Multifocal lenses in coral reef fishes

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Summary

The optical properties of crystalline lenses were studied in eleven species of coral reef fish from the Red Sea in Eilat, Israel. Three species each of diurnal planktivores, nocturnal planktivores and diurnal herbivores constituted three groups of animals with little within-group variability. In addition we studied two predators, which differed with respect to body size, prey preference, hunting method and diel activity period. All species studied have multifocal lenses. There were statistically significant differences in the optical properties of the lenses between the first three groups and between the predatory species. The properties of the lenses correlate well with known complements of visual pigments and feeding habits. Lenticular zones focusing ultraviolet light were found in two diurnal planktivores. The optical properties of the lens seem to be specifically adapted to the visual needs of each species.

Key words: physiological optics, color vision, chromatic aberration, spherical aberration, visual pigments, Red Sea.

Introduction

The refractive power of a typical fish eye resides exclusively in the powerful spherical lens, since the cornea is thin and has fluid of similar refractive index on both sides (Matthiessen, 1886). All lenses suffer from a number of optical aberrations. In spherical lenses, asymmetric aberrations such as astigmatism and coma are absent or at least of minor importance. There are, however, important symmetric aberrations. A homogeneous spherical lens focuses light passing through its periphery closer to the lens than light rays nearer to the optical axis (longitudinal spherical aberration, LSA). Fish lenses, however, are inhomogeneous gradient index lenses. Protein concentration and thus refractive index is highest in the center and decreases gradually towards the periphery. The refractive index profile is roughly parabolic and corrects a typical fish lens rather well, although not completely, for LSA (Maxwell, 1854; Matthiessen, 1882; Matthiessen, 1886; Kröger et al., 1994).

Chromatic defocus originates from the prismatic effects of single lenses. Short wavelengths (ultraviolet to blue) are refracted more strongly and consequently focused closer to the lens than long wavelengths (red). The phenomenon is known as longitudinal chromatic aberration (LCA) (Kröger and Campbell, 1996; Born and Wolf, 1999). This can cause a serious problem in fish eyes since depth of focus is short in optical systems with small *f*-numbers (*f*-number=focal length/diameter of aperture), as in most fishes. In addition, most teleosts do not constrict their pupils even in bright light, since light flux is regulated by mechanisms located in the retina and retinal pigment epithelium (Walls, 1942; Douglas, 1982; Burnside and Nagle, 1983). LCA thus usually exceeds depth of focus, which means that fish lenses should only be able to focus a narrow spectral range on the retina, even in bright light when color vision is possible and advantageous.

It has been shown in the African cichlid fish *Astatotilapia* (formerly *Haplochromis*) *burtoni* that the lens has residual LSA of complex shape. This LSA leads to several discrete focal lengths for monochromatic light and such lenses are therefore called 'multifocal' lenses. If polychromatic light impinges on an *A. burtoni* lens, the lens focuses the wavelengths maximally absorbed (λ_{max}) by the cone photoreceptors at the same depth (Kröger et al., 1999). LCA is thus corrected for by accurately tuned LSA, which in turn is dependent on the refractive index profile within the lens. Multifocal optical systems have been demonstrated in some other freshwater fishes (Kröger et al., 1999; Malkki and Kröger, 2005) and a variety of terrestrial vertebrates (Kröger et al., 1999; Malmström and Kröger, 2006). We present here the first evidence for multifocal lenses in marine teleosts. In addition we extend the study of multifocal lenses to correlations between the optical properties of the lenses and the ecologies of different species.

Tropical and sub-tropical coral reefs are among the most colorful habitats on Earth (Chiao et al., 2000) and support large varieties of animal species with different lifestyles (Dubinsky, 1990). Clear water and proximity to the surface allow the use of the full spectrum of light, including the ultraviolet (UV) range. Color vision can provide a wealth of information in such an environment and it is known that at least some coral reef fishes have well-developed color vision systems at the retinal

level (Losey et al., 2003; Marshall et al., 2003). Different lifestyles and diel activity periods are expected to have led to different retinal and optical adaptations. In the present work we used recently described optical methods (Malkki and Kröger, 2005) to investigate how the optical systems are matched to the capabilities of the retinas. In addition, we studied whether there are lifestyle-specific characteristics in the optical properties of fish lenses.

Materials and methods
Animals

Fish from 11 species were investigated, representing four different lifestyles: diurnal herbivores, diurnal planktivores, nocturnal planktivores and predators. The first three groups consisted of three species each and there was little within-group variability in body sizes and shapes, foraging strategies and diel activity periods. In the latter group (predators) there were two species different in these respects. All animals were caught by SCUBA divers, using hand nets, in shallow waters not deeper than 10 m on or near a coral reef in Eilat (the Gulf of Aqaba) under permit no. 18267 from the Israeli Natural Parks Authority. Characteristics of the selected species are summarized in the Appendix.

The animals were kept at the Inter University Institute of Eilat, Israel, in outdoor tanks with a continuous supply of unfiltered seawater. Most fish were studied on the same day they were caught; a few animals were kept for up to 1 week.

Optical investigations

The methods we used have been described in detail elsewhere (Malkki and Kröger, 2005). Here we briefly present their essential features. All investigations were performed during daytime, i.e. on light-adapted fishes.

Lens examination in vivo

Photoretinoscopy. In the form used here, photoretinoscopy (Fig. 1A) was developed by Schaeffel and co-workers (Schaeffel et al., 1987; Schaeffel et al., 1993). It can be used with live animals and gives an indication at what distance, relative to the camera, the eye is focused. If the eye has a multifocal optical system, ring-like structures are visible in photoretinoscopic images (Kröger et al., 1999). With this method we could detect animals with eyes that were optically aberrant from the general pattern present in each particular species. Such deviations may be caused, for example, by intraocular parasites (Malkki and Kröger, 2005).

All caught fish were screened using photoretinoscopy and aberrant individuals discarded. One fish at a time was kept in a small glass tank with unfiltered seawater. After allowing the fish to acclimatize for about 5 min, each eye was videotaped for approximately 1–1.5 min and typical frames were later exported using Adobe Premiere 6.0 software.

Lens examination in vitro

The fish was sacrificed by rapid cerebral section and pithing. Its total length (*TL*, tip of snout to end of tailfin) and standard length (*SL*, tip of snout to base of tailfin) were measured to the nearest mm. One eye was excised, while the other one remained in place and was kept moist with seawater until the first eye had

been completely processed. During extraction of the lens the excised eye was immersed in phosphate-buffered saline (PBS; Na^+=7.58 g l^{-1}, Cl^-=4.88 g l^{-1}, HPO_4^{2-}=0.757 g l^{-1}, $H_2PO_4^-$= 0.259 g l^{-1}, pH 7.2, osmolality 290 mOsm) in order to prevent dehydration of the lens.

Schlieren photography. Schlieren photography (Fig. 1B) was adapted for use on excised animal lenses by Jagger and Sands (Jagger and Sands, 1996; Jagger and Sands, 1999). These workers used a single-pass design and monochromatic infrared light. We used a double-pass system and white light from a standard cold-light laboratory lamp run at 3200 K (Malkki and Kröger, 2005). Our setup allowed for correct focusing of the fish lens and the resulting images give indications of the spectral ranges of light being brought to focus by the lens.

Immediately after extraction, the lens, suspended by its retractor lentis muscle, was immersed in a small plastic tank containing PBS. The sutures of the lens were used as landmarks to align the optical axis of the lens with the axis of measurement. Thereafter, several photographs were taken using a digital color
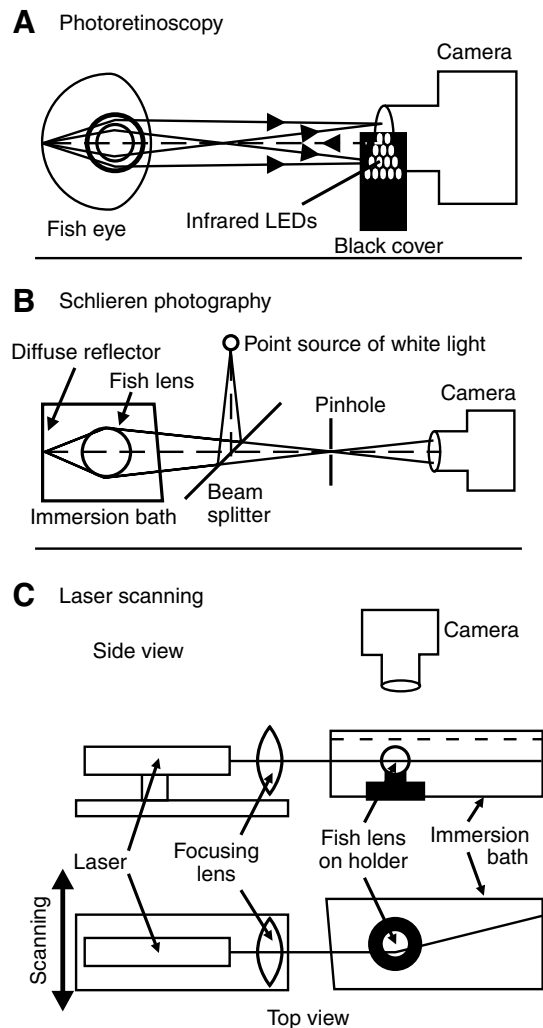

Fig. 1. Schematic sketches of the optical methods. Photoretinoscopy (A) was applied on live animals, while excised lenses were studied with schlieren photography (B) and laser scanning (C). For technical details, see Malkki and Kröger (Malkki and Kröger, 2005).

camera (Sony DSC-F 707) with varying distances between the fish lens and the diffuse reflector (Fig. 1B).

Laser-scanning. The LSA of a lens was quantitatively determined using laser-scanning (Fig. 1C). Our setup was a modification of the system used previously (Kröger et al., 1994). Refinements increased speed and resolution of measurement, and included semi-automated analysis of the raw data (Malkki and Kröger, 2005). Data were obtained by scanning a thin laser beam of a wavelength of 547 nm (diode-pumped solid state laser) through a meridional plane of the lens. The results are equivalent to a transverse section through the symmetrical wavefront aberrations of the lens and can be directly compared with schlieren images, in which variation in focal length is indicated by variation in color.

The lens was carefully placed on a plastic holder in the laser scanning unit (Fig. 1C). To correctly align the lens with the laser beam, the direction of small grooves in the lens capsule was determined using a light microscope. A small amount of polysterene microbeads (diameter 100 nm) was added to the PBS used for immersion. The microbeads scattered some light, which made the laser beam visible. Each lens was scanned twice and an average LSA recorded.

From the video sequence of each scan, 200 frames were exported to TIFF (tagged image file format, a non-compressed format) images using Adobe Premiere 6.0. From these frames, the LSA was determined by using custom-written software (Malkki and Kröger, 2005). LSA curves were generated by plotting back center distance (BCD), i.e. the distance between the center of the lens and the intercept of the exit beam and the optical axis, as a function of beam entrance position (BEP), i.e. the lateral distance between the optical axis and the entrance beam. All data were normalized to equatorial lens radius (R_e), such that the results from lenses of different sizes could be pooled or compared.

We averaged the LSA curves across the optical axis over both halves of each lens because we were only interested in spherical

aberration, which is a symmetrical aberration. Each lens was treated as an independent measurement because intra-animal variance is higher than inter-animal variance in this kind of measurement (Kröger et al., 2001). LSA curves were plotted with 90% confidence intervals, because if two such intervals do not overlap, the probability of the average curves being identical is less than 5%. Relative focal lengths were determined from averaged LSAs. The BCDs were weighted for their BEPs since peripheral regions of the lens contribute more to the image than central regions (Kröger and Campbell, 1996).

After all optical experiments on a lens were complete, its diameter was measured with calipers to the nearest 0.1 mm. The entire procedure from sacrifice of the fish to completion of all measurements on both lenses lasted between 45 and 60 min per fish.

Results

In each species, 9–13 lenses were studied, each examined with all available methods (Table 1). All studied species had multifocal lenses (Figs 2 and 3). However, the optical designs of the lenses differed between species (Figs 2–4).

The peripheries of the lenses of diurnal herbivores (*Siganus luridus*, *Siganus rivulatus*, *Acanthurus nigrofuscus*) are red on schlieren photographs, which indicates that long wavelengths were focused by these zones (Fig. 2A). Many differently colored rings are visible, especially in the large-eyed *S. luridus*. The LSA curves show less detail because of the method's lower spatial resolution (Malkki and Kröger, 2005). There is relatively little variation in BCD in the LSA curves, which indicates that UV light is not focused on the retina.

The LSA curves of the diurnal planktivorous damselfishes (*Chromis viridis* and *Dascyllus marginatus*) showed steep rises in BCD for beams of high entrance positions (Fig. 2B). High BCDs in a lens zone mean that such a zone focuses short wavelengths on the retina, because focal length decreases with decreasing wavelength. On schlieren photographs, the

Table 1. *Basic data on the animals studied*

Species	Order/Family	No. animals	No. lenses	*SL* (mm)	Lens diameter (mm)	Normalized focal length (R_e)
Diurnal planktivores						
Chromis viridis	Perciformes/Pomacentridae	5	10	35±1	1.3±0.1	2.38
Dascyllus marginatus	Perciformes/Pomacentridae	8	13	39±7	1.5±0.2	2.40
Pseudanthias squamipinnis	Perciformes/Serranidae	7	12	67±17	1.9±0.2	2.36
Nocturnal planktivores						
Apogon cyanosoma	Perciformes/Apogonidae	7	12	49±2	2.8±0.2	2.28
Apogon exostigma	Perciformes/Apogonidae	5	10	40±3	2.1±0.2	2.28
Cheilodipterus novemstriatus	Perciformes/Apogonidae	5	9	37±5	1.8±0.2	2.27
Diurnal herbivores						
Siganus luridus	Perciformes/Siganidae	6	12	155±20	4.6±0.8	2.39
Siganus rivulatus	Perciformes/Siganidae	6	10	156±39	3.9±0.9	2.39
Acanthurus nigrofuscus	Perciformes/Acanthuridae	6	12	120±9	3.3±0.2	2.38
Predators						
Fistularia commersonii	Syngnathiformes/Fistularidae	6	12	653±52	6.8±0.8	2.69
Pterois miles	Scorpaeniformes/Scorpaenida	6	12	127±43	3.9±1.1	2.38

Among the predators, *F. commersonii* is diurnal to crepuscular and *P. miles* is crepuscular to nocturnal.

Standard length (*SL*) and lens diameter values are means ± s.d. Normalized focal lengths were calculated from the average LSA of each species. R_e is the equatorial radius of the lens.
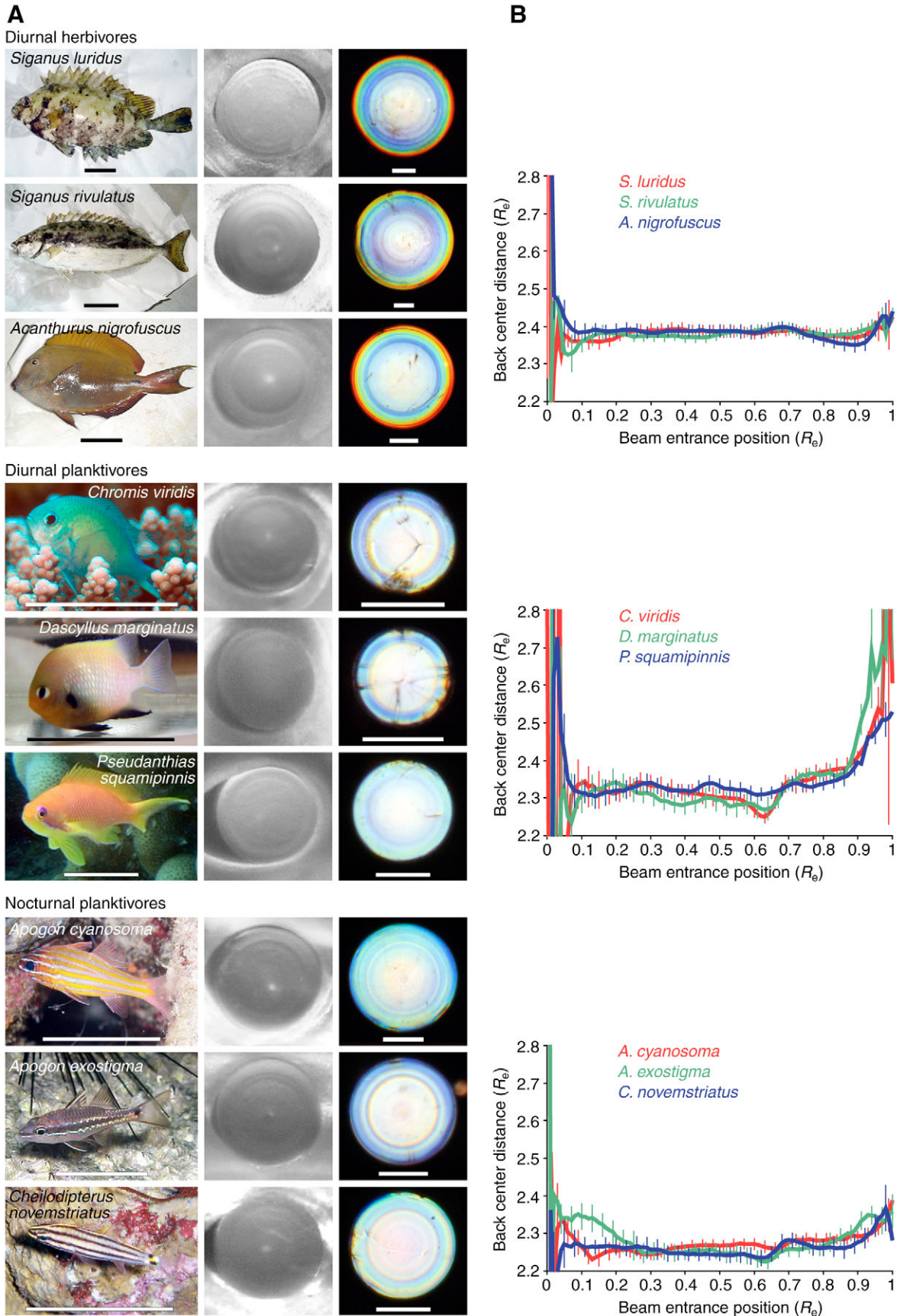
Fig. 2. See next page for legend.

Fig. 2. (A) (Left) Diurnal herbivores, diurnal planktivores and nocturnal planktivores; scale bars, 5 cm. Grayscale pictures of eyes (centre) are from photoretinoscopy. Ring-like structures indicate multifocal lenses. Color pictures of lenses (right) are from schlieren photography. The colors roughly indicate the spectral ranges that are brought to focus by different zones of the lenses; scale bars, 1 mm. (B) LSA curves for each species, means ± 90% confidence intervals. The results are normalized to R_e, i.e. the equatorial radius of each lens.

peripheries of the lenses appeared dark or were invisible. This indicates that such a zone focuses light of shorter wavelength than the visible range, i.e. UV light. A similar curve shape of the LSA, although with a less pronounced rise in BCD in the periphery of the lens, was found in the sea goldie (*Pseudanthias squamipinnis*). There was no peripheral dark zone in schlieren photographs of the lenses of this species (Fig. 2).

In the lenses of nocturnal planktivores (*Apogon cyanosoma*, *Apogon exostigma*, *Cheilodipterus novemstriatus*) there was considerably less variation in BCD, indicating smaller differences in focal length between different lens zones. There was no steep rise in BCD for high BEPs and dark outer rings were absent in schlieren photographs (Fig. 2). This indicates that variation in focal length within each lens is less pronounced in the nocturnal species compared to their diurnal counterparts and that UV light is not brought to focus on the retina. Normalized focal lengths were shortest in this group (Table 1).

The LSA curves obtained from the three groups of herbivores and planktivores were similar within each group, but differed significantly between groups (Fig. 3).

The predators (*Fistularia commersonii* and *Pterois miles*) were considerably different from each other in body shape and lifestyle (see Fig. 4A, Table 1, and the Appendix). However, schlieren photographs of *F. commersonii* and *P. miles* lenses are similar and show red peripheral rings (Fig. 4A). These zones of short focal length for monochromatic light were also detected by the laser-scanning method as sharp dips in the LSA curves at about 0.98 R_e (Fig. 4B). The LSA curves obtained from *F. commersonii* and *P. miles* are similar in shape, but also show that mean focal length is about 13% longer in *F. commersonii* (Fig. 4, Table 1).

Discussion

All of the species investigated have multifocal lenses, which is consistently indicated by ring-like structures in photoretinoscopic images, variations in BCD in the LSA curves, and particularly evident from the colorful schlieren images (Figs 2 and 4).

These colors, however, have to be interpreted with care. The camera used was designed to record images in about the same way as the human eye does. We furthermore adjusted the distance between the fish lens and the diffuse reflector behind it (Fig. 1) such that the center of the fish lens appeared white to the camera. The white point, however, is different for fishes if the animals have visual pigments that are spectrally different from the camera's color channels. Red, green and blue rings in schlieren images therefore do not indicate that the corresponding zones of a fish lens are used to focus wavelengths that fit into the camera's red, green and blue color channels. It is indicated instead that these zones focus relatively long, intermediate and short, respectively, wavelengths within the sensitivity range of the investigated species.

Furthermore, there was little overlap between the color channels of the camera, such that similar wavelengths could be detected by different color channels (Malkki and Kröger, 2005). A wavelength that would appear orange–yellow to a human
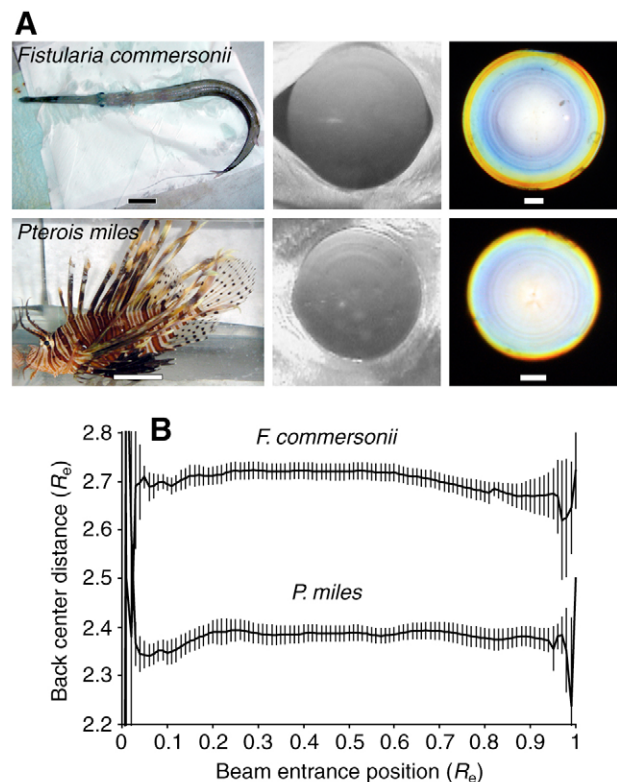
Fig. 4. (A) Two predatory species. Scale bars as in Fig. 2. (B) LSA curves ± 90% confidence intervals (CI). Note that, except for the very centers of the lenses where the accuracy of the method is low, the 90% CI of the LSA curves do not overlap. This indicates significant differences (*P*<0.05) between species in the optical properties of the lenses. Note also that the lenses of *F. commersonii* and *P. miles* bring to focus similar spectral ranges, but at different relative distances from the lenses.
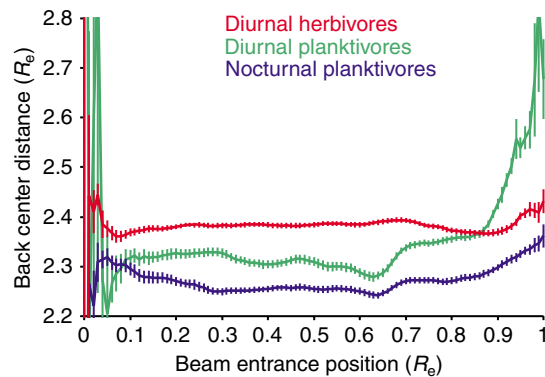
Fig. 3. Average LSA curves obtained from three groups of coral reef fishes having lifestyles that are similar within each group, but different between groups. Values are means ± 90% confidence intervals (CI). Note that the CI do not overlap in large regions, indicating statistically significant differences (*P*<0.05) between the groups.

observer may have been detected by the red channel, while a wavelength that to a human looks yellow–green was recorded as pure green. Such border cases were favored by our method, since we always tried to make the center of the lens look white on schlieren photographs by adjusting the lens' distance to the diffuse reflector behind it (Fig. 1). If the lens center appeared white, the lens focused on the reflector several wavelengths that stimulated all three color channels of the camera. The sharp spectral borders between the color channels of the camera may also have made some colored rings appear more steeply bordered than they would have appeared to a human or fish eye.

Correlations between known photopigment absorbances and the optical properties of the lenses

In light-adapted retinas of most fish species, the cone inner and outer segments are in the most vitread position close to the outer limiting membrane, while the rods are in a more sclerad position and protected from light by pigment granules in protrusions of retinal pigment epithelium cells (e.g. Douglas, 1982; Burnside and Nagle, 1983). The retinas of the studied animals were therefore functionally all-cone, such that the absorbances of the rods are irrelevant for the discussion of the results.

Furthermore, fish crystalline lenses are usually spherical and optically radially symmetric, such that all directions of incidence of light are functionally virtually identical (Matthiessen, 1882; Matthiessen, 1886). It is therefore irrelevant where in the retina different spectral cone types occur as long as there is some region in the retina where all cone types are present. From the literature and our results, we have no reason to assume that this is not the case in any of the species we studied.

Diurnal herbivores

The group of diurnal herbivores consists of two rabbitfishes (Siganidae) and one surgeon fish (Acanthuridae) all of similar size (Table 1). Cone absorbances are known for *S. rivulatus* with λ_{max} being 440, 450, and 512 nm (A. Chaouat and N. Shashar, unpublished observation). Of three *Acanthurus* species previously studied (Losey et al., 2003), only one cone pigment is described for one species, two pigments for another species, and three pigments for the third species. It seems to be a general trend that the diurnal herbivores among the coral reef fishes have limited ranges of spectral sensitivity, with UV-sensitive cones being absent. This is in agreement with findings that the lenses and/or corneas of *A. nigrofuscus* and three *Siganus* species from Australian coral reefs are opaque for UV light (Siebeck and Marshall, 2001).

Limited spectral sensitivity is apparently in contradiction to the colorful schlieren images obtained in this study (Fig. 2). However, it should be kept in mind that the colors in schlieren images cannot be interpreted as absolute wavelengths and that similar wavelengths may be detected by different color channels of digital cameras (Malkki and Kröger, 2005). The LSA curves show gradual decreases in BCD between 0.7 and 0.9 R_e, which is most prominent in *A. nigrofuscus* lenses that also had the most well-defined red rings in schlieren images (Fig. 2). Because of the limited spectral resolution of schlieren photography and limited spatial resolution of the laser-scanning technique

(Malkki and Kröger, 2005), it is unclear whether there really are well-defined peripheral zones that focus relatively long wavelengths and how much these wavelengths differ from the wavelengths that are focused by other zones of the lenses. It is clear from our results, however, that the optical properties of the lenses differ between diurnal coral reef fishes with different food preferences (compare the results from diurnal herbivores and planktivores shown in Fig. 2).

Diurnal planktivores

Among the diurnal planktivores, there are the damselfishes (Pomacentridae) *C. viridis* and *D. marginatus*. The retinas of pomacentrids usually have high cone densities, and damselfishes are regarded as having acute color vision (McFarland, 1991; Hawryshyn et al., 2003). *Chromis viridis* has four cone pigments with λ_{max} ranging from the near-UV to the green range of the spectrum (367, 478, 493 and 524 nm) (Hawryshyn et al., 2003). No such data are available for *D. marginatus*, but closely related species have been studied and at least three other *Dascyllus* species have four spectral cone types with λ_{max} similar to those of *C. viridis* (Hawryshyn et al., 2003; Losey et al., 2003). The steep rises in BCD for peripheral BEPs and dark outer zones in schlieren photographs (Fig. 2) suggest that the periphery of the lens is used to focus UV light in both species. Many species of the zooplankton readily absorb UV light (Johnsen and Widder, 2001) and are therefore detectable as dark objects against back-scattered UV light (Browman et al., 1994; Losey et al., 1999; Losey et al., 2003). The ability of damselfish lenses to focus UV light suggests that the animals also have acute vision in this range of the spectrum. In trout (*Salmo trutta*), the cone ratio is 2:1:1 (long- and middle-wavelength sensitive, short-wavelength sensitive, UV-sensitive, respectively) (Bowmaker and Kunz, 1987). If damselfishes also have such high relative numbers of UV-sensitive cones, high spatial acuity in the UV range would not come as a surprise.

The lens of *P. squamipinnis*, which is a sea bass (Serranidae), is opaque to UV light (Siebeck and Marshall, 2001). This agrees with our observations that the LSA curve of this species shows least variation in BCD of all studied diurnal planktivores and that a dark outer ring is absent in schlieren photographs (Fig. 2). *Dascyllus marginatus* and *P. squamipinnis* share a similar depth distribution (see the Appendix) and time of activity (Rickel and Genin, 2005). They differ, however, in their food preferences, with the former having a higher appetite for Appendicularia, which appear transparent in the visible part of the spectrum (Rickel, 2005) and thus UV vision may improve the ability of *D. marginatus* to detect its prey organisms.

Nocturnal planktivores

The nocturnal planktivores are the most homogenous group in this study, with all species being cardinalfishes (Apogonidae; Table 1). The results from laser-scanning and schlieren photography show that the optical properties of the lenses are similar, but not identical between species (Fig. 2). Visual pigment absorbances are only known for the closely related *Apogon kallopterus*. The species has three spectral types of cone with sensitivities clustering in the blue and blue–green range (λ_{max} 441, 494 and 516 nm) (Losey et al., 2003). This agrees well with the rather colorless schlieren

photographs and flat LSAs obtained from these species (Fig. 2), which indicate that only a limited spectral range can be in focus on the retina. Furthermore, *A. annularis* cardinalfish were not able to detect prey organisms smaller than 0.9 mm in diameter (Holzman and Genin, 2005). It appears that the nocturnal planktivores have sacrificed wide spectral sensitivity and high spatial acuity for high sensitivity. They could therefore afford to minimize relative focal length of the lens (Table 1). Short relative focal length means that the *f*-number of the eye is small and thus the light gathering ability high. It also means that depth of focus is short, such that LCA is particularly destructive, and that image magnification is low, which may limit spatial resolution.

Comparisons between groups

The most complex lenses, with regard to variation in BCD, were found in diurnal planktivores living in shallow water (the damselfishes), presumably because these animals need to see planktonic prey against a background of scattered UV light. This range of the spectrum seems to play a crucial role in the sensory worlds of these species, and their lenses have evolved the ability to focus very short and rather long wavelengths at the same distance from the lens. This is an impressive achievement because color dispersion and thus LCA increases almost exponentially in the UV range of the spectrum (Hecht, 2002). Large differences between the focal lengths of the lens (Fig. 2) are necessary to correct for LCA.

Nocturnal planktivorous cardinalfishes (Apogonidae) and the diurnal planktivorous sea goldie *P. squamipinnis* (Serranidae) do not have UV sensitivity. Consequently, their lenses showed less variation in BCD. The lenses are, however, undoubtedly multifocal, which agrees with the fact that even these species have several spectrally different cone types. The same is true for the diurnal herbivorous species. In contrast to planktivorous fishes that use the periphery of the lens to focus short wavelengths, herbivorous species use the periphery of the lens to focus long wavelengths. The reason for this difference may be found in the need for distance estimation. The accommodative state of the eye may be used to judge distance to an object of interest. Such a mechanism has been shown to guide the predatory tongue strikes of chameleons (Harkness, 1977; Ott et al., 1998). The periphery of the lens has a short depth of focus, which means that wavelengths focused by this region of the lens can be used for distance estimation of high accuracy. While many diurnal planktivorous fishes that forage close to the surface are interested in UV-absorbing zooplankton, herbivorous fishes graze green to red algae growing on rocks and dead corals. This type of food should not present a UV-specific contrast.

Predators

The predatory species in this study have different diel activity periods. The cornetfish (*F. commersonii*) is diurnal to crepuscular and the lionfish (*P. miles*) is crepuscular to nocturnal (Golani, 1999). The species also differ markedly in body shape (Fig. 4) and foraging activities (Appendix). We therefore expected considerable differences in the optical properties of the lenses.

Surprisingly, judging from the schlieren images, *F.* *commersonii* and *P. miles* seem to have almost identical lenses. Laser-scanning revealed, however, that there is a sizable difference in focal length (Fig. 4, Table 1). The lenses of *F. commersonii* had an average relative focal length of 2.69 R_e, while focal length was 2.38 R_e in *P. miles* (Table 1). Long focal length is equivalent to high optical magnification of the image and low light-gathering ability. This agrees with the differences in lifestyle and body shape between these species. *Fistularia commersonii* is mainly active during the day and performs rapid strikes from a longer distance and, because of its fused and extremely elongated jaws (Helfman et al., 1997), has a longer distance between the eyes and the mouth opening. The fish therefore benefit from high image magnification. *Pterois miles*, on the other hand, has a shorter operating distance and forages at lower light intensities. Correspondingly, the species has eyes of lower image magnification and higher light-gathering ability. Both species, however, seem to have about the same needs with regard to the spectral composition of the light that is focused on the retina, as are indicated by the similar appearances of the lenses on schlieren photographs (Fig. 3).

General discussion

Each species seems to have a particularly adapted lens and there are also lifestyle-dependent differences between different groups of species. The multifocal properties of fish lenses thus are specific adaptations and not by-products of mechanisms controlling lens development. Some optical features of the lenses can be interpreted as adaptations to the visual needs of the animals, while the adaptive values of others, such as the dips in the LSA curves between 0.6 and 0.7 R_e in *C. viridis*, *D. marginatus*, *A. exostigma* and *C. novemstriatus* (Fig. 2), remain elusive.

It has previously been demonstrated that multifocal optical systems exist in freshwater teleosts (Kröger et al., 1999; Malkki and Kröger, 2005) and a variety of terrestrial vertebrates (Kröger et al., 1999; Malmström and Kröger, 2006). This study adds eleven species of marine teleosts to the growing list of vertebrates that are known to have multifocal optical systems. Such optical systems seem to be widespread among vertebrates. It may be that multifocal lenses are an original vertebrate trait that has secondarily been lost in terrestrial diurnal species, such as humans. The firefly squid (*Watasenia scintillans*) is one of a few cephalopod species known to have the potential for color vision and a well-developed, relatively large eye of the camera type. It has, however, a monofocal lens, and the problem of LCA is solved by a banked retina (Kröger and Gislén, 2004). The lenses of other invertebrates having well-developed lens eyes, e.g. spiders, have not been studied so far, but most of these eyes are so small that chromatic defocus may not be a problem.

Conclusions

Multifocal lenses are present in a variety of coral reef fishes and their optical properties correlate well with the fishes' lifestyles and cone absorbances. According to our results and those of previous studies, multifocal optical systems appear to be common among teleosts and terrestrial vertebrates. Multifocality of the lens may therefore be a trait of old evolutionary origin that is characteristic for vertebrates.

Appendix

Species descriptions

Descriptions of the fishes are summarized from other publications (Randall, 1986; Khalaf and Disi, 1997; Golani, 1999).

Diurnal planktivores

Chromis viridis (Cuvier 1830), common name: blue-green chromis. Occurs in shallow waters down to approx. 12 m in schools within branching corals. Standard length (*SL*) 3–8 cm, maximum total length (*TL*) 9.5 cm.

Dascyllus marginatus (Rüppell 1829), common name: blackbordered damselfish. *SL* 3–5 cm, maximum *TL* 6 cm. Abundant down to depths of 12 m, occurs down to 30 m. Lives in schools of up to 20 individuals within and around a coral head. Feeds exclusively on zooplankton.

Pseudoanthias squamipinnis (Peters 1855), common name: sea goldie. *SL* females: 8–10 cm, males 12–15 cm. Lives in schools reaching hundreds to thousands of individuals near the reef front. Abundant down to 15 m in depth, occurs down to 35 m. Feeds exclusively on zooplankton.

Both *D. marginatus* and *P. squamipinnis* have been shown to be visual predators on zooplankton. They escape into shelter as soon as there is insufficient light for detecting their prey. They are also relatively confined in their movements and capture planktonic prey from the water body by short bursts of rapid swimming (Rickel and Genin, 2005).

Nocturnal planktivores

Apogon cyanosoma (Bleeker 1853), common name: yellowstriped cardinalfish or Fanas (Arabic). *SL* 2–6 cm, maximum *TL* 7 cm. Inhabits lagoons and reef related areas down to depths of 50 m. Often found near *Diadema* sea urchins. Forages at night for zooplankton and small fishes.

Apogon exostigma (Jordan and Seale 1906), common name: narrowstripe cardinalfish. Maximum *TL* 11 cm. Found near coral reefs down to 25 m. Forages at night for zooplankton.

Cheilodipterus novemstriatus (Rüppell 1838), common name: Indian Ocean twospot cardinalfish. Maximum *TL* 12 cm. Forages at night for zooplankton, gastropods and small fishes. Found down to 10 m depth.

These fishes spend the day hours in hidden, unlit sites and start to forage at dusk. Their eyes are highly sensitive and *Apogon annularis*, another nocturnal planktivore, is able to successfully prey on planktonic organisms during moonless nights at depths of at least 2–3 m (Holzman and Genin, 2003).

Diurnal herbivores

Siganus luridus (Rüppell 1829), common name: dusky spinefoot. *SL* 5–25 cm, maximum *TL* 30 cm.

Siganus rivulatus (Forsskål 1775), common name: marbled spinefoot. *SL* 10–22 cm, maximum *TL* 30 cm.

Both rabbitfishes occur down to 40 m. They have crossed the Suez Canal and established populations in the eastern Mediterranean.

Acanthurus nigrofuscus (Forsskål 1775), common name: brown surgeonfish. *SL* 8–18 cm, maximum *TL* 22 cm. Limited to a maximum depth of 25 m.

All species in this group are exclusively herbivorous, although small invertebrates, which are likely to occur on the consumed algae, are occasionally found in gut contents. Algae are grazed from rock surfaces and dead coral areas.

Predators

Fistularia commersonii (Rüppell 1835), common name: cornetfish. *SL* 40–120 cm, maximum *TL* 150 cm. Often found high in the water column near the reef or above adjacent sandy habitats, or a few cm away from the bottom in shallow waters down to 10 m. The animals swim slowly in the water body and perform rapid lunges at small fishes using strong tail undulations. The diel activity period is diurnal to crepuscular.

Pterois miles (Bennett 1828), common name: lionfish. *SL* 10–40 cm, maximum *TL* 50 cm. Found down to a depth of 50 m. A nocturnal to crepuscular predator that occasionally feeds during the day. Lionfishes swim slowly with all fins widely spread and 'herd' small fish to within striking range, and then lunge and engulf prey using opercular suction. Several lionfish may collaborate in herding and attacking schools of prey.

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