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The optics of the growing lungfish eye: Lens shape, focal ratio and pupillary movements in *Neoceratodus forsteri* (Krefft, 1870)

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Abstract

Lungfish (order Dipnoi) evolved during the Devonian period and are believed to be the closest living relatives to the land vertebrates. Here we describe the previously unknown morphology of the lungfish eye in order to examine ocular adaptations present in early sarcopterygian fish. Unlike many teleosts, the Australian lungfish *Neoceratodus forsteri* possesses a mobile pupil with a slow pupillary response similar to amphibians. The structure of the eye changes from juvenile to adult, with both eye and lens becoming more elliptical in shape with growth. This change in structure results in a decrease in focal ratio (the distance from lens center to the retina divided by the lens radius) and increased retinal illumination in adult fish. Despite a degree of lenticular correction for spherical aberration, there is considerable variation across the lens. A re-calculation of spatial resolving power using measured focal ratios from cryosectioning reveals a low ability to discriminate fine detail. The dipnoan eye shares more features with amphibian eyes than with most teleost eyes, which may echo the visual needs of this living fossil.

Keywords: Dipnoi, Optics, Development, Longitudinal spherical aberration of the lens, Matthiessen's ratio

Introduction

The Australian lungfish *Neoceratodus forsteri* is the only member of the ceratodontid family of lungfishes within the order Dipnoi and currently believed to represent the closest living relatives to the land vertebrates (Yokobori et al., 1994; Tohyama et al., 2000; Venkatesh et al., 2001; Brinkmann et al., 2004). However, the comparative evolutionary complexity of the sensory systems of dipnoan fish is unknown and very little information exists regarding the gross structure of the lungfish eye (Günther, 1871; Kerr, 1902; Grynfeltt, 1911 cited by Rochon-Duvigneaud, 1943; Walls, 1942; Rochon-Duvigneaud, 1943). In the case of the Australian lungfish, *N. forsteri*, this may be the result of their limited distribution in Southeast Queensland, Australia, and their current protected status by the Australian government.

Recent work on the retina of the Australian lungfish *Neoceratodus forsteri* has shown that the eye is designed for increased sensitivity but poor spatial resolving power (Bailes et al. 2006*a*, 2006*b*). The characterization of at least four morphological types of photoreceptors, some containing colored intraocular filters, and the expression of all five vertebrate visual opsins within the retina, also provides the potential for colour vision (Robinson, 1994; Bailes et al., 2006*a*; Bailes et al., unpublished). The size and morphology of lungfish photoreceptors show more similarities to retinal photoreceptors in urodele amphibians than teleosts (Bailes et al., 2006*a*), while it is unknown if the optics of the lungfish eye follows the *bauplan* of teleosts or of amphibians.

This study describes the ocular dimensions, the lens optics with respect to longitudinal spherical aberration, and pupillary movements in *N. forsteri*. Accurate lens measurements also enable a recalculation of the spatial resolving power of *N. forsteri* that was originally calculated using a Matthiessen's ratio of 2.55 (Bailes et al., 2006*b*), which assumes a spherical lens and constant focal proportions throughout development.

Materials and methods

A total of eight hatchlings, seven juveniles, and two sub-adults of *Neoceratodus forsteri* (Krefft, 1870) were kindly donated by Jean Joss of Macquarie University, Sydney, Australia. Developmental stages of lungfish were classified as follows: <5 cm in total length (TL) are hatchlings; 5–35 cm in TL are juveniles; 35–75 cm in TL are sub-adults and >75 cm in TL are sexually mature adults (Brooks & Kind, 2001). The eyes of one sub-adult and three adults were also generously donated by Michael Bennett of the University of Queensland, Australia for morphometric data. Three adults were caught with hook and line from the Mary River, Queensland (Queensland Fisheries Management Authority Permit No. PRM01599G). Animals were sacrificed with an overdose (>20

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mL/L) of benzocaine (Sigma-Aldrich, Inc., St Louis, MO) dissolved in acetone (50 g/L), and spinal cord section in accordance with the ethical guidelines of the University of Queensland (AEC no. ANAT/436/04/ARC). Five juvenile animals used for pupillary movement experiments were maintained in freshwater aquaria at the University of Queensland under a 12:12 light/dark cycle and fed a diet of commercial fish food. The eyes of five African lungfish *Protopterus dolloi* between 40 and 50 cm in TL (fixed) were kindly donated by Richard Puzdrowski of the University of Houston, Texas, United States.

Ocular dimensions

The dimensions of the eye and lens were measured along with information on total body length (TL, from snout to tail tip) and body weight. The axial length of the vertebrate eye has a linear allometric relationship on a log-log plot with body weight (Howland et al., 2004). The best-fit value log-log regression for axial length of the Australian lungfish eyes was compared to the slope and intercept of the published data on fish from Howland et al. (2004). Axial length in *N. forsteri* was also compared to African and South American species of lungfish (*Protopterus dolloi* and *Lepidosiren paradoxa*) using an unpaired *t*-test. Axial length of the eye and body weight for these two other lungfish species were calculated from eye diameter (Ali & Anctil, 1973) based on the assumption that the relationship between equatorial (rostral-caudal) eye diameter and axial eye length and between total body length and body weight is the same as *Neoceratodus forsteri* (linear regression of the axial length = 0.75(equatorial eye diameter) + 0.38; $r^2 = 0.87$; $n = 19$; $\log_{10}$ body weight = 2.71($\log_{10}$ total body length) −4.32; $r^2 = 0.94$; $n = 24$). A graph of the relationship between the total length and body weight of *N. forsteri* used in this study was compared to that derived from a study of more than 2000 Australian lungfish in the wild (Brooks & Kind, 2001). There was no significant difference between our data and the slope ($p = 0.081$) or intercept ($p = 0.085$) of the weight/length relationship of wild fish.

The left eye of a juvenile and both right and left eyes of an adult fish were frozen in OCT compound in liquid nitrogen (Sakura Finetechnical Co. Ltd., Tokyo, Japan) immediately following excision of the whole eye. Sections were cut every 30 μm on a Leica CM3050S cryostat (Leica Microsystems AG, Wetzlar, Germany) and the remaining block face photographed using a Sony cybershot DSC-F828 digital camera (Sony Corp., Tokyo, Japan) fitted with a macro lens. Ocular dimensions were measured from the digital photograph representing the widest diameter (or geometric center) of the lens (Sivak, 1978). Radii of curvature were calculated from the formula:

$$r = (y^2/2s) + (s/2) \quad (1)$$

where r is the radius of curvature, y is half of the chord of the curved surface when assumed to be spherical, and s is the sagittal depth of the chord. From these dimensions, the focal ratio (fr) was also calculated as follows:

$$fr = \mathrm{PND}(0.5a) \quad (2)$$

where PND is the posterior nodal distance (as measured from cryosections), or the length between the center of the lens and the back of the retina at 90° from the plane of the cornea and a is the axial length of the lens.

Two eyes were dissected and light micrographs taken using an Olympus SZ-CTV microscope, an Olympus DP70 digital camera and DP70-BSW software (Olympus Corp., Tokyo, Japan). The magnification of the ocular media was recorded by placing the excised lens and cornea over a two lines per mm Rhonchi grating and measuring the size of the magnified parallel lines from digital micrographs.

Pupillary movements

Four juvenile animals were placed into a Perspex container (22 × 9 × 15 cm) and held in place with polystyrene blocks. Animals were then dark-adapted for 60 min following Douglas et al. (2002). All experiments were carried out at the same time of day to minimize any circadian effect on pupillary responses. Shortly before 60 min of dark adaptation, a Sony DCR-HC85E digital video camera (Sony Corp., Tokyo, Japan) was activated and both the overhead fluorescent and fiber optic lights (situated 5 cm away from the tank) simultaneously turned on. A high light level at the cornea (2000 cd m^{-2}, Konica-Minolta LS-110 luminance meter, Konica-Minolta Holdings Inc., Toyko, Japan) was used to elicit the maximum pupillary response to white light. Filming of the pupillary response continued for 60 min. Still images grabbed every 30 s were downloaded onto a PC using ImageMixer version 1.5.0.1E (Pixela Corp., Osaka, Japan) and external eye and pupillary area were measured. External eye area was measured and calibrated from a still photograph, which included a scale, and all subsequent measurements were related to the known external eye area to allow for any movements the fish made during filming.

Longitudinal spherical aberration of the lungfish lens

The laser-scanning set-up was based on that described in Malkki and Kröger (2005). In brief, the right and left lenses from the eye of an adult lungfish were placed on a black platform forming the base of a Perspex container with the anterior face of the lens facing a thin glass window at the front of the container. The aspherical nature of the adult lungfish lens meant that the vertical midpoint could be determined as the widest point of the lens in profile through the Perspex walls of the chamber within which the lens was placed. The edges of the lens could similarly be determined, as typically, flakes of pigment were still attached, derived from ligamentous attachments between the lens and optic cup at both the dorsal and ventro-temporal edges. The container was filled with physiological saline solution (Krizaj et al., 1998). A drop of milk powder was added to visualize a helium-neon laser beam (532 nm, Leadlight Technology Inc., Taiwan) from a laser pen attached to a motor, which moved the laser beam through the glass window at the vertical midpoint and across the lens diameter in the meridional plane. A Sony DCR-HC85E digital video camera (Sony Corp., Tokyo, Japan) with a macro lens was secured above the lens-holding chamber. Digital footage was downloaded onto a PC and still images were grabbed every 0.5 s to ascertain the back vertex distance (BVD) at different beam entry positions. BVD was calculated by overlaying each still photograph individually onto a photograph of the laser passing through the center point of the lens (determined by measuring the angle of beam entry before and after the lens) and measuring the distance from the back of the lens to the point where the laser crossed the center point beam. Distance from the lens edge was normalized so that the center point was 0 and the edges spread from +1 to −1 following Douglas et al. (2002).

Spatial resolving power

The theoretical spatial resolving power of *N. forsteri* has previously been calculated based on both photoreceptor and ganglion cell spacing at various developmental stages (Bailes et al., 2006*a*), assuming Matthiessen's average fish focal ratio of 2.55 (Matthiessen, 1880). The calculation based on ganglion cell spacing is regarded as more accurate than that based on cone spacing due to the possible summation of photoreceptor input onto retinal ganglion cells. Following the determination of the path length ratio in *N. forsteri* in this study, spatial resolving power is re-calculated using the formula:

$$v = d[1/(S\sqrt{3})] \quad (3)$$

Where d is the distance subtended by one degree on the retina from the formula:

$$d = (2\pi \mathrm{PND})/360 \quad (4)$$

PND (lens center to retina distance) was taken from frozen sections (Table 1). S represents the cell-to-cell spacing assuming hexagonal packing and that ganglion cells are the limiting factor for spatial resolution using the formula:

$$S^2 = 2/(D\sqrt{3}) \quad (5)$$

where D represents the maximum density of ganglion cells per mm^2 (Hart, 2002). The maximum density of ganglion cells in a juvenile 22 cm in TL has previously been calculated as 2446 cells mm^{-2}, while in an adult 125 cm in TL the maximum density is 1190 cells mm^{-2} (based on retrograde labeling of ganglion cells from the optic nerve; Bailes et al., 2006*b*).

Results

Eye morphology and morphometry

The adult Australian lungfish *Neoceratodus forsteri* has laterally-placed eyes and a circular pupil (Fig. 1). Eyes of juveniles are positioned more dorsally and become increasingly lateral with growth. The axial length of the eye increases in a linear relationship to total body length y = 0.007x + 3.83; $r^2 = 0.64$; $n = 20$, and a linear log-log relationship with body weight y = 0.17x + 0.87; $r^2 = 0.93$; $n = 16$ (Fig. 1B). The equatorial diameter of the eye also increases in a linear relationship to total body length but at a faster rate than the axial length of the eye y = 0.012x + 1.72; $r^2 = 0.89$; $n = 34$ (data not shown). A comparison of eye size between the Australian and lepidosirenid (African *Protopterus dolloi* and South American *Lepidosiren paradoxa*) lungfish species shows that in similar-sized individuals, Australian lungfish eyes are significantly larger ($P < 0.0001$, unpaired t-test). However, the axial length of the eyes of *N. forsteri* is relatively smaller than the average axial eye length for a number of other fish species (including representatives of Petromyzontiformes, Chondrichthyes, and Actinopterygii orders of fishes; Howland et al., 2004), although this is much less pronounced in juveniles (Fig. 1B). The slope and intercept of the allometric growth of the axial length of the eye in *N. forsteri* is significantly different to the generalized fish regression line (slope: $F = 45.96$, $p < 0.0001$; intercept: $F = 191.9$, $p < 0.0001$). Like the eye, the lungfish lens (axial length) increases with total body length in a linear relationship (y = 0.0023x + 1.599; $r^2 = 0.84$; $n = 13$; Fig. 1C).

The cornea is comprised of two layers, the outermost of which is distinctly yellow in both juveniles and adults. No noticeable difference in the intensity or distribution of the yellow pigment was observed in a dark-adapted juvenile. The cornea has no magnification properties (Fig. 1D). The conjunctiva, sclera and iris are heavily pigmented with melanin granules and the iris contains gold-reflecting material, which is mostly obscured by the pigmented layer. A thin circle of reflecting material protrudes through the dark pigment around the rim of the iris (Fig. 1). The lens is not spherical but flattened antero-posteriorly and is attached to the orbit via a dorsal suspensory ligament. The lens axial length is between 5.27% and 8.86% less than the equatorial diameter in adults. A second attachment between the lens and optic cup originates from the anterior portion of the lens on the ventro-temporal side near the limbus (Fig. 1D). This attachment is muscular and the distal portion contains pigment granules. The lens of the adult is yellow, which appears to increase in concentration towards the lens center. The lens magnifies evenly from the center to the periphery, and prominent line sutures are visible over the lens surface with little magnification (not shown). The eye of the juvenile is almost spherical, becoming more ellipsoidal in adult fish, so that the eye appears less convex in adults (Fig. 2). Ocular dimensions of the juvenile and adult are summarized in Table 1.

Pupillary movements

On exposure to white light after dark-adaptation, the relative pupil size (pupillary area (A)/maximum dilated pupillary area (A_{max})) of *Neoceratodus forsteri* constricts by an average of 37 ± 7% ($n = 4$) after 45 min (Fig. 3). The average initial reduction in pupil

Table 1. *Ocular dimensions in Neoceratodus forsteri*

	Radius of curvature (mm)		Dimensions						
	Anterior cornea	Retina	Corneal thickness (mm)	Anterior chamber depth (mm)	Lens diameter (equatorial) (mm)	Lens axial length *a* (mm)	% difference in lens dimensions	Posterior nodal distance (PND; mm)	Focal ratio (PND/(0.5*a*))
Juvenile	2.11	2.18	0.17	0.17	2.14	2.07	3.04	2.82	2.72
Adult	6.87–9.63	6.28–6.3	0.37–0.41	0.5–0.91	5.54–5.64	5.14–5.25	5.27–8.86	6.15–6.42	2.39–2.44

Adult values show the range observed from the left and right eye of the same animal. All dimensions are based on measurements of the geometric center of the eye from frozen sections of a juvenile (22 cm in TL) and adult (127 cm in TL) lungfish.

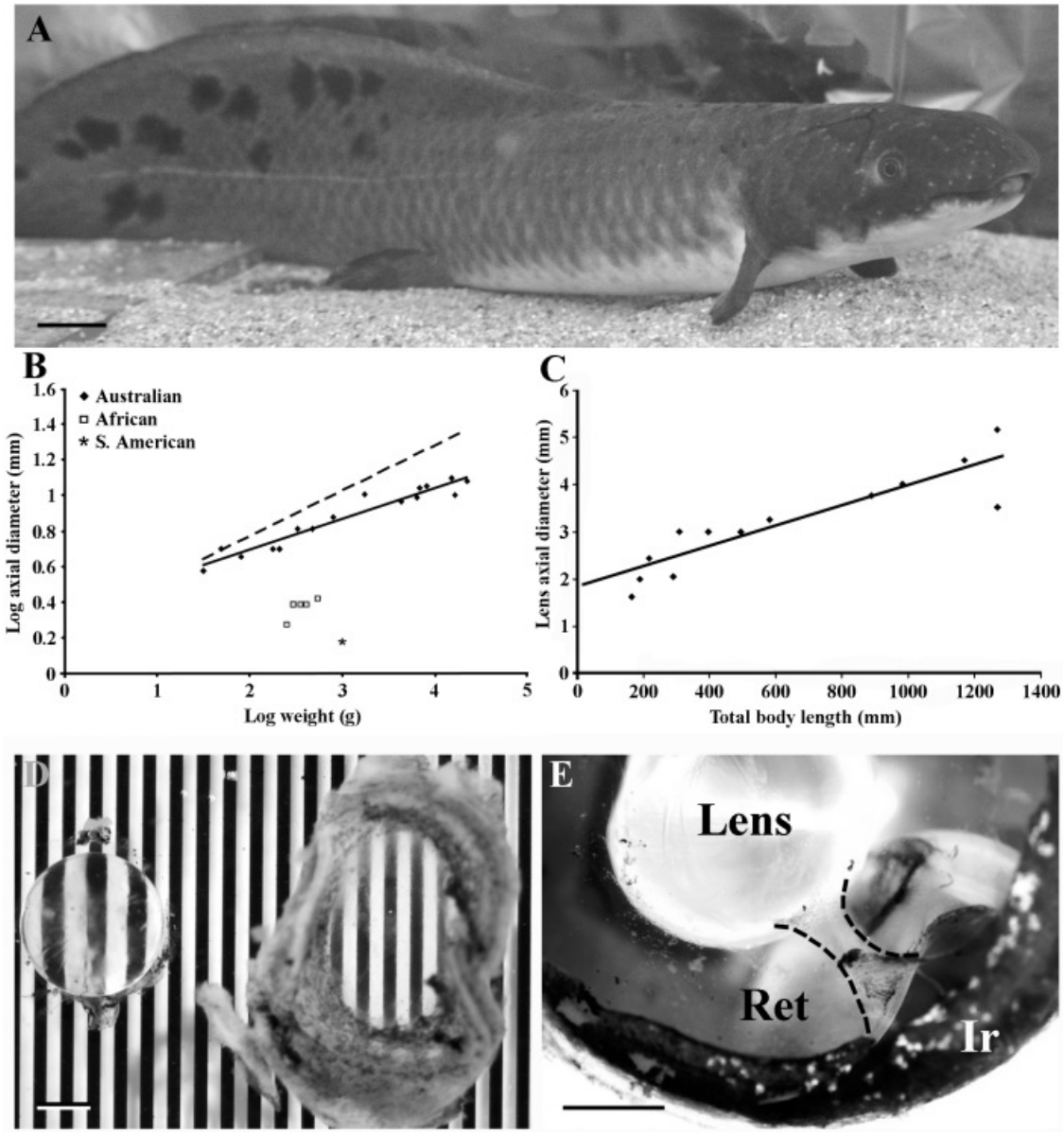

Fig. 1. General ocular features in the Australian lungfish *Neoceratodus forsteri*. (**A**) A juvenile lungfish in a typical position propped on its front fins. Scale bar = 2 cm. (**B**) Comparison of axial eye length in relation to body weight in representative lungfish and the average axial eye length regression line from a range of fish (dotted line; y = 1.0266 + 0.2562x; Howland et al., 2004). Data have been transformed into a log-log plot. The Australian lungfish eye is significantly larger than the South American (*Lepidosiren paradoxa*; Ali & Anctil, 1973) and African lungfishes (*Protoperus dolloi*) of similar size. The $\log_{10}$ axial length of the eye of *N. forsteri* significantly increases with $\log_{10}$ body weight in a linear relationship (continuous line; y = 0.17*x* + 0.87; $r^2 = 0.93$; $p < 0.0001$; $n = 16$). (**C**) Changes in lens axial length during growth in *N. forsteri*. The lens increases with body length in a linear relationship ($y = 0.0023x + 1.599$; $r^2 = 0.84$; $n = 13$). Lens growth in juveniles is faster than in larger individuals, unlike the steady increase in eye growth seen in (B). (**D**) Freshly excised lens (left) and cornea (right; from a juvenile *N. forsteri*) placed on a Rhonchi ruling (2 lines/mm) viewed from the posterior surface. Note that the lungfish lens magnifies the parallel lines right to the periphery, whereas the cornea has no magnification. Scale bar = 2 mm. **E**. The ventral portion of the lungfish lens *in situ* within the eyecup (from a subadult). The removal of the cornea and most of the iris (Ir) enabled the exposure of the lens attached to a ventral anterior ligament, (outlined in dashed black lines) overlying the retina (Ret). Dorsal is uppermost whereas temporal is towards the left. Scale bar = 500 μm.

size shows a one-phase exponential decrease with half the maximum constriction ($t_{0.5max}$) complete at nine minutes.

A comparison was made between the diameter of the constricted and dilated pupil of a juvenile (22 cm in TL) and the lens equatorial diameter from frozen sections of the eye from the same animal. The constricted and dilated pupil had a diameter of 1.74 and 2.29 mm, respectively, while the lens diameter was 2.14 mm. A slight concentric aphakic gap of 0.07 mm therefore exists at full pupil dilation, while the constricted pupil covers 0.16 mm of the lens periphery, or 34% of the lens cross sectional area. The range

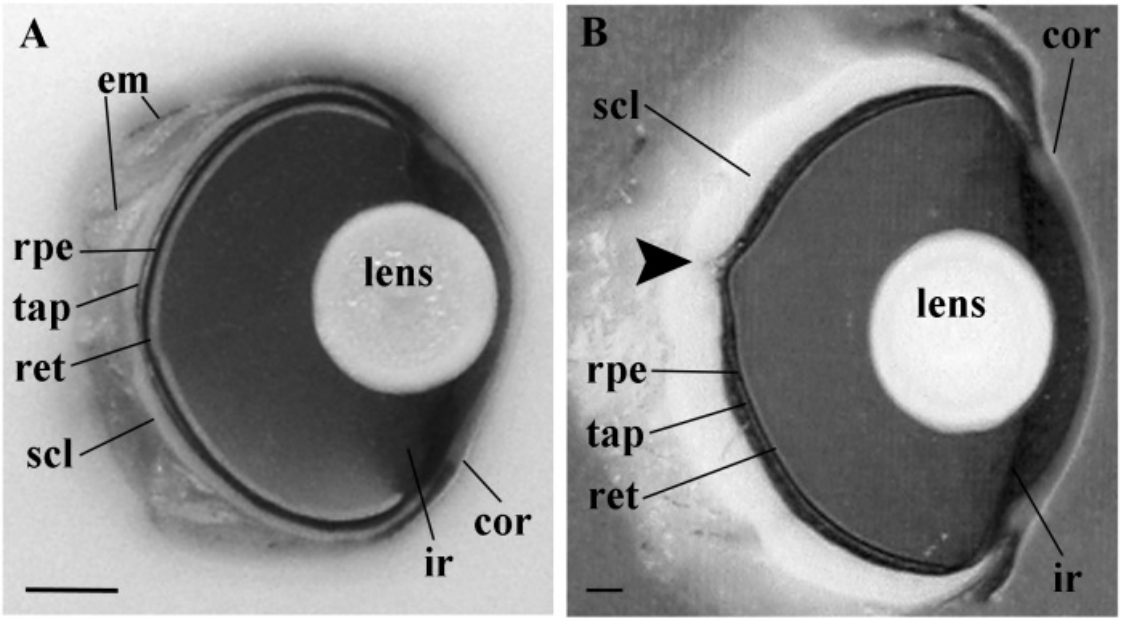

Fig. 2. Frozen sections of the geometrical center of the eye of *Neoceratodus forsteri*. (**A**) Juvenile eye. The retina tapers inwards towards the lens at the periphery. Extraocular eye muscles (em) are visible in the juvenile eye posterior to the sclera (scl). (**B**) Adult eye. The lens is flattened antero-posteriorly and is proportionally closer to the retina than in the juvenile. Occasional indentations of the retina into the sclera (arrowhead) can be seen in transverse sections in addition to the optic disc (not pictured as the optic nerve is slightly temporal). All photos are axial cross sections of the left eye (dorsal uppermost) and the iris (ir) is constricted in both. Both juvenile and adult eyes have an even, intensely yellow anterior half of the cornea (cor), a red tapetum (tap) and a dark layer of retinal pigment epithelium (rpe). Scale bars = 1 mm.

of pupillary movement was also compared to the lens axial length. The F-number of the lungfish juvenile lens (F = PND/A where the PND is the posterior nodal distance of the lens from the lens center to the retina, and A is the aperture diameter) is 1.24–1.55 (dilated to constricted pupil).

Spherical aberration of the lens

When the back vertex distance (BVD) is plotted for each beam entry position, a straight vertical line would indicate a lens perfectly corrected for longitudinal spherical aberration, while deviations from the line show positive (under corrected, light focused behind the retina) or negative (over corrected, light focused in front of the retina) aberration. As light moves from the periphery towards the central axis through the adult lungfish lens, it first crosses the central axis further away from the more central beams, indicating positive spherical aberration (Fig. 4). Rays towards the center do not continue this trend and instead are focused nearer to the lens, showing a correction for spherical aberration that fluctuates somewhat non-monotonically, including a "jump" in BVD at the central point of the lens. The difference between the two lenses is large. The BVD for the two adult lenses is between 3.76 and 4.98 (focal path length 6.38–7.93), equivalent to a lens power of 169 dioptres (D) in the left lens and 208 D in the right lens. The maximum difference in BVD across the lens is approximately 15% of the focal path length.

Spatial resolving power

The theoretical spatial resolving power calculated using the maximum ganglion cell density (Bailes et al., 2006*b*), with the focal path length ascertained from the PND in frozen sections, ranges from 1.31 cycles degree^{-1} (juvenile 22 cm in TL) to 2.03 cycles degree^{-1} (adult 125 cm in TL). When the optical focal length—as ascertained from the average back vertex distance in ray tracing in an adult 117 cm in TL—is used, theoretical spatial resolving power increases to 2.31 cycle's degree^{-1} in adult fish.

Discussion

Structure of the lungfish eye

Neoceratodus forsteri is the only extant member of the ceratodontid family of lungfishes and has significantly larger eyes than lungfish in the lepidosirenid family. Larger eye size in vertebrates generally indicates increased acuity (Hughes, 1977; Lisney & Collin, 2007) and therefore ceratodontid lungfish may also have the potential for higher spatial resolving power, with vision playing a more important role in *N. forsteri* than in the Lepidosirenidae (Walls, 1942). Alternatively, larger eyes in a species of the same order may indicate nocturnal behavior as large eyes can increase photon capture and hence retinal illuminance, an advantage in dim light conditions. Whereas *N. forsteri* possesses larger eyes than the other members of the order, their eyes are relatively small when compared to those of other fishes (Fig. 1; Howland et al., 2004). It should be noted, however, that Howland et al. (2004) found fish eye size to be so variable that they could not draw any general conclusions regarding their relative size to those in other vertebrates. This is mostly because of the variety in body plans amongst fishes, where elongated fish such as lampreys and eels (and hence lungfish) may obey different allometric relationships than other fish (Howland et al., 2004). A comparison of relative eye size may

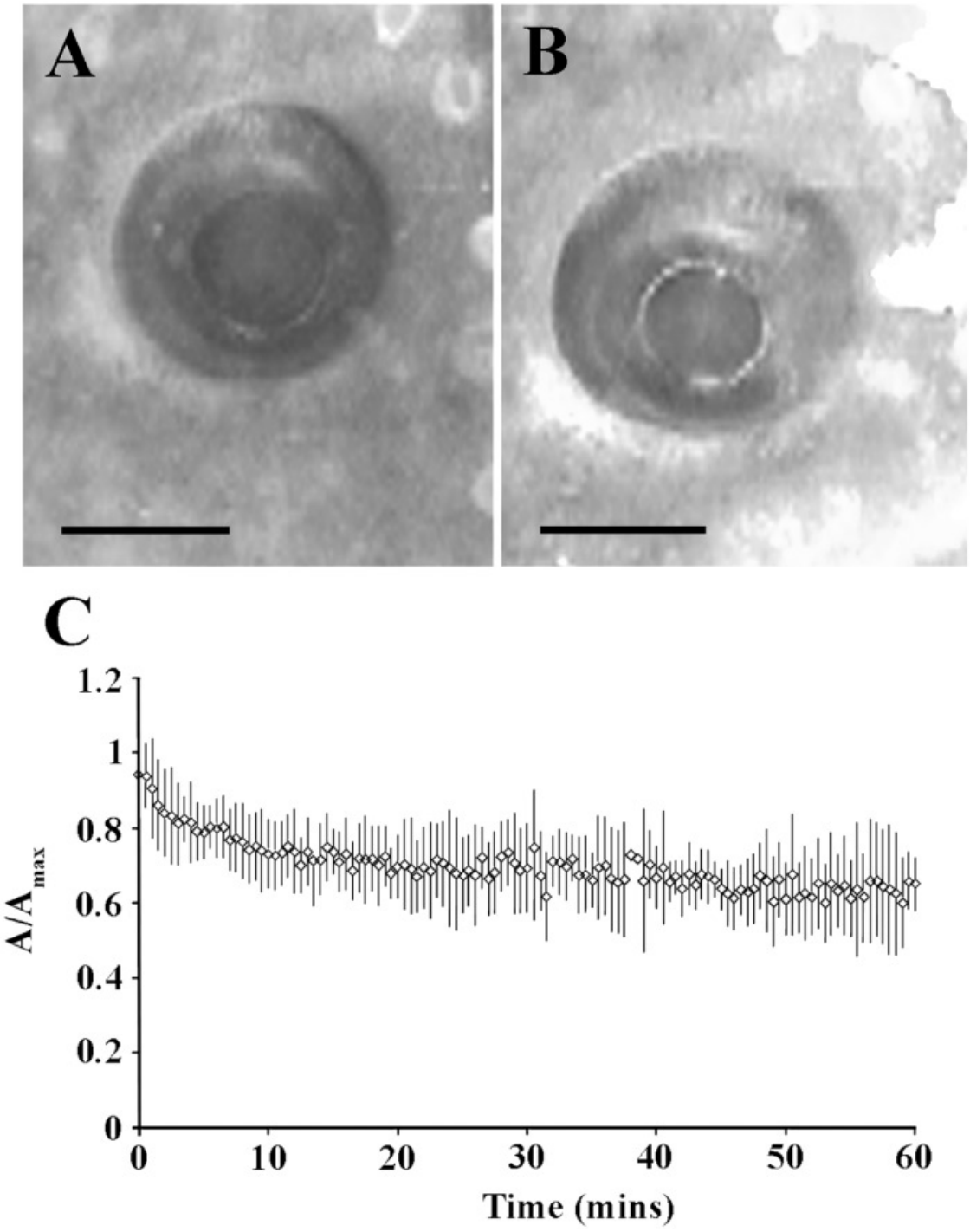

Fig. 3. Pupillary constriction in *Neoceratodus forsteri* eyes in bright white illumination. (**A**) Still image captured from video of the dilated pupil in a juvenile lungfish after one hour dark adaptation. (**B**) A fully-constricted pupil after one hour in white light. Scale bars = 2 mm. (**C**) Time course of the pupillary response to white light in juvenile lungfish. The relative pupillary aperture as a fraction of the maximum pupillary aperture after dark adaptation is plotted against time over 1 h. Average values of four juveniles are shown with 95% standard error bars ($A/Amax = 0.24\ e^{-0.076t} + 0.65$; $r^2 = 0.84$).

have more relevance between closely related species, such as between lungfish families.

The overall shape of the eye becomes more ellipsoidal and flatter in adult lungfish. Amphibia undergoing metamorphosis often possess a larval eye that is spherical, while the adult eye may become markedly more elliptical, depending on the species' environment (Möller, 1951; Duke-Elder, 1958; Sivak et al., 1985; Mathis et al., 1988; Sivak, 1988). The change in the dimensions of the eye in the newt *Notophthalmus viridescens* (with a relative reduction in axial length of the lens increasing from 1.7 to 6.6% of

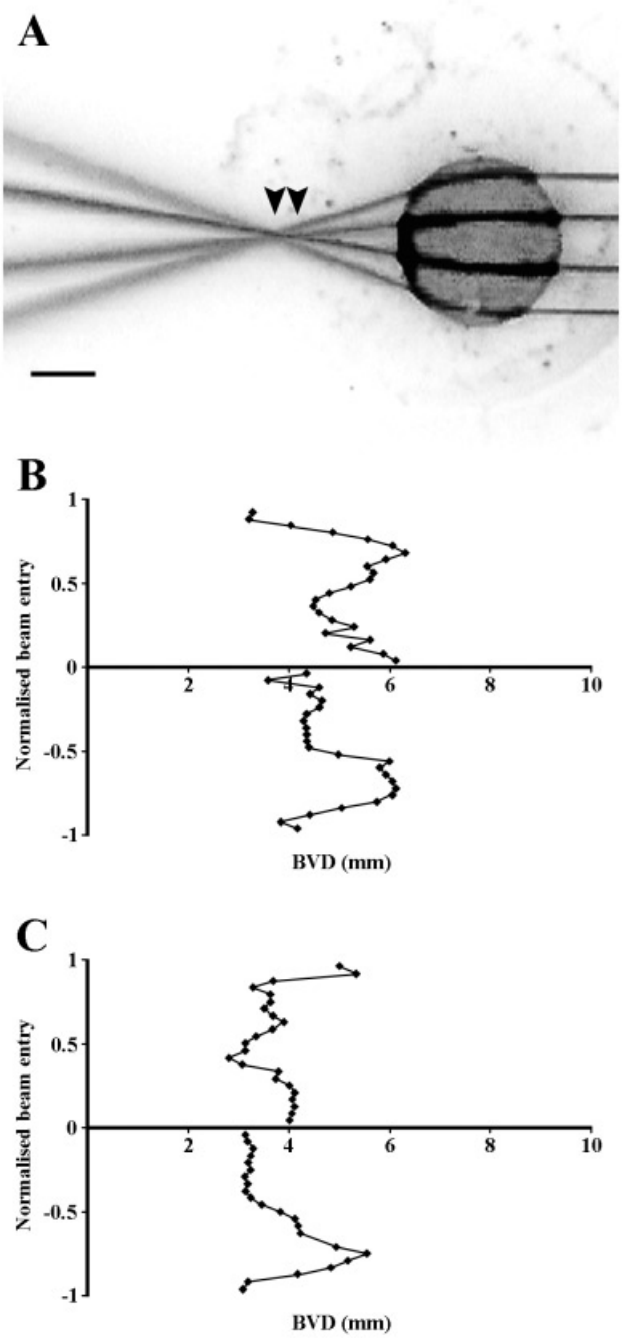

Fig. 4. Optical quality of the lens of *Neoceratodus forsteri*. (**A**) Ray tracing of a laser beam (532 nm) through a freshly excised lungfish lens. Note that beams entering the lens nearer the central axis are focused closer to the lens than peripheral beams (arrowheads). Scale bar = 2 mm. (**B**, **C**) Graphs showing the back vertex distance (BVD) for the left (B) and right (C) lenses of an adult lungfish. The center of the lens is somewhat corrected for longitudinal spherical aberration, while the periphery shows varying degrees of positive aberration. The point of beam entry has been normalized so the edges of the lens are represented by +1 and −1 and the center is 0.

the equatorial lens diameter; Sivak et al., 1985) between its aquatic larval phase and the land adult form is comparable to the ontogenetic changes observed for the eye in *N. forsteri*. However, *N. forsteri* does not spend much, if any, time out of water (Illidge, 1893) and does not undergo any period of metamorphosis. It has been suggested that this extant species may be neotenic (Joss, 1998).

The lens of the adult Australian lungfish eye is elliptical, in disagreement with an early account of a spherical adult lens (Günther, 1871). The lens of lepidosirenid lungfish may also prove to be elliptical as opposed to "approximately spherical" as described for *Lepidosiren paradoxa* (Rochon-Duvigneaud, 1943). The cornea of *N. forsteri* also shares features with cartilaginous fish, possessing a covering of dense microvilli as opposed to the pattern of corneal microridges seen in all teleost fish examined to date (Collin & Collin, 2000, 2006).

Aspherical lenses such as those observed in this species of lungfish have been described in a number of aquatic species, e.g., lampreys (Collin & Fritzsch, 1993; Collin et al., 1999), elasmobranchs (Rochon-Duvigneaud, 1943; Gilbert, 1963) and actinopterygians including some teleosts (Munk, 1984, 1986; Pettigrew & Collin, 1995; Douglas et al., 2002; Bantseev et al., 2004). Despite these examples of aspherical fish lenses, the "ideal" aquatic eye is consistently described as possessing a spherical lens, as this allows for greater refraction in the absence of any corneal power due to the similarity of the refractive indices of the cornea and the surrounding water. A spherical lens is found in most teleosts as well as a number of aquatic amphibians, reptiles, birds and mammals, while their terrestrial cousins exhibit a more elliptical lens shape (for reviews see Walls, 1942; Rochon-Duvigneaud, 1943; Duke-Elder, 1958; Sivak, 1990). Lens shape may therefore also depend on analogous visual needs or shared visual behavior.

Two structures hold the lungfish lens in place; a suspensory ligament and an anterior ventro-temporal attachment, which appears muscular. It was previously thought that all lungfish genera lack any accommodatory structures based on examination of the eye of *Protopterus annectens* (Munk, 1964). It is unknown if the lungfish lens has the ability to accommodate (the pupil conceals the lens edge in bright light unlike in teleosts), or if the anterior muscle enables retractive (as in teleosts: Beer, 1894 cited by Tamura, 1957; Walls, 1942; Somiya, 1987), or protractive (as in amphibians: Douglas et al., 1986 and references therein) lens movement.

Lungfish behavior and visual ecology

The overwhelming gaps in the knowledge of the biology of *N. forsteri* come from a lack of well-documented, thorough investigations into lungfish behavior. Lungfish eggs are found in dense, shallow macrophyte beds in river tributaries, which probably represent suitable microhabitat for young juveniles (Bancroft, 1911; Kemp, 1984; Brooks, 1995; Brooks & Kind, 2001). New hatchlings are negatively phototactic (J. Joss, Pers. Com.), although this developmental stage was not available for this study. Older juveniles are highly active in late afternoon and evening and the few that have been caught in the wild were again caught in an environment of shallow water with macrophytes and wood debris. As lungfish grow, their habitat seems to change (Brooks & Kind, 2001) and adult lungfish are more commonly reported in open river water. They are reportedly crepuscular and can be seen at the river surface, particularly when mating. Changes in dentition also occur developmentally, changing from cone-like teeth thought to aid in the capture of small invertebrates in juveniles to crushing tooth plates for immobilizing larger prey (e.g., small fish, amphibia, crustacea) and plant material in adults (Kemp, 1977, 1986). The ontogenetic change in eye shape in lungfish may therefore

have functional significance to the change in habitat by altering the focal ratio. A more ellipsoidal eye design also increases streamlining in water and suggests that active prey capture is more important in adult lungfish than in juveniles (Jamieson, 1971). However, no appreciable change in retinal photoreceptor or ganglion cell distribution between juveniles and adults has been revealed (Bailes et al., 2006*b*).

Pupillary movements

The pupil of the Australian lungfish exhibits small but significant constriction when exposed to bright, white light (2000 cd m^{-2}), which is mostly complete after 10 min. This is in contrast to the pupils of many teleost fishes, which are generally considered immobile (Muntz & Wainwright, 1977), although the number of examples of species with highly mobile apertures is ever increasing (Brown-Séquard, 1859; Bateson, 1890; Young, 1933; Walls, 1942; Rubin & Nolte, 1982; Douglas et al., 1998, 2002). Most teleost species that exhibit notable pupillary movements can fully constrict the pupil within a few seconds (*Opsanus tau* and *Lophius piscatorius*: Rubin & Nolte, 1982; *Porichthys notatus*: Douglas et al., 1998), although catfish pupillary constriction takes up to 40 minutes (*Liposarcus pardalis*: Douglas et al., 2002). *Liposarcus pardalis* lives in freshwater rivers in South America and probably inhabits a similar environment to that of *N. forsteri*. In amphibians, some degree of pupil mobility is found in most species examined to date (Barr & Alpern, 1963; Cornell & Hailman, 1984; Henning et al., 1991). Pupil constriction is generally slower in this group and bears a similarity to the speed and magnitude of pupillary constriction in *N. forsteri*. Dark-adapted pupils reach maximum closure in bright light after 10–20 min in ranid frogs and urodelans (e.g., *Triturus alpestris* and *Pleurodeles waltli*; Cornell & Hailman, 1984) and constrict from 49% to 65% of the dilated pupillary area (*Pleurodeles waltli* and *Rana pipiens*: Cornell & Hailman, 1984). Trends in the magnitude and speed of the pupillary response can be seen between different phylogenetic groupings, and in this regard, the eye of *N. forsteri* shares more similarities with amphibians than teleost fishes. However, examples such as the catfish indicate that the visual environment and visual demands may have more influence on the pupillary response strategy than the phylogenetic relationships between groups.

African lungfish *P. annectens* were observed to show pupillary constriction more than 100 years ago, although no further investigation was ever undertaken (Steinach, 1890). Unlike in *N. forsteri*, the pupil in *P. annectens* constricts in an uneven manner to resemble a horizontal slit upon light exposure when emerging from an aestivation cocoon (a physiological adaptation to drought that does not occur in *N. forsteri*). Pupillary constriction in most vertebrates is the result of the contraction of the sphincter pupillae muscle within the iris (Walls, 1942) and yet Rochon-Duvigneaud (1943) reported the absence of a sphincter pupillae muscle in lepidosirenid lungfish. In *Protopterus* spp. the contractility of epithelial cells of the *pars iridica* may be involved (Walls, 1942), although further anatomical investigation of the iris in all dipnoan species is needed to clarify the structures responsible for pupillary mobility.

Optical quality of the lungfish lens

The lungfish cornea lacks any magnification properties, similar to teleost fishes, where the lens is the only refractive element of the eye (Sivak, 1982). Changes in lens quality therefore have marked ramifications on the optical quality of the image reaching the photoreceptors (for reviews see Walls, 1942; Fernald, 1988; Sivak, 1990). The adult lungfish lens exhibits positive (under-corrected) spherical aberration in the periphery, while becoming somewhat corrected toward the lens center. This correction can be attributed to a gradient of refractive index within the lens. In teleost fish, spherical aberration varies both interspecifically and developmentally and it has been suggested that more between-lens variation occurs in less visual species (Sivak & Kreuzer, 1983; Kreuzer & Sivak, 1984). The difference and degree of aberration within lungfish lenses may therefore indicate less dependence on vision than species with highly-corrected lenses such as pike *Esox lucius* (Kreuzer & Sivak, 1984) and the orange-spotted sunfish *Lepomis humilus* (Bantseev et al., 2004). A strikingly similar pattern of aberration, and therefore lens refractive index, to *N. forsteri* is observed in catfish *Pterygoplichthys etentaculus* (Douglas et al., 2002) and the freshwater sturgeon *Acipenser fulvescens* (Bantseev et al., 2004), both of which inhabit slow moving, freshwater rivers and may have similar visual needs. Some of the non-monotonic quality of aberration toward the center of the lens and the "jump" in BVD at the centermost point in *N. forsteri* lenses may be a direct result of the prominent lens sutures in this species, which can cause lens disorder and asymmetry in focal length (Sivak et al., 1994).

A second explanation exists for the inconsistent pattern of longitudinal spherical aberration (LSA) seen in the lungfish lens. Malkki and Kröger (2005) have shown that great variations in LSA can indicate multiple focal lengths that correct for longitudinal chromatic aberration. Chromatic aberration is considered to be the largest contributing factor to the distortion of the image reaching the teleost retina (Sivak, 1974; Fernald, 1988), averaging approx. 4.6% of focal length (442–632.8 nm: Kreuzer & Sivak, 1985). The crucian carp *Carassius carassius* has multifocal lenses that are well-corrected for longitudinal chromatic aberration and exhibit variation in BVD exceeding 10% of the focal length (Malkki & Kröger, 2005), while the variation in BVD in lungfish lenses is 15%. Multifocal lenses may decrease contrast between objects, but this can be compensated for by increased spatial resolution of colour images (Kröger et al., 1999). Enhanced resolving power of chromatic images may be of benefit to lungfish, with recent evidence showing that they could be capable of tetrachromatic vision, with four cone opsins expressed in the retina (H.J. Bailes et al., unpub. data).

Juvenile and adult lungfish eyes possess noticeably yellow corneas, while a yellow core within the adult lens is also evident. Longitudinal chromatic aberration is highest at short wavelengths of light and one possible function of yellow corneas and lenses is the reduction of chromatic aberration by absorbing the shorter wavelengths (Tansley, 1965; Muntz, 1972, 1975; Orlov & Gamburtzeva, 1976; Appleby & Muntz, 1979; Muntz, 1982; Heinermann, 1984). Yellow filters are predominantly found in diurnal fish living in high light environments because the subsequent light absorption will lead to a loss of scotopic sensitivity (Walls & Judd, 1933; Heinermann, 1984; Douglas & McGuigan, 1989; Siebeck et al., 2003), adding to evidence that *N. forsteri* is, at least in part, active in bright light conditions.

Changes in focal ratio and optical implications for spatial resolving power

The decrease in focal ratio from juveniles to adults will result in an increase in retinal illumination with growth. Changes through development have also been described for a number of teleosts and

an elasmobranch (*Forsterygion varium*: Pankhurst et al., 1993; *Acanthopagrus butcheri*: Shand et al., 1999; *Danio rerio*: Easter & Nicola, 1996; *Raja elanteria*: Sivak & Luer, 1991). Shand (1994) found a general trend that nocturnal teleosts possess lower focal ratios (down to 2.26), supporting the hypothesis of a functional increase in retinal illumination in dim light conditions brought about by a decrease in the posterior nodal distance (PND) with development.

The increase in retinal illumination with lungfish growth may be reflected in changes in habitat. Juvenile fish are found in shallower water, where illumination will be greater than in the open river where adult fish are more commonly seen. In addition, adult fish are active at all times of the day, especially late afternoon and evening (Kemp, 1986), and the ability to adapt to a wide range of lighting conditions may be beneficial. All photoreceptor types increase in length and diameter with growth, which will also lead to greater light sensitivity in adult fish (Bailes et al., 2006*a*).

The increase in retinal illumination produced by focal ratio changes during growth in *N. forsteri* may be a developmental phenomenon, where compression of older lens material in the lens core has yet to occur in juveniles (increases in lens compression occurs with age in skate *Raja elanteria*; Sivak & Luer, 1991). In this study, the change in focal ratio between juvenile and adult lungfish is calculated from frozen sections. This method undoubtedly preserves the size and relationship of ocular structures more accurately than traditional histological processing and we believe the consistency of the technique used is a good indication of developmental differences in lungfish eye structure. However, it is not possible to completely exclude differential effects of freezing on different sized eyes and the effects of anesthesia on lens accommodation.

In juvenile *N. forsteri*, a circumlental aphakic space, although small (only 0.07 mm between the lens edge and pupillary margin), exists at maximal pupillary dilation. An aphakic space is present in a number of teleosts, where the pupil diameter exceeds the lens diameter (Sivak, 1980). A circumlental aphakic space produces a substantial increase in the size of the maximally illuminated central part of the retina, thereby increasing the detection of laterally-placed objects that would otherwise fall below the absolute threshold of the eye (Munk & Frederiksen, 1974). This aphakic space may be absent in adult fish, as the juvenile lens is growing at a faster rate than the rest of the eye, indicating the lens is proportionately smaller in young fish than in adults.

The increase in theoretical spatial resolving power calculated from maximum ganglion cell density between the juvenile and adult is different according to whether the measured lungfish-specific focal ratio is used (spatial resolving power increases from 1.31 cycles degree^{-1} to 2.03 cycles degree^{-1} using the anatomical PND, or 2.31 cycles degree^{-1} using optical focal lengths) or a Matthiessen's focal ratio of 2.55 is used (1.43 cycles degree^{-1} in the juvenile to 1.65 cycles degree^{-1} in the adult; Bailes et al., 2006*b*). This is because of a decrease in focal ratio with growth in lungfish and indicates that adult lungfish may have a higher spatial resolving power than previously predicted (Bailes et al., 2006*b*). Similar species-specific changes in lens growth should be considered when spatial resolving power is calculated for other primitive fish species for which the optical parameters are unknown. The difference between using the anatomical PND and optically measured focal lengths may be due to individual variation, but it should be noted that the optical focal length was derived from an average value, which includes the positive spherical aberration characteristic of the edges of the lens.

In conclusion, Australian lungfish have significantly larger eyes than other extant lungfish and may possess multifocal lenses, which would be an advantage for chromatic vision. The eye of *N. forsteri* shares many features with the amphibian eye design (such as a slow pupillary response and a flattened lens) and shows a developmental change in eye position and shape. Given the unique phylogenetic position of *N. forsteri* as the closest extant relative to land vertebrates, it is interesting that *N. forsteri* has a range of optical features found in both fish and amphibians. The Mary River is close to the known range of *Ceratodus* in the Cretaceous era (Kemp & Molnar, 1981) and could represent a typical lungfish habitat. However, Queensland rivers known to support lungfish populations today represent an important human agricultural resource with the growth of the cane farming industry and may have a significantly altered spectral and micro habitat from Australian rivers in the Cretaceous. Further work on the behavior of this species will be invaluable in interpreting functional adaptations in the visual system of this living fossil.

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