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Retinal specializations in the blue marlin: eyes designed for sensitivity to low light levels

Kerstin A. Fritsches[A,C], *N. Justin Marshall*[A] *and Eric J. Warrant*[B]

[A]Vision, Touch and Hearing Research Centre, School of Biomedical Sciences, University of Queensland, Brisbane, Qld 4072, Australia.
[B]Department of Cell and Organism Biology, University of Lund, Lund, Sweden.
[C]Corresponding author. Email: kerstin.fritsches@uq.edu.au

Abstract. The large eyes and well-developed visual system of billfishes suggest that vision is an important sense for the detection and interception of prey and lures. Investigations of visual abilities in these large pelagic fishes are difficult, however anatomical studies of billfish eyes and retinas allow prediction of a number of visual capabilities. From the density of ganglion cells in the blue marlin (*Makaira nigricans*) retina, visual acuities of less than 10 cycles per degree were derived, a surprisingly low visual resolution given the absolute size of the marlin eye. Cone photoreceptors, on the other hand, were present in high densities, resulting in a presumed summation of cones to ganglion cells at a ratio of 40 : 1, even in the area of best vision. The optical sensitivity of the marlin eye was high owing to the large dimensions of the cone photoreceptors. These results indicate that the marlin eye is specifically adapted to cope with the low light levels encountered during diving. Since the marlin is likely to use its vision at depth, it is suggested that this line of research could help estimate the limits of vertical distribution based on light level.

Extra keywords: acuity, billfish, optical sensitivity, retinal anatomy.

Introduction

> 'Now that he had seen him once, he could picture the fish swimming in the water with his purple pectoral fin set wide as wings and the great erect tail slicing through the dark. I wonder how much he sees at that depth, the old man thought. His eye is huge and a horse, with much less eye, can see in the dark.' *The Old Man and the Sea* by Ernest Hemingway.

Almost 50 years after Hemingway wrote these lines, we are still largely in the dark about what marlin can see. Do they see in colour? How sensitive are their eyes to low light levels? How much detail can the marlin resolve in the world around it? The large eye is one of the most striking features of the marlin, suggesting vision is a crucial sense for behaviours such as prey detection and capture. Also, the optic tectum, the part of the brain devoted to vision, is well developed in this group of fish, suggesting vision is a dominant sense (Kawamura *et al.* 1981). The large body size, the remote habitat and the inability to keep billfish in captivity severely limit our options to find out what they see. However, much information can be gathered from anatomical investigations of the billfish eye and its retina, the nervous tissue lining the back of the eye. We investigated visual parameters in the eye of the blue marlin (*Makaira nigricans*), including retinal cell size and cell density, to calculate important visual capabilities such as the sensitivity of the eye to light and its acuity.

The acuity of an eye determines how much detail can be resolved, which is important for the detection of objects at distance or identification of finer detail if the target is close. A crucial determinant of the acuity of a fish's eye is the anatomical design of its retina, namely the density of cells transmitting the visual information. After passing through the optical components of the eye (the cornea and lens), light quanta (photons) reach the retina and are first absorbed by the photoreceptors. These light-sensitive cells transform the light information into neural impulses. Through a complicated network of intermediate cells, the neural signal is then received by the ganglion cells, which transmit the signals to the visual centres in the brain. Although the density, size and shapes of the photoreceptors influence the perception of an image, the density of the ganglion cells determines the maximum spatial resolution of the eye. It is the number of ganglion cells per unit area of the retina that provides the 'bottleneck' for the fineness of an image reaching the brain (Pettigrew *et al.* 1988; Collin 1989). We therefore investigated neurones in the ganglion cell layer of the blue marlin retina in order to estimate the highest spatial resolution one could expect in this species.

Diurnal billfishes such as the blue marlin are known to utilize a habitat that can stretch several hundred meters in the vertical water column. When diving down from the water surface, light levels are reduced by about 1.5 log units every 100 m in clear ocean water (Clarke and Denton 1962), which

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means that only 0.003% of the light available at the surface reaches the eye at a depth of 300 m. From archival tagging data, we know that marlin make deep dives to 200 m and beyond (Block *et al.* 1990; M. Hinton, unpublished data), and are likely to feed at these depths (Abitia-Cardenas *et al.* 1999). Presuming that vision plays an important role during feeding, one would expect the marlin eye to show adaptations that improve its sensitivity to low light levels. While a large eye and pupil are themselves important adaptations to low light levels (Walls 1942; Hughes 1977; Motani *et al.* 1999), the retinal design can also show a number of strategies that increase sensitivity. Increasing the dimensions of photoreceptors and extensively pooling retinal cells are strategies to improve photon catch at depth, and these adaptations are especially noticeable in animals inhabiting the mesopelagic zones of the deep sea (Locket 1977; Land 1981; Warrant 2000; Warrant *et al.* 2002). From the results presented here, we show that the blue marlin retina is specifically designed to cope with vision at low light levels. We suggest that this line of investigation could have important implications for the determination of a light-limited vertical distribution in the blue marlin.

Materials and methods

Investigation of the retinal anatomy

For this study, we used seven blue marlin caught by recreational game fishers in Hawaii and Australia. The fish were killed immediately after capture and the eyes fixed by immersion between 10 min and 6 h post-mortem.

For the investigation of ganglion cell distribution, the cornea and lens were removed and the eye immersed for 2 h in a periodate-lysine-paraformaldehyde fixative (2% paraformaldehyde, 0.1 M L-lysine and 0.01 M sodium periodate; Sigma; see Sagar *et al.* 1986). For the dissection, the viscous vitreous was carefully removed and the retina taken out of the calcified eye-cup through the pupil. The dark pigment layer was then removed and the retina was mounted onto a gelatinized glass slide (120 × 170 mm) with the ganglion cell layer pointing upwards. Flattening of the retina was achieved by inserting radial cuts in the retina. The ganglion cells were stained by topically applying highly concentrated cresyl violet (0.5%) with a paintbrush (under visual control through a microscope) for up to 2 h. The retina was dried overnight and then put under a coverslip.

For photoreceptor density maps, the retina was dissected from the eye before fixation and the pigment layer carefully removed using a paintbrush. The retina was then immersed in a 4% paraformaldehyde/0.1% glutaraldehyde solution in 0.1 M phospate buffer and fixed for 2 h. Subsequently, the retina was flattened by inserting radial cuts, mounted with the photoreceptor layer pointing upwards in 50% glycerol in phosphate buffer (0.1 M) and the preparation sealed with nail polish.

The technique for creating ganglion cell and photoreceptor density maps was largely derived from Collin and Pettigrew (1988*a*), and only the variations to their technique are described here. The outline of the retina was magnified and copied onto graph paper with the aid of an overhead projector (Hanimex, LVQ). The retina was then viewed through a microscope (Leitz Dialux 20), to calibrate the drawing on graph paper to the true positions of the retina on the microscope stage using landmarks on the retina. Owing to the large size and sparse distribution of the ganglion cells, counts were made at 10× magnification in most parts of the retina, increasing the magnification to 25× in the high-density areas. Counts were obtained every 4 mm, and in high-density areas, every 1 mm, resulting in more then 300 points sampled. All clearly identifiable neural elements were counted within the ganglion cell layer irrespective of size. From the data points of ganglion cell density, isodensity lines were extrapolated manually (Stone 1981). Shrinkage induced by the whole-mount method has been found to be less then 2% and was therefore negligible (Mednick and Springer 1988). Retinal whole-mounts for the investigation of ganglion cell densities were also generated for several other species of billfish, including *Makaira indica, Tetrapturus audax* and *Istiophorus platypterus*. In these species, a thick fibre layer precluded cell counts in large parts of the retina and so no meaningful cell counts were obtained from these retinas.

In two whole-mounted retinas, cone cell counts were obtained with a similar procedure to that used for the ganglion cell counts. However, owing to the small size of the photoreceptors, the cells were counted at 100× magnification at the level of the inner segment of the photoreceptor layer. Since the retina was unstained, Nomarski optics facilitated the differentiation of the photoreceptor outline. With the preparation of photoreceptor whole-mounts, we observed a larger rate of shrinkage (estimated at 17%), presumably owing to fixation of the free-floating retina after removal from the eye. The cell counts were corrected for this shrinkage.

Measurement of photoreceptors

Pieces of fixed retina from two fish were placed on a microscope slide and separated into small pieces using hypodermic needles. The preparation was then placed under a coverslip and examined under the microscope. This 'squash' preparation resulted in many small pieces of retina with loose photoreceptors of which both inner and outer segments were visible. Outlines of 20–30 of these twin cones selected from each of four areas of the retina were drawn manually using a camera lucida set-up. From these drawings, the length and width of both the inner and outer segments of the twin cones were measured using Image Tool (freeware, http://www.ddsdx.uthscsa.edu/ (accessed July 2003), University of Texas).

Acuity

From the maximum ganglion cell density found in the billfish retina, the theoretical peak acuity can be calculated. The acuity for the blue marlin can be derived following the reasoning of Collin and Pettigrew (1989). Matthiessen's ratio (Matthiessen 1882) states that in teleost fish, the focal length (f), the distance from the centre of the lens to the retina, is $2.55r$, where r is the radius of the lens. For the blue marlin, $r = 9.5$ mm (*M. nigricans* IV), which gives $f = 24.2$ mm. From this value of f, we can calculate α, the angle subtending 1 mm on the retina. This is simply given by $\tan\alpha = 1\text{ mm}/f$, which gives $\alpha = 2.36°$. The visual acuity, expressed in cycles per degree, is then given by:

$$Acuity = \frac{1}{2}\left(\frac{\sqrt{n}}{\alpha}\right)$$

where n is the number of cells per mm^2 of retina (Collin and Pettigrew 1989). The highest ganglion cell density in the blue marlin retina was found to be 1600 cells mm^{-2} (*M. nigricans* IV), giving acuity of 8.5 cycles per degree. This value accounts for the fact that two ganglion cells are needed to resolve one black and white cycle of a striped grating pattern. In other words, the marlin is able to resolve up to 8.5 black and white stripe cycles per degree of visual space.

Optical sensitivity

The absolute optical sensitivity of an eye can be calculated using Land's equation (Land 1981). In this calculation, optical sensitivity is the number of photons absorbed by one receptor per unit of luminance in the extended visual field that is being imaged. The factors influencing the optical sensitivity (S) are the size of the circular aperture (A), the focal

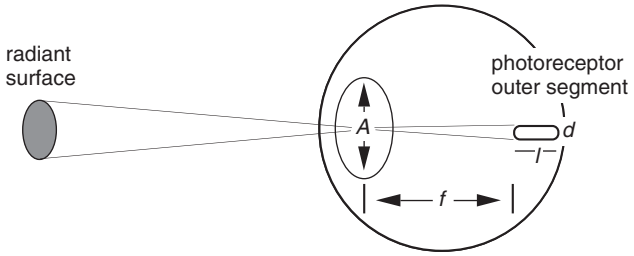

Fig. 1. Schematic drawing of the parameters required for calculating the optical sensitivity of the eye (modified from Warrant and Nilsson 1998). *A*, Diameter of the pupil aperture; *f*, focal length; *l*, photoreceptor length; *d*, photoreceptor diameter.

length of the eye (f), as well as the receptor diameter (d) and length (l), and the fraction (F) of incident light absorbed by each photoreceptor (Fig. 1).

$$S(\mu m^2 sr) = (\pi/4)^2 \times A^2 \times (d/f)^2 \times F$$

The focal length (f) was calculated from Matthiessen's ratio and the radius of the lens of the fish as described above. In fish, the diameter of the pupil (A) can be approximated as the diameter of the lens (Fernald 1990) since the majority of fish do not have pupillary movements (Collin 1997). Also, as billfish do not have a large aphakic gap, the pupil is approximately circular. The fraction (F) of incident light absorbed by each photoreceptor was calculated in two different ways. The commonly used formula $F\lambda_{max} = 1 - e^{-\kappa l}$ (Land 1981; Warrant and McIntyre 1990) only considers monochromatic light at the preferred wavelength of the photoreceptor. A recent modification of this expression allows one to calculate F using white light, which contains all wavelengths of the visible spectrum and is the predominant form of light in most habitats ($F_{\text{white light}} = \kappa l/(2.3 + \kappa l)$; Warrant and Nilsson 1998). The value for the photoreceptor absorption coefficient (κ) for bony fishes was estimated at $0.035\ \mu m^{-1}$ (Land 1981; Partridge 1990; Warrant and Nilsson 1998). The source of light is considered to be extended, as provided by a broad visual surrounding rather than a point source.

Results

Anatomy of photoreceptors

We identified three types of photoreceptors in the retina of the blue marlin: rods, single cones and twin cones. Although rods were visible, the light-adapted state of the animal and the poor condition of the tissue available for sectioning precluded closer investigation of rod density and shape. The predominant cone type is the twin cone, which consists of a pair of equally large cones with long outer segments, joined at the inner segments. In the whole-mount preparation, which provides a tangential view of the retina, smaller single cones were also visible (Fig. 2*c*).

Ganglion cell topography and acuity

The ganglion cell layer (Fig. 2*b*) in the blue marlin retina showed two high-density areas (Fig. 3*a*), which indicate that these are the best areas of vision in the eye. These regions are also called area centralae. The peak ganglion cell density in the temporal area centralis was found to be 1600 cells mm^{-2}, resulting in peak acuity of 8.5 cycles per degree in this animal. A further high-density area occurred in the nasal part of the retina, however the maximal density of cells in this area was lower, showing peak counts of 470 cells mm^{-2}. The temporal

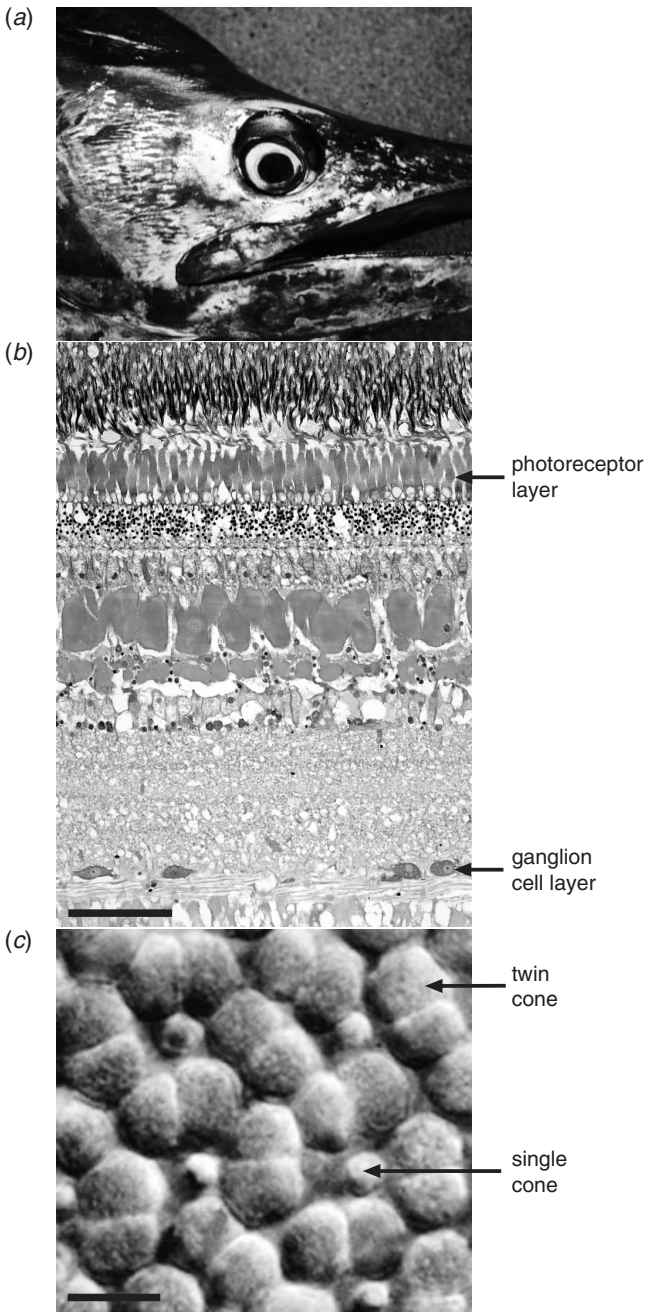

Fig. 2. (*a*) Photo of a marlin head showing the prominent eye. For example, a blue marlin with a fork–jaw length of 262 cm (161 kg in weight) has an eye diameter of 82 mm, a pupil diameter of 24 mm, lens diameter of 20 mm and a calculated focal length of 25.5 mm (personal observations). (*b*) Transverse histological section through the retina of a blue marlin, revealing both the photoreceptor and the ganglion cell layer (scale bar = 100 μm). (*c*) Single and twin cone photoreceptors as seen in a retinal whole-mount (scale bar = 25 μm).

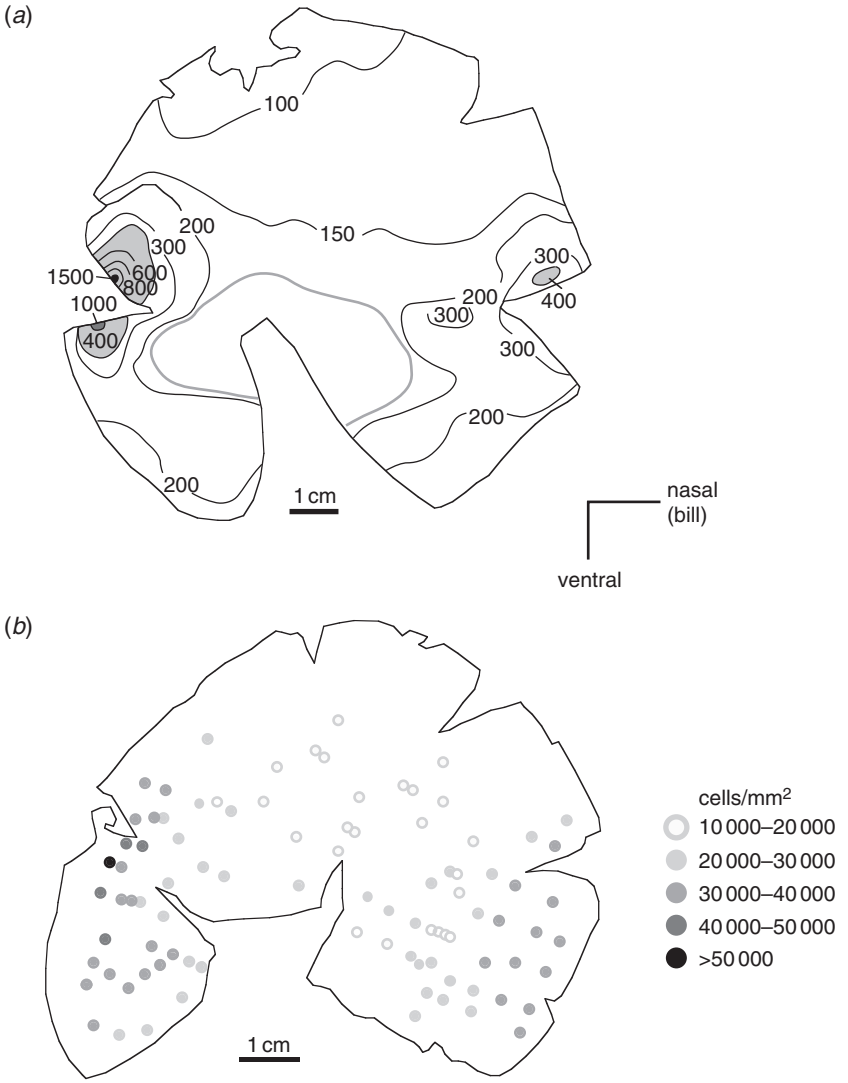

Fig. 3. (*a*) Whole-mount of the retina of *Makaira nigricans* IV showing the ganglion cell topography (right eye, densities in cells mm^{-2}). The area of highest cell density (area centralis) is located in the temporal retina. The hemispheric shape of the retina *in vivo* means that this area images what is ahead of the marlin along the bill. A second higher density area is found in the nasal retina. Cell density also increases from the dorsal to the ventral retina. The grey line indicates an area where optic fibres precluded cell counts. (*b*) Topographic map of the density of cones (both single and twin cones) in the retina of *M. nigricans* VII. The density increases from the dorsal to the ventral retina. Higher densities are also found in the nasal retina, while the peak density of cones is located in the temporal retina.

retina views the world ahead of the animal, while the nasal retina views what is behind the fish. The overall density of ganglion cells outside the high-density areas also increased from the dorsal to the ventral retina, rising from less than 100 cells mm^{-2} to more than 200 cells mm^{-2}. The second blue marlin retina showed a similar pattern of ganglion cell densities, although the densities were higher overall. The peak density was found to be 2070 cells mm^{-2} in the temporal retina; however, the smaller size of the eye of this animal resulted in similar peak acuity of 9 cycles per degree.

Cone density

Most studies of cone densities in fish are obtained from transverse and tangential sections of selected areas of the retina. These time-consuming histological procedures restrict the number of data points for a detailed density map. With the whole-mount technique employed in this study, the cone density of the marlin retina could be obtained in up to 95 places per retina, providing a detailed map that discloses variations of cone density and cone types throughout the retina (Fig. 3*b*). Similar to the ganglion cell densities, the density of cones

Table 1. Calculation of the optical sensitivity of the eyes of the blue marlin (*Makaira nigricans*) and, for comparison, the blue tuskfish (*Choerodon albigena*)

Anatomical data from Engström (1963), Ali and Anctil (1976) and Collin and Pettigrew (1989). The sensitivity of the marlin eye was calculated for a photoreceptor of average size and for the largest twin cone found (maximum). For all three cases, sensitivity was calculated for the preferred wavelength λ_{max} and for white light

	Lens diameter (mm)	Focal length (mm)	Receptor diameter (μm)	Receptor length (μm)	Fraction of λ_{max} absorbed (1)	Fraction of white light absorbed (2)	Sensitivity (μm^2 sr) Calculated with 1	Sensitivity (μm^2 sr) Calculated with 2
Blue marlin (average)	19.0	24.2	2.9	57.0	0.86	0.46	2.8	1.5
Blue marlin (maximum)	19.0	24.2	4.0	72.0	0.92	0.52	5.6	3.2
Blue tuskfish	4.8	6.1	3.0	15.0	0.41	0.19	1.4	0.6

increased from the dorsal to the ventral retina. More cones could be found in the temporal and nasal parts of the retina and high densities of up to 62 000 cells mm^{-2} were found in the temporal periphery. This peak density results in a visual acuity at the photoreceptor level of 53 cycles per degree. It is possible that even higher densities of cones exist in the temporal area centralis, as suggested by counts of the ganglion cell density (Fig. 3*a*). However, in the available preparations, it was not possible to count cone cells in the far temporal periphery.

The general topography of cones and ganglion cells match well with respect to the location of the high-density areas (Fig. 3*a*, *b*). However, when comparing numbers of cones and ganglion cells in similar-sized retinas, we found a convergence ratio of cones to ganglion cells of around 100 : 1 cells mm^{-2} in the general low ganglion cell density areas such as those in the centre of the retina. In the temporal area centralis, the convergence ratio remained at 40 : 1 cells mm^{-2}. Hence, even in this high acuity area, a significant number of cones were pooled together to form the receptive field of one ganglion cell.

Photoreceptor size and the optical sensitivity of the marlin eye

The calculation of the optical sensitivity shows the extent to which a photoreceptor captures photons from an extended light source of given intensity (Land 1981; Warrant and Nilsson 1998). Optical sensitivity depends on the design and optics of the eye and the size and shape of the photoreceptor. Since the *f* number (focal length/aperture diameter) of most teleosts is approximately constant (Fernald 1990), the anatomically defined optical sensitivity of the fish eye is entirely determined by the diameter and length of the outer segments of the photoreceptors.

The optical sensitivity can be calculated both for the preferred wavelength of a photoreceptor (λ_{max}) and for white light. Given their vertical range of several hundred meters (Block *et al.* 1990; Holland *et al.* 1990), blue marlin encounter both near-monochromatic light at depths close to their λ_{max} (Munz and McFarland 1975; Fritsches *et al.* 2000) and white light close to the surface of the ocean. The fraction of light absorbed is largely dependent on the length of the photoreceptor, and, interestingly, the marlin showed a close-to-optimal outer segment length for the absorption of the preferred wavelength λ_{max} ($F\lambda_{max} = 0.86$ for the average photoreceptor, $F\lambda_{max} = 0.92$ for the longest outer segment found; Table 1). For comparison, the blue tuskfish, an inhabitant of shallow reefs, had shorter outer segments that allowed only a fraction of 0.41 to be absorbed at λ_{max}. Wavelengths other than λ_{max}, which are present in white light, are absorbed at a lower rate. This results in a reduced fraction of light absorbed and hence a reduced sensitivity of the eye in the presence of white light (Warrant and Nilsson 1998). In both billfishes and tuskfishes, the optical sensitivity to white light was reduced by approximately half of the sensitivity calculated for the preferred wavelength.

The dimensions of twin cones changed throughout the retina, revealing larger photoreceptors in the dorsal part of the eye than in the ventral part (Fig. 4*a*, *b*). The length of the cone outer segments varied considerably; this could be owing to the difficulties in finding outer segments, which were intact for their full length. However, the thickness of the outer segment is the important measure for the optical sensitivity of the eye since light reaches the receptor perpendicular to the cross-section of the outer segment. Hence, the larger the diameter of the outer segment, the more photons are sampled. The dimensions of the cones in the blue marlin retina showed an adaptation to suit the light conditions encountered in the different parts of the eye (Fig. 4*a*, *b*). The cones located in the ventral retina, which receive images of the bright water above the fish, were thinner (2.5 ± 0.6 μm) and hence less sensitive. In the dorsal retina, which views the dark water below, the cones were noticeably larger (3.7 ± 0.6 μm) and showed twice the optical sensitivity. This is clearly an adaptation to the lower light levels below the fish (Fig. 4*c*).

Discussion

Topography of the blue marlin retina

It has been shown in numerous studies that the structure of the visual surrounds shape the regional specializations of cell

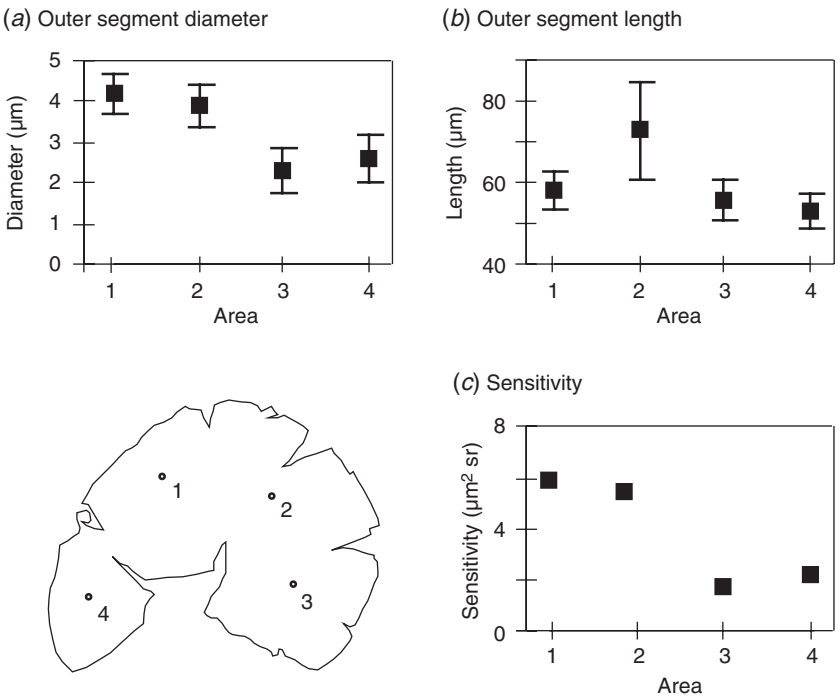

Fig. 4. Twin cone dimensions and optical sensitivity in different parts of the retina. Inset: location of the different areas of measurement in the retina of *Makaira nigricans*. (*a*, *b*) Measurements of the diameter and length of the outer segments in two dorsal retinal areas (1 and 2) and two ventral areas (3 and 4). (*c*) Optical sensitivity in the four areas based on the absorption at the preferred wavelength (λ_{max}). Note that the sensitivity is twice as high in the dorsal retina.

densities in fish (Ahlbert 1968; Collin and Pettigrew 1988*a*, 1988*b*; Collin 1997). The blue water habitat of billfishes provides a featureless environment and objects of interest can be located in any direction in the water column. As a result, blue marlin show a circular area of highest acuity in the temporal retina viewing the world ahead of the animal. The position of this area in both eyes will improve distance judgement and the resolution of objects ahead of the fish. A second peak density area in the nasal retina receives images from what is behind the marlin. While the temporal area centralis identified in this study is most likely used in feeding, the nasal area of higher cell density might allow the billfish to better observe the movement of other predators in order to avoid collisions. A study of cone densities in tunas, dolphinfishes and two species of billfishes, has also shown high cone density areas in the temporal retina, however the extensive survey was limited to few data points per retina (Tamura and Wisby 1963).

High-density areas have been found in a number of predatory reef fish (Collin and Pettigrew 1988*a*, 1988*b*; Collin 1997) and are indicative of a well-developed visual system. High-density areas also require highly developed spontaneous eye movements that point the area of best vision towards objects of interest (Walls 1942). Fast and frequent eye movements in marlin were evident in some of the close-up footage of hunting marlin (G. Harvey, unpublished data).

Estimates of acuity

Previous calculations of the visual acuity of billfishes were derived from cone cell separations (Tamura and Wisby 1963) and have resulted in acuity 3–4 times higher than the values calculated from peak ganglion cell densities in this study. The simple reason for this finding is a convergence of cone cells to ganglion cells in the area centralis in all areas of the marlin retina. A close relative of the billfish, the tuna shows a behavioural acuity of 8–10 cycles per degree, while the cone separation predicts an acuity 3 times higher (Nakamura 1968). This supports the view that ganglion cell density is a more appropriate morphological indicator of acuity than counts derived from cones, since ganglion cells represent the 'bottleneck' for information from the eye to the brain (Pettigrew *et al.* 1988).

The accuracy of ganglion cell counts is often questioned owing to the presence of displaced amacrine cells in the ganglion cell layer (Stone 1981). With conventional histological staining methods, it is impossible to distinguish ganglion cells from the non-ganglion cells. Hence, it would be more accurate to refer to the ganglion cell maps presented here as maps

of neural elements in the ganglion cell layer, assuming that the densities obtained are higher than those of the ganglion cells alone. These ganglion cell maps are intended to provide an estimate of the acuity of the animal and the topography of the retina, and to identify regions of specialization. As Collin and Pettigrew (1989) pointed out, the error introduced by inaccurate cell counts is unlikely to be larger than estimates of acuity obtained from behavioural measurements. Furthermore, in those teleosts studied so far, the topography of the cell densities was not altered when retrograde labelling identified non-ganglion cells, and visual acuities had to be revised only marginally (Collin and Pettigrew 1988*c*).

Since the internal dimensions of the marlin eye are as yet unknown, we used a commonly applied estimate for the focal length in teleost fishes, Mathiessen's ratio, to calculate both the acuity and optical sensitivity. Traditionally stated as a constant of 2.55, this ratio of focal length and lens radius ranges from 2.2 to 2.8 among a number of marine and freshwater fishes measured (Mathiessen 1982; Fernald 1990). Within this range of possible focal length in the marlin, the resulting values for acuity and optical sensitivity do not change significantly. However, further research is planned to investigate the optical properties of the marlin eye.

Considering the large size of the billfish eye, we were surprised to find a relatively low acuity, which seems to contradict a general rule that larger eyes have better acuity. During ontogeny, larger fish show a better acuity than smaller fish, both morphologically and behaviourally (Hairston *et al.* 1982; Blaxter 1986; Fernald 1991; Zaunreiter *et al.* 1991; Miller *et al.* 1993; Pankhurst *et al.* 1993; Shand 1997). This also appears to hold within different species of fish of various sizes, since larger fish with larger eyes have higher acuity (Collin and Pettigrew 1989; Zaunreiter *et al.* 1991; Shand 1997). Following this rule, large pelagic fish with large eyes should have even higher acuity, which has also been argued for ichthyosaurs (Humphries and Ruxton 2002), extinct animals that had exceptionally large eyes (Motani *et al.* 1999). Assuming that the photoreceptor size does not increase proportionally with eye size, larger eyes would allow a finer sampling grid owing to much larger focal length. However, as shown here, the strong convergent relationship of cones to ganglion cells results in a greatly decreased spatial resolution. For instance, the eye of the blue marlin is almost 4 times the size of the eye of the blue tuskfish (*Choerodon albigena*), but the acuity derived from peak ganglion cell counts of the tuskfish is 15 cycles per degree (Collin and Pettigrew 1989), compared with 8.5 cycles per degree in billfish.

It therefore seems that, at least in the case of billfishes, large eyes do not equate to high acuity, which could be explained by visual constraints in their aquatic habitat. Even in the clear tropical water habitat of the blue marlin, particles in the water introduce scatter, resulting in a 'haze' that degrades contrast with increasing distance (Lythgoe 1979; Jagger and Muntz 1993). Compared with land vertebrates, teleosts (i.e. Collin and Pettigrew 1989), marine mammals (Schusterman and Balliet 1970) and octopus (Muntz and Gwyther 1988) show limited acuity, indicating that there is a general constraint for acuity in the aquatic environment. The pronounced pooling of photoreceptor input into ganglion cells allows billfishes to maintain the same 'optimal' spatial resolving power at lower light intensities, which is important for hunting at depth. We suggest that maintaining the same acuity at lower light levels might be a strategy that limits absolute acuity in many marine animals.

The marlin retina is designed for sensitivity

The retinal anatomy of the blue marlin shows a number of adaptations that increase the sensitivity of the eye, a strong indication that the animal has evolved to cope with low light levels. Sensitivity is improved by a high convergence ratio of cones to ganglion cells, even in the area centralis, which also shows this hard-wiring spatial pooling. This is in addition to any further pooling of ganglion cell signals that might occur at higher levels in the visual system. Most shallow-water fish studied so far live in brightly lit environments and only have a slight convergence of cones to ganglion cells in the area of best vision (O'Connell 1963; Wagner 1978; Shand 1997). On the other hand, many deep-sea species adapted to very low light intensities show a grouping of photoreceptors within reflective tapetal cups. These groups act like macroreceptors that improve the photon catch (Locket 1977; Collin *et al.* 1998).

In this study, we used twin cones to estimate the optical sensitivity of the billfish eye. This was partially owing to the fact that little information is available yet on rod morphology in this fish. However, it is noticeable that twin cones are the largest and most dominant receptor in the billfish retina, suggesting their importance for sensitivity, while the rods are very thin. Relatively large numbers of twin cones are found in fish that inhabit deeper water, leading to the conclusion that this cone type is more sensitive to light than single cones (Lyall 1957; Tamura 1957; O'Connell 1963). Identical twin cones are likely to be involved in both brightness and colour discrimination (Burkhardt *et al.* 1980). Furthermore, spatial resolution (see Zaunreiter *et al.* 1991) and hence motion perception (Gegenfurter *et al.* 1999), is better in cones than in rods. This makes twin cones suitable candidates for optimal function in the interface between bright and dim light vision, and could be important for an animal that dives quickly through a range of light levels.

Owing to the unusually large size of the cones, billfish probably have one of the highest absolute optical sensitivities among the teleost fishes. This optical sensitivity ($S = 5.6\ \mu m^2$ sr) is not as high as that found in some deep-water invertebrates such as a deep-sea shrimp (*Oplophorus*: $S = 3300\ \mu m^2$ sr) or the bathypelagic ostracod *Giantocypris* ($S = 6100\ \mu m^2$ sr) (Land 1981). However, these invertebrates achieve high optical sensitivities by having an eye with a large relative

aperture. Optical sensitivities in humans range over 3 log units, depending on the pupil size and whether the sensitivity is considered for peripheral or foveal photoreceptors (for the fovea in bright light $S = 0.023\ \mu m^2$ sr; Land 1981). With very few exceptions, the pupils of fish do not constrict (Guthrie and Muntz 1993; Collin 1997), so changes in the aperture in response to bright light are not possible. For teleosts, the one way to improve optical sensitivity is to enlarge the photoreceptors, which appears to be the strategy adopted by billfish. These anatomical improvements in photon catch can, however, only improve the sensitivity of the eye by a factor of 1000 which is relatively low compared with light intensities that vary 1000 million times between day and night (Warrant 1999). A more effective way to increase photon catch is achieved by summing photons in space and time (Snyder *et al.* 1977; Lythgoe 1979). Using a mathematical model (Warrant 1999), we plan to use the anatomical data presented here to explore the importance of summation for billfish vision at low light levels. This approach will allow us to estimate the minimum light levels at which a marlin is still capable of using its visual system.

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