

Vision in Lungfish

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ABSTRACT

All three extant genera of lungfish, Australian, African and South American, appear to possess unremarkable, even ‘degenerate eyes’ when viewed externally. The eyes of the Australian lungfish, *Neoceratodus forsteri*, are slightly larger than those of the other species (seven African species in the genus *Protopterus* and the single South American species *Lepidosiren paradoxa*). *N. forsteri* seems to be the most visually-oriented of the extant lungfishes. All three genera of lungfish, however, possess remarkable and beautiful retinal adaptations, including, coloured oil droplets, multiple cone spectral sensitivities and large photoreceptor inner segments, making them more closely aligned in design to modern amphibians and other terrestrial animals, than to teleosts. The tetrapod-like retinal features of *N. forsteri* provide the capability for tetrachromatic colour vision and add to the debate on the phylogenetic origin(s) of lungfish. They also suggest that the complex colour vision system of vertebrates on land, exemplified by birds, may have first evolved in the aquatic environment or at least close to the time when aquatic life emerged onto land. Other ocular adaptations in dipnoans include a non-spherical lens, the anatomical mechanism for accommodation, a mobile pupil and giant retinal cells. This eye design suggests a need to increase light flux, rather than for a reliance on high spatial acuity, a conclusion supported by the relatively low ganglion cell densities. Future work should certainly aim at a better understanding of the visual biology, behaviour and ecology of all lungfish, especially in light of their disappearing habitat worldwide. Both African and South American species also need a full description of their visual system

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before they are properly consigned to being 'less well developed', than *N. forsteri*.

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INTRODUCTION

An ability to breathe air, the possession of lobe-fins, and an evolutionary position possibly closer to amphibians than to fish, places the lungfishes (Dipnoi) in an iconic position within the Osteichthyes. Several aspects of eye design in lungfishes suggest that they possess a visual system much more like that of a land dwelling vertebrate such as a bird, reptile or amphibian. The potential for complex tetrachromatic colour vision, spectral coverage from the UV to deep in the red region of the spectrum and coloured filters to tune the light incident on the retina has recently been realised (Robinson 1994; Bailes *et al.* 2006a). Although Bailes and colleagues have provided a recent and thorough examination of the visual system of the Australian lungfish, *Neoceratodus forsteri*, components of this surprisingly un-fish-like visual system have been known for more than a century (Krefft 1870; Gunther 1871; Schiefferdecker 1886; Kerr 1902; Grynfeldt 1911; Walls 1942). Robinson (1994) revived interest in lungfish vision by a more complete description of the most surprising aspect of their vision, the coloured oil droplets and ellipsoids housed within the photoreceptors, and by suggesting the potential for tetrachromatic colour vision in *N. forsteri*. These sorts of coloured filters, while present in the eyes of many land vertebrates, are rare among fish (Walls 1942; Douglas and Marshall 1999), although the coelacanth *Latimeria chalumnae*, a close relative of the lungfish, is one example where a large oil droplet is associated with one of its three large single cones (Locket 1973).

A gaping hole in our understanding, that will unfortunately permeate this chapter, is the lack of work on the visual systems of the African and South American species of lungfishes. In general, due to their small eye size (Fig. 1) and comparatively reduced complexity in a number of ocular features, these species are thought to be less 'visual' than the Australian lungfish. It is, however, possible that this is an expression of our ignorance rather than a reflection of the real situation. Wherever possible in this chapter we refer to known work on *Lepidosiren paradoxa* and *Protopterus* species, but the majority of what is summarised here concerns *N. forsteri*.

Retinal morphology and the optics of the eyes of *N. forsteri*, along with visual ecology and some scant observations on visual behaviour, indicate that this visual system is designed for increased sensitivity rather than for high acuity. This conclusion is supported by the findings of very large or "monstrous" photoreceptors described by Walls (1942), a general feature shared by all species of lungfishes. Conversely, the relatively small eyes, in particular those of the Lepidosirenidae (this includes both African and South American species) suggest that sensitivity per se is not of importance to the sensory biology of the lungfishes and that other

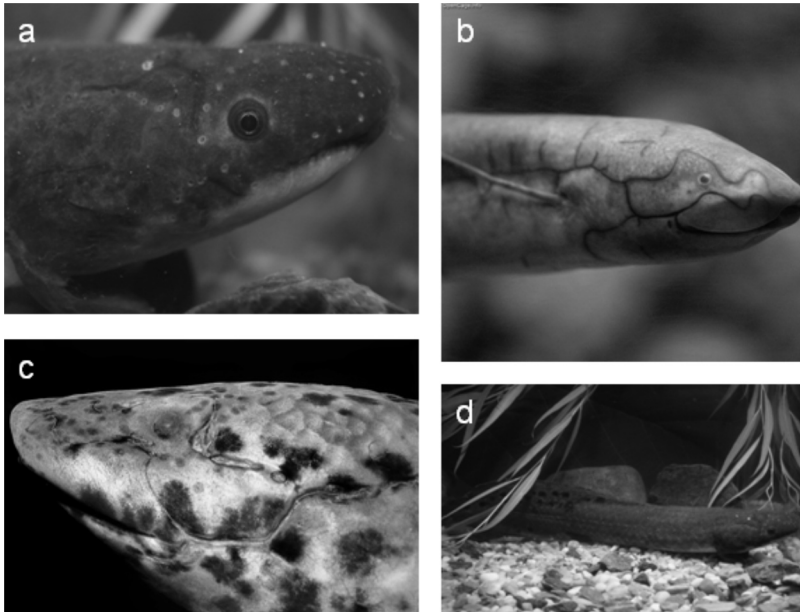


Fig. 1 The heads and eyes of lungfish. (a) Juvenile Australian lungfish, *Neoceratodus forsteri*, at around two years of age (25 cm total length). The body proportions are more similar to the adult at this stage. Note relatively large eye and lateral line pores. (Photograph Justin Marshall and Helena Bales). (b) South American lungfish, *Lepidosiren paradoxa*. Note relatively small eye and pigmented lateral line canals on head. (Photographic source – [www.opencage.info/ Image 800_8532](http://www.opencage.info/Image/800_8532)). Size unknown. (c) African lungfish *Protopterus annectens*. Note relatively small eye in this species also. (Photographic source – www.Photovault.com). Size unknown. (d) Same fish as (a) demonstrating eye/body proportions.

Color image of this figure appears in the color plate section at the end of the book.

factors such as the presence of dense coloured oil droplets in their inner segments may have driven the apparent need for sensitive photoreceptors (Partridge 1989).

Several aspects of lungfish vision add to the debate on the relationship of lungfish to teleost fish and urodele amphibians (Robinson 1994; Carroll 1988, 1997; Joss 1998; Pough *et al.* 1999; Takezaki *et al.* 2004; Bailes *et al.* 2006a, 2007a,b). While this is the subject of another chapter (Chapter 20), it is worth noting in passing here the striking resemblance between what we think of as an advanced terrestrial vertebrate colour vision system and that of the lungfish visual system (Bailes 2006; Bailes *et al.* 2006a; Hart *et al.* 2008). Molecular analysis of the visual pigments or opsin genes also places lungfishes closer to the amphibians than teleost fishes (Bailes *et al.* 2007b).

The complexity of the lungfish colour vision system contributes to the discussion on the evolution of colour vision in vertebrates (Tresize and Collin 2005; Lamb *et al.* 2007), suggesting that the capacity for colour discrimination first evolved

in water and was retained after emerging onto land, although specific classes of photoreceptors have subsequently been lost. The suggestion of neoteny in lungfish (Joss 1998) could also point towards the retention of visual characteristics evolved on land in a now largely aquatic animal.

Prior to the recent work of Bailes and colleagues, fewer than ten publications existed on lungfish eyes. Gunther (1871), Schiefferdecker (1886), Kerr (1902) and Grynfeldt (1911) provided early anatomical descriptions. Walls (1942) reviewed this early work and went on to provide what is still considered the best source of comparative morphological study on the eyes of the extant species. More recently, Munk (1969), Locket (1970), Ali and Anctil (1973, 1976), Pow (1994) and Robinson (1994) have contributed morphological studies, largely based on individual species. A common theme of these investigations is that the retina and choroid in all species described are considered thin, while the cells of all retinal layers are very large. Walls (1942) implies that the eyes of the *L. paradoxa* and *Protopterus* sp. are degenerate, at least when compared to *N. forsteri*, while Ali and Anctil (1976) rank them according to complexity in the ascending order South American, African and Australian species. Walls (1942) states:

“The dearth of knowledge about *Lepidosiren* is of no great importance, since this form is in the same family as *Protopterus*. But *Neoceratodus* deserves a thorough investigation, for this large fish has none of the appearances of degeneracy characteristic of the Lepidosirenidae. Its relatively large eye may have, in particular, a mechanism of accommodation: and its cone oil droplets may be coloured in life. But the animal is reputedly nocturnal (in captivity, at least), and may not have retained such things even though some diurnal ancestor may have had them.”

GROSS MORPHOLOGY OF THE EYE

In all species, the eyes are laterally-placed in the head and possess circular-shaped pupils (Fig. 1; Walls 1942; Bailes *et al.* 2007a). The eyes are not remarkably coloured or protruding and the iris is generally pigmented to match the pale brown colour of the body. Eyes in members of the Lepidosirenidae are small, i.e. 1.5 mm in a 50 cm fish in *L. paradoxa* (Ali and Anctil 1973) and 2 mm in a 30 cm fish in *Protopterus dolloi* (Pfeiffer 1968). The eyes of *N. forsteri* are proportionally larger i.e. 14 mm in a 127 cm fish, and the axial length of the eye increases with total body length according to a linear relationship (Bailes *et al.* 2007a). Compared to an “average teleost fish”, the axial length of the eye in *N. forsteri* is, however, relatively small (Howland *et al.* 2004; Bailes *et al.* 2007a).

CORNEA, LENS AND PUPIL

In *N. forsteri*, the non-refractile cornea consists of two layers; inner definitive (scleral) cornea and an outer secondary spectacle (Walls 1942), the outer layer

being evenly pigmented yellow, which is especially dense in the adults (Fig. 2). As the eye grows, the the lens and globe become increasingly elliptical in shape (Fig. 2) but retains an even magnification from centre to periphery. Functionally, the result is a decrease in focal ratio and an increase in illumination on the retina, suggesting increased sensitivity is needed (Bailes *et al.* 2007a). Aspherical lenses are known in deep-sea teleosts, most elasmobranchs and lampreys with a variety of explanations given (Munk 1984; Sivak 1990; Collin *et al.* 1999; Douglas *et al.* 2002). The lens of the adult lungfish is also yellow, with an increasing concentration of pigment

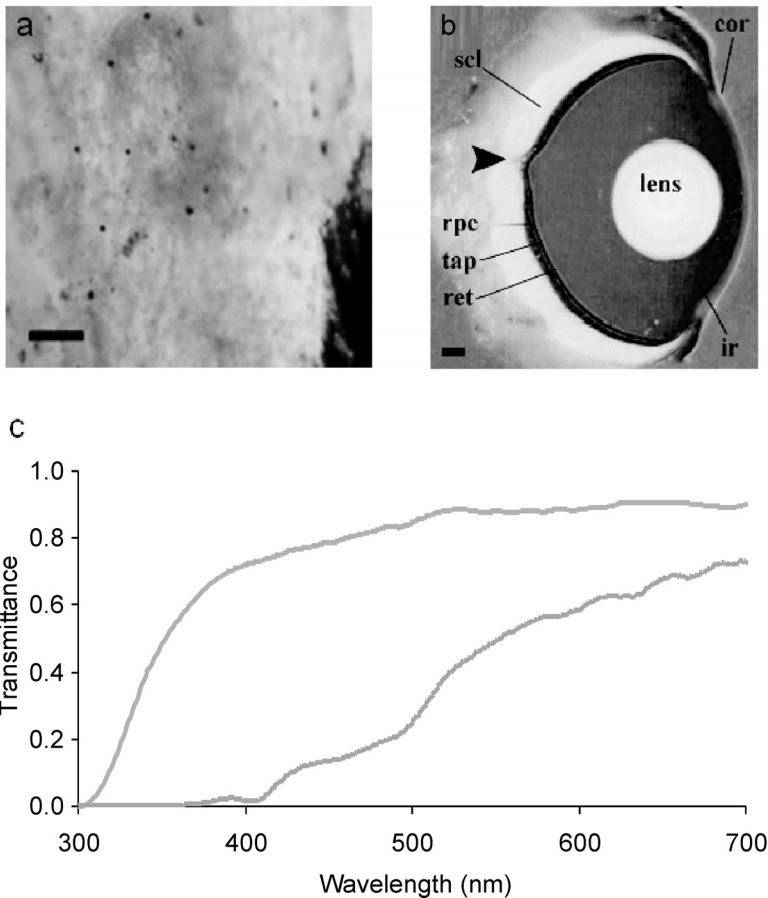


Fig. 2 Some optical features of the eye of *Neoceratodus forsteri*. (a) Freshly dissected cornea of adult lungfish with transmitted light to show intense yellow pigment. Scale 250 μ m. (b) Frozen hemisected left eye of adult (127 cm total length), close to the geometrical centre. Note slight flattening of the lens. scl – sclera, ir – iris, cor – cornea, tap – tapetum, rpe – retinal pigment epithelium, ret – retina. Retinal indentation (arrow) close to optic nerve head. Scale – 1 mm.

Color image of this figure appears in the color plate section at the end of the book.

located towards the lens centre (Bailes 2006). Possible functions of a yellow lens and cornea are discussed in sections on the retina and colour vision.

A muscular attachment has been found to secure the lens of *N. forsteri* anteriorly, suggesting that the lens could accommodate and focus on near or far objects in the environment (Bailes 2006; Bailes *et al.*, 2007a). However, this has yet to be shown directly in *Protopterus annectens* and other species (Walls 1942; Munk 1964).

The optical performance of the lungfish lens and eye appears rather poor with the likelihood of both spherical aberration (more marked in the periphery of the lens) and hyperopia reducing the ability to discriminate fine detail (Bailes *et al.* 2006b; Bailes *et al.* 2007a). However it is also possible that accommodatory lens movement and the use of multiple focal lengths to correct for spherical aberration (Malkki and Kröger 2005) are present in *N. forsteri*, both mechanisms providing improved spatial (and chromatic) vision (Bailes *et al.* 2007a). The spatial resolving power of the eye is discussed in more detail below along with the changes in retinal ganglion cell density. It is worth noting here, however, that if the eye does remain hyperopic at rest, this will be an over-estimate due to the defocused image. The larger eyes of *N. forsteri* compared to other lungfish species, may in fact be optimized both for enhanced sensitivity and/or spatial resolution within its environment (Land 1981; Howland *et al.* 2004).

Most of the teleosts described with pupillary responses are benthically-oriented (Walls 1942; Rubin and Nolte 1982; Douglas *et al.* 1998; Douglas *et al.* 2002). The finding of pupillary movement in *N. forsteri* reveals that this ability is a primitive characteristic for vertebrates, a suggestion confirmed by the finding of a mobile pupil in the southern hemisphere lamprey, *Mordacia mordax* (Collin *et al.* 2004). In lungfish, pupillary contraction is noted in *Protopterus annectens* (Steinach 1890, Walls 1942), where on release from aestivation, the eye has a slit-shaped pupil that rounds up after several hours. Whether this slit is only present when the fish lies within the protection of its mud cocoon is not known, but apparently no intraocular eye muscles are involved in the slow pupillary response (Walls 1942). Other animals with slit pupils narrow the slit with increasing light intensity (Walls 1942). The pupillary response in *N. forsteri* is also slow, taking at least 30 minutes, but being mostly complete after 10 minutes (Bailes *et al.* 2007a). During constriction of the adult pupil, the retinal illumination changes by a factor of two and the F-number of the eye changes from 0.47 to 0.64 for the dilated and constricted states, respectively (Bailes 2006). Contraction is described as similar to that observed in amphibians, almost all of which are known to show a slow pupillary response (Walls 1942; Cornell and Hailman 1984), one factor that initially suggested the possibility of a close relationship between lungfishes and amphibians (Walls 1942).

GENERAL DESCRIPTIONS OF RETINAL LAYERS AND SURROUNDING TISSUES

General descriptions exist for the retinae of all three lungfish genera, based mainly on light and some electron microscopical studies. Walls (1942) describes a *Protopterus* species, probably mostly *P. aethiopicus*. Walls describes 'huge' retinal pigment epithelial (RPE) cells making the pigment epithelium as thick as the sclera and its rudimentary (thin) choroid. "All retinal elements are monstrous," he reports, where the outer nuclear layer possesses two rows of nuclei of rod and cone soma. The inner nuclear layer contains four rows of nuclei, the outer plexiform layer is very thin and the inner plexiform layer is thick. Horizontal cells are "slenderly fibrous" or possibly not present at all. A single row of ganglion cells exists.

Ali and Anciaux (1973, 1976) describe the retina of *Lepidosiren* as essentially similar to that of *Protopterus*. The retinal cells are both large and sparse and the photoreceptor, bipolar and ganglion cell populations are among the largest in any fish. No retinal mosaic was observed, as often seen in teleost fishes, and indeed is not considered present in the other two species although no nearest neighbour analysis was undertaken. After light and dark adaptation, no retinomotor movements (the exchange in retinal position of rods and cones on a diurnal cycle) were noted, in agreement with similar observations for *Protopterus* (Pfeiffer 1968). Walls (1942), however, makes a reference to the possibility of retinomotor movements in *Lepidosiren*, in connection with light flux regulation, but without any reference. In the outer plexiform and inner nuclear layers of the *Lepidosiren* retina, apparently horizontal and amacrine cells could not be distinguished from bipolar cells, but their presence was not eliminated. Retinal cell distribution was largely uniform in the four eye quadrants (dorsal, ventral, nasal, temporal) with the number of rods about the same as the number of cones over the retina.

Locket (1970), Pow (1994), Robinson (1994) and Bailes *et al.* (2006a) provide a more detailed description of the retinal morphology in *Neoceratodus forsteri*. Again, all cell types are large although the entire retina is thin (Walls 1942) where the nuclear layers are placed between two thin plexiform layers. Bailes *et al.* (2006a) note up to four sub-layers in both outer and inner nuclear layers. As in the other species, there is no retinal vasculature and no retinomotor movement of photoreceptors. The choroid has large blood vessels and a rete mirabile within a meshwork of cells containing reflective material that comprise a tapetum lucidum, as identified with transmission electron microscopy (Bailes *et al.* 2006a). The tapetum elicits a reddish-pink reflex from the dorsal two thirds of the eye, although the red colour probably is the result of the large red cone oil droplets housed within the inner segments of a sub-population of photoreceptors. Below the tapetum lucidum lies a darkly-pigmented, non-reflective strip of tissue. Vitread to the choroid, large RPE cells extend processes between all photoreceptor

types to the external limiting membrane. Using immunohistochemical methods, Pow (1994) identified two types of horizontal cells, bipolar cells (displaced to the outer nuclear layer and lying within the inner nuclear layer), seven different types of amacrine cells within the inner nuclear layer (four were noted by Bailes *et al.* 2006a), two types of glial cells and both normal and displaced types of ganglion cells. Bailes *et al.* (2006b) identified displaced amacrine cells in the ganglion cell layer by retrograde labeling and four types of ganglion cells. Pow (1994) also demonstrated the retinal distribution of retinal neurotransmitters, such as glutamate and GABA, along with other amino acids.

Previous work, summarised by Walls (1942), Locket (1970) and Pow (1994) describe bipolar cell processes called Landolt's clubs in *Protopterus* and *Neoceratodus*. These structures contain two centrioles and a cilium and are also observed in urodele amphibians and elasmobranchs (summarised in Locket 1970). Their function, however, is speculative.

PHOTORECEPTOR MORPHOLOGY AND DISTRIBUTION

The presence of oil droplets, including the rods of the lepidosireniform species, and the large size of all retinal cells are the two key observations arising from the early body of work on lungfish (Fig. 3; Gunther 1871; Schiefferdecker 1886; Kerr 1902; Grynfeldt 1911; Walls 1942; Munk 1969; Ali and Anctil 1973). Oil droplets, while present in amphibians, birds reptiles and non-placental mammals, are rare in fish (Walls 1942; Douglas and Marshall 1999). Notable exceptions are the relative of lungfishes, the coelacanth, *Latimeria chalumnae* and some species of early ray-finned fishes such as chondrosteans (sturgeon and paddle-fish; Walls 1942; Locket 1973). The lamprey, *Geotria australis* also contains coloured inclusions within three of its five photoreceptor sub-types. These receptors contain yellow inclusions localized within the endoplasmic reticulum of the myoid region (Collin *et al.* 2003). Oil droplet-like inclusions are also part of the retina of the downstream and upstream migrants of *Mordacia mordax* (Collin *et al.* 2004) and *G. australis* (Collin and Trezise 2004), respectively but these do not contain any coloured filters and are ellipsosomes formed from mitochondria. Double cones are present in *Protopterus* sp. (Fig. 3) but, despite previous suggestions (Munk 1964, Pow 1994), they probably do not appear in *N. forsteri* (Bailes *et al.* 2006a) or in *L. paradoxa* (Walls 1942; Ali and Anctil 1973, 1976). The unequal double cones of *Protopterus* sp. possess an oil droplet in the principle (larger) member of the double cone (Fig. 3). Unfortunately, the colours of oil droplets of the lepidosireniforms are not well known as these are largely described from sectioned material only, where the oil droplets may have faded. In the older literature, they are usually described as colourless (summarised in Walls 1942), but this may not be accurate as there are accounts of red oil droplets in both *Protopterus* (Locket 1999) and *Lepidosiren* (Nicol *et al.* 1972). It would be particularly interesting to learn the colour of the rod oil droplet (Fig. 3) as this and other photoreceptor features have led to the suggestion that these rods are in fact modified cones (Walls 1942).

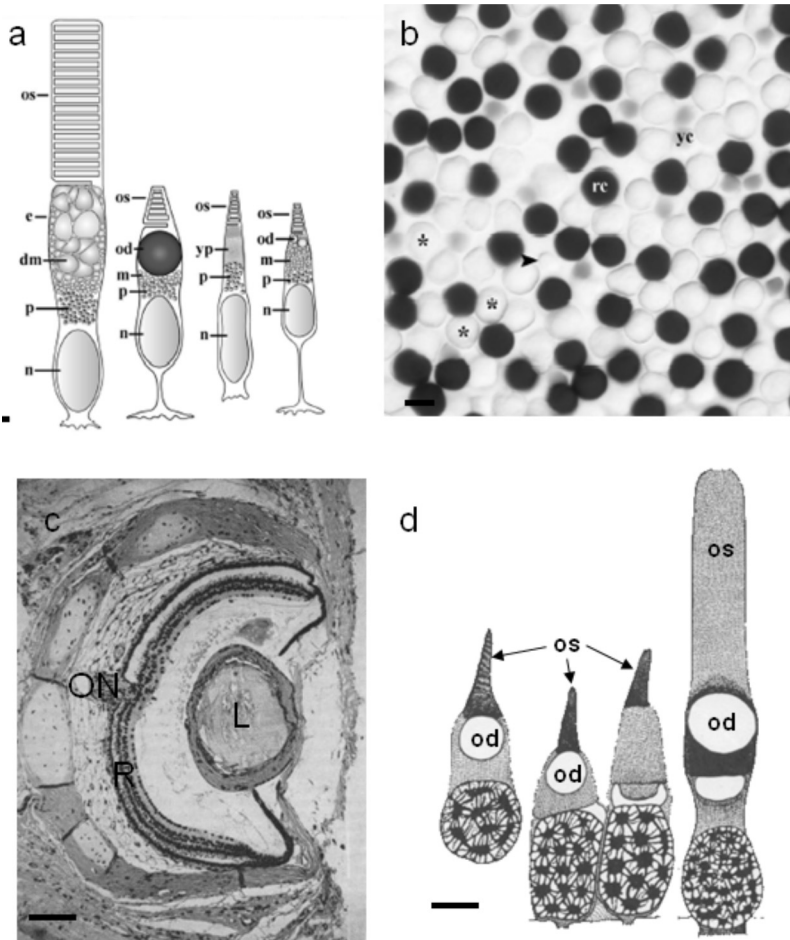


Fig. 3 Photoreceptors and retina of lungfish. (a) Schematic diagram of four morphologically distinct photoreceptors in *Neoceratodus forsteri* from left to right, rod, red cone, yellow cone, clear cone (after Bailes 2006). dm - distended mitochondria, e - ellipsosome, os - outer segment, p - parabaloid, n - nucleus, od - oil droplet, m - myoid, n - nucleus (for explanation of all terms see Walls 1942; Bailes 2006). Scale 10 μ m. (b) Retinal whole-mount of fresh *Neoceratodus forsteri* retina showing all four morphological photoreceptor types. Large clear photoreceptors are rods (asterisks), red and yellow photoreceptor inclusions clearly distinguish these cone types. Arrow marks one clear cone type, being notably smaller than the rods. Scale 10 μ m. (c) Section through the eye of *Lepidosiren paradoxa*. (From Ali and Ancil 1973, with kind permission). Note thin retinal layers. R - retina, L - lens, ON - optic nerve. scale 250 μ m. (d) Schematic diagram of the photoreceptors of *Protopterus aethiopicus*. Note large oil droplet in the rod (far left). Apparent double cone (centre) and single cone (left) (after Walls 1942). Abbreviations as (a). Scale 10 μ m.

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Through a combination of techniques including whole-mount and electron microscopy, five morphological types of photoreceptor, four cone types and a rod, have been identified and extensively characterised in *N. forsteri* (Fig. 3; Robinson 1994; Pow 1994; Bailes *et al.* 2006a). The large rods (around $18 \times 40 \mu\text{m}$ outer segments) make up around 53% of the photoreceptor population. The most abundant cone type (34% of the total population) contains a red oil droplet in the inner segment, with an outer segment close to $15 \mu\text{m}$ long with basal diameter of $6 \mu\text{m}$. The next most frequently found cone (10% of the photoreceptors) possesses a granular, yellow elipsoid pigment and outer segments around $4 \times 10 \mu\text{m}$ in dimension. The third, and least abundant cone type (3% of the photoreceptors), has clusters of clear oil droplets in the ellipsoid and the outer segments are around $4 \times 3 \mu\text{m}$ in size. Both molecular biology and microspectrophotometry (MSP) identify a fifth type of photoreceptor in juvenile lungfish with a spectral sensitivity peaking in the ultraviolet (UV). Morphologically, these cells appear much like the clear oil droplet category just described, although a little smaller (Table 1).

The large rods of lungfish are more like those of amphibians than teleosts in size and 'doom' the animal 'forever to low visual acuity' (Walls 1942). Teleosts, on the other hand, tend to possess many tiny rods and larger cones (Ali and Anctil 1976). Spatial resolving power calculated based on cone spacing is equally poor at around 3.3 cycles per degree in adults (Bailes *et al.* 2006a). In a range of marine teleosts, cone-density based spatial resolving power varied from 4.2 to 14.3 cycles per degree (Tamura 1957). More accurate estimates of spatial resolving power come from ganglion cell mapping as detailed below (Collin and Pettigrew 1989; Bailes *et al.* 2006b).

The distribution of all *N. forsteri* photoreceptors has been mapped in some detail (Bailes 2006). For all cone types, there is an increase in density mostly in the dorso-temporal retina but also in the ventro-nasal retina (Fig. 4, Bailes *et al.* 2006a). The rods are concentrated within a weak horizontal streak of increased density, matching that of the ganglion cells. This distribution of photoreceptors has been taken to imply an interest in objects in front of the fish and possibly an interest in a local horizon such as the sand/water interface.

Large photoreceptors generally imply a need for higher sensitivity rather than high spatial resolution (Land 1981). *N. forsteri* is reported to be active nocturnally or during crepuscular periods (Grigg 1965a; Kemp 1986) and this may explain the size of both cones and rods in these fishes.

GANGLION CELLS AND VISUAL ACUITY

A single row of ganglion cells is described in both *Protopterus* and *Lepidosiren* (Walls 1942, Ali and Anctil 1976). *Lepidosiren* has one of the highest ratios of photoreceptors to retinal ganglion cells of any vertebrate and contains a total of only 1,500 ganglion cells in its retina (Ali and Anctil 1973, Northcutt 1977).

Table 1 Photoreceptor spectral properties of juvenile and adult *N. forsteri*

<i>Juvenile</i>	<i>Rod</i>	<i>Clear UVS</i>	<i>Clear SWS</i>	<i>Yellow MWS</i>	<i>Red LWS</i>
Visual pigment mean λ -max	540	374	481	558	624
Filter mean λ -cut		<330	<330	534	563
Spectral sensitivity peak	535	383	484	584	631
Opsin type	Rh1	SWS1	SWS2	Rh2	LWS
<i>Adult</i>	<i>Rod</i>		<i>Clear SWS</i>	<i>Yellow MWS</i>	<i>Red LWS</i>
Visual pigment mean λ -max	540		481	558	624
Filter mean λ -cut			<330	534	591
Spectral sensitivity peak	574		517	584	656
Opsin type	Rh1		SWS2	Rh2	LWS

Values are to the nearest nm wavelength. λ -cut wavelength of intercept at the maximum measured absorbance by a line tangent to the absorbance curve of the filter at half maximum measured absorbance (Hart and Vorobyev 2005; Bailes *et al.* 2006a). Visual pigment data for the UVS cone is from a juvenile fish only. This cone type probably does not exist in adults. Peak spectral sensitivities are calculated from the filtering effect of the oil droplets and ocular media on the visual pigment. Note effect of more yellow cornea and larger oil droplets in the adult is to shift spectral sensitivities to longer peak values. Opsin localisation in the retina is assumed from spectral sensitivity rather than *in-situ* labeling and therefore are likely rather than certain (Bailes *et al.* 2006a).

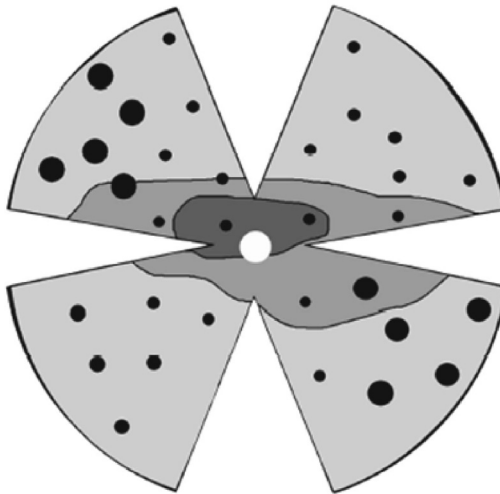


Fig. 4 Schematic representation of the topographic distribution of ganglion cells and cone photoreceptors derived from a retinal wholmount in *Neoceratodus forsteri*. The schematic retinal cup is flattened by making 4 cuts, dorsal (uppermost), ventral, nasal (right hand side) and temporal. The optic nerve head is represented by the clear area in the centre. Ganglion cell density is plotted as iso-density contours and shaded areas and shows a horizontal streak of increased density (darkest grey – 1.2×10^3 cells per mm, light grey – 0.048×10^3 cells per mm). Cone density, which is lowest towards the middle of the retina (1.85×10^3 cells per mm) and highest in dorso-temporal retina (3.49×10^3 cells per mm) is represented by black circles. Scale 1 mm.

This is thought to be an indication that the retina has stopped developing during growth and has remained larval-like or neotenic. In contrast, Bailes *et al.* (2006b) describe around 50,000 ganglion cells in *N. forsteri* divided into four categories, along with one type of amacrine cell that ‘invades’ the ganglion cell layer. They go on to map their distribution in detail (Fig. 4) demonstrating that a horizontal streak of higher ganglion cell density, matching that of the rods, lies across the meridian of the eye. Spatial resolving power based on ganglion cell spacing is low, averaging 1.7 cycles per degree. As the ganglion cells represent the final input to the brain, these estimates, rather than estimates based on rod or cone densities, are considered more accurate. Compared to highly visual teleosts, whose peak ganglion cell density can reach more than one hundred times that observed in *N. forsteri*, lungfishes clearly do not need to resolve fine detail (Collin and Pettigrew 1988). Ganglion cell density in *N. forsteri* is comparable to other riverine fish with benthic lifestyles, such as catfish (Dunn-Meynell and Sharma 1987). The horizontal streak may help stabilise the eye on the local horizon, the sand/water interface, and may even help in prey localisation as the fish sweeps the substrate for food (Bailes 2006). However, more in situ behavioural observations are needed to reliably begin to predict the importance of specific parts of the visual field to the survival of this species. Other aquatic vertebrates, such as teleosts, elasmobranchs, anurans and cetaceans also exhibit horizontal streaks and increased interest in the local horizon is often given as the driving force behind such retinal design (Hughes 1977; Collin, 2008 and see <http://optometrists.asn.au/ceo/retinalsearch> for a new comparative database of retinal maps).

Although, as with the other genera of lungfishes, the ganglion cell layer in *N. forsteri*, is relatively thin (7–10 μm), the presence of four different types of ganglion cells implies a degree of visual complexity not previously expected (Walls 1942; Bailes *et al.* 2006b).

OPTIC NERVE AND CENTRAL PROJECTIONS

The optic nerve in *Protopterus* is described as slender and simple, while in the other two genera, the optic nerve is divided into fascicles (Walls 1942). Northcutt (1977) notes that “the visual system of lepidosirenid lungfish is one of the most reduced visual systems in living vertebrates, as reflected by the small number of optic fibres.” Bailes *et al.* (2006b) describe the optic nerve in *N. forsteri* as unpleated and divided into around 15 large fascicles. Each large fascicle is divided into smaller fascicles of axons surrounding a group of glial cells. In one adult examined (125 cm total length), there were 74,100 axons in the optic nerve of which around 26% were unmyelinated. In a comparison with the retinal ganglion cells counted in retinal wholemount, 62% of the axons in the optic nerve were derived from ganglion cells. The remaining axons may represent a retinopetal population of cells or be the result of axonal branching (Bailes *et al.* 2006b).

Retinofugal projections (projections to the visual centres of the brain) have been described for all genera of lungfishes by Northcutt (1977, 1980). In common with the retina and eye, *Protopterus* and *Lepidosiren* both possess an apparently simple pattern of projections with no ipsilateral retinal projections. However, *N. forsteri* exhibits both contralateral and ipsilateral projections, the latter often associated with stereopsis or at least binocular cross-talk (Pettigrew 1991; Northcutt 1980). The complexity of the larger eye of the Australian lungfish is also reflected in the neurons and brain areas concerned with vision, with a large differentiated dorsal thalamus and pre-tectum and a clearly laminated optic tectum (Northcutt 1980; Robinson 1997). These features imply a more complex visual repertoire. The lungfish brain is closer in structure to both the coelacanth (*Latimeria chalumnae*) brain and the amphibian brain than that of teleost fishes (Northcutt 1977, 1980, 1986).

VISUAL PIGMENTS, OIL DROPLETS AND SPECTRAL SENSITIVITIES

In almost every fish examined, morphologically-distinct photoreceptors contain different visual pigments and usually, therefore, possess different spectral sensitivities (Loosey *et al.* 1999; Kusmic and Gualtieri 2000; Bowmaker and Loew 2008). Microspectrophotometry (MSP, Dartnall 1975) of the outer segments of the retina in adult *N. forsteri* (individuals 105 and 110 cm total length) has revealed a rod and three cone types with a maximal absorbance, or λ -max, of 534 nm (rod), 481 nm (short wavelength sensitive, clear oil droplet cone), 560 nm (medium wavelength sensitive, yellow ellipsoid cone) and 624 nm (long wavelength sensitive, red oil droplet cone) (Fig. 5). In juveniles (24, 28, 32 cm total length), a fourth type of cone was found with maximal absorbance in the ultra violet UV region of the spectrum at 375 nm (Fig. 5). This cone type shares the morphology of the short wavelength sensitive cone in the adult, including the presence of several clear oil droplets, although these UV sensitive photoreceptors are probably slightly smaller (Bailes 2006a; Hart *et al.* 2008). Such diversity and a large spectral range of photoreceptors argues against the idea that these animals are crepuscular/nocturnal, slow moving and not very reliant on vision. Rather, this sort of complexity invokes a diurnal existence with visual demands requiring a finely tuned colour vision system.

In common with several fish species, most notably the salmonids (Hawryshyn *et al.* 1989; Beaudet *et al.* 1997; Bowmaker and Loew 2008), it seems most likely that the UV cone is lost in adulthood. MSP can be a haphazard technique in terms of photoreceptor sampling and can miss photoreceptor types all together, especially if they are located in discrete retinal regions and are particularly rare and/or small. It is therefore possible that UV cones persist in the adult, however the fact that UV wavelengths are also blocked from reaching the retina by an

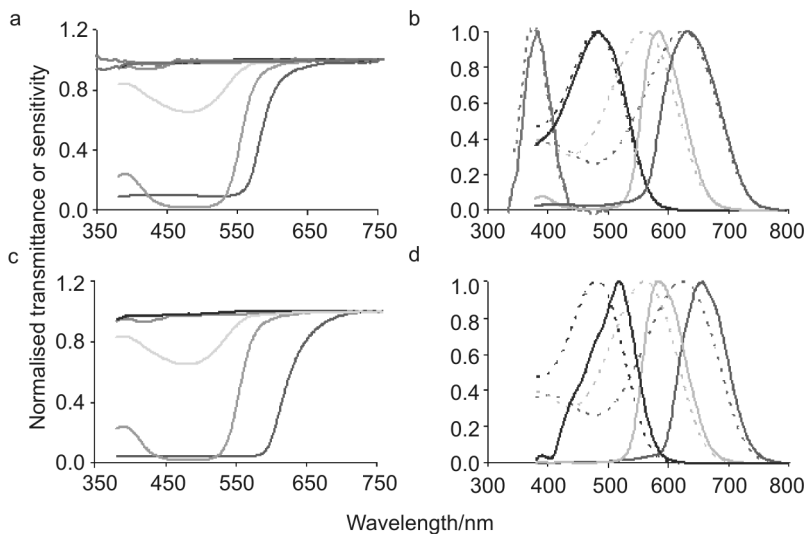


Fig. 5 Oil droplet and visual pigment spectral characteristics (a) Transmittance of oil droplets and yellow pigment in juvenile *Neoceratodus forsteri* photoreceptors relative to maximum transmission at 750 nm. The red oil droplet absorbs strongly below its λ -mid at 585 nm (red line), the yellow ellipsoid pigment λ -mid is at 556 nm (orange line) and the clear oil droplets of both UV (purple line) and short wavelength sensitive clear cones (green line) have negligible absorbance from 350–750 nm. (b) Visual pigment sensitivities of four cones (dotted curves) and the actual photoreceptor spectral sensitivities resulting from filtering by oil droplets and ocular media (Fig. 2) in juvenile *Neoceratodus forsteri*. Visual pigment curves are a nomogram based on peak absorbance (Table 1) and a vitamin A2 template, except for the UV sensitive photoreceptors which do not fit a template well and are just averaged direct measurements (Hart *et al.* 2008). Note loss of spectral sensitivity overlap and peak sensitivities pushed to longer wavelengths. (c) As (a) but for adult *Neoceratodus forsteri*. The red oil droplet is larger and absorbs more light, its λ -mid pushed longer at 623 nm. Yellow and blue pigments are the same as the juvenile. (d) Visual pigment sensitivities of three cones (dotted curves) and the actual photoreceptor spectral sensitivities resulting from filtering by oil droplets and ocular media (Fig. 2) in adult *Neoceratodus forsteri*. Visual pigment curves are a nomogram based on peak absorbance (Table 1) and a vitamin A2 template. Note loss of spectral sensitivity overlap and peak sensitivities pushed to longer wavelengths. Unlike the juvenile (b), the denser yellow adult ocular media (Fig. 2) absorbs strongly below 400 nm.

Color image of this figure appears in the color plate section at the end of the book.

increasingly yellowing cornea and lens in the adult, supports the notion that these photoreceptors are no longer useful to the mature fish (Figs. 2, 5, 7; Hart *et al.* 2008). The behavioural function of an apparent shift from potential tetrachromacy to potential trichromacy (Fig. 7) needs a clearer understanding of the environmental factors driving this change and of the behavioural ecology of these lungfishes. Possession of UV sensitivity may indicate a more surface biased existence, where

UV is still abundant even in green/brown water, or that a UV-specific task needs to be performed such as planktivory (Losey *et al.* 1999; Loew *et al.* 1993).

The yellow cornea and the yellow lens, particularly notable in adult *N. forsteri*, modifies the spectrum of light reaching the retina (Figs. 2, 5). The cornea and lens of juveniles filters out light below 330 nm, while in the adult, this moves up to around 400 nm. Variable UV filtering by ocular media is common in fishes (Kondrachev *et al.* 1986; Douglas and McGuigan 1989; Douglas and Thorpe 1992; Thorpe and Douglas 1993; Thorpe *et al.* 1993; Douglas and Marshall 1999; Siebeck and Marshall 2001; Siebeck *et al.* 2003) and is often thought to prevent the potentially damaging UV wavelengths from reaching the delicate retinal tissue. Increasing the density of yellow, short wavelength absorbing pigment within the ocular media during ontogeny is common in fishes (Thorpe and Douglas 1993) and indeed other animals including humans. Yellowing may be a result of an ageing eye as well as screening this damaging irradiation from reaching an increasingly large and therefore sensitive retina (Douglas and McGuigan 1989; Whitmore and Bowmaker 1989; Thorpe and Douglas 1993; Douglas and Marshall 1999).

All adult visual pigments contain a vitamin A2-based chromophore and this is typical of many freshwater fishes (Lythgoe 1979; Kusmic and Gualtieri 2000; Bowmaker and Loew 2008). The A2 containing visual pigments display longer wavelength sensitivities than their A1 equivalent and this appears to be of ecological benefit due to the largely longer wavelength dominated 'green' world of streams and lakes as opposed to the blues of oceanic waters (Lythgoe 1979). The UV sensitive cones of the juvenile lungfish, break this trend and indeed the 375 nm λ -max visual pigment in the juveniles was not a good fit to the A2 chromophore templates (template fitting is used to determine λ -max and chromophore type, Govardovski *et al.* 2000). A1 templates also did not fit the UV visual pigment absorbance curve well, implying secondary filtering or another factor as yet unknown may determine the absorbance in these photoreceptors (Fig. 5).

Each of the four morphological types of cone photoreceptor in *N. forsteri* contains a different oil droplet visual pigment combination (Table 1; Bailes *et al.* 2006a). Oil droplets, and other coloured inclusions in the eyes of many vertebrates, act as filters, tuning the spectral sensitivity provided by the visual pigment alone. The transmittance of oil droplets and the ocular media (cornea and lens) of *N. forsteri* have also been measured using MSP and spectrophotometry (Table 1; Fig. 5, 6; Hart *et al.* 2008). Both the clear and the intensely coloured red oil droplet and yellow ellipsoid of *N. forsteri* are similar to the coloured and clear oil droplets and ellipsoidal inclusions of birds and reptiles and the coloured retinal inclusions of the lamprey *Geotria australis* (Partridge 1989; Douglas and Marshall 1999; Collin *et al.* 2003; Bailes *et al.* 2006a; Hart *et al.* 2008). The oil droplets of amphibians, non-placental mammals and sturgeons are mostly colourless and this is often said to be the case for *Lepidosiren* and *Protopterus* (Walls 1942, see above). However, red oil droplets in both *Protopterus* (Locket 1999) and *Lepidosiren*

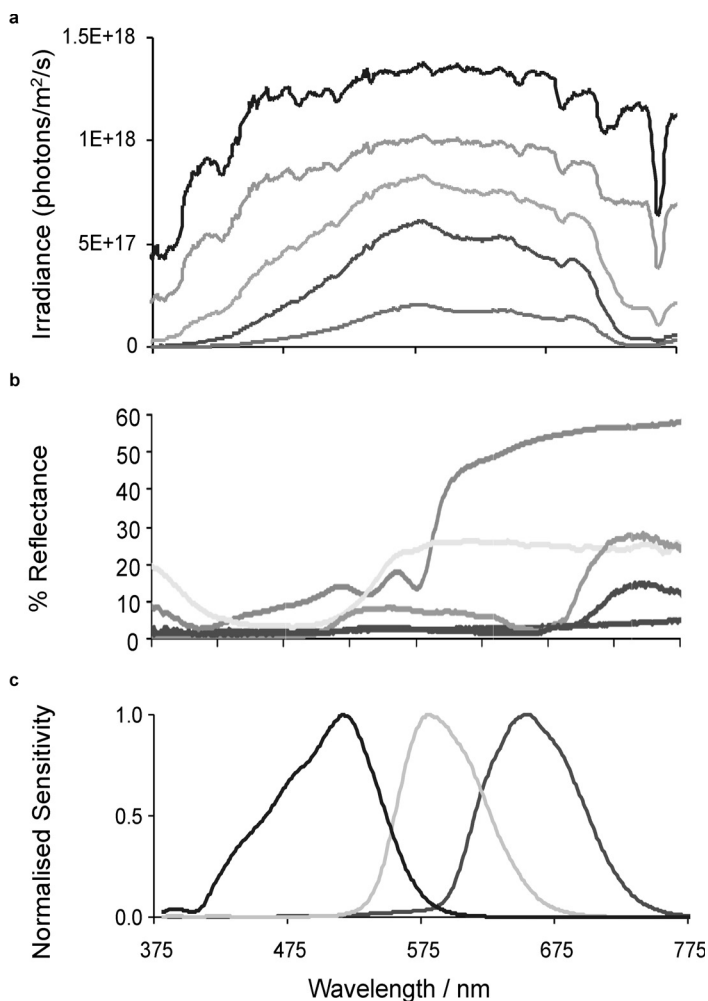


Fig. 6 Aspects of visual ecology in *Neoceratodus forsteri*. (a) Irradiance of light in the Mary River (Queensland, Australia), known habitat of *Neoceratodus forsteri* at: the surface (blue line), 0.05 m (bright green line), 0.5 m (mid-green line), 1 m (dark green line), 1.25 m (kaki line). (b) Reflectance of objects in the environment of *Neoceratodus forsteri*: gravid female belly (orange line), non gravid female belly (yellow line), macrophytes from Mary River (green lines – see Hart *et al.* 2008 for details), submerged log from Mary River (brown line). These and other objects are plotted in the colour space of adult and juvenile in Fig. 7. (c) Adult spectral sensitivities (as Fig. 5b) included here for direct comparison with illumination in habitat and to complete complement of components needed for visual ecology characterisation; spectral sensitivities, illuminant on objects of interest and reflectance from objects of interest. Using these 3 factors is the first step towards the colour vision system models of Fig. 7 (Kelber *et al.* 2003).

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(Nicol *et al.* 1972) are now thought likely from tapetal examinations. Clearly, a careful examination of these retinæ is needed and of particular interest will be the colour, if any, of the large oil droplets reported in the rods of these species (Fig. 3; Kerr 1902; Rochon-Duvigneaud 1941; Walls 1942; Ali and Anctil 1977).

Coloured photoreceptor inclusions generally act as long-wavelength-pass cut-off filters, absorbing wavelengths of light most strongly below their so called λ -cut (Table 1; Liebman and Granada 1975; Bowmaker and Knowles 1977; Partridge 1989; Hart and Vorobyev 2005). The functional result of this is examined in more detail in the visual ecology section below. Here, we just demonstrate the way in which the spectral sensitivities of the photoreceptors are sharpened and shifted to longer wavelengths (Fig. 5). In short, the spectral tuning by these filters improves the spectral discrimination ability of the owner's colour vision system (reviewed in Kelber *et al.* 2003). The composition of oil droplets and ellipsoids is comprehensively described elsewhere and is generally thought to be lipid and/or carotenoid based, as are the yellow pigments in the cornea and lens (Muntz 1972; Walls and Judd 1933; Wald and Zussman 1937; Meyer 1965; Johnston and Hudson 1976).

One result of filtering, by both ocular media and coloured photoreceptor inclusions, is a reduction in sensitivity of sometimes 90% or more (Douglas and Marshall 1999). The dimensions of the large and therefore most sensitive cone (and in the lepidosirenid lungfish, rod), may be a response to the quantal flux reduction imposed by the oil droplets and ellipsoids, along with a need to retain complex colour vision in the potentially light limited world of lungfish. If the currently observed activity patterns of *N. forsteri* are indeed crepuscular or nocturnal as suggested (Kemp 1986), any colour vision system that needed to retain colour discrimination in these challenging light conditions would certainly need to operate with 'monstrous' photoreceptors in order to catch enough photons (Land 1981; Warrant 1999; Warrant and Locket 2004). Night colour vision has recently been shown in a number of invertebrates (Kelber and Henique 1999; Kelber *et al.* 2003; Warrant 1999) and photoreceptor enormity is certainly in evidence there.

MOLECULAR BIOLOGY OF THE VISUAL PIGMENTS

Molecular characterisation the visual pigments of *N. forsteri* reveals the presence of all five known vertebrate opsin families: Rh1 (medium wavelength sensitive 1, found in rods), Rh2 (medium wavelength sensitive 2, found in cones), LWS (long wavelength sensitive, found in cones), SWS1 (UV/violet/short wavelength sensitive 1, found in cones) and SWS2 (blue/short wavelength sensitive 2, found in cones) (Yokoyama 2000a,b, 2002; Bowmaker and Hunt 2006; Bailes *et al.* 2007b). Table 1 shows the distribution of these within the 4 morphologically-distinct photoreceptors. The opsin is the protein part of the visual pigment molecule and its tertiary structure is one of the factors determining the spectral tuning of the visual pigment (Bowmaker and Hunt 1999; Bowmaker and Loew 2008).

This sort of opsin diversity is now being found in several fish lineages and interestingly, fish seem to be able to 'choose' when to express a particular opsin within a photoreceptor, both in an ontogenetic and a phylogenetic context (Bowmaker and Hunt 1999; Spady *et al.* 2006). It is, at least in part, the spectral environment and visual ecology of the species that determines the loss, duplication or retention of these genes during evolution (Bowmaker and Hunt 1999; Bowmaker and Loew 2008; Trezise and Collin 2005).

Interestingly, it was the use of molecular techniques that first suggested the presence of a SWS1 UV/violet sensitivity in lungfish and this was later confirmed with microspectrophotometry (MSP) (Bailes 2006; Bailes *et al.* 2007b; Hart *et al.* 2008). From both molecular and MSP evidence, it now seems likely that adults lose the fourth UV sensitive cone type and that this cone is retained in juveniles only (Bailes *et al.* 2007b; Hart *et al.* 2008). Similar loss of UV sensitivity during ontogeny is known in several fish species, notably the salmonids, and reflects changes in visual demands often associated with migrations and differing visual environments during different life stages (Hawryshyn *et al.* 1989; Archer and Hirano 1996; Beaudet *et al.* 1997; Bowmaker and Hunt 1999; Bowmaker and Loew 2008).

Phylogenetic analysis of the sequences of amino acids of the opsins from a range of vertebrates, places lungfish opsins closer to amphibian opsins than to those of teleost fish, again lending support to this evolutionary scenario (Bailes *et al.* 2007b). Partial sequences of Rh1 and LWS genes have been found in African lungfish, *Protopterus* sp. (Venkatesh *et al.* 2001; H.J. Bailes, W.L. Davies, A.E.O. Trezise, S.P. Collin – unpublished data) but more work is needed on this species and in *Lepidosiren* to determine the complement of opsin families present and their level of expression. As *N. forsteri* is generally regarded as plesiomorphic and the lepidosirenids derived and neotenic, determining the relationship of opsins within the three lungfish genera would add useful data to the current debate on their phylogenetic relationship and that of their nearest neighbours (Bailes *et al.* 2007b).

HABITAT, BEHAVIOUR AND VISUAL ECOLOGY

The visual behaviour of all lungfish species is poorly understood and it has even been stated that vision is of little importance to them (Dean 1906; Walls 1942; Kemp 1986; Simpson *et al.* 2002). Other sensory systems, including the lateral line and electroreception are well developed and some focus has been placed on these modalities (Watt *et al.* 1999; Jørgensen and Kemp, pers comm and see Chapters 18 and 20). *N. forsteri* is said to be mostly sedentary showing an increase in activity in the late afternoon and during the night, however the majority of these observations are from captive individuals (Grigg 1965a, Kemp 1986). While they can move fast if necessary (Kemp 1986), nothing is known about visual

guidance of such behaviour. The diet of adult *N. forsteri* includes molluscs, fish, crustaceans and amphibians as well as some plant matter and this could suggest a visual component to prey capture (Kemp 1986). Olfaction and gustation are likely to play a significant role in feeding behaviour as the fish have been observed snuffling around in the weeds looking for food (Kemp 1986, Kind 2002). Live food is preferred by juveniles (Kemp 1986), although this observation is based largely on captive breeding programmes rather than field observations and, due to their poor spatial resolution, it is unlikely that they visually pursue prey (Bailes 2006; Bailes *et al.* 2006b). Courtship and spawning may include visual input since these behaviours are relatively elaborate and involve circling near the surface followed by spawning over apparently carefully selected macrophyte beds containing *Vallisneria gigantea*, *Hydrilla verticillata* and *Nitella* sp. (Grigg 1965a; Kemp 1984; Brooks and Kind 2001). Gravid females also become bright orange on the belly and this is likely to be a strong visual signal, especially in mid to long wavelength dominated fresh water systems (Lythgoe 1966, 1968a, b; Loew and McFarland 1990; Bowmaker and Loew 2008). A more detailed examination of how such colours may appear to *N. forsteri* is included below (Fig. 7).

An extensive survey of Australian lungfish movements and habits has all but failed to find any juveniles and, aside from a few sightings, this is a lasting mystery (Kind 2002). While it has been suggested that juveniles may spend much of their first few years buried in mud, Kemp (1986) suggests that the 'absence' of juveniles is due to inadequate capture mechanisms or a small fish's ability to avoid nets and escape. Kind's survey did determine that the average depth which adults inhabit was around 2 m but with considerable variation (Kind 2002). While a walk up the Mary or Fraser rivers might give the impression that *N. forsteri* lived in predominantly green, murky water, these fish are known from a wide variety of water types, use very shallow tributaries for spawning and are seen around surface waters (Grigg 1965a; Kemp 1986; Brookes and Kind 2001). That is, they clearly spend some time in broad spectrum, brightly-lit (possibly clear?) habitats in which a complex colour vision system, including UV wavelengths, may be useful.

The photic habitat of *N. forsteri* in the Mary River (Queensland) has been quantified, and in common with many algae/chlorophyll-laden freshwater systems, is largely green (Fig. 6). The spectral range of this species is clearly influenced by its light availability, as is the case for most fish (Fig. 6; Lythgoe 1966, 1968a,b, 1979, Bowmaker and Loew 2008). How UV may be of importance to juveniles, or even if it is truly lost in adults remains unresolved, although colours in the lungfish habitat, including body colours, contain a UV component (Fig. 6). This reflection in the UV is not remarkable as it can be found in other, apparently specific UV, signalling systems (Losey *et al.* 1999; Losey 2003; Hausmann *et al.* 2003; Marshall *et al.* 2003a, b; Siebeck 2004).

Protopterus is also found in a wide variety of natural habitats, both lentic and lotic, and is known to be an omnivorous carnivore (Greenwood 1986). Both

African and South American lungfishes are very poorly documented in terms of any behaviour that could be called visually driven. Unlike their Australian relative, they are capable of aestivation during drought and all lungfish species can flop about out of water, breathing air (Grigg 1965b; Burggren and Johansen 1986). *N. forsteri* is even said to 'walk' using its lobed fins but given the body weight of adults at several 10s of kilograms and the rudimentary musculature of these fins, this is not likely (Grigg 1965a, b; Kemp 1986). There are no reliable reports of any lungfish using their eyes for visually guided behaviour out of water, so the tenuous hope of lungfish complex colour vision being specifically for any out of water behaviour is questionable.

In short, we do not know much about where any species of lungfish spends its time and almost nothing about what it is doing while it is there. Given our new knowledge of the colour vision of Australian lungfish and the recent characterisation of the visual ecology of this animal (Bailes 2006; Hart *et al.* 2008), it is possible to predict detection and discrimination capabilities of potentially important colours to lungfish. As part of this, the function of the oil droplets and yellow ellipsoid pigments also becomes clear (Figs. 5, 7). These predictions using established visual models are now discussed in the context of oil droplet function and likely tri- or tetrachromacy in lungfish.

As previously demonstrated in birds (with visual systems also consisting of four cone types for colour vision, each with unique combinations of visual pigment and oil droplet), oil droplets function to expand the colour space of the animal (Fig. 7; Govardovski 1983; Vorobyev 2003 and see Kelber *et al.* 2003 for a good review of colour models and visual systems). In brief, by narrowing the spectral sensitivity of the naturally broad-band visual pigment contained in the cone to which they are associated, and by spacing the spectral sensitivities evenly in the available spectrum (Fig. 5), oil droplet filtering increases the distance of the loci spectra occupying each animal's colour space. As colour discrimination is, broadly speaking, proportional to distance between loci, this potentially allows finer spectral discrimination (Kelber *et al.* 2003 and Fig. 7) as well as improved colour constancy (Osorio *et al.* 1997; Neumeyer *et al.* 2002).

While we are not sure what colours may be of importance to lungfish, Fig. 7 plots the spectra of objects found in habitat of the Australian lungfish, i.e. plants, rocks, sand, logs and the lungfish itself, in the modelled colour space of both juvenile and adult *N. forsteri* (Bailes 2006; Hart *et al.* 2008). For the adult, this is done with oil droplets present and theoretically removed to show the contribution of these coloured retinal filters. Object spectra chosen include potential spawning-site plants and the colour of the female's ventral surface. The latter is known to change colour from a dull yellow to bright orange as the female becomes more gravid (Kemp 1984, 1986) and this may act as a colour signal to the males. While this is currently supposition, it is interesting that the position in *N. forsteri* colour space occupied by a ripening female's belly colours allows them to be particularly well discriminated (Fig. 7).

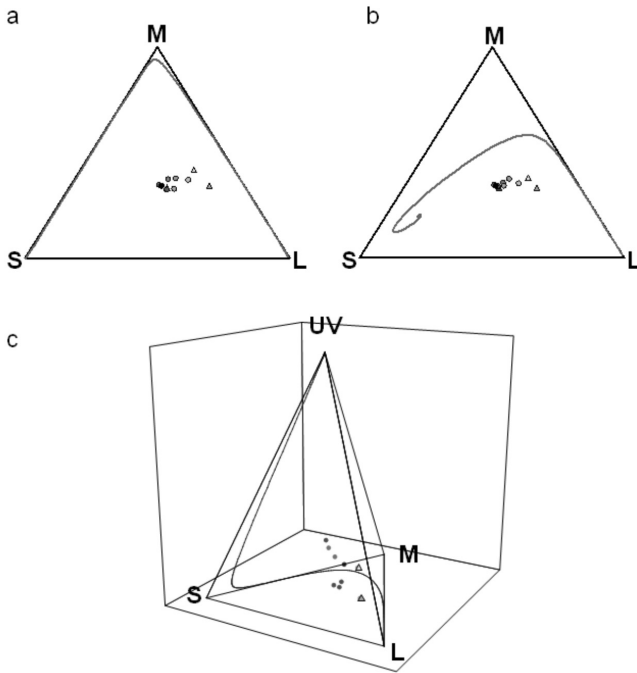


Fig. 7 Modelled colour vision performance in *Neoceratodus forsteri* examining coloured objects from Mary River environment (see Kelber *et al.* 2003 for review and references therein for methods). (a) Colour space based on Maxwell triangle for three cone sensitivities in adult *N. forsteri*. Although behavioural proof is required, this suggests trichromacy. The centre of the triangle is the achromatic point (occupied by flat spectra of e.g. white, grey and black) and areas towards the edges of the triangle are occupied by increasingly saturated spectral chromaticity loci up to the blue line – the monochromatic locus. The shape of the monochromatic locus is set by the spectral sensitivity characteristics and a comparison of (a) and (b) here demonstrates the way filtering by coloured ocular inclusions (oil droplets, ellipsoid pigment and ocular media) expands this space, thus improving the potential colour vision. Each symbol in the triangle (and tetrahedron in (c)) is the position occupied by one reflectance spectrum (Fig. 6b). The corners of the triangle; S, M and L plot the spectral loci of maximal stimulation of the short wavelength, medium wavelength and long wavelength photoreceptors respectively. Thus, for example, red spectra will plot near the L corner. Colour coding same as spectra in Fig. 6b. Female lungfish belly, gravid – orange triangle, female lungfish belly, non-gravid yellow triangle, macrophytes – green circles, log – brown circle, rocks and sand – grey circles (not plotted in Fig. 6b). (b) As (a) except for the hypothetical adult lungfish spectral sensitivities in the absence of filtering by coloured ocular inclusions (Govardovski 1983). Note the contraction of colour space delimited by the monochromatic locus (blue line) and the lesser separation of the loci of the coloured objects from lungfish habitat. (c) Three dimensional tetrahedral colour space of juvenile, potentially tetrachromatic, *N. forsteri*. Conventions and colours the same as triangles, UV, denotes the chromatic locus of the UV sensitive cone (Fig. 5b).

Color image of this figure appears in the color plate section at the end of the book.

Required input parameters to this model are environmental light in photons, reflectance measurements of colours and spectral sensitivity (calculated from visual pigment absorbance and the filtering effects of both oil droplets and cornea; Figs. 5, 6). These, along with the relative photoreceptor number, allow an accurate assessment of the performance of different colour vision systems (Vorobyev and Osorio 1989; Kelber *et al.* 2003). A comparison of the juvenile and adult (Fig. 7) does not shed light on any added advantage of potential tetrachromacy for the juveniles, however, given the paucity of knowledge regarding visual behaviour and habitat of either, this is no surprise.

It is clear that the general envelope and long wavelength bias of the cones in adult *N. forsteri* are well matched to the relatively long wavelength light prevalent in the freshwater habitat (Fig. 6) as is the case for many fish species, both freshwater and marine (Levine and MacNichol 1979; Lythgoe 1979; Loew and McFarland 1990).

Whether the adult and juvenile *N. forsteri* possessed truly trichromatic and tetrachromatic colour vision would require behavioural proof (Neumeyer 1992; Vorobyev *et al.* 1998; Kelber *et al.* 2003). Both forms of colour vision are known in freshwater fish (Neumeyer 1991, 1992, 1998), but they lack the added complexity of oil droplet filtering, a visual attribute of the terrestrial vertebrates. The dimensionality and details of the lepidosireniform lungfish colour vision (if any) are unknown, although the presence of (probably) coloured oil droplets certainly makes work on these species an exciting future project. Why Australian lungfish, with apparently poor spatial resolution and a desire to increase sensitivity with its large photoreceptors then 'invests' in the same sort of complexity for colour vision as a bird is another mystery. Colour vision often has rather poor spatial resolution (Vorobyev *et al.* 2001), but this does limit the sorts of task performed to those involving large colourful objects. Perhaps judging the quality of a big female with a bright orange belly and the correctly coloured patch of water-weed to lay eggs on are what has driven this colour vision system? On the other hand, it may be a neotenic survivor from a previously truly terrestrial amphibian. While this is unlikely to be resolved, visual systems are metabolically expensive and the fact that lungfish retain apparently complex colour vision suggests that they still use it. After the recent revelations regarding this remarkable fish's visual sense, it is clearly even more imperative that we both find out more and work to prevent the disappearance of lungfish through habitat destruction (Chapter 20).

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