Functional changes of the visual system of the damselfish *Dascyllus marginatus* along its bathymetric range

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ABSTRACT

Shallow-water zooplanktivorous fish rely on their vision for foraging. In shallow water, feeding efficiency decreases in dim light and thus the fish cease foraging at crepuscular hours. Creatures living in the lower parts of their depth ranges are expected to be exposed to limited light levels for longer hours. However, observations of the zooplanktivore *Dascyllus marginatus* showed little change in foraging duration down to 40 m deep. We asked whether the visual system's functionality changes with depth along the depth range of this damselfish; we examined eye and retina anatomy for changes in visual acuity and light sensitivity and used the optomotor response to test for spatial and temporal light summation. We found only minor changes in the anatomy of the eye that are not expected to affect visual sensitivity or acuity. However, behavioural experiments showed that the deeper water fish's test performance exceeded those of fish in shallow water under lower light levels. We found that deeper water fish responded to the optomotor test at lower light levels and also had more discriminating visual acuity in low light, which can increase their potential reactive distance. The plastic adaptive ability of the visual system to low light levels may explain the fish's ability to inhabit deeper reef habitats and thus expand their depth range limits.

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1. Introduction

Diurnal zooplanktivorous fish rely on their vision for prey capture. Rickel and Genin [1] have shown, for three diurnal zooplanktivore species, that in dim light (i.e. at dusk and at dawn) feeding rates, feeding efficiency and the reactive distance to prey items are substantially reduced. These reductions were also shown for other predatory fish and may reflect a more general pattern [2]. Dusk and dawn are important foraging times as large amounts of demersal zooplankton ascend at dawn and descend back to the bottom at dusk [3]. Some zooplanktivorous coral reef fish have a wide bathymetric range [4] exposing different fractions of the population to various intensities of light. Fish living at the limits of their bathymetric distribution are exposed to low light levels for longer periods than their shallow-water counterparts, especially in the winter and in cloudy conditions. For example, Rickel [5] found that fish leave their

shelter at dawn and return to it at dusk when light levels reach ca. $6\,\mu E\,m^{-2}\,s^{-1}$, and their foraging success drops. Rickel's data [5] reveals that at 35 m depth light levels of $6\,\mu E\,m^{-2}\,s^{-1}$ are reached about an hour later in the morning and about an hour and a half earlier in the evening than in shallow water. We would therefore expect that, if no adaptation to deep water exists, fish would begin their foraging activity later in the deep reef and would return to their cover earlier, compared with the shallow-water fish. However, our observations (Brokovich, unpublished data) on *Dascyllus marginatus* suggested the existence of a very small difference (only ca. 10 min) in the actual foraging times between shallow- (5 m) and deep water (40 m) fish of the same species (Brokovich, unpublished data). This observation raises questions regarding the ability of the visual system to function and adapt to dim light environments.

Visual perception involves, among other things, functional components of light sensitivity, spatial resolution (to enable detail discrimination and object detection), and temporal resolution (relates to motion detection) [6,7]. Wagner and Kroger [8] demonstrated that fish have considerable plasticity in their visual system and especially adaptive developmental plasticity of colour vision. Foraging successfully in relatively low light can be achieved anatomically by enhancing

the physical attributes of the eye such as pupil and lens size and/or by enhancing quantum catch efficiency in the retina, by regulating the photopigment density, outer segment length and cell density [8,9], as well as increasing the photoreceptors' light sensitivity [10]. Increased sensitivity to light can also be achieved neurologically by either increasing the effective connectivity of horizontal cells with several photoreceptors to combine their output [8] (spatial summation of light); or by using slower sampling duration of the environment to create temporary summation of photons [11,12]. Temporal resolution is the capacity to identify identical images as separate when presented within the field of view of an animal at a given time frequency. At relatively low frequencies, the series of images are perceived as separate; at intermediate frequencies, the series produce a sensation of movement of the elements in the image. The critical flicker fusion frequency, or flicker fusion threshold, is the frequency at which the images appear fused into a single structure [6]. Kröger et al. [13] and Wagner and Kröger [8] have shown that the retina can adapt to changes in the light spectrum in addition to its intensity. Rearing fish under monochromatic blue light resulted in longer cones and changes in the connectivity of horizontal (neural) cells to photoreceptors. Tropical seawater can be regarded as a blue filter; therefore fish living in deeper habitats will be exposed to more blue light relative to other wave lengths, which may result in cone elongation. Retinal adaptation or, for short periods of time, acclimatization to low light, by means of wider or longer photoreceptors or a neural combination of a number of photoreceptors, may be beneficial in enhancing light sensitivity. However, this adaptation mechanism can have a negative effect on the visual abilities of the fish, especially on visual acuity (VA). VA, also known as the resolving power of the eye, can be defined as the reciprocal of the minimum angle formed at the eye by two objects that appear as separate (minimum separating angle, MSA). To distinguish between two point stimuli, the light reaching the eye must be separated by reaching different photoreceptors. Two photoreceptors will thus make a minimum unit for stimuli detection. The photoreceptors' width and the focal length of the eye (in fishes correlated to lens size) will determine the anatomical MSA (aMSA) [14]. Although larger individuals among the fish may have larger cones (up to a given limit), which in itself increases aMSA, the lens size increases with body size leading to an increase in the eye's focal length and thus to a decrease of the aMSA [15–18]. These changes grant the fish an increased striking distance to their prey and a better choice of prey items through ontogeny [19]. Shand [15] showed that for the same lens size the aMSA for different species are similar.

As mentioned above, in addition to anatomical variations of the eye structure, it may be possible to apply spatial and temporal summation in the neurological pathway to improve light sensitivity [8,20]. Spatial summation will enlarge MSA whereas temporal summation will reduce temporal resolution. Spatial and temporal summation can be evaluated using behavioural experiments, such as the optomotor test, which uses the fish's intrinsic tendency of fixing the background on its retina and staying stationary with respect to it [21]. Behavioural MSA (bMSA) was found to be larger than aMSA [6,22–24], which indicates that in most visual systems spatial summation does occur. Therefore, behavioural studies of visual acuity are likely to yield a more accurate estimate of an animal's functional visual abilities, compared with its anatomical visual acuity [22].

Our aim in this study was to elucidate the intraspecific differences in the visual system of D. marginatus between individuals that live and forage near the surface (<5 m) and those that live and forage at depth (40 m). Specifically, we examined anatomical changes in the eye and retina between various depths and tested for spatial and temporal summation abilities using behavioural responses. As we observed deeper water fish to forage in lower light levels than their shallow counterparts, we predict that the visual system of the deep fish can adapt to low light levels.

2. Methods

2.1. Study area and species

We conducted the study in the northern tip of the Gulf of Aqaba, Red Sea. The downwelling irradiance attenuation coefficient with depth in this area has a yearly average of 0.0726/m at the PAR nm range [25]. We studied the pomacentrid fish D. marginatus (Rüppell, 1829) as a model animal (Fig. 1) because it is a very common diurnal zooplanktivore which was studied extensively in the shallow water of the Gulf of Aqaba [1,5,26] [4]. D. marginatus can be found in many branching coral species, mostly Acropora spp. and Stylophora pistillata from near the coastline down to 42 m depth [4,26]. D. marginatus recruits from the plankton to the reef from June to December and although there is no data regarding recruitment to the deeper reef or on possible movements of juveniles along the depth gradient, young juveniles may move between corals over short distances [26]. Adults, however, stay within specific corals and are organized in stable territorial harems [27].

Rickel and Genin [1] showed that in dim light such as at twilight time, the feeding rate and reactive distance of D. marginatus in the shallow reefs are positively correlated with light levels. At a depth of 42 m (the foraging limit of D. marginatus), the ambient illumination is only 4.5–5% of that at 5 m depth and consists of a narrow spectrum of mainly blue and green light (Fig. 2). D.
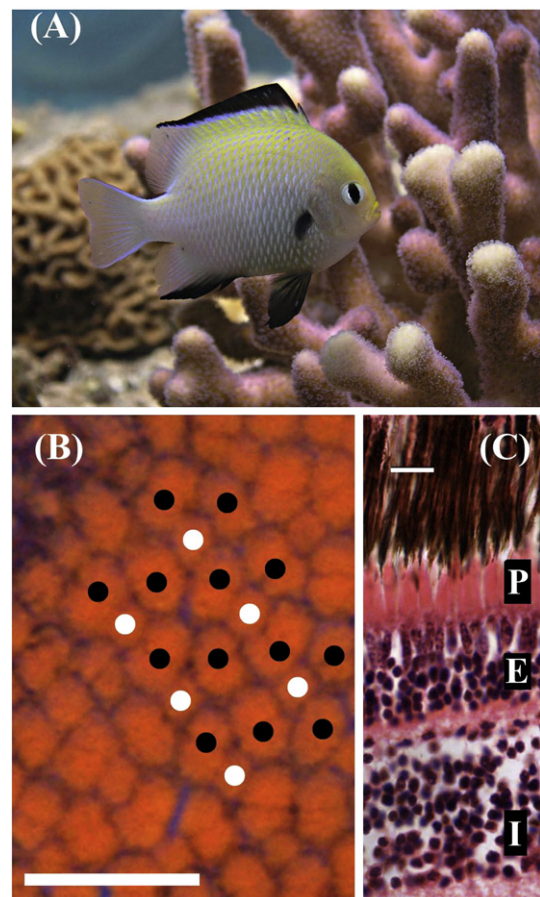

Fig. 1. (A) Photo by O. Polak of Dascyllus marginatus (Rüppell, 1829). Total length ~5 cm; (B) tangential retinal section on the level of the cones outer segments from D. marginatus. A regular square-type cone mosaic is evident with 4 pairs of double cones (black dots) with single cones in between (white dots); (C) a micrograph of a long section of the dorsal retina of D. marginatus. P = photoreceptors (cones), E = external nuclear layer, I = inner nuclear layer. White scale bars in both B and C are 10 μm long.
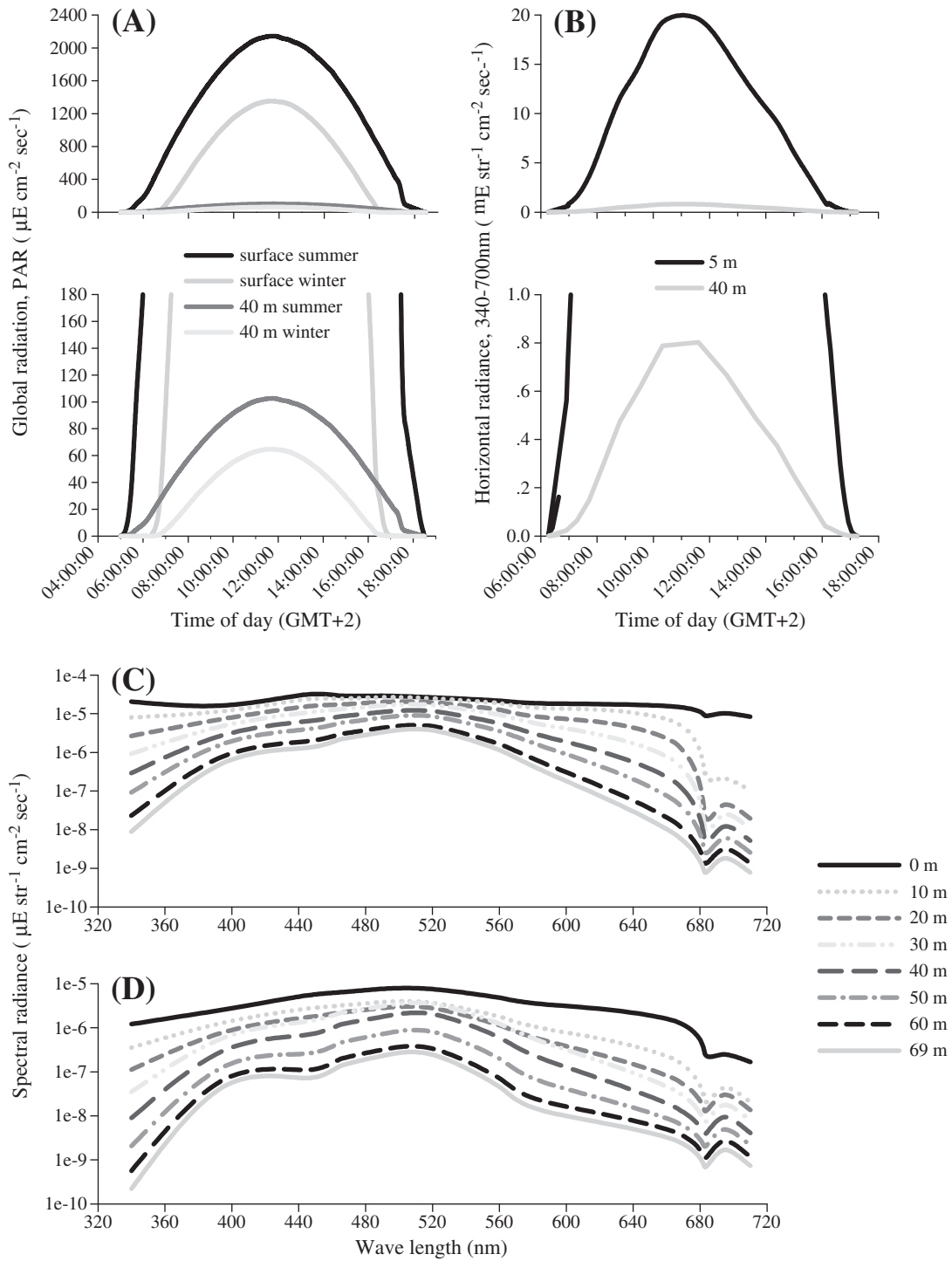
Fig. 2. Light and spectral measurements. (A) Global radiation in typical summer and winter days at the surface and calculated for 40 m in Elat. Bottom section shows a blow-up of the lower 5% range of the top figure. Dial light data were obtained from the IUI database at http://www.meteo-tech.co.il/eilat-yam/eilat_daily_en.asp from mid August and mid December 2007 and converted to PAR using calibration measurements of G.D. Calculations of light at depth were made with the year averaged attenuation coefficient of Kuguru et al. [25]; (B) Dial horizontal radiance calculated for 5 m and 40 m from data of the PRR800 Biospherical Instruments underwater spectral radiometer. Bottom section shows a blow-up of the lower 5% range of the top figure. (C) Changes in downwelling light spectrum with depth as measured with the PRR800. (D) Changes in horizontal light spectrum with depth as measured with the PRR800.

marginatus has a long reproduction and recruitment season, which enabled us to simultaneously catch fish of different size groups. The abundance, the relatively easy capture, the high site fidelity of the adult, and the wide depth range of the species, made this species a good candidate for depth-related research.

2.2. Fish sampling and processing

2.2.1. Anatomical examination

Fish were captured by placing a plastic bag on the coral colony and injecting an anaesthetic into it (clove oil–ethanol 1/4 v/v), under

Israel's Nature and Park Authority permit number 24905/2006 and 28878/2007. Altogether, 40 individual fish of different sizes from each of three depths 5 m, 20 m and 40 m were captured. Fish were sampled from all depths in the same dive. Immediately after the dive fish were sacrificed using an overdose of clove oil.

Fish were all measured by a digital calliper to the nearest 0.01 mm and digital scale to the nearest 0.01 g. Measurements included weight (W), total length (TL), eye diameter (ED) and pupil diameter (PD). Subsequently, the lens was carefully dissected and removed from one eye and its diameter (LD) was measured. LD measurements were done under a dissecting scope. We measured details of at least 19 fish from each depth.

To study retinal morphology, we removed the other eye whole and preserved it in 5% formaldehyde (~12% Neutral Buffered Formalin), for histological cross sections. Formaldehyde-fixed tissue was dehydrated in graded ethanol (from 70% to 100%), cleared in solvent for histology (Frutarom), and infiltrated and embedded in Merck paraffin (melting point of 56 °C). Sections were cut at 6 μm, and stained with Mayer's hematoxylin and Putt's eosin.

2.2.2. Fish sampling for behavioural experiments

Fish were captured from depths of 4 m and 40 m, using clove oil. Fish were transferred immediately after the dive to tanks with natural light illumination schedule and spectrum, and an open water system. Shallow-water fish were placed in opaque white tanks under direct sunlight. The tanks were 70% covered and maintained at a light level during the day equal to the conditions at 4–5 m. Deep fish were placed in a shaded blue walled aquarium under ca. 1% of natural sunlight illumination. Fish were kept at least three days in the tanks prior to the experiments, to recuperate from their oceanic removal. Fish were given supplement food at the end of each day. At the end of the experiments all fish were returned to their home corals. All dives were performed using technical diving using both open circuit scuba and Megalodon™ closed circuit rebreathers (Innerspace Systems).

2.3. Optomotor apparatus

To test for spatial and temporal summations, we used an apparatus similar to the one described by Darmaillacq and Shashar [28] with minor changes. The apparatus consisted of a 20 cm diameter cylindrical glass aquarium standing on a small motionless pedestal (Fig. 3). The aquarium was surrounded by a rotating cylindrical drum 41 cm in diameter with a black base. The drum was used to attach various strips of alternating black and white vertical stripes to

produce the optomotor response. A pair of black and white stripes is defined as the minimal response unit (one cycle) and creates an angle to the eye, depending on the distance of the fish from the cycle. The black and white stripes in each strip were of equal width; we used a variety of stripe widths for the experiments. Given the distance between the striped pattern and the holding tank, each stripe could have the width of 4.26–0.23° from the point of view of the fish in the holding tank. (Note that the bMSA is not calculated from the centre of the apparatus, but from the actual distance of the fish to the pattern at the time of the experiment. Therefore, when the fish was not in the centre we calculated according to the shortest distance to the pattern.). The minimum width still causing the fish to respond would determine the minimal separating angle for the specific light level. The drum could be turned clockwise or counter clockwise, using a variable speed electric motor, without moving the aquarium. The motor was controlled via a power source with a calibrated dial. The aquarium was illuminated by three 12 V car headlight bulbs, which were also controlled (to produce different illumination intensities) by a similar power source. A semi-opaque diffuser was used to distribute the light evenly in the measurements chamber. We placed a video camera above the aquarium and a monitor was placed next to the apparatus. To enable videotaping the fish even at low light levels we placed 20 infrared LEDs (850 nm, ROHM) above the aquarium. The whole apparatus was placed in a small measurement chamber covered throughout by a black screen, to block outside light and to reduce visual and other inputs/distractions to the tested fish.

2.4. Optomotor experiments

Individual fish were transferred from the holding tanks to the experimental chamber and left to acclimatize within the optomotor apparatus for 20 min prior to testing. At this time fish were maintained at light levels somewhat higher than in the experiment (ca. 1.41 $\mu E \, sr^{-1} \, cm^{-2} \, s^{-1}$) and were kept ventilated by an air stone. Ventilation periods were used approximately every 30 min during the experiments.

Clear cut rules were established to determine positive or negative response. We defined a positive result as a fish swimming (or turning) with the direction of the turning drum at a similar speed for at least half a circle AND stopping its movement as the drum stops. Fish were tested for both clockwise and counter clockwise rotations. If the fish responded only to one direction they were retested for both directions. A positive result was either a response for both directions or at least responding twice in the same direction. Drum direction for
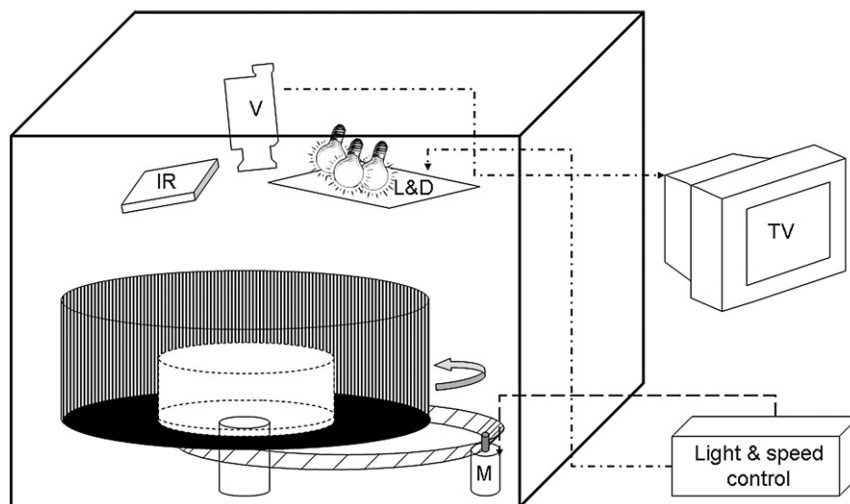

Fig. 3. A general scheme of the optomotor apparatus. The glass aquarium is in the centre on a small pedestal. The rotating drum around it is connected to an electric motor (M) via a rubber belt. Above the aquarium we placed an infrared sensitive video camera (V), 20 infrared LEDs (IR) and 3 light bulbs behind a semi transparent diffuser (L&D). Drum speed and light intensity were controlled by a changeable power source. The video camera was connected to an outside monitor (TV).

the initial trial was decided randomly. Preliminary experiments were performed to test for validity of scoring by the chosen observer (EB). Three different observers scored the same fish independently. The observers scored the fish behavioural responses similarly. Subsequent scoring was performed by a single observer (EB).

Two experiments were conducted. The first was to test for spatial summation by finding the minimum separating angle at different light levels. The second experiment tested for temporal summation by finding the flicker fusion speed. This is the maximal speed at which fast moving stripes appear as single moving stripes rather than a gray smear. Trials for spatial summation consisted of rotating stripes of various widths at a constant low speed of 2.25 rpm. For each stripe width we repeated the experiment under descending light levels, until the fish no longer responded, noting the minimum light per width. Stripe widths for each experiment were assigned arbitrarily and we also alternated the order of the subjects. We used 8 individuals from 40 m and 4 individuals from 4 m. Minimum separating angle per light level was calculated as bMSA = 2 arctan(s/d), where s is the width of a single stripe and d is the shortest distance to the fish's eye closer to the moving apparatus. Fish were also tested using white sheets and in complete darkness, as controls. Based on the bMSA and zooplankton prey size (p) we calculated the expected distance (d) at which the fish would be able to detect a non illuminated prey item (size 500 μm): d = p/tan(0.5 bMSA).

To examine temporal summation we chose a single pattern with wide stripes that resulted in very strong and clear optomotor responses (covering 3.5° calculated from the centre of the holding tank). We started the experiment while rotating the drum at a high speed (48 rpm), creating a visual gray smear and getting no response from the fish. We slowly decreased the speed every 30 s until the fish responded by swimming with the stripes. At this time (defined as the flicker fusion threshold), we noted the speed of the drum. Flicker speed (i.e. flicker frequency) was calculated as the number of stripe cycles per second [6,29]. We repeated this experiment under different light levels alternating between the directions of the turn and test objects. We tested 4 individuals from 4 m and 5 individuals from 40 m. Positive results were determined as with the spatial summation experiment, i.e. fish responded to both directions or at least twice to a specific direction.

2.5. Light measurements

Light levels were measured using a PRR800 (Biospherical Instruments Inc.) underwater spectral radiometer having 38 channels (19 in a cosine collector for irradiance and 19 narrow 10° sensors for radiance measurements) at UV–VIS–IR (300 nm–900 nm). We specifically examined light photon fluxes in the 340–700 nm range, which coral reef fish are sensitive to [30]. Light levels within the apparatus were measured pointing at a totally white surface on the drum, simulating the light reaching the fish's eye, while the radiometer's sensing head is placed at the centre of the optomotor apparatus. The radiance measured per channel was converted to μmol photons $sr^{-1} cm^{-2} s^{-1} nm^{-1}$; the total radiance (I) was then calculated by interpolating and integrating the entire spectrum from the radiance at 12 different wave lengths between 340 and 700 nm. This procedure allowed us to produce a calibration curve for the voltage of the illuminating light bulbs and the tank's illumination.

On December 23rd 2008, from 12:29 to 12:53, we cast the spectral radiometer from a boat both in horizontal and vertical positions to measure ambient light levels and downwelling light (both radiance and irradiance), from the surface to 70 m. Light was measured 5 times each second and a slow descending and ascending rates were used, so that 7 measurements/m were made. Sun position at the time of casting was ca. 34.36–32.86° altitude and 166.5–160.3° from the north (http://www.aa.usno.navy.mil/data/docs/AltAz.php). Measurements were averaged around each meter (e.g. 7 measurements between

0.5 and 1.5 were averaged for 1 m), for 12 wave lengths between 340 and 700 nm and then integrated as before, to calculate total radiance or irradiance per depth. From these measurements we calculated the attenuation coefficient for horizontal and downwelling light. To follow the dial changes in light, we attached the same spectral radiometer to the IUI jetty at approximately 1.5 m underwater, facing south, and took multiple measurements throughout the day. Using the dial data together with the attenuation coefficient for horizontal radiance, we calculated the ambient horizontal radiance at 5 m and 40 m during the dial cycle. Sun position at noon was 37.6° above the horizon.

2.6. Data analysis

Light attenuation with depth (K_d) was calculated for both horizontal and downwelling light along the depth gradient by fitting an exponential decay model to the data. K_d was used to calculate the dial light intensities at depth.

Visual acuity (VA) was defined as the reciprocal of the minimum separating angle (aMSA) in minutes of arc. It was calculated following Job and Bellwood [31] from the average cell width (w) and the appropriate focal length (f) of the eye:

$$\text{Sin}\,\alpha \approx \alpha\,(\text{minutes}) \;=\; 2w/f \times (60 \times 180/\pi),$$

where α is the aMSA, w is the average photoreceptor width and f is the focal length of the eye. Focal length is generally calculated as lens radius multiplied by the generalized 2.55 Matthiessen's ratio [31,32]. Following Shand et al. [33] and pers. communication with Ronald Kröger, we calculated the aMSA based on 90% of the lens radius.

Light sensitivity of the photoreceptors is dependent on pupil area, focal length, photoreceptor dimensions and absorbing capabilities of the pigment. Sensitivity for white light was calculated according to Warrant and Nilsson [34]:

$$S = \left(\frac{\pi}{4}\right)^2 A^2 \left(\frac{w}{f}\right)^2 \left(\frac{kl}{2.3 + kl}\right)$$

where A is the pupil diameter, f the focal length, w the photoreceptor diameter, k the absorption coefficient of the photopigment and l the photoreceptor outer segment's length. We set k for 0.03 μm^{-1} as an average for bony fishes [34].

Measurements of the photoreceptors' outer segments were accomplished using a camera (Nikon Digital sight DS-5M and DS-L1) mounted on a microscope (Nikon E600) ×1000 magnification. Measurements were performed on a computer screen using the camera's own image analysis program (UTHSCSA Image Tool for Windows 3.0, University of Texas Health Science Station, San Antonio, TX) (Fig. 1).

For the calculations of aMSA and optic sensitivity we measured the photoreceptor width of 16 fish from 5 m, 12 from 20 m and 17 from 40 m. Many fish species have a region of high cone density (RHCD, [35]), either in the form of a pit (fovea) or without a pit (area centralis) [35,36]. We measured cell width in the centre of the cross section and in two areas on both sides. 9–39 measurements were made for each area in each eye (depending on quality of histology sections). The results were tested with STATISTICA software [37], using repeated measure two-way ANOVA, with depth as one factor and the three regions as repeated measures. Further calculations were made only with the central cells. For the calculations of the photoreceptors' light sensitivity we measured the length of photoreceptors' outer segments of 17 fish from 5 m, 16 fish from 20 m and 18 fish from 40 m. We measured 16–21 cells for each eye (one eye per fish).

Allometry relations were investigated using Standardized Major Axis regression (SMA) as both axes have inherent errors to the data

[38]. Data was log-transformed when appropriate and tested for differences between slopes with the SMATR computer program [39]. When a common slope was found we tested for a difference in elevation using WALD statistic with the same program.

3. Results

3.1. Light measurements

The calculated attenuation coefficient (K_d) from horizontal radiance measurements along the depth gradient was 0.072/m ($r^2 = 0.993$, $p < 0.0001$) and the attenuation coefficient for down-welling radiance (L_d) was 0.041/m ($r^2 = 0.996$, $p < 0.0001$), showing horizontal radiance attenuation along the depth gradient to be ca. twofold faster than for downwelling radiance. The K_d for downwelling irradiance (E_d) was 0.071/m ($r^2 = 0.994$, $p < 0.0001$), which is only slightly lower than the year average K_d for downwelling irradiance attenuation [39]. Therefore, we consider our light measurements to be representative of the prevailing underwater light field in the Gulf throughout the year. These results show average ambient illumination at 40 m to be only ~5% of that at 5 m (Fig. 2).

3.2. Lens and pupil size (LD and PD)

Lens diameter (LD) was linearly correlated to fish length by the equation:

$$LD_{mm} = 0.231 + 0.0333\ TL_{mm}; r^2 = 0.966, p < 0.001.$$

We found no differences in lens diameter between individuals from the three depths (SMA, $p > 0.05$) (Fig. 4).

We measured 24, 25 and 28 pupil diameters (PD) of fishes from 5, 20 and 40 m respectively. We found no difference between depths in the relation of pupil size to length (slope angle). However, the 40 m fish had a smaller pupil on average (SMA, $p < 0.01$) (Fig. 4). The equations were:
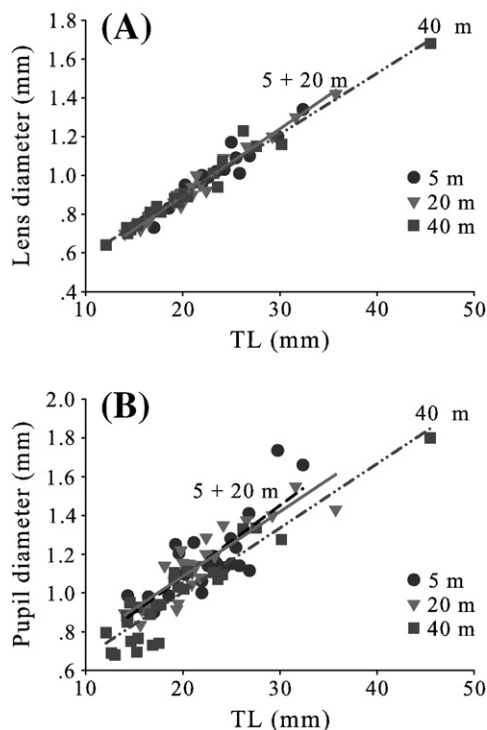
$PD_5 = 0.04607\ TL + 0.1395$, ($r^2 = 0.639$); $PD_{20} = 0.03749\ TL + 0.3298$, ($r^2 = 0.792$); and $PD_{40} = 0.03557\ TL + 0.2935$, ($r^2 = 0.869$).

3.3. Retinal anatomy

3.3.1. Photoreceptors

D. marginatus displays a square mosaic pattern of cones in the retina (Fig. 1). We did not find a concaved fovea in this fish. We found the central area of our sections to have ca. 16% more cells per mm than the outer area of each retina at all depths (repeated measure ANOVA, $p \ll 0.0001$). The localized increase in cell density indicates possible changes in cone density and size through the retina. Subsequent analysis was performed only with the central cells. No significant relation between fish length and cone width or length was found (SMA) (Fig. 5A and B). There was no difference in cone width between individuals from different depths. We found no significant difference between the slopes and elevation of the line of best fit (i.e. fish from different depths had a common slope). We found that the cones were ca. 7.8% shorter in 40 m fish averaging 9.49 μm ± 0.17 while at 5 and 20 m they averaged 10.26 μm ± 1.06 and 10.25 μm ± 1.03 respectively (one way ANOVA, $p < 0.04$). However, this difference in cone length seems to result from the fact that the 40 m fish were somewhat smaller (i.e. they shift towards the intercept along the common slope, $p = 0.001$).

3.3.2. Visual acuity and light sensitivity

aMSA declines with total fish length, which means that visual acuity increases with size (Fig. 5). We found no differences in this aMSA/size relationship between depths. An exponential decay fits the data best. aMSA $= 27.58\ e^{-0.0314\ TL}$; $r^2 = 0.81$. aMSA also declines with lens size following the equation: aMSA $= 32.31\ e^{-0.87\ LD}$; $r^2 = 0.76$.

Photoreceptor sensitivity to light averaged 0.35 ± 0.08 SD and did not change with fish length or with depth (SMA and one way ANOVA) (Fig. 5). This result implies that, within the depth range we examined, the probability of photon capture is independent of depth or body length and suggests that the shorter cells and the smaller pupils of deep fish are compensated by the focal length or by the photoreceptor width [7].

3.4. Behavioural experimental results

Fish did not turn with the rotating stripes in total darkness and did not react to a rotating totally white strip (controls). Behavioural minimum separating angle (bMSA), as calculated from the optomotor behaviour trials, increased in low light levels, especially for shallow fish. We noted that fish from 40 m start to have an advantage over the 4 m fish at light levels of ca. $2.25e^{-7}$ μE sr^{-1} cm^{-2} sec^{-1} where they still have a very low bMSA (Fig. 6). It should be noted that, at dim light, 40 m fishes #1, 2, 3 and 7 had a much lower bMSA than any of the shallow-water fish.

Flicker fusion speed decreased with decreasing light, from 35–65 cycles per second to 5–10 cycles per second. Thus, fish sampled their environment more slowly in dim light environments. We also noticed that shallow fish have faster flicker fusion speeds in medium to high light environments (Fig. 6).

4. Discussion

For some fish species the visual system has high adaptive plasticity and can adapt to spectral deprivation and low light levels [8,13]. However data is lacking regarding *in situ* adaptation of the visual system to different depths, by the same species. In this study we asked whether and how the visual system of a coral reef fish adapts to low light environments. We found that the anatomical changes in the eye between depths are small and are likely to have little effect on light sensitivity. If at all, fish coming from the deep waters had shorter cones and smaller pupils than shallow-water fish. We found that *D. marginatus*
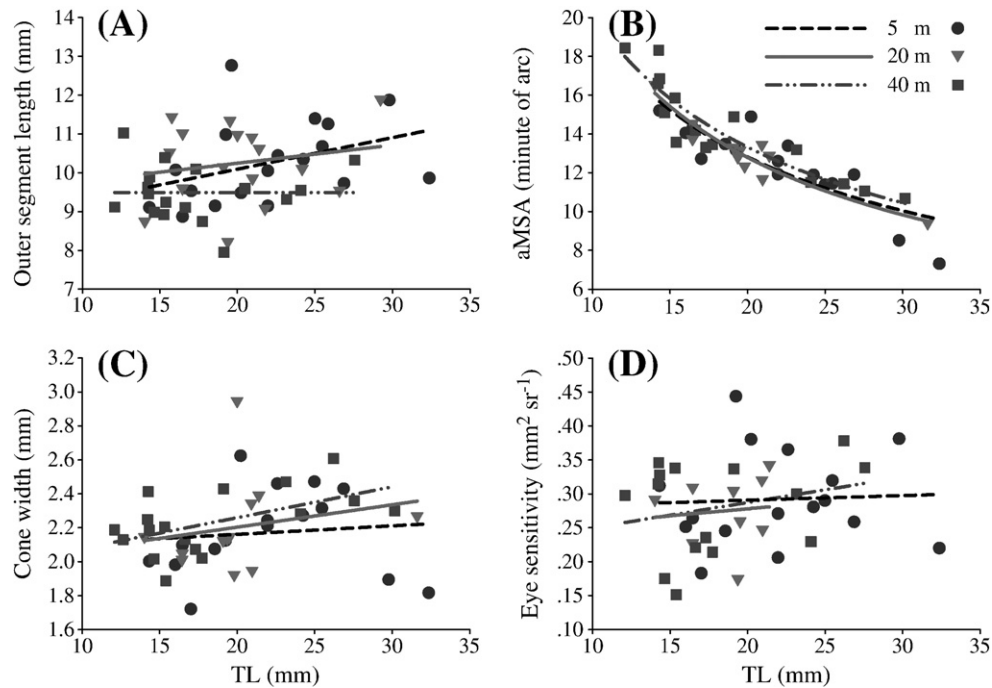
Fig. 4. Eye attributes as a function of size. (A) Lens diameter as a function of total length; (B) pupil diameter as a function of total length.

Fig. 5. Retinal measurements and calculations as a function of total length: (A) cones length, (B) cones width, (C) calculated anatomical MSA and (D) calculated light sensitivity of the photoreceptors.

has the ability for both spatial and temporal summations of light. We showed that except for the lowest light level measured, some of the deep water fish also have a slower sampling rate of the environment (low temporal resolution), compared with shallow-water fish. Low temporal resolution could increase their sensitivity to light but may affect their ability to detect movements. Most deep water fish reacted to the optomotor apparatus at much lower light intensities than their shallow-water counterparts, indicating higher light sensitivity. At low light levels some of the deep fish also had a smaller behavioural minimum separating angle (bMSA), which corresponds to higher visual acuity. This low bMSA gives these deep fish an advantage in dim light, where they can locate the prey from greater distances (Fig. 6E and F), effectively extending their reactive distance and reactive volume [40] and thus, their chances of catching their prey.

The average pupil size of individuals from 40 m was relatively smaller than shallower water individuals. A small pupil could decrease the photoreceptor's light sensitivity but, on the other hand, it could increase the depth of field of the eye, which may help focus on close objects [41]. It is apparent from our observations that deep water fish are able to forage at very low light levels and are able to utilize zooplankton from first light. Anatomical MSA consistently overestimates bMSA (e.g. [23,24]). The actual minimum size of the visual unit ('pixel' size) may depend on the convergence ratio of ganglion cells to cones and may be adjustable across the retina [42–45]. In D. marginatus the smallest bMSA, which was achieved at the highest light intensities we experimented with, was about four times larger than the aMSA (reduction in visual acuity). The bMSA suggests a basic pixel size for this species of ca. 4 cells (possibly a 4 × 4 cells unit), which could be composed of a double cone unit plus a corner cone and a central cone (Fig. 1). As we found no anatomical changes, which could lead to either higher visual acuity in deeper fish (narrower cones or larger lens) or higher light sensitivity, we believe that some of the deeper fish are able to keep the minimum pixel size under low light intensities and with minimal increasing of its size by spatially summing a few units together (e.g. Fig. 6B; fish 2). This ability of high visual acuity under low light suggested that deep water fish have higher light sensitivity than the shallow-water individuals.

There are a number of mechanisms which can increase the sensitivity to dim and bluish light besides anatomical changes and spatial and temporal summations. Some examples are: a shift in the neural horizontal cell response to shorter wavelengths [8]; optimizing the absorbance of the photoreceptor pigment to the environmental light [46], also at the individual level [47]; and increasing the sensitivity levels of photoreceptors by way of molecular mechanisms [10,48]. Ultraviolet and polarized light sensitivity of the eye may also enhance the foraging success of zooplanktivores [30,49,50]. McFarland and Loew [51] have shown that in pomacentrids, UV sensitive cells may shift in sensitivity through ontogeny as adults move to deeper water. This acclimatization response may also be present in D. marginatus living in deeper waters. The exact mechanisms enhancing visual sensitivity in D. marginatus call for further investigation.

Some fish are known to have areas in the retina with higher resolving power, either in the form of a pitted fovea or just as denser photoreceptors or ganglion cells named area centralis [35,36,44,45]. It is possible that due to our limited sections we only detected an area centralis and not a fovea, where cone density would be higher and anatomical MSA even smaller than we found [36]. As for the changes in the visual system with ontogeny, we found that the cone density for this species did not change with age (size) and that the visual acuity increased as a result of the increase in lens size with fish growth. We showed that the aMSA for this fish in relation to lens size is described by an extremely similar equation to that of Shand [15]. Collin and Partridge and Collin and Shand [42,43] suggested that the minimum visual unit (i.e. pixel size) would be the retinal ganglion cell and its dendritic arbor. Lee and Stevens [52] found that the density of retinal ganglion cells of goldfish and zebra fish increases with fish size. On the other hand Pankhurst and Eagar [53] found no change in ganglion density with size in several species, including the coral reef spiny damsel, *Acanthochromis polyacanthus*. If ganglion density increases with size for D. marginatus then the ratio of cones to ganglion cells decreases with size. Fewer cones per ganglion cell will decrease the "pixel" size, therefore further increasing acuity with fish growth.
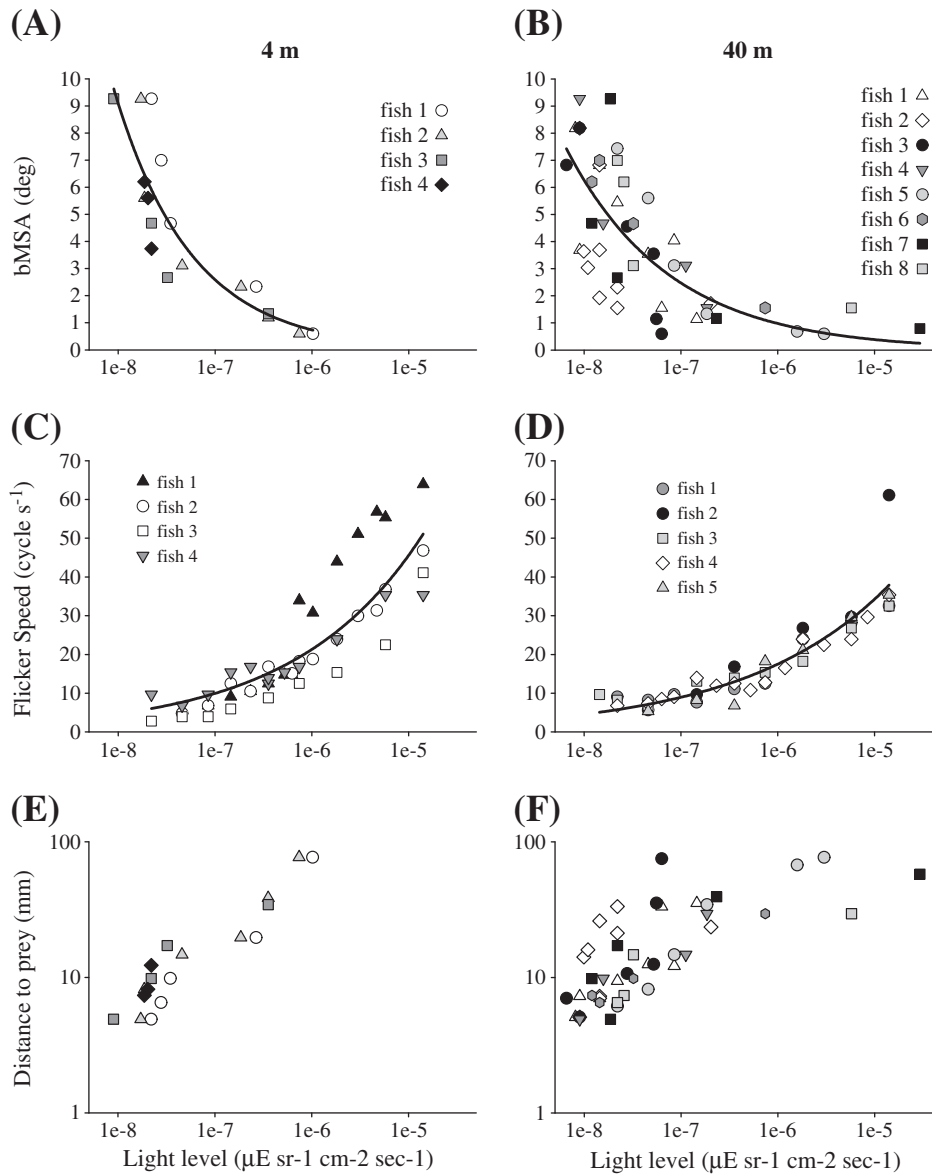
Fig. 6. Behavioural MSA of 4 and 40 m fish (A and B); flicker fusion threshold of 4 and 40 m fish (C and D); the maximal calculated distance at which fish will see a dark zooplankton 500 μm in cross section diameter (E and F). In E and F the legend is identical to A and B. Regression lines were calculated on all the fish together.

Our findings demonstrate how the plasticity of the visual system enables fish to extend their bathymetric range. Fish at the lower limits of the range will have to adapt to lower light levels. Fish species with better adaptation abilities will be able to penetrate deeper habitats. It is possible that juveniles recruiting to the deep reef may have various degrees of light adaptation plasticity, which may affect their survivorship. It is also possible, though this remains to be researched, that species with a wide bathymetric range have greater visual plasticity. Although we found changes in the visual system of some deep water individuals, they were not shared by all. Individuals living in the deeper environments without the same plasticity level as their counterparts may find it more difficult to feed at certain times. It remains to be examined whether these differences between individuals affect their physical state or even their growth rates.

Acknowledgments

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