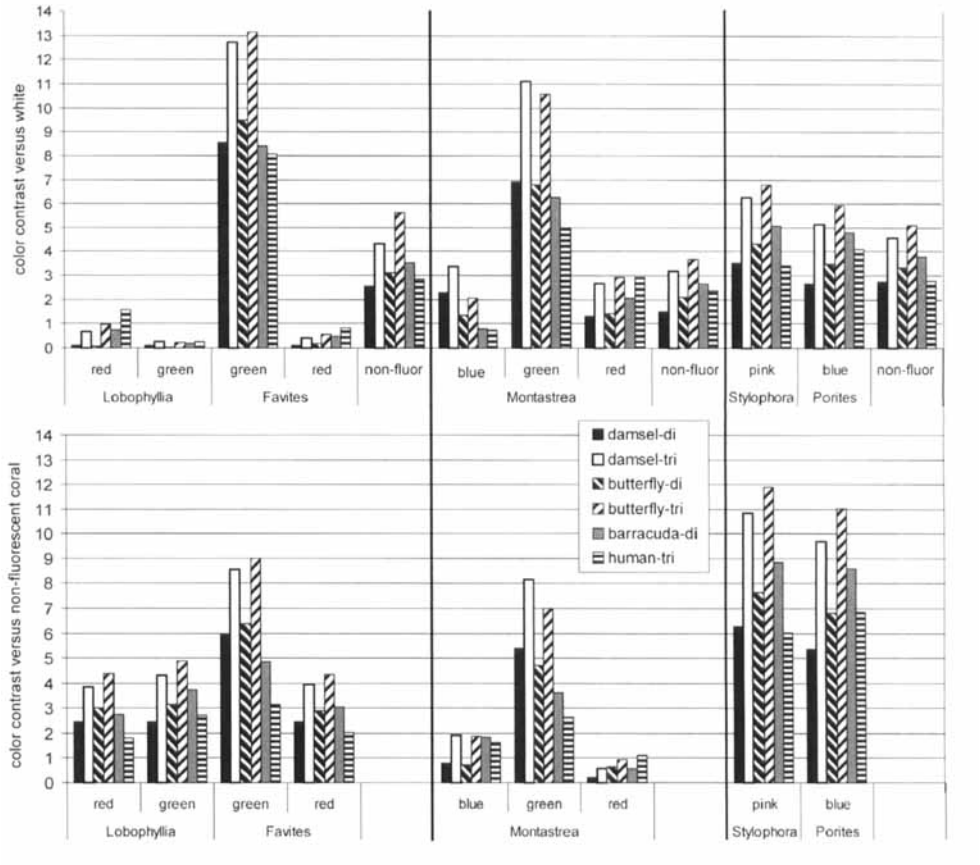

Figure 3. Color contrasts of the investigated corals within different visual systems (see legends). Upper panel: colorfulness, measured as the contrast between the object and white standard under ambient illumination. "non-fluor" bars indicate colorfulness of the modeled nonfluorescent coral at the location where the real corals were studied (three separate locations/depths for *Lobophyllia/Favites*, *Montastrea* and *Stylophora/Porites*). Lower panel: contrasts between real coral colors and a modeled nonfluorescent coral at a particular location. The contrasts are scaled in units of "just noticeable distance" (see methods).

it appears most likely that the color contrasts in these species were due to a similar collection of fluorescent GFP-like proteins as in *M. cavernosa* (Fig. 1).

The main coloration in pink *Stylophora pistillata* and blue *Porites sp.* (Fig. 1N, O) is due to absorption by nonfluorescent GFP-like chromoproteins, or pocilloporins (4,25). *In situ* fluorescence measurements (Fig. 1P) suggest the presence of a green fluorescent protein in both corals (Fig. 1P), although it was inconspicuous due to intense chromoprotein absorption and masking by the relatively full-spectrum light at 7 m depth where the corals were found. The pink color in *S. pistillata* seems to be enhanced by the red fluorescence from uncharacterized red fluorescent protein emitting near 600 nm. It is possible that the red fluorescence actually may come from the "kindled" form (25,26) of the same chromoprotein that provides the overall pink coloration.

Colorfulness

We estimated the colorfulness of a coral as the color difference between its spectrum and the spectrum of uniformly reflecting white surface illuminated by the ambient light underwater (see Methods, Eqs. [4] and [5]). From our results (Fig. 3) it is immediately apparent that trichromatic visual systems are more sensitive to colors than the dichromatic ones. This is not surprising provided the extra dimension of the color space in trichromatic versus dichromatic vision. Interestingly, the colors of *Lobophyllia* as well as the thecal area in *Favites* appear the least colorful (grayish) even within the human visual system, despite apparent colorfulness of the corals on the photograph (Fig. 1A). Why then did we pick these corals as a model if it was not supposed to look colorful to a human underwater? When the human Weber fraction was reset to a more characteristic value of 0.02 (22), all the *Lobophyllia* and *Favites* colors were distinguished except the green in the *Lobophyllia* theca (not shown). Another factor that aided color viewing for us underwater was that we used color-correcting red filters for photography and also on the diving mask. These filters are designed to compensate for the overabundance of blue light underwater and to bring out color details defined within a longer-wavelength region of visible spectrum (greens and reds). The most colorful signals for fishes are the green of *Favites* and *Montastrea*. The pink and blue colors of *Stylophora* and *Porites* also appear quite colorful to fishes. All these colors would still be well distinguishable even if the fish Weber fraction was reset from 0.05 to the worst realistically possible value of 0.1: This would decrease the calculated distances by the factor of 2 (see Methods), which would not be nearly enough to make these contrasts less than the value of 1, "just noticeable difference."

To see whether the studied corals would be perceived differently from a coral displaying typical brown coloration due to symbiont photosynthetic pigments, we calculated the color contrasts between the measured spectra and the inferred radiance spectrum of a hypothetical nonfluorescent coral. To model such a spectrum at a required depth we used the reflectance of a nonfluorescent *Montastrea annularis* and the radiance of the reflective white standard recorded *in situ*. The calculations indicate that fluorescent greens of *Favites* and *Montastrea* and nonfluorescent pink/blue of *Stylophora* and *Porites* still appear very contrasting versus nonfluorescent background. The most remarkable fact, however, is that some fluorescent parts of *Lobophyllia* and *Favites*, as well as blue and red morphs of *Montastrea*, tend to appear less colorful

Table 1. Contrasts between coral colors within visual systems of fish and human, scaled in the units of "just noticeable distance" (see methods). Values exceeding 1 signify noticeable contrast and are printed in bold.

Organism	Vision model	*Lobophyllia* red/green	*Favites* red/green	*Montastrea cavernosa* morphs			*Stylophora/Porites* pink/blue
				Red/green	Red/blue	Green/blue	
Damselfish	di	0.00	**8.44**	**5.62**	**1.00**	**4.62**	0.89
Damselfish	tri	0.90	**12.43**	**8.60**	**1.79**	**7.91**	**1.29**
Butterflyfish	di	0.13	**9.28**	**5.38**	0.05	**5.44**	0.83
Butterflyfish	tri	**1.20**	**12.70**	**7.94**	**1.48**	**8.55**	**1.19**
Barracuda	di	0.97	**7.91**	**4.21**	**1.26**	**5.48**	0.25
Human	tri	**1.84**	**7.27**	**2.79**	**2.27**	**4.25**	**2.17**

(*i.e.* more similar to the luminant spectrum) to fish than the nonfluorescent model (Fig. 1). This is unlikely to be the consequence of different symbiont densities in corals measured *in situ* and the model *M. annularis*, because our calculations took into account only the spectral shape while disregarding the absolute intensity of coloration. Instead, this counterintuitive discoloration effect must be attributable to cyan or green fluorescence present in these corals (Fig. 1) adding to the radiance in the green spectral region that is depleted in the brown nonfluorescent coral. In other words, the presence of cyan and/or green proteins may mask the brown coloration of symbionts. It is tempting to speculate that this might be a mechanism to conceal the algae within the coral host from the eyes of herbivores.

Color discrimination

The color contrasts for the visual systems of three fishes and a human are presented in Table 1. They were calculated in the same way as colorfulness described above by comparing the signals from corals between each other. As expected, the contrasts involving intense green of *Favites* and of the green morph of *Montastrea* are well seen by all. The blue and red morphs of *Montastrea* can be told apart by all except dichromatic butterflyfish. The pink *Stylophora* and blue *Porites*, on the other hand, would tend to appear the same to fishes not endowed with trichromatic vision, unless the fishes have a Weber fraction less than 0.05. The greatest challenge proved to be resolving red and green colors of *Lobophyllia*: among fish, only trichromatic butterflyfish would see the contrast exceeding the detection threshold, although barracuda come very close despite being a dichromat. The smaller Weber fraction would help barracuda and trichromatic damselfish to distinguish these colors as well, but there would be still no hope for the dichromatic damsel and butterflyfish for any realistic value of Weber fraction.

The contrast between red and blue morphs of *Montastrea* is of special interest because it may reflect the ability of a visual system to see red color (red morph has both cyan and red fluorescent proteins, while blue morph has only cyan, Fig. 1L). Notably, this contrast exceeds detection threshold for all but the dichromatic butterflyfish, which is unexpected, as fish lack a specialized long-wave visual pigment for the red color. It is possible, however, that the perceivable colorfulness of the red and blue morphs as well as visual contrast between them may be to some extent due to the subtle differences of spectral shapes in the shortwave region rather than the presence/absence of the red component in the radiance spectrum (Fig. 1K). This explanation is especially probable for damselfish, which has its visual pigment sensitivities very well positioned for acute color discrimination in the blue-violet region.

It is clear from our results that trichromatic vision distinguishes colors better than dichromatic, just as expected from theory. Apart from this, visual capabilities of various fishes are surprisingly similar. There are only two notable differences, which are the above-mentioned inability of dichromatic butterflyfish to discriminate between blue and red *Montastrea* morphs and slightly better overall performance of barracuda in comparison to other dichromatic models. This result may be an indication of visual adaptation of fish to different lifestyles, although it is rather contrary to our initial expectations (see "Fish vision systems" in the "Materials and Methods" section above). It is important to note that trichromatic human systematically performs worse than trichromatic fish and in most cases worse even than dichromatic barracuda (Fig. 3). It is tempting to speculate that this might signify fish adaptation to view coral colors, although it may be a consequence of adaptation of fish vision to ocean environment in general.

CONCLUSIONS

Our modeling of fish visual response to the coral radiances measured *in situ* suggests that GFP-like proteins may indeed confer colors perceivable by reef fish. The most vivid coloration is provided by fluorescent greens and nonfluorescent chromoproteins such as in pink *Stylophora* and blue *Porites*. Surprisingly, fluorescent cyan and green proteins may also produce an opposite "discoloration" effect, counterbalancing the brown color of symbiont pigments and thereby making the coral appear less colorful to a fish eye. We speculate that this might be a way for the coral to hide its endosymbionts from the eyes of herbivores. These observations indicate that the GFP-related colors of corals may indeed be relevant for the visual ecology of the reef fishes. There are subtle differences in color-perception abilities between fishes occupying different ecological niches, although this subject obviously requires further study. It is tempting to speculate that the evolution of diverse coral colors in early Mesozoic (3) might have prompted the specialization of the fish visual systems in the process of adaptation to the environment, eventually leading to appearance of very unusual color signaling displayed by present-day reef fishes.

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