

## RESEARCH ARTICLE

# Adaptation in the optical properties of the crystalline lens in the eyes of the Lessepsian migrant *Siganus rivulatus*

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### SUMMARY

Vision is an important source of information for many animals. The crystalline lens plays a central role in the visual pathway and hence the ecology of fishes. In this study, we tested whether the different light regimes in the Mediterranean and Red Seas have an effect on the optical properties of the lenses in the rivulated rabbitfish, *Siganus rivulatus*. This species has migrated through the Suez Canal from the Red Sea and established a vital population in the Mediterranean Sea. Longitudinal spherical aberration curves and focal lengths of the fish lenses were measured by laser scans and compared between the two populations. In addition, rivulated rabbitfish from the Mediterranean Sea were exposed to colored light (yellow, green and blue) and unfiltered light for periods of 1 or 13 days to test for short-term adjustments. Lens focal length was significantly longer (3%) in the Red Sea population. The shorter focal length of the Mediterranean population can be explained as an adaptation to the dimmer light environment, as this difference makes the Mediterranean eyes 5% more sensitive than the eyes of the Red Sea population. The difference may be due to genetic differences or, more likely, adaptive developmental plasticity. Short-term regulatory mechanisms do not seem to be involved.

Key words: Lessepsian migration, adaptation, fish, lens.

### INTRODUCTION

Vision is an important source of information for many animals and a variety of different eye types have evolved (Land and Nilsson, 2002). Vertebrate eyes are generally similar in design to a photographic camera. However, a good camera objective consists of a number of lenses to correct for various kinds of optical aberrations, whereas there are at a maximum only two refractive elements in a vertebrate eye: the cornea and the crystalline lens. In aquatic vertebrates, the cornea interfaces with water on its outer surface and aqueous humour on its inner surface. Both media have relatively high refractive indices (RIs) and, if the cornea is thin, which is the case in most species, its refractive power is negligible (Matthiessen, 1893; Mandelman and Sivak, 1983). The task of focusing light on the retina is thus left to the lens alone.

Among the aquatic vertebrates, the lenses of bony fishes (teleosts) have received the most attention, mainly because of easy access to fresh material and the simple geometries of the lenses, which are typically spherical (Pumphrey, 1961; Walls, 1964; Sivak and Luer, 1991). Spherical lenses made of homogenous materials (e.g. glass) suffer from longitudinal spherical aberration (LSA). Maxwell was first to note that fish lenses are almost free of LSA (Maxwell, 1854). He suggested that LSA is greatly reduced in fish lenses by an RI gradient, with the highest RI in the center of the lens and the lowest RI at the surface.

Another optical problem arises from dispersion: the RI of any transparent medium, except for a vacuum, is wavelength dependent. In consequence, optical systems focusing a wide range of wavelengths (polychromatic light) suffer from longitudinal

chromatic aberration (LCA): light of different wavelengths is focused at different distances from the optical system, which in a vertebrate eye leads to chromatic blur on the retina. The blurring effect is most pronounced in optical systems with small  $f$ -numbers (i.e. the system's focal length divided by its aperture diameter). Fishes have powerful lenses with normalized focal lengths of 2.2–3.3 lens radii ( $R$ ) (Matthiessen, 1882; Kröger et al., 2009). In many species, the iris is unresponsive to changes in light levels and does not cover any significant part of the lens, such that the aperture diameter of the optical system is identical to the lens diameter.

Although focal length is a measure of how strongly a lens focuses (or defocuses) light, it also affects the image brightness, resolution and depth of field. A lens with a short focal length focuses light from a larger spatial angle onto a given photoreceptor cell than a lens with a long focal length. A decrease of  $a\%$  in focal length will result in an  $a^2\%$  increase in image brightness. However, because the image is focused on a larger area by a lens with a longer focal length, its resolution is higher because of the fact that the photoreceptor cells do not proportionally expand with the image size. Here, an increase of  $a\%$  in focal length will result in an  $a^2\%$  increase in image resolution. Finally, more objects that are found outside the lens' field of focus are brought into focus for a lens with a longer focal length. The precise relationship between focal length change and depth of field depends on other lens parameters not considered in this study.

Many fish lenses have several focal lengths when examined with monochromatic light, i.e. they are multifocal. The distances between the focal points along the optical axis are equal to the focal length

differences due to LCA between the wavelengths of highest importance to the animals. This means that these wavelengths are correctly focused on the retina, and multifocality has been identified as a mechanism compensating for the defocusing effect of LCA (chromatic defocus). A multifocal lens creates a well-focused color image on a background of defocused light that has passed through 'wrong' zones in the lens (Kröger et al., 1999b). Compensation for chromatic defocus by the multifocal principle is common in fishes (Kröger et al., 1999b; Malkki et al., 2003; Karpestam et al., 2007; Gustafsson et al., 2008; Kröger et al., 2009; Schartau et al., 2009) and tetrapods (Malmström and Kröger, 2006; Hanke et al., 2008; Lind et al., 2008).

The multifocal properties of fish lenses show species-specific adaptations suitable for different visual needs (Malkki et al., 2003; Karpestam et al., 2007; Kröger et al., 2009). The most basic factors defining the demands that a multifocal fish lens has to meet are: (1) the light spectrum available in the environment and (2) the spectral sensitivities of the photoreceptors in the retina. It has been shown under laboratory conditions that the optical properties of fish lenses are actively fine-tuned (Kröger et al., 2001; Schartau et al., 2009). Rearing fish under different light regimes for long periods of time (10 months) induced optical changes in the lenses of the South American cichlid *Aequidens pulcher* (Kröger et al., 2001). In another study, optical changes in the lens between day and night have been detected in the African cichlid *Astatotilapia burtoni* (Schartau et al., 2009). These changes occur in parallel to retinomotor movements: from a functionally all-cone retina (color vision) at daytime to an all-rod retina (grayscale vision) at nighttime (Douglas, 1982; Burnside and Nagle, 1983; Kröger and Wagner, 1998; Burnside, 2001). In accordance with these changes in the spectral sensitivities of the active photoreceptors, the animals switch from a multifocal lens at day to a monofocal lens at night (Schartau et al., 2009).

We investigated whether the optical properties of fish lenses may change in response to naturally occurring differences between the visual environment the fish migrated from and the one it migrated to. The Suez Canal (opened 1869) has enabled animals to migrate from the Red Sea to the Mediterranean Sea (Lessepsian migration) (Por, 1978), allowing previously separated species to live in both areas (Golani, 1993; Bilecenoglu et al., 2002; Streftaris et al., 2005). The rivulated rabbitfish, *Siganus rivulatus* Forsskål 1775, has migrated from the Red Sea to the Mediterranean Sea (Ben-Tuvia, 1964) and is well established from the eastern Mediterranean Sea to the Tunisian coast and Adriatic Sea (George, 1972; Dulcic and Pallaoro, 2004). The two populations occur at similar depths in both seas (down to 30 m) and have the same herbivorous diet, favoring *Ulva* spp. (sea lettuce) (Harmelin-Vivien, 1981; Spanier et al., 1989; Lundberg and Golani, 1995; Golani and Darom, 1997; Harmelin-Vivien et al., 2005; Golani et al., 2007).

The environments differ markedly between these two habitats. The Red Sea is surrounded by deserts that generate small amounts of freshwater run-off with low nutrient content. The nearshore depth profile is steep and the water is almost free of suspended particles and algae. The water is clear blue and poor in nutrients, allowing for the growth of coral reefs. Seasonal variation is minimal, with very low precipitation and almost cloud-free skies. In contrast, the eastern Mediterranean Sea borders coastal areas with fertile soils and high agricultural activity. Rivers contribute large amounts of run-off carrying suspended particles and dissolved nutrients. The Nile run-off in particular sweeps along the eastern Mediterranean coast, driven by the Coriolis force. The depth profile is shallow with rocky to sandy bottoms and wave action keeps particles in

suspension, increasing the turbidity of the water. Coral reefs are absent. Variations because of weather and season are pronounced, with overcast skies in winter and clear skies in summer. This results in generally less direct sunlight in winter. All of these factors contribute to a generally darker light environment in the shallow coastal regions of the eastern Mediterranean Sea than near the coasts of the northern Red Sea (Fig. 1).

We compared the optical properties of *S. rivulatus* lenses between populations from both seas. Control experiments in the laboratory were performed to safeguard against short-term adjustments of the optical properties of the lens.

## MATERIALS AND METHODS

Rivulated rabbitfish were caught in the eastern Mediterranean Sea, offshore the Maritime College of Michmoret, Israel, in August 2007. The fish were placed either in an aquarium for no longer than 20 min or kept in an underwater basket for as long as 1 h, at which point the fish were moved to indoor aquaria supplied with oxygenated filtered seawater. Lighting was through multiple windows located close to the aquaria and supplemented with fluorescent lamps. *Siganus rivulatus* is a diurnal herbivorous species and the animals were fed algae from the local coast. The Red Sea population had been sampled similarly in an earlier study by our group (Karpestam et al., 2007). Experimental transfer of fish between the Mediterranean Sea and the Red Sea is prohibited by the Israeli Nature Protection Authority to avoid biological contamination and ecological misfortune.

The main differences between the present study and that of Karpestam et al. (Karpestam et al., 2007) were the laser wavelengths used in the scans (547 nm vs 534 nm in the current experiments) and the camera model used to record the scans (Sony DCR TRV140E vs Sony HDR-HC7E in the current experiments). The results reported in this study are more precise because the newer camera had higher resolution. The use of different wavelengths led to a systematic bias in focal length because of LCA. The magnitude of this effect was estimated from measured LCA in fish lenses (Kröger and Campbell, 1996). The two main differences are more formally inspected below.

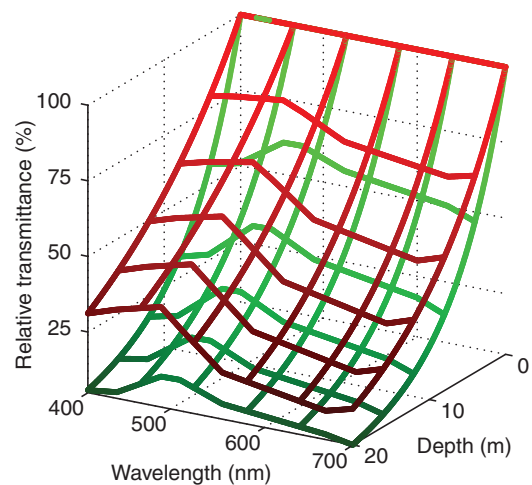


Fig. 1. Spectra of transmitted downwelling light in the coastal northern Red Sea (red mesh, taken near the Marine Biological Laboratory, Eilat, Israel) and the coastal eastern Mediterranean Sea (green mesh, taken off Givvat Olga, Israel, courtesy of Shai Shalev). Measurements were taken in winter in shallow waters where *Siganus rivulatus* occurs, using an underwater spectral radiometer (Biospherical Instruments PRR800). Note that any given depth is reached by more light of all wavelengths in the Red Sea.

To examine the effects of short-term exposure to different light environments, a laboratory experiment was conducted. Forty-nine fish from the Mediterranean Sea were divided into six groups, each containing between five and nine individuals. The groups were placed in aquaria with or without a covering colored filter for different amounts of time. We used a midrange band-pass filter (green; Lee Filters, Andover, Hampshire, UK) or no filter for 1 day. Two more groups were kept under the same conditions for 13 days. In addition, we exposed one group each to short-pass (blue, cellophane) and long-pass (red, cellophane) filtered light for 1 day. See Fig. 2 for the transmittance curves of the filters used.

All measurements were performed during daytime (before noon). The fish were individually killed by rapid decapitation and pithing. Fish handling and experiments were performed in accordance with the guidelines of Ben Gurion University for animal welfare and experiments with animals. The eyes were excised, and each lens was extracted from the eye through a large section in the cornea and immersed in phosphate buffered saline (pH 7.4, 290 mosmol). The optical properties of the fish lenses were determined by laser scans (Malkki and Kröger, 2005). Each lens was scanned in a meridional plane parallel to the optical axis with a thin 534 nm laser beam. Beam paths were recorded with a digital video camera. The video sequences were processed using a custom-written program that detected the laser beams in the video footage [this program was tested in previous studies (Gagnon et al., 2008; Schartau et al., 2010a; Schartau et al., 2010b)]. For diagrams detailing the optical setup used in this study, see fig. 2 in Kröger et al. (Kröger et al., 1994) and fig. 4 in Malkki and Kröger (Malkki and Kröger, 2005).

The analysis resulted in an LSA curve for each lens that describes the deflection of the laser beam as a function of where the beam enters the lens. The dependent variable of this function is the distance between the center of the lens and the point where the exiting – deflected – beam intercepts the optical axis (back center distance, BCD), and the independent variable is the lateral distance between the optical axis of the lens and the entering beam (beam entrance position, BEP). BEP values range between  $0R$ , i.e. the center of the lens, and  $1R$ , i.e. the surface of the lens, whereas typical BCD values range between  $2.2$  and  $3.3R$  (indicating f-numbers of lenses of  $1.1$ – $1.65$ ). BCD values for BEPs smaller than  $0.3R$  or larger than  $0.95R$  were excluded from the analysis because the laser scanning method has relatively low resolution close to the optical axis and

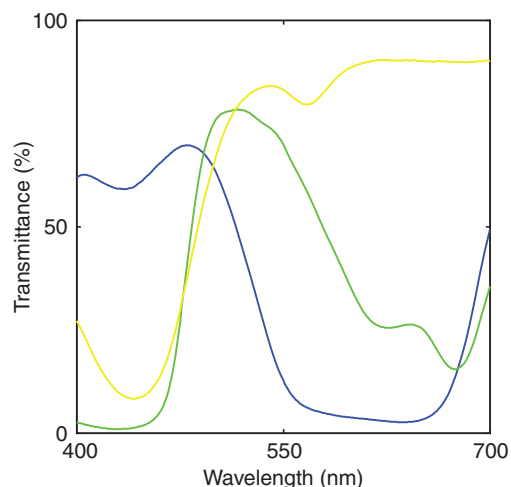


Fig. 2. Relative transmittance of the three colored filters used. The filters are color-coded: yellow, long-pass; green, midrange band-pass; blue, short-pass.

in the outer periphery of the lens (Malkki and Kröger, 2005). Furthermore, the central region of the lens contributes little to retinal illumination because of its small area, and most of the incident energy is lost by reflection for BEPs larger than  $0.95R$  (Sroczyński, 1977).

There were seven groups in this study: the Red Sea population and the six groups from the Mediterranean population. These include four 1 day exposure groups, three with colored filters and one without a filter, and two 13 day exposure groups, one with the green filter and one without a filter. The optical properties of the lenses were quantified as the focal lengths and LSA curve shapes of the lenses.

The lens focuses a cone of light on the retina if the entire aperture is illuminated. The results from scanning a lens in a meridional plane with a thin laser beam are equivalent to an axial section through such a cone of light. To determine the mean focal length of a lens, we interpolated between measured beam paths and let the number of beams used for analysis increase by a square-root function towards the periphery of the lens. The focal lengths of the lenses were normalized to lens radius and compared between groups using the Kruskal–Wallis test (MATLAB v.2008a, The MathWorks, Natick, MA, USA).

Left and right eye LSA curves were averaged for each fish. To maintain a balanced test, five (the number of samples in the smallest group) randomly chosen replicates from each group were included in the analysis. The shapes of the LSA curves were compared using analysis of similarities (ANOSIM) on the resemblance matrix of the two-stage analysis of the curves with Primer-E 6 software [see Clarke et al. (Clarke et al., 2006) for a detailed explanation of the statistical reasoning behind two-stage analysis of transect curves]. Each BCD value was associated with two factors: the group the fish came from and its BEP value. A resemblance matrix (Bray–Curtis similarity) was calculated from the (square-root-transformed) BCD values. A second stage analysis (2STAGE in Primer-E 6) was performed on this resemblance matrix with the filter period as the outer factor (the factor of interest) and BEP as the inner factor (Sommerfield and Clarke, 1995). This was done to calculate the statistical difference between distance estimations (from the resemblance matrix) for all possible combinations of distance pairs across BEP (i.e. per unique BEP value). This resulted in a resemblance matrix whose columns and rows represented the individual levels of the outer factor. This matrix was analyzed with a one-way ANOSIM to test for significant differences between the groups (Clarke and Green, 1988).

## RESULTS

There was a statistically significant difference between the groups in the focal lengths of the lenses (Kruskal–Wallis,  $P=0.008$ ). Mean focal length of the lenses was  $2.38R$  in the Red Sea population, whereas the lenses from the Mediterranean fish had a 3% shorter mean focal length of  $2.32R$  (pooled across the Mediterranean groups) (Fig. 3). A *post hoc* test (Tukey's honestly significant difference criterion) showed that the focal lengths of the lenses were significantly longer in the Red Sea population than in the Mediterranean population, whereas keeping fish from the Mediterranean Sea in spectrally unfiltered or filtered light for 1 or 13 days had no effect on the focal length of the lens. Lens radius was  $3.9\text{ mm} (\pm 0.9\text{ s.d.})$  for the Red Sea population and  $3.5\text{ mm} (\pm 0.3\text{ s.d.})$  for the Mediterranean population.

The different environments in the Mediterranean Sea and Red Sea had no effect on the shape of the LSA curves for BEPs between  $0.30$  and  $0.95R$ . Differences in LSA curve shape were also absent

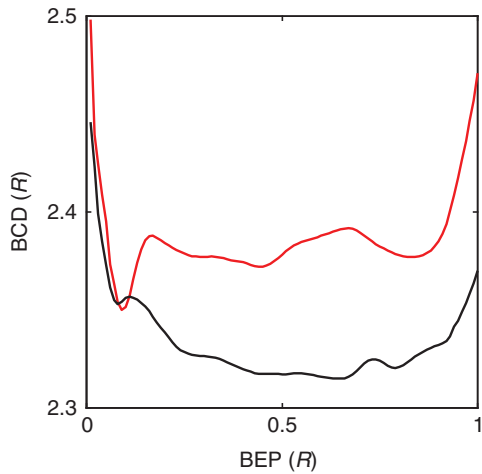


Fig. 3. Mean longitudinal spherical aberration curves of *S. rivulatus* lenses from the Mediterranean Sea (black line) and the Red Sea populations (red line). The x-axis is the beam entrance position (BEP) whereas the y-axis is the back center distance (BCD) of the laser beam; both are in lens radius (R) units.

between the groups experimentally exposed to different light environments (Fig. 4). For all groups, ANOSIM returned a global *R* of  $-0.21$ ,  $P=1.00$ .

**DISCUSSION**

The opening of the Suez Canal offered an excellent opportunity to observe evolution in progress. The passage connects the Mediterranean Sea and Red Sea, allowing fish species to colonize new habitats if they manage to adapt. *Siganus rivulatus* migrated from the Red Sea, with rocky shores and clear blue water, and has successfully established a vital population in the eastern Mediterranean Sea, with mostly sandy bottoms shaped by the particle loads of the Nile. Adaptations of the visual system to the new

environment may have played an important role in the success of the migrants.

Ideally, we would have performed a detailed quantitative comparison of the underwater visual environments in both seas. However, seasonal and daily variations, mainly in the Mediterranean Sea, thwart such efforts, unless detailed long-term monitoring programs are in place in both seas. Persistent differences in the visual environments are likely because of the vastly different geological conditions. In comparison to the Red Sea, light levels should be lower and the available spectrum narrower in the eastern Mediterranean Sea. This is confirmed by the measurements performed in winter (Fig. 1).

Relative focal length (focal length normalized to lens radius) was shorter in the Mediterranean population than in the Red Sea *S. rivulatus*, and this can be explained as an adaptation to a generally dimmer environment in the newly colonized habitat. Fish eyes, as most vertebrate eyes, emmetropize by developmental adjustment of eye size to the focal length of the optical system (Kröger and Wagner, 1996; Kröger et al., 1999a). It is therefore a reasonable assumption that the images are in focus on the retina in both populations. Light gathering ability is proportional to the inverse square of the *f*-number (Land and Nilsson, 2002) and shorter relative focal length means that the *f*-number is smaller. The difference in focal length of 3% makes the eyes in the Mediterranean group approximately 5% more sensitive than those of the Red Sea group. Contrast detection and spatial acuity are compromised under dim light conditions, but higher sensitivity of the eye counteracts these negative effects, allowing the animal to be active under dimmer light. The smaller *f*-numbers of the eyes in the Mediterranean group suggest that the animals have adapted to lower light levels. It has been suggested that the cut-off frequency of signals does not differ markedly between different underwater light environments (Johnsen et al., 2004). This indicates that the decrease in image resolution (proportional to the square decrease in focal length  $\sim 5\%$ ) and depth of focus may act as trade-off factors balancing image brightness and contrast with image acuity in the visual system of the migrant fish.

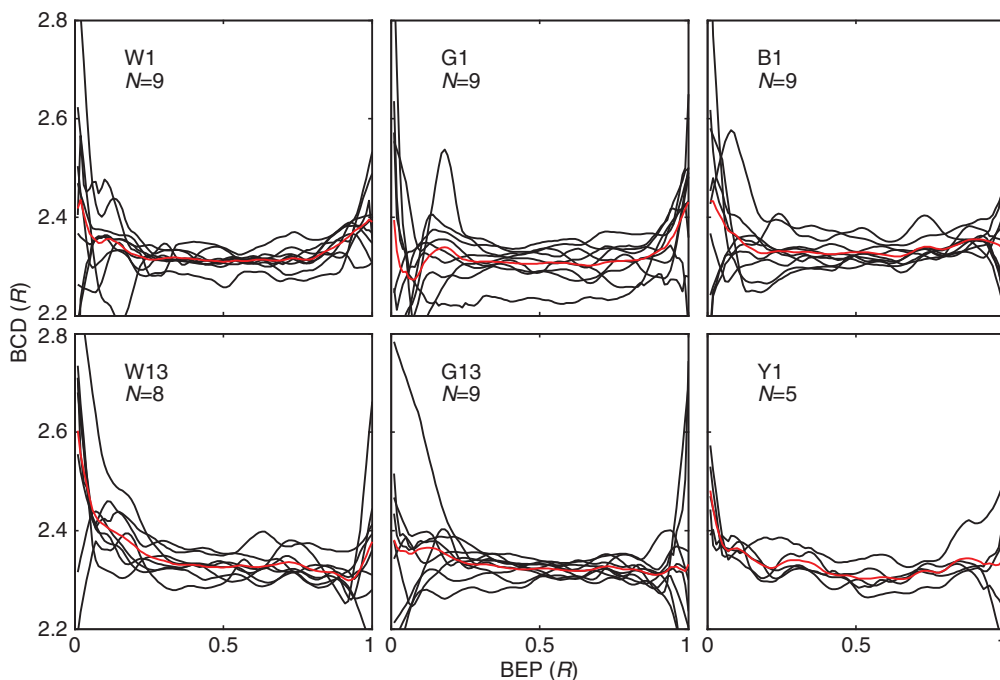


Fig. 4. Longitudinal spherical aberration curves of *S. rivulatus* lenses from the Mediterranean Sea. The x-axis is the beam entrance position (BEP) whereas the y-axis is the back center distance (BCD) of the laser beam; both are in lens radius (R) units. Black lines are the individual scans of each fish; red lines are the mean LSA curves in each group. Capital letters indicate the filter color used in each group: W, no filter; G, green; Y, yellow; B, blue. Numbers following those letters indicate the number of days that group was exposed to the respective light regime (1 or 13 days). *N*, number of replicates (fish).



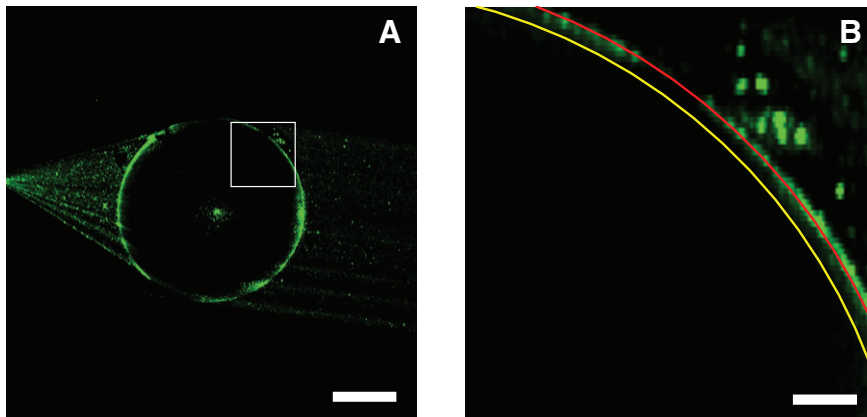


Fig. 5. A compound image of a Mediterranean *S. rivulatus* lens assembled from the video frames of laser scanning footage. Notice the distinct circular shape of the lens that is visible because of light scattering at the outer surface of the lens capsule. Scale bar, 1 mm. (B) A magnified part of the image in A (indicated by the white square in A). The green pixels are the interface between the lens and the medium and the lens surface is marked by the red line. The yellow line depicts a lens of a size equivalent to that of the Red Sea population. Note the absence of any green pixels close to or under the yellow line. Scale bar, 150  $\mu$ m.

The use of a longer laser wavelength in the earlier study (Karpestam et al., 2007) was expected to result in a longer focal length because of LCA. The results of model calculations indicate that the longer laser wavelength accounts for a 0.3% longer focal length (Kröger and Campbell, 1996; Gagnon et al., 2010). The difference in laser wavelength can thus not explain a 3% longer focal length as observed in this study. Differences in light sensitivity between the cameras used could have resulted in a consistent difference in judging lens diameter. If one camera is more sensitive than the other, lens diameter might appear larger because of light scattering at the lens surface and glare in the images. Higher laser beam intensity may result in the same bias. Overestimation of lens size results in shorter BCD values after normalization to lens radius, which in turn leads to shorter focal lengths. We calculated the difference in lens radius necessary to account for the observed difference in focal length. Fig. 5 shows that this difference would constitute a deviation too large to be explained by glare.

The LSA curves had similar shapes in all groups, indicating that the multifocality of the lens had not changed. The rivulated rabbitfish has three different photopigments [with wavelengths of maximum absorbance of 440, 450 and 512 nm (A. Chaouat and N.S., unpublished observations); also cited in Karpestam et al. (Karpestam et al., 2007)]. The absence of ultraviolet-sensitive cones and the high cut-off in the transmittance (>400 nm) of the ocular media in three other (Australian) rabbitfishes (Siebeck and Marshall, 2001) indicate that ultraviolet light is not used by these species. This reduces the need for compensation of LCA by a multifocal lens. Accordingly, the LSA is relatively flat in the herbivorous rivulated rabbitfish, in contrast to the highly structured LSAs of zooplanktivores and predators from the same environment (Karpestam et al., 2007).

We can exclude that adjustment of focal length occurred by activation of a short-term regulatory mechanism such as the one responsible for changes in lens optics between day and night in *A. burtoni* (Schartau et al., 2009). The lenses of fish from the Mediterranean Sea were unaffected by exposure to surface light levels and a full spectrum for 1 to 13 days. Likewise, exposure to skewed (red and blue filters, 1 day) or narrowed spectra (green filter, 1 and 13 days) had no effect on the optical properties of the lens. Kröger et al. showed that the optical properties of cichlid lenses had changed after rearing the fish in monochromatic light for a period of 10 months, demonstrating the existence of a mechanism regulating the optical properties of the lens in response to the light environment (Kröger et al., 2001). However, these changes affected multifocality

rather than focal length and exposure times were considerably longer than those in our study.

The differences in lens focal length between the Mediterranean and Red Sea populations may be the result of genetic changes. The Suez Canal opened in 1869, so the passage has been accessible for approximately 140 years. The *S. rivulatus* populations of the Red Sea and eastern Mediterranean Sea are indistinguishable in mitochondrial and genomic DNA, which indicates that either the initial number of migrating fish was sufficiently high to avoid a founder effect and a rapid speciation or that the migration of fish is a continuous process (Bonhomme et al., 2003; Hassan et al., 2003). These hypotheses are supported by the parasite faunas of both populations being the same (Diamant, 1998). However, because the genes controlling the focal length of the lens are unknown, genetic differences between the populations cannot be ruled out as the cause for the observed difference.

Alternatively, the difference in lens focal length may be an example of the large eco-physiological plasticity suggested for this species (Hassan et al., 2003), and may have come about by the action of regulatory mechanisms that fine tune the optical properties of the lens to the visual environment experienced during development. Such mechanisms have been shown to exist in cichlids (Kröger et al., 2001). Adaptive developmental plasticity allows species to adapt quickly, from one generation to the next, at least to some degree. Genetic adaptation may be the next step and, in due course, lead to the origin of a new species.

In conclusion, the changes occurring in the lens of *S. rivulatus* may be due to genetic changes or adaptive developmental plasticity. Their main adaptive value seems to be a tuning of the *S. rivulatus* eye to lower light levels in the Mediterranean Sea, allowing for better vision in the new habitat colonized by this migrating species. The difference is due to a change in the RI profile of the lens, as the spherical shape of the lens was unchanged. Such adaptations may require a considerable amount of time, certainly more than 13 days in an individual adult migrant; alternatively, lenses in this species may be unchangeable after the initial development.

#### LIST OF SYMBOLS AND ABBREVIATIONS

ANOSIM	analysis of similarities
BCD	back center distance
BEP	beam entrance position
LCA	longitudinal chromatic aberration
LSA	longitudinal spherical aberration
<i>R</i>	lens radius
RI	refractive index

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