Other-Worlds: Encounters with the visual perception of lungfishes through science and art

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ABSTRACT

Lungfishes are large, freshwater, lobe-finned fishes that have the ability to breathe both dissolved and atmospheric oxygen through gills and “primitive” lungs, respectively. Six species of lungfishes remain worldwide; four in Africa (*Protopterus* spp.), one in South America (*Lepidosiren paradoxa*) and one in Australia (*Neoceratodus forsteri*).

Lungfishes are the closest living descendants of the ancestors of all terrestrial vertebrates, and are vital to the study of the evolution of vision in tetrapods because of their association with animals that made the remarkable transition from water onto land.

This unique interdisciplinary study explores the visual perception and experience of lungfishes through biological science and visual art, while also tracing the history of human cultural interactions with this significant animal. Scientific data generated through anatomical, electrophysiological and behavioural experimentation contributes to the emerging field of lungfish visual ecology, and artistic research provides a more sensorial speculation of the lungfish visual experience in order to reflect on how we (humans) produce knowledge of non-humans and the limitations of these methods.

To begin, a cultural history of non-human animal visual perception is investigated to understand and acknowledge the limitations of technologies and our anthropocentric perception of reality. Human narratives from the nineteenth century to present day are traced to reveal the similarities and differences in previous attempts to uncover non-human animal perceptual capabilities, with a particular focus on Jakob von Uexküll and his concept of the *Umwelt*. A collaborative art project titled ‘*Inperception*’ (2012) that endeavoured to interpret the *Umwelten* of Australian lungfishes through contemporary scientific knowledge and art is discussed as a case study.

Diverse cultural narratives are discussed, revealing a long history of the lungfish as an “in-between” and “monstrous” animal. This thesis shows that human understandings of lungfishes are influenced by a myriad of factors that include, but are not limited to, the evaluation of scientific data. Personal and cultural biases effect how we make sense of non-human “Others”. These inescapable biases, formed by ingrained beliefs, assumptions and ideologies, force a particular understanding between what we “see” and what we “know” about lungfishes. The crucial importance of recognising these biases and anthropocentric limitations in any attempt to understand the lungfish *Umwelt* and the historical and cultural contexts of scientific exploration more broadly is revealed.

Having established the limitations and complexities involved in “knowing” the perception of a non-human “Other”, the biological “machinery” of the lungfish visual perception is described by two scientific studies. The first presents the results of anatomical investigations of the retinal photoreceptor cells of two previously unexamined species, *P. dolloi* and *L. paradoxa*, in order to determine their capacity for colour vision. These findings were compared with previous studies of *N. forsteri* in order to present a more comprehensive understanding of the visual ecology of the three lungfish genera. Previous anatomical studies of *N. forsteri* have shown that lungfishes possess the retinal machinery to process colour. This study revealed the presence and ultrastructure of one rod and two cone photoreceptor types in juvenile *P. dolloi* and characterised one rod and one cone photoreceptor type in *L. paradoxa*. The topographical distribution of the red cone in a juvenile *P. dolloi* is also described. The presence of large oil droplets (or intracellular inclusions that act as filters) in all three species suggests that the lungfish visual system is adapted for high sensitivity. The absence of double cones in all three species however, strongly suggests that double cones may have developed after the evolutionary separation of tetrapods from *P. dolloi*.

In addition, the characterisation of the retinal machinery of *L. paradoxa* by this study implies that, although both *N. forsteri* and *P. dolloi* possess the machinery to process colour, *L. paradoxa* does not have the potential for colour vision.

The second scientific study investigated the visual perception of a unique group of juvenile *N. forsteri* whose eyes were not externally visible. New findings are presented that begin to uncover how a unique group of fish experience the world without the ability to form images, resulting from behavioural, circadian, electrophysiological and anatomical studies that compared these abnormal *N. forsteri* with normal Australian lungfishes. The abnormal fishes all possessed subcutaneous eyes capable of photo-transduction, although possibly not spatial vision. Clear corneas were absent in the eyes of all these abnormal *N. forsteri*, although they all possessed the retina and photoreceptor complement typical of their species. Variation in the functional integrity of the eye between individuals was evident however, as behavioural phototaxis was not demonstrated by all specimens.

Finally, a number of artworks and exhibitions generated through the scientific and artistic research that extended and complicated issues surrounding the lungfish *Umwelt* are discussed. Presented chronologically, these creative outcomes reveal my shifting understandings of the limitations of technology, objectivity and human perception and cognition in attempting to interpret the *Umwelt* of such a different animal. The first set of artworks, ‘*Third Eye*’ and ‘*In Focus*’, produced in 2013 present the “machinery” of the lungfish eye generated through biological dissection, macro-photography and light microscopy. These black and white, abstracted micrographs of retinæ and photoreceptors are aestheticised scientific representations produced in my attempt to discover the “truth” of the lungfish eye. A series of sculptural works produced in 2014 and collectively titled ‘*The Forms*’ explores role of aesthetics and manipulation in the

production and presentation of scientific images through transmission electron microscopy (TEM), camera-less photography (photograms) and material manipulation. My frustrated attempts to negotiate the gaps between technology, measurement, representation and experience are manifest in these objects. Most recently, the 2015 multi-media exhibition, '*Cernentia: exploring the visual perception of lungfishes without eyes*' provided visitors with a multi-sensorial insight into the investigation of the abnormal *N. forsteri* visual system and a speculative experience of the perceptual world of these unique animals. Collectively, these artworks trace my own journey with lungfishes and offer new encounters with the lungfish *Umwelt* outside of the mechanistic realm of biological science. Importantly, they reveal the anthropocentric lens through which an *Umwelt* of a non-human animal "Other" is explored.

This unique interdisciplinary thesis unravels the variety and complexities of the visual perception of an important group of non-human animals – lungfishes. The methodologies of three disparate disciplines – biological science, history and visual art – are integrated in an attempt to understand the complexity of how non-human animals perceive their environment, whilst recognising the limitations of each discipline. In a society which is characterised by increasing specialisation, there is a need for scholars who can communicate and speculate on the "Other" animal from a rigorous and highly informed scientific and cultural/artistic position. The unique interdisciplinary and inter-sensory approach offered by this thesis is therefore, vital when human relations with our ecology are in crisis and in need of re-negotiation.

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STATEMENT OF CANDIDATE CONTRIBUTION

In accordance with the University of Western Australia's regulations regarding Research Higher Degrees, this thesis is presented as a series of chapters that may be prepared for publication.

This thesis is an account of research undertaken by the candidate during the period of 10th February 2011 to 31st of August 2015 while enrolled as a full-time postgraduate student in The School of Anatomy, Physiology and Human Biology, and The School of Animal Biology at the University of Western Australia.

Lepidosiren paradoxa and *Protopterus dolloi* specimens were donated by Glenn Northcutt and Guido Westoff, respectively. Except for the occasional assistance from the candidate's supervisors and other academics of the lab (Shaun P. Collin, Nathan S. Hart, Jan Hemmi, Michael Archer, and Ionat Zurr) in the experimental set-up, training for experimental procedures and analyses; all experiments and data analyses were conducted by the candidate.

The custom software used to analyse electrophysiological data was created by Nathan S. Hart. The custom software used to analyse behavioural and circadian data was created by the candidate and Jan Hemmi.

A critical review of each chapter upon completion was provided by the candidate's supervisors.

The artwork '*Inperception*' (2012) was a collaborative project between the candidate and two other artists (Shannon Williamson and Devon Ward), and is credited accordingly. The construction of the headset for the artwork '*Bloop*' (2015) was in collaboration with Devon Ward and the candidate, and is credited accordingly.

The content of this thesis is the candidate's own composition, and all relevant sources are acknowledged. This thesis has not been submitted for any other degree in this or another institution.

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LIST OF ABBREVIATIONS

μm	Micrometres
μV	Microvolts
λ_{max}	Maximum absorbance
<i>a</i>	Aperture
<i>a</i>	Axial length of the lens
AC	Alternating current
Ag/Ag-Cl	silver/silver chloride
c/cor	Cornea
cc	Clear cone
cFFF	critical flicker-fusion frequency
CO ₂	Carbon dioxide
DAFF	Department of Agriculture, Fisheries and Forestry
E	Eyeless <i>Neoceratodus forsteri</i>
EM	Electron microscopy
ERG	Electroretinography
EPBC	Environment Protection and Biodiversity Conservation Act 1999
<i>f</i> _r	Focal ratio
g	Grams
<i>gfp</i>	Green fluorescent protein
H ₂ O	Water
Hz	Hertz
ir	Iris
INL	Inner nuclear layer
ipl	Inner plexiform layer
is/IS	Inner segment of photoreceptor cell
JITL	Jindalee International Pty Ltd
kV	Kilowatts
LED	Light-emitting diode
LM	Light microscopy
ln	Lens
LWS	Long-wavelength sensitive
M	Molarity
m	Mitochondria
ms	Milliseconds
MS222	Tricaine methane sulfonate
MSP	Microspectrophotometry
MWS	Medium-wavelength sensitive
N	Normal <i>Neoceratodus forsteri</i>
n	Sample size
N/A	Not available
NA	Numerical aperture

NaHCO ₃	Sodium bicarbonate
ND	neutral-density filter
nm	Nanometres
O ₂	Oxygen
od/OD	Oil droplet
on	Optic nerve
ONL	Outer nuclear layer
OP	Oscillatory potentials
opl	Outer plexiform layer
os/OS	Outer segment of photoreceptor cell
p	Paraboloid
Pax6	Paired box protein Pax-6
PC	Personal computer
PFA	4% paraformaldehyde in 0.1 M phosphate buffer
PND	Distance between the centre of the lens and the back of the retina at 90° from the posterior nodal distance
PR	Photoreceptors
Qld	Queensland, Australia
<i>r</i>	Radius of curvature
rc	Red oil droplet containing cone
rd	Rod
r/ret	Retina
rgc/RGC	Retinal ganglion cells
rpe/RPE	Retinal pigment epithelium
scl	Sclera
SEM	Standard error of the mean
SD	Standard deviation
SWS	Short-wavelength sensitive
TEM	Transmission electron microscopy
TL	Total length
UV	Ultra-violet
UVS	Ultra-violet sensitive
VEPs	Visually evoked potentials
yc	Yellow-pigment containing cone

I
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**GENERAL
INTRODUCTION**
.....

For human beings, colour vision has largely aesthetic values. If it is present in lower animals....what does it do for the animals? What was the incentive for its evolution?

(Walls, 1942)

This research project makes an original contribution to scientific, artistic and cultural literature by investigating and communicating, to the expert and the public, the subjective visual experience of non-human animals through science and art. The processes of unravelling visual perception through scientific and cultural methodologies are burgeoning fields of research. While science is able to provide a general and mechanistic understanding of the visual system, it is always informed by an anthropocentric perception of reality, cultural frames of reference, and data generated and interpreted by humans. Similarly, cultural and artistic inquiries acknowledge anthropocentric limitations and aspire to provide a holistic non-human perspective, but lack the objectivity of scientific methodology to conceptualise a reality that may be very different from our own. All three disciplines have much to contribute to the discourse surrounding visual perception. However, the disparity between academic “cultures” through lack of communication and integration results in considerable gaps in knowledge about the visual experience of non-human animals.

This research focuses on lungfishes as model organisms to explore the visual perception of non-human animals because of their importance in the evolution of vision.

Lungfishes are living fossils and evolved approximately 410 million years ago during the Devonian period (early Lochkovian), the “Age of Fishes”, according to the International Union of Geological Sciences (Clack et al., 2011) (Long, 2015). They are large, freshwater fishes that have the ability to breathe atmospheric and dissolved oxygen through “primitive” lungs and gills. (Brinkmann et al., 2004, Amemiya et al.,

2013). The lungfishes' evolutionary position makes them vital to the study of the evolution of vision in land vertebrates (tetrapods) as they are the closest surviving descendants of those animals that made the transition from water onto land.

This thesis investigates how the sciences and the arts (often opposing disciplines) explore the visual perception of lungfishes by simultaneously undertaking scientific and artistic experiments to uncover, represent and interpret the visual experience of these animals. In addition, it discusses the general anthropocentric and discipline-specific limitations inhibiting deeper understanding of the visual experience of non-human animals. This discussion of limitations results in opportunities when these different academic "cultures" are forced or encouraged to communicate. Therefore, a more informed view of the lungfish's visual experience is sought by uncovering limitations and opportunities provided by previous methods.

i.1 NON-HUMAN ANIMAL PERCEPTION

We polish the animal mirror to look at ourselves

(Haraway, 1991)

Scholars from the sciences and the arts¹ have attempted to critically uncover the visual perception of non-human animals. Scientific scholars focus on quantitative measurements of perceptual capabilities in other organisms, and rely on direct logical extrapolations from experimental data to address non-human visual perception. Early

¹ I refer to both humanities and visual art disciplines here

scientific discoveries that tested the presence and type of colour vision of non-human animals were achieved by direct observation. John Lubbock and Karl von Frisch were pioneers in this research, and used behavioural criteria to determine whether non-human animals were able to differentiate colours (Lubbock, 1889, Von Frisch, 1914). The “grey card experiment” created by Frisch, established that honeybees (*Apis mellifera*) associate colour with food, and can distinguish blue and yellow from thirty shades (intensities) of grey (Von Frisch, 1914). Experiments following the same design have subsequently confirmed the ability to discriminate colour in a variety of animals, including butterflies (belonging to the families Nymphalidae, Pieridae, Satyridae and Papilionidae), hermit crabs (*Eupagurus anachoretus*), shrimp (*Crangon vulgaris*) domestic chickens (*Gallus gallus*) (Kelber et al., 2003), and the giant shovelnose ray (*Glaucostegus typus*) (Van-Eyk et al., 2011).

Techniques such as microscopy, microspectrophotometry (MSP) and electrophysiology have also been useful in understanding the mechanistic basis of vision and identifying differences in visual performance of many species. These techniques enable the identification and characterisation of different kinds of retinal cells and other retinal specialisations, and can define the range of wavelengths to which an animal is optimally sensitive, and whether that animal has the potential for colour vision. The potential for colour vision and the description of visual specialisations have been reported in many organisms including the crayfish (*Orconectes virilise*) (Wald, 1967), the southern hemisphere lamprey (*Geotria australis*) (Collin et al., 2003, Collin et al., 2004), the turtle (*Pseudemys scripta elegans*) (Kolb and Jones, 1987), the human (*Homo sapiens*) (Bowmaker and Dartnall, 1980), the tawny owl (*Strix aluco*) (Bowmaker and Martin, 1978) and the duck (*Anas platyrhynchos*) (Jane and Bowmaker, 1988).

However, the design, conduct and interpretation of such scientific experiments have anthropocentric limitations that are often overlooked or ignored. Scientific studies attempt to objectively explain the visual experience of other organisms, but the data is always collected and understood by human-mediated apparatuses, be it the human body or human-created technological instruments, making the endeavour fundamentally and inescapably anthropocentric.

Non-scientific scholars that have attempted to address non-human visual perception place a greater focus on the anthropocentric problems associated with seeing through the eyes of the “Other”. Jakob von Uexküll was one of the first researchers to describe non-human worlds holistically through observational experiments that analysed animal behaviour. Uexküll believed that biological science gave little regard to the subjective perceptual response of an animal to and within its environment. With this in mind he constructed the theory of the *Umwelt* (Uexküll, 2010). Uexküll suggested that each individual constructs its own environment (*Umwelt*) that is dependent on perceptions, actions and relationships within/to that environment. He conceptualised the *Umwelt* (plural: *Umwelten*) as a soap bubble surrounding each organism. Uexküll’s main objective was to observe and consider the animal and environment as interdependent and complex, instead of deconstructing the animal into its mechanical constituent parts, as a purely scientific approach tended to do. Although the *Umwelt* theory had merit opening up radical new ways of interpreting and conceptualising non-human animal perceptions, it drew significant criticism from the scientific community due to the unfalsifiable and fundamentally subjective nature of its ideology.

Philosopher Thomas Nagel recognized the problems inherent in exploring the subjective visual experience of other organisms. Nagel discussed the conscious experience of individuals occurring “in countless forms totally unimaginable to us” because the

experience is exclusive to the organism, and one must “*be* that organism” in order to understand it (Nagel, 1974). Although he acknowledged the human mind as the limiting factor preventing full comprehension of another perceptual world, Nagel did not dismiss the futile task of attempting to bridge the subjective character of experience. Instead, he suggested removing human imagination from the interpretation, perhaps through objective experimentation, as a way to reveal parts of “the subjective character of experiences in a form comprehensible to beings incapable of having those experiences” (Nagel, 1974). However, Nagel did not engage with science or suggest and/or articulate how such experiments could take place.

Non-scientific research into the visual perception of animals rarely engages with science or the mechanisms and concepts surrounding what are known about visual systems, while scientific research rarely acknowledges the anthropocentricity and limitations of the field. This research project acknowledges the importance of the scientific investigation of non-human animal visual experiences, but also recognises the anthropocentric limitations of the scientific method highlighted by non-scientific scholars, and attempts to combine them for a deeper understanding of the lungfish visual experience. Chapter 1 explores this through a discussion of several historical scientific and non-scientific attempts to uncover animal perception. With a particular focus on the similarities and differences of the two fields, the collaborative art project ‘*Inperception*’ (2012) endeavoured to interpret the *Umwelten* of Australian lungfishes through contemporary scientific knowledge and art. In order to uncover the mechanisms of lungfish perception, chapter 3 and chapter 4 utilizes contemporary scientific approaches (anatomical dissection, transmission electron microscopy, electrophysiology, behavioural and experiments on circadian rhythms) to describe aspects of the visual system of the spotted African (*Protopterus dolloi*) and South

American (*Lepidosiren paradoxa*) lungfishes, and compares the visual perception of normal and abnormal Australian lungfish (*Neoceratodus forsteri*).

i.2 LUNGFISHES AND THEIR VISUAL SYSTEM

If it hadn't been for the obstinacy of our great-uncle N'ba N'ga, we would have long since lost all contact with the aquatic world. Yes, we had a great-uncle who was a fish, on my paternal grandmother's side, to be precise, of the Coelacanthus family of the Devonian period...

at any season of the year all we had to do was push ourselves over the softer layers of vegetation until we could feel ourselves sinking into the dampness, and there below, a few palms' lengths from the edge, we could see the column of little bubbles he sent up, breathing heavily the way old folk do, or the little cloud of mud scraped up by his sharp snout, always rummaging around, more out of habit than out of the need to hunt for anything.

We lay on a sloping bank, all three of us: my great-uncle was nearest the water, but the two of us were half in and half out, too, so anyone seeing us from the distance, all close together, wouldn't have known who was terrestrial and who was aquatic.

(Calvino, 1968)

Lungfishes are ideal to explore the visual perception of non-human animals through the sciences and arts because of their unique evolutionary position as “in-between” creatures. These animals diverged from the main vertebrate stock around 410 MYA and along with the coelacanth (*Latimeria chalumnae*), encompass the surviving lobe-finned

fishes (Sarcopterygii) (Hart et al., 2008, Clack et al., 2011). They have the ability to breathe dissolved and atmospheric oxygen through gills and “primitive” lungs, respectively, existing between water and land. Linked to humans by being the closest living relatives to the tetrapods (Brinkmann et al., 2004, Amemiya et al., 2013), they are vital to the study of the evolution of vision in terrestrial animals.

Six extant species of lungfishes remain worldwide. Four of these are located in Africa, one in South America and one in Australia. The South American species (*L. paradoxa*) and all African species (*Protopterus* spp.) belong to the family Lepidosirenidae (Walls, 1942). *N. forsteri* is the only surviving species belonging to the family Ceratodontidae, and is considered to be the most basal of all lungfishes with a single lung used only during periods of increased activity or in areas of poor water quality (Kind, 2011).

i.2.1 Morphology

N. forsteri was originally described by Krefft (1870), who thought this large fish to be an amphibian “allied to the genus *Lepidosiren*” because of its comparative morphology and dentition. Morphologically, adult Australian lungfishes are characterised by having a wide, flat head, a thick heavy body, large scales that decrease in size towards the tail, a diphyccercal tail, and paddle shaped fins (Fig. i.1) (Kemp, 1986, Kind, 2011).

Fig. i.1. Living specimens of *Neoceratodus forsteri*. **(A)** An adult *N. forsteri* about to rise to the surface. Note the paddle shaped pelvic fins and diphycercal tail. Scale bar = 1 cm. **(B)** Two living juveniles of *N. forsteri*, illustrating the body proportions and differences in morphology when compared to the adult **(A)**, relatively smaller pelvic fins and rounder heads. The dorsal fin also reaches further forward. Scale bar = 1 cm. Reproduced with kind permission from Wiley-Liss Inc. (Kemp, 1986).

L. paradoxa is morphologically similar to *Protopterus* spp. (Mlewa et al., 2011).

However, its body length is longer and slimmer, with smaller scales, and reduced pectoral and pelvic fins for sensory functions when compared to *N. forsteri* (Berra, 2001, Fonesca de Almeida-Val et al., 2011). Juvenile *L. paradoxa* are predominantly black and spotted yellow which fades to a brown/gray colour as they mature (Figs. i.2 and i.3).

Fig i.2. Immature South American lungfish (*Lepidosiren paradoxa*). Photo credit: SE Temple, SP Collin, NS Hart and RG Northcutt (2010).

Fig. i.3. Adult South American lungfish (*Lepidosiren paradoxa*). Photo credit: SE Temple, SP Collin, NS Hart and RG Northcutt (2010).

Protopterus spp. are identified by their elongate bodies. *Protopterus* larvae also possess external gills, a trait shared with *L. paradoxa* but not with *N. forsteri*. As adults, *P. aethiopicus* and *P. dolloi* lose these external gills, while *P. amphibius* and *P. annectens* retain them. *P. dolloi* possesses a brown colouration and is relatively slender when compared to *P. aethiopicus*, which has a more cylindrical body and larger adult size (Bailey, 1994).

i.2.2 Distribution and habitat

At the time of European settlement, Australian lungfish (*N. forsteri*) populations were thought to reside only in the Burnett and Mary River basins. The origin of the Brisbane River populations are still under debate. *N. forsteri* populations are now also found in the North Pine, Logan, Albert and Coomera Rivers, and Enoggera, Gold Creek and Manchester reservoirs, however these are almost certainly recent unreported movements and translocations (Kemp, 1995, Kind, 2011). This species occurs in small groups mainly in still or slow flowing water where dense macrophytes are present (Allen et al., 2002) at a depth between 3 and 10 meters, and remain sedentary within a restricted area of one or two pools (Kemp, 1995).

The South American lungfish is found in the neotropics of South America, namely Argentina, Bolivia, Brazil, Colombia, French Guiana, Paraguay, Peru, and Venezuela, primarily in the Amazon River basin, but also the tributaries of the Para-Paraguay river systems (Fonesca de Almeida-Val et al., 2011). They inhabit stagnant and lentic waters of swamps and lakes that are populated with vegetation and low oxygen conditions where the temperature ranges from 24° - 28°C (Fonesca de Almeida-Val et al., 2011).

All *Protopterus* species inhabit both lentic (lakes, or standing water) and lotic (river, stream, or running water) environments that extend throughout most of the continental African landmass, including all of the Rift Valley lakes, with the exception of Lake Malawi, the major river systems of tropical Africa and a number of localities therein (Greenwood, 1987). *P. annectens* and *P. dolloi* are predominantly West African, while *P. aethiopicus* and *P. amphibius* are considered East African taxa (Mlewa et al., 2011). However, *P. aethiopicus* also occurs in the Zaire basin and *P. annectens* can extend east into the middle and lower Zambezi systems as well as the Limpopo system. Although African lungfishes are known to reside in open waters of lakes and river systems, they are generally associated with the shallow areas and swamps surrounding the margins of these water bodies including marginal grass-, water lily, and papyrus swamps.

i.2.3 Diet

All lungfishes are opportunistic omnivores. In their natural habitat, these fish forage among macrophytes and ingest large quantities of substrate particles and various plants, which pass through the digestive tract relatively intact (Günther, 1871). They have also been reported to feed on terrestrial plant seed pods and molluscs, crustaceans, worms, tadpoles and small fishes (Kemp, 1986, Kemp, 1995, Kind, 2011). The adult South American lungfish diet consists of small bony fishes, snails, clams, shrimp and some algae and terrestrial flora (Berra, 2001, Fonesca de Almeida-Val et al., 2011). African lungfishes are considered to be the most carnivorous of all the Dipnoan fishes (Greenwood, 1987). They consume a variety of prey including molluscs, crustaceans, small fishes, worms and aquatic insects as well as plant material found in their natural habitat (Curry-Lindahl, 1956).

The diet of Australian lungfishes changes over time. Juvenile *N. forsteri* tend to be sit-and-wait carnivorous predators, whereas adults are opportunistic omnivores (Kind, 2011). Evidence of this change in diet within *N. forsteri* is evident in dentition; juveniles possess sharp grasping teeth that change to broad crushing plates in adults (Kemp, 1977).

i.2.4 Aestivation

Five of the six lungfish species undergo aestivation, with *N. forsteri* being the only exception. Aestivation can be described as a “light” state of dormancy, characterised by the construction of a cocoon, withdrawal into a state of torpor, and characteristic cardio-respiratory and metabolic changes (Fishman et al., 1987). The process enables lungfishes to avoid damage from high temperatures and desiccation during the dry season without leaving the swamps for permanent water, like other fishes (Greenwood, 1986).

L. paradoxa and *P. dolloi* employ different methods of aestivation. The South American species creates a burrow 0.5 metres into the muddy clay substrate of the swamp by forcing its way vertically or obliquely using undulating body movements (Carter and Beadle, 1930) and leaving several holes to facilitate air-breathing (Fonesca de Almeida-Val et al., 2011). There the fish lies coiled in an oval expansion of the burrow and lowers all metabolic function. The burrow’s substrate is predominantly clay which is characteristically impervious to water. This provides the fish with an ideal burrow that is partially filled with water to prevent it from drying out (Carter and Beadle, 1930). Although *L. paradoxa* does not form a mucous seal over its water-filled burrow, the burrow is sufficient to sustain the fish throughout the dry season (Fonesca de Almeida-Val et al., 2011).

Aestivation patterns in *Protopterus* vary between species (Greenwood, 1987), *P. annectens* being the most dependant on aestivation (Johnels and Svensson, 1954, Greenwood, 1987, Mlewa et al., 2011). With the arrival of the dry season and the resulting decrease in water level, *P. annectens* (40 mm and over in length) individuals excavate a vertical burrow into the still submerged mud of the swamp bed by body movements and biting into the soil (Johnels and Svensson, 1954). Once the burrow is between 30 – 250 mm in depth (depending on the fish's length), the fish turns 180° on its long axis, creating a bulb-shaped chamber approximately twice the width of its body in diameter, and rests with its snout pointing upwards (Greenwood, 1987). Once this chamber is formed, the fish makes its mouth into a tube in order to breathe until the water table falls below the snout level of the fish, whereby it will cease respiration and remain coiled in the chamber. As the ambient water decreases, the fish secretes mucus that, as the mud surrounding the chamber dries, hardens to produce a waterproof cocoon that completely engulfs the lungfish apart from the respiratory opening in the snout (Mlewa et al., 2011). *P. annectens* remains in this state until the water level rises again and the cocoon is submerged by the coming wet season. An individual can aestivate for periods up to 7 or 8 months. However, some captive and laboratory reared lungfishes have been reported to have aestivated for 7 years (Smith, 1931, Greenwood, 1987, Mlewa et al., 2011). There is little evidence that on *P. aethiopicus* aestivates in the wild, although their burrows have been found in various places including north of Lake Kyoga, Uganda, and closely resemble those described for *L. paradoxa* (Wasawo, 1959). *P. dolloi* has developed a different approach to surviving the dry season, burrowing a vertical shaft into the raft-like substrate of its swamp habitat. This shaft is partially filled with water that is rich in organic matter, with a high CO₂ and low O₂ content. *P. dolloi* inhabits this burrow until the next wet season (Greenwood, 1987). Along with the cessation of respiration during aestivation, physiological processes characteristic of

aestivation in *Protopterus* include reduced metabolic rate, renal shutdown and mechanisms that prevent the harmful effects of fat and protein metabolism while fasting without access to water (Fishman et al., 1987, Mlewa et al., 2011). Aestivation allows the lungfish to permanently occupy seasonal habitats and decreases the mortality rates and resource limitations of migrating, non-resident species.

i.2.5 The visual system of lungfishes

The aquatic vertebrate eye (Fig. i.4) is adapted to detect light and form images in a range of light environments. Light first passes through the transparent cornea, differing from terrestrial vertebrates by imparting only limited refractive power to the eye due to the similarities of the refractive indices of the cornea and the surrounding water. A much larger and more highly refractile crystalline lens in aquatic vertebrates compensates for this lack of refractive power (Charman, 1991, Collin and Collin, 2001). After entering the eye through the cornea, light passes through the lens to and strikes the photoreceptor cells in the retina located at the back of the eyecup. Photoreceptor cells transform light energy into a biochemical signal through a process called phototransduction, and these visual signals are transmitted through the interneurons of the retina (bipolar, horizontal and amacrine cells) to reach the ganglion cells. The axons of ganglion cells are then responsible for conveying this information to the visual centres of the brain via the optic nerve.

The rods and cone photoreceptors are not only morphologically distinct, but have different physiological properties in order to operate effectively under different levels of illumination. Rods are used for vision in low light conditions, as they are highly sensitive and saturate in bright light. Cones are used for vision in bright light because they saturate only under very intense illumination. If two or more types of

photoreceptor (usually cones) that are maximally sensitive to different parts of the visible spectrum are present in the retina, their outputs can be compared in order to perceive colour because a single photoreceptor type cannot distinguish between changes in intensity and changes in wavelength². The relative stimulation of photoreceptors with differing spectral sensitivities must be compared by higher order neurons (such as the retinal ganglion cells or output cells that send messages to the brain) in order to discriminate objects on the basis of spectral reflectance ('colour') rather than just intensity.

Humans have three different types of cone photoreceptors and one type of rod photoreceptor. These cones have peak sensitivities ($\lambda_{\max}$ values) at 562 nm (long-wavelength sensitive [LWS], or red), 533 nm (medium-wavelength sensitive [MWS], or green), and 420 nm (short-wavelength sensitive [SWS], or blue), while the single rod photoreceptor type is MWS with a $\lambda_{\max}$ of 497 nm (Bowmaker and Dartnall, 1980). Opponent comparisons of the relative stimulation of the different types of cone photoreceptors enable us to perceive colour.

² This is known as the theory of univariance. See RUSHTON, W. 1972. Review lecture. Pigments and signals in colour vision. *The Journal of Physiology*, 220, 1-31..

Fig i.4. Schematic representation of a fish (teleost) eye and the direction of incoming light. Modified from (Walls, 1942).

Due to the relatively small size of the lungfish eye in comparison to its body and its predominantly nocturnal lifestyle in captivity, it was assumed that vision was unimportant for these animals (Bancroft, 1928). However, recent evidence suggests that the visual system of *N. forsteri* is highly specialised, with the retina adapted for increased dim-light or scotopic sensitivity (the possession of large photoreceptors, a reflective tapetum lucidum and high degree of spatial summation) whilst still capable of functions typically associated with bright-light or photopic vision (the potential for tetrachromatic colour vision, coloured oil droplets/spectral filters within the photoreceptors) (Bailes et al., 2006). These characteristics are rare within actinopterygian fishes, being more commonly associated with strongly diurnally-active terrestrial vertebrates (Walls, 1942, Bailes et al., 2007, Hart et al., 2008, Marshall et al.,

2011). The sophistication of its ocular system suggests that the visual sense of *N. forsteri* is crucial for many aspects of its behavioural ecology (Bailes et al., 2007).

Considerable gaps in our knowledge about the visual system in lungfishes are evident, and although there has been considerable interest in *N. forsteri* over the last ten years, the South American and African species have been somewhat neglected. Therefore, this project investigated and compared the visual systems of these lungfish species with that of *N. forsteri* to determine if the complex visual system of *N. forsteri* is universal among this group of ancient organisms.

i.2.5a The lungfish retina

The retinae of lungfishes have been described as “simple”, although containing very large cells (Locket, 1970). The retinae of South American and African lungfishes previously characterised by light microscopy revealed a well pigmented epithelial layer, with rod and cone photoreceptors possessing oil droplets. The “monstrous” rods and cones are difficult to distinguish by size, as their morphology is similar (Kerr, 1902, Walls, 1942, Pfeiffer, 1968, Ali and Anctil, 1973). All lungfish retinae contain both rods and cones. *Protopterus* spp. appear to have single and double cones (Pfeiffer, 1968), unlike *L. paradoxa* and *N. forsteri*, which show no evidence of double cones (Ali and Anctil, 1973, Bailes et al., 2006). In the case of *N. forsteri*, the rod (53%) and cone (47%) proportions are similar, suggesting the animal balances both scotopic and photopic³ visual requirements (Bailes et al., 2006). Conversely, the number of rods is inferior to cones in the case of *L. paradoxa* (Ali and Anctil, 1973). *N. forsteri* photoreceptors are composed of a single rod type and three (adults) or four (juvenile) morphologically and spectrally distinct cone types (Fig. i.5). The retina of this species

³ Scotopic is vision under low light conditions, and photopic is vision under bright light conditions.

displays low spatial resolving power, and increased scotopic sensitivity with the possession of large rod photoreceptors (up to 20 μm in diameter and 38 μm in length) and a tapetum lucidum⁴ (Bailes et al., 2006).

Fig i.5. A light micrograph of an Australian lungfish (*Neoceratodus forsteri*) retina illustrating the morphological differences of the rod (rd), red oil droplet containing cone (rc), clear cone (cc) and yellow-pigment containing cone (yc) photoreceptors (PR). The retina consists of different cell layers such as the retinal pigment epithelium (RPE), photoreceptors (PR), outer nuclear later (ONL), inner nuclear layer (INL) and retinal ganglion cells (RGC). Arrowheads indicate oil droplets. Scale bar = 20 μm . Modified and reproduced with kind permission by Wiley-Liss Inc. (Bailes et al., 2006).

⁴ The tapetum lucidum is an intraocular reflecting structure that is responsible for the “eye-shine” phenomenon in many animals. It enhances visual sensitivity by providing photoreceptors with a second opportunity for photon-photoreceptor stimulation in low light conditions. See BRAEKEVELT, C. R. 1990. Fine structure of the feline tapetum lucidum. *Anatomia, histologia, embryologia*, 19, 97-105. and OLLIVIER, F., SAMUELSON, D., BROOKS, D., LEWIS, P., KALLBERG, M. & KOMÁROMY, A. 2004. Comparative morphology of the tapetum lucidum (among selected species). *Veterinary ophthalmology*, 7, 11-22..

i.2.5b Spectral characteristic of *N.forsteri* retinal photoreceptors

Five visual pigment opsin genes, orthologous to the five visual opsin groups in other jawed vertebrates have been characterised in *N. forsteri*. Along with a single type of rod photoreceptor with a wavelength of maximum absorbance ($\lambda_{\max}$) of 540nm, the juvenile *N. forsteri* visual system contains four individual cone photoreceptor types with visual pigments with $\lambda_{\max}$ value at 366 nm (ultra-violet sensitive [UVS]), 479 nm (SWS), 558 nm (MWS) and 623 nm (LWS) (Hart et al., 2008). All five photoreceptor types are observed only in juveniles. The adult fish loses the UVS photoreceptor, perhaps as a result of increased filtering of short wavelengths by the ocular media (cornea) in adults that prevents UV light from entering the eye (Hart et al., 2008). This suggests that juvenile *N. forsteri* have the potential for tetrachromatic vision. However, the influence of all characterised photoreceptor types on lungfish behaviour remains to be investigated.

In addition to the five distinct photoreceptor types, *N. forsteri* also possess red oil droplets in the ellipsoid of their LWS cones, one or multiple colourless oil droplets in the inner segments of their UVS and SWS cones, and yellow ellipsoidal pigmentation in their MWS cones. The yellow pigmentation and red oil droplets shift peak sensitivities of the photoreceptor types to 584 nm and 656 nm, respectively (Hart et al., 2008).

As depicted in Fig. i.6, spectral filters such as intracellular oil droplets enhance colour discrimination (Vorobyev, 2003) but also decrease in the absolute quantum catch of the visual pigment, reducing overall light sensitivity (Hart et al., 2008). Although their behavioural function is unknown, the presence of spectral filters within lungfish photoreceptors suggests that these animals may lead a more diurnal lifestyle than

previously thought. In addition, *N. forsteri* also go through ontogenetic changes in their diet, from sit-and-wait carnivores in their juvenile years to opportunistic omnivores as adults (Kemp, 1977). These changes in lifestyle may be involved in the ontogenetic shifts observed in the lungfish retinal photoreceptors.

Fig. i.6. The object colour solid volumes for juvenile (**A, B**) and adult (**C, D**) *Neoceratodus forsteri*, with (**A, C**) and without (**B, D**) the spectral filtering affects of yellow ellipsoidal pigmentation and red oil droplets in medium- (MWS) and short-wavelength sensitive (SWS) cones. The tetrachromatic colour space of juvenile lungfish (**A, B**) is shown by two projections of the spaces identified by the quantum catches of the ultraviolet-wavelength sensitive (UVS), SWS and MWS cones and also the SWS, MWS and long-wavelength sensitive (LWS) cones. Note that there is an increase in the volume of object-colour solids in both juveniles (**B**) and adults (**D**) with spectral filters relative to those projections without spectral filters (**A** and **C**, respectively). Reproduced with kind permission from Shaun P. Collin. (Hart et al., 2008).

The visual machinery of lungfishes, predominantly at the ocular and retinal levels, is investigated through scientific experimentation in chapter 3 and chapter 4 of this thesis. Chapter 3 reveals aspects of the morphology, density and topographic distribution of retinal photoreceptors in *L. paradoxa* and *P. dolloi*, and chapter 4 investigates and compares the visual perception of abnormal *N. forsteri* lacking external eyes with normal *N. forsteri* possessing external eyes. These two chapters reiterate that science is an important tool and methodology to make sense of lungfishes' visual perception. However, as Bruno Latour says, "scientific activity is just one social arena in which knowledge is constructed" (Latour and Woolgar, 1986) and Donna Haraway reminds us that, "biology is a discourse, not the living world itself" (Haraway, 1992). Therefore, chapter 2 of this thesis delves into historical and contemporary stories about lungfishes to show that many factors, aside from the evaluation of scientific data, influence our understanding of lungfishes. Just as scientific research is one "social arena" in which knowledge about lungfish visual perception can be attained, cultural ideologies and assumptions also influence how humans comprehend the visual experience of lungfishes. Chapter 5 brings scientific and artistic investigation together by contextualising a number of artworks that create new encounters and relationships with non-human animals through the exploration of perception. These artworks poetically and sensorially extended the complicated understandings and limitations surrounding the lungfish *Umwelt* to provide speculation that lies outside the mechanistic realm of scientific research.

i.3 CREATING AN INTERDISCIPLINARY PLATFORM

“To deny the reality or logical significance of what we can never describe or understand is the crudest form of cognitive dissonance”

(Nagel, 1974)

The sciences and the arts have both made important contributions to how we understand the visual experience of non-human animals. However, the two fields rarely understand or communicate with one another on equal terms. The differing research methodologies adopted by each field create discipline-specific boundaries that, while enabling depth of disciplinary investigation, may restrict understanding, especially when the subject is non-human animal perception; a problematic topic because the reality of the beholder is subjective. Chemist C.P. Snow (1959) was one of the first to describe science and the arts (“literary intellectuals”) as “two cultures”. He wrote:

constantly I felt I was moving among two groups – comparable in intelligence, identical in race, not grossly different in social origin, earning about the same incomes, who had almost ceased to communicate at all, who in intellectual, moral and psychological climate had so little in common that...one might have crossed the ocean.

(Snow, 1998)

According to Snow, non-scientists described scientists as “shallowly optimistic, unaware of man’s condition” and lacked “imaginative understanding”. To scientists, practitioners of the arts were “lacking in foresight”, “anti-intellectual, (and) anxious to restrict both art and thought to the existential moment” (Snow, 1998). Snow described the sciences and arts cultures as “ignorant specialists” that, through their beliefs and oftentimes

misinterpretations of the opposing culture, became self-impoverished as a result (Snow, 1998). Snow's main concern was the communication gap formed by the disparity between the two cultures to the detriment of society and to further knowledge: "Closing the gap between our cultures is a necessity in the most abstract intellectual sense, as well as in the most practical" because they are "of great importance for our intellectual and moral health" (Snow, 1998). Snow recognized a need to address the methodological limitations of each culture so he proposed the emergence of a "third culture" to close this unbridgeable gap. In Snow's "third culture", the "literary intellectuals" would reach out to scientists and "some of the difficulties of communication will at last be softened: for such a culture has, just to do its job, to be on speaking terms with the scientific one" (Snow, 1998).

Other, more contemporary theorists also recognized a need to address the limitations within the scientific and non-scientific fields. Literary agent and author John Brockman used Snow's "third culture" to describe a space where members of the "science culture", such as Richard Dawkins, Brian Goodwin, Marvin Minsky, Roger Penrose and Stephen Jay Gould, could communicate directly with the general public through books and the media (Brockman, 1991). In Brockman's "third culture", Snow's "literary intellectuals" are replaced rather than cooperated with. He writes, "third-culture thinkers tend to avoid the middleman and endeavour to express their deepest thoughts in a manner accessible to the intelligent reading public" and are "taking the place of the traditional intellectual in rendering visible the deeper meanings of our lives, redefining who are and what we are" (Brockman, 1996).

Theorists and philosophers Donna Haraway, Giles Deleuze and Félix Guattari approach the limitations of the sciences and the arts by addressing the gap between humans and non-human animals in order to better understand the world. For them (and others in the

budding field of critical animal studies), attempting to “perceive” like the “Other” (non-human animal) involves tearing down pre-conceived notions about what it is to be human. They believe that there is a growing need for humans to reconnect with the environment and become “more worldly” (Haraway, 2008). Deleuze and Guattari described this process as “becoming animal”; the human being’s opportunity to think themselves “other-than-in-identity” and provide “the way out, the line of escape” (cited in Baker, 2000). Haraway described this discourse as a way to understand that the human species is part of – and interdependent on – the environment as all animals are, and refute the anthropocentric assumption that we have dominion over it (Haraway, 1991, Haraway, 2008).

The importance of this process of “becoming animal” lies in the opportunity to think differently about humans and animals. It is to leave the purely human and directly engage with the “Other” (be it animal or its environment, for the purpose of this thesis) to gain new understanding (Deleuze and Guattari, 1987, Baker, 2000). Deleuze and Guattari write,

the relationships between animals are object not only for science but also of dreams, symbolism, art and poetry, practice and practical use...[and] are bound up with the relations between man and animal, man and woman, man and child, man and the elements, man and the physical and microphysical universe (Deleuze and Guattari, 1987)

This engagement can be done in many ways that should not be exclusive to any single discipline, and when investigating subjective experience, the objectivity of science is “a direction in which understanding can travel” (Nagel, 1974). Snow also spoke of this potential, stating that, “the clashing point of two subjects, two disciplines, two cultures – of two galaxies, so far as it does – ought to produce creative chances” (Snow, 1998).

The underlying philosophy that grounds Haraway, Deleuze and Guattari is posthumanism. Posthumanism began as cynicism at how “aimless and arbitrary” human intellect appears in the natural world. This is more evident as we become more aware that the humanist perspective that privileges humanity above all other forms of existence will not prevent our extinction (Heidegger, 1993, Wolfe, 2010). This causes us to realise that there is an urgent need for a new kind of thought that is less anthropocentric, and positions humans as part of, connected to, and interdependent on the environment. If humanism is the belief that humans are the pinnacle of existence, then posthumanism is one that rethinks the position of humanity, and recontextualises it in terms of other species (Wolfe, 2010). Posthumanist scholar Cary Wolfe (2010) observed that posthumanism is “acknowledging that we are fundamentally prosthetic creatures that have co-evolved with various forms of technicity and materiality, forms that are radically ‘non-human’ and yet have nevertheless made the human what it is.” The posthuman aspires to understand the world from multiple, heterogeneous perspectives and manifest him/herself in consideration with the different identities.

This thesis creates an interdisciplinary platform that is different from the “third culture” Snow predicted and Brockman has created. Here, rather than Snow’s “literary intellectuals” repairing communication, I connect science with art, and the humanities. Like members of Brockman’s “third culture”, a part of this research does involve scientific experimentation and directly communicating it to a general audience, but it does not do this by casting the arts aside. Instead, the arts are an integral part of the methodology. Deleuze and Guattari’s “becoming animal” is purely philosophical, and although they suggest a way to leave the human by inventing an “animal personality”, they do not engage with scientific concepts that might be useful to accomplish it. Haraway, who has a PhD in primatology, engages with both the sciences and the arts to further human knowledge about our connection to non-human animals. Unlike Haraway

however, this research also involved art-making as a tool to access the visual experience of lungfishes, and embody the process of communication beyond the verbal and cognitive to other senses (sight, sound touch etc.).

Instead of creating a new definition for a “third culture” or “space” that connects science and the arts, and humans and non-human animals, this research project is posthumanist in its attempt to understand the visual perception of lungfishes from multiple heterogeneous perspectives (scientific, philosophical/cultural, and art-making). The success of this posthumanist endeavour is considered in the general discussion chapter which integrates all the thesis chapters and considers the strengths and weaknesses of combining the “two cultures” in this way. In addition, each chapter conforms to its discipline-specific language and style conventions to respect and maintain discipline conventions (e.g. chapter 3 and chapter 4 follow scientific convention with methodology, results and discussion, while chapters 1, 2 and 5 are purposefully narrative driven).

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CHAPTER 1

.....

THE VISUAL *UMWELTEN* OF LUNGFISHES THROUGH ART AND SCIENCE

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1.1 INTRODUCTION

Aristotle famously inferred that the whole is greater than the sum of its parts⁵. He may have been contextualising this in terms of metaphysics, but the same applies to scientific research and the study of non-human animal behaviour. The process of unravelling the visual perceptual experience of non-human animals using anatomical, physiological and behavioural approaches is an established yet burgeoning field of scientific research. Scientists have relied on mostly anthropocentric, technologically unaided observation and logical extrapolations when investigating the visual reality of the non-human animal. However, technological advances over the last 100 years have allowed scientists to further uncover the visual reality of non-human animals.

Techniques such as electron microscopy and electrophysiology unravel the anatomical and physiological mechanisms of vision, and behavioural experiments enable us to observe how non-human animals react to visual stimuli within their environment.

Although it is possible to obtain a mechanistic understanding of non-human perception by the exploration of visually guided behaviours, and neuronal circuitry within the eye and brain, there are still large gaps in our understanding of how to conceptualise a reality that is fundamentally different from our own, if only because our understanding of non-human perception is constructed and understood by our embedded human perception and its inherent biases and limitations.

This chapter explores some of the scientific and non-scientific history of the visual perception of non-human animals from the nineteenth century to present day by drawing on some of the similarities and differences researchers shared in their attempts to uncover non-human animal perceptual capabilities. By recognizing the limitations of technology and our anthropocentric perception of reality, this chapter will attempt to

⁵ See Aristotle, *Metaphysics* Book VIII 1045a1-2ff

reconcile (and agitate further) some of these fundamental issues through a combination of art and science. With a particular focus on Jakob von Uexküll and his concept of the *Umwelt*, this will be illustrated by an analysis of ‘*Inperception*’ (2012); a collaborative artwork between myself and two other artists to interpret the *Umwelten* of Australian lungfishes. This project proposed that an interdisciplinary approach to the exploration of the visual reality of non-human animals enables a more sensorial and environmentally contextualised speculation of this reality than strict reliance on the reductionist findings of visual philosophy.

1.2 VISUAL PERCEPTION STUDIES: A HISTORICAL OVERVIEW

1.2.1 Jakob von Uexküll and the *Umwelt*

Jakob von Uexküll (1864 – 1944) was one of the first to attempt a description of non-human animal perceptions holistically by way of experimental methodologies which included careful observation and analysis of animal behaviour. He differed from other scientists of his era by objecting to the mechanistic view that he believed conventional biology had adopted, a view that gave little regard to the non-human animal’s perceptual response to and within its environment (Sagan, 2010). Uexküll, who criticised the Cartesian view that non-human animals were nothing more than automations, said, ‘Whoever wants to hold on to the conviction that all living things are only machines should abandon all hope of glimpsing their environments’ (Uexküll, 2010).

Uexküll suggested that each organism constructs its own perceptual environment, which he called the *Umwelt* (plural: *Umwelten*). This *Umwelt* is dependent on the organism’s perceptions, actions, and relationships to and within its physical environment

(Buchanan, 2008). *Umwelten* are depicted by Uexküll as a series of ‘soap bubbles’ encircling each organism, where context and reality are determined by the individual’s behaviour and perception, and to which no other organism has full ‘access’ or complete comprehension because it is completely subjective (Uexküll, 2010). Uexküll firmly believed that all animals were capable of identifying meaning from cues that originated from the environment beyond an instinctual or innate level. As Buchanan (2008) described, ‘Instead of interpreting organisms based on natural selection...Uexküll sought to understand them with respect to the designs that they represented in relation to meaningful signs’. This has always been considered a radical approach in the attempt to understand and relate to non-human perception.

Uexküll’s theory of the *Umwelt* as an organism-environment unit opened up radical new ways of interpreting and conceptualising non-human animal perception. However, its conception drew a large amount of criticism from the scientific community, primarily because his theories were seen as unfalsifiable and too subjective to be useful in science. Despite this justified criticism, Uexküll was one of the first researchers to recognise the importance of studying non-human animal perception (including visual perception) and its relationship to the animal’s environment, and developed experiments to test non-human animal cognition and capabilities. Uexküll’s contribution to the understanding of beings as active agents, although lacking scientific integrity due to its subjective nature, is still a useful conceptual tool in the approach and interpretation of visual perception in non-human animals.

1.2.2 Visual perceptions and behaviour: Lubbock, von Frisch, and Morrison-Scott

Early scientific experiments that tested the colour vision capabilities of non-human animals were conducted by observation of the animals' behaviour. John Lubbock and Karl von Frisch were pioneers in developing behavioural criteria to establish whether non-human animals possessed the ability to differentiate colours (for full review on behavioural tests on animal colour vision, see Kelber et al., 2003). Lubbock observed that various species of *Daphnia* (planktonic crustaceans) prefer yellow to white or unfiltered light (Lubbock, 1889). He also claimed that honeybees (*Apis mellifera*) associate colour with food (Lubbock, 1889), and this was later confirmed by Frisch. Frisch established that honeybees discriminate blue and yellow from 30 different shades of grey (Von Frisch, 1914). This experiment, coined the 'grey card experiment', is still used today to determine colour discrimination in non-human animals. An animal is first taught to associate a reward with a colour. This colour is then placed within a sample of many shades of grey. At least one of these grey shades has a similar brightness to the test colour, which renders brightness as an unreliable cue. In order to gain the food reward, the animal must identify the colour by hue alone. In an experiment of similar design, Morrison-Scott's 1954 study on the satin bowerbird (*Ptilonorhynchus violaceus*), observed colour preferences of male birds by the choice of coloured fabrics with which they preferred to adorn their bowers (Morrison Scott, 1937). Whether the bowerbird or honeybee colour preference is a product of instinct and learned behaviour, or determined by the relationship between animal and its *Umwelt* is still unknown.

Behavioural experiments following the same design principle of von Frisch's grey card experiments have confirmed the ability to discriminate colour in a number of species including various species of butterflies (belonging to the families Nymphalidae, Pieridae, Satyridae and Papilionidae), hermit crabs (*Eupagurus anachoretus*), shrimp

(*Crangon vulgaris*) domestic chickens (*Gallus gallus*) (Kelber et al., 2003), and the giant shovelnose ray (*Glaucostegus typus*) (Van-Eyk et al., 2011).

Lubbock, Frisch, and Morrison-Scott's findings were solely based on human observation, and as Uexküll argued, did not take into account the full subjectivity of the non-human animal and its environment because both were continuously being defined through human perception and its inescapable bias. This anthropocentrism is based on an assumed correlation between stimulus and behaviour, and between the meanings of expressed behaviour. This meaning is usually based on human experience, which has led to assumptions about animal behaviour that may not be consistent with what the animal is actually or fully experiencing. For instance, the human expression of a smile to be one of pleasure, happiness and amusement can be a gross misinterpretation in other animals such as canids and primates. In wolves, the horizontal retraction of lips across the teeth can be a submissive gesture to dominant members of the group, and the vertical lip retraction given by dominant animals is a sign of aggression (Fox, 1970). Similarly, bared teeth displays of chimpanzees illustrate submission and/or fear in response to aggression or uncertainty regarding the intent of conspecifics (Waller and Dunbar, 2005).

Are we able to ascertain and explain the experience of a non-human animal, and its visual capabilities, if we cannot see or experience them? New scientific technologies may be able to overcome some of the limitations of behavioural studies and describe non-human animal visual perceptions more accurately by pointing to the anatomical and physiological attributes that enable an organism to perceive.

1.3 VISUAL PERCEPTION: CONTEMPORARY

In addition to behavioural studies, techniques such as electron microscopy, microspectrophotometry, and electrophysiology have been used since the middle of the 20th century to study the vision of human and non-human animals. These techniques have proven to be particularly useful in understanding the mechanistic basis of vision and interspecific differences in visual ability/performance. Although other techniques, such as molecular genetics, are increasingly being used to explore the sensory worlds of other non-human animals, this review will focus on these three methods in particular. See the general introduction for a review of the vertebrate eye and its components.

1.3.1 Anatomical, physiological, and behavioural methodologies

Microscopy enables the identification and characterisation of the different kinds of retinal photoreceptor cells based on morphological characteristics such as size, shape and ultrastructure. Light microscopy is typically used to characterise cells to a resolution of microns (10^{-6} m), while electron microscopy enables cells to be examined at an even higher resolution of nanometers (10^{-9} m) (Williams and Carter, 1996). Transmission electron microscopy (TEM), which passes an electron beam through the retinal tissue, allows for ultrastructural differentiation in cell morphology, i.e. the size of mitochondria (organelles within cells that are primarily responsible in generating cellular energy) within the outer segment, and the number and type of connections (synapses) between photoreceptors and bipolar cells.

Physiological techniques such as microspectrophotometry (MSP) and electroretinography (ERG) are able to characterise photoreceptor cells by function. MSP allows the precise measurement of spectral absorption characteristics in the visual

pigments and intracellular spectral filters found in individual photoreceptors (Liebman and Entine, 1964, Liebman, 1972, Brown, 1968). This information not only defines the range of wavelengths to which an animal is sensitive but also reveals whether multiple spectral types of photoreceptor are present in the retina, and thus, the potential for colour vision.

ERG is used to quantify the response of an animal's eyes to visual stimuli (Brown, 1968). Not limited to ophthalmologists who routinely utilize this technique on humans to diagnose various retinal diseases, ERG can be applied to other animals by measuring the massed electrical response of the eye to light stimuli in order to determine its absolute retinal sensitivity, spectral sensitivity and temporal responses properties (Blough et al., 1972, Goldsmith, 1986). ERG can also determine how sensitive the eye is to temporal changes in illumination; potentially revealing how fast an animal can react to predators or prey under different conditions of illumination (dark or light) (Cavonius, 1964). This is often determined by identifying the critical flicker-fusion frequency (cFFF); the frequency at which a train of light flashes can no longer be seen as individual flashes, but appears as a continuous light stimulus (Hecht et al., 1933, Jenssen and Swenson, 1974). The photopic cFFF of the human eye in bright light is 50 to 70 Hz (Bowles and Kraft, 2012). In contrast, some birds have a much higher photopic cFFF, which enables them to resolve flicker greater than 100 Hz (Nuboer et al., 1992). This difference may be because birds often move quickly when flying and need superior motion vision relative to humans. Superior motion vision is achieved by improving the temporal response properties (reflected by a high cFFF) of the retina, otherwise moving objects would appear to 'blur' as they move across the visual field. At low light intensities, cFFF is reduced, and so is the ability to see motion. This is why cricket and baseball matches, for example, are abandoned in poor light because the cFFF of the

scotopic rod system is much lower than the photopic cone system (Bowles and Kraft, 2012).

1.4 CASE STUDY: THE LUNGFISH *UMWELT*

As previously discussed, lungfishes are large, freshwater dipnoan⁶ fishes that have the ability to breathe dissolved and atmospheric air through gills and “primitive” lungs. Both internal and external nostrils are found in lungfishes; an uncommon feature of fishes, which usually possess two pairs of external nostrils. Lungfishes are classified as Sarcopterygii, along with coelacanths and tetrapods (Clack et al., 2011), and through a whole genome analysis of the African coelacanth, it was recently confirmed that they are the closest living ancestors of all terrestrial vertebrates (Amemiya et al., 2013). By investigating the only living source to the origin of vision on land, many lines can be drawn to connect animal *Umwelten*, may they be human or not.

Dipnoan fish are important in the debate surrounding the evolution of vision in terrestrial vertebrates because their visual adaptations and evolutionary history places them in the divide between life in the water and on land. In general, the visual machinery of lungfishes resembles that of terrestrial vertebrates, which suggests that several of the ocular characteristics these animals possess may have evolved in shallow water before the transition onto land (Collin, 2010). One adaptation is the corneal surface microprojections which are important for vision on land and are present in both terrestrial vertebrates and in the Australian lungfish (*Neoceratodus forsteri*). These appear as a thick carpet of microvilli extending over the dermal cornea, which stabilise

⁶ ‘Dipnoan’ originates from the Greek *dipnoos*, meaning ‘double-breathing’.

the corneal tear film in terrestrial vertebrates, necessary for clear aerial vision (Collin and Collin, 2001).

Six species of lungfishes are extant worldwide; four in Africa (*Protopterus* spp.), one in South America (*Lepidosiren paradoxa*) and one in Australia (*N. forsteri*). Previous anatomical studies have shown that lungfishes possess a number of different types of large photoreceptors in their eyes, demonstrating that they have the retinal machinery to process colour (Walls, 1942, Pfeiffer, 1968, Ali and Anctil, 1973). Although the full complement of photoreceptors has yet to be established in the South American and African lungfishes, the retina of the Australian lungfish – which is considered to be the most primitive of the extant lungfishes – has been studied in detail (Marshall, 1986, Tokita et al., 2005, Bailes et al., 2006a, Bailes et al., 2006b, Bailes et al., 2007b, Bailes et al., 2007a).

As previously discussed, there are up to five spectrally distinct types of retinal photoreceptors present in the Australian lungfish: one type of rod ($\lambda_{\max}$ 540nm) and four types of cones (UVS $\lambda_{\max}$ 366nm, SWS $\lambda_{\max}$ 479nm, MWS $\lambda_{\max}$ 558nm and LWS $\lambda_{\max}$ 623nm). UVS cones are only found in the retinae of juvenile *N. forsteri*, suggesting that sensitivity to ultraviolet light is lost during maturation (Bailes et al., 2006a, Bailes et al., 2007a, Hart et al., 2008). The possession of four types of cones by the juvenile lungfishes implies that they have the potential for tetrachromatic vision; unusual for an animal that has previously been considered predominantly nocturnal or crepuscular⁷ (Dean, 1906, Grigg, 1965, Kemp, 1986).

L. paradoxa and *Protopterus* spp. possess coloured and colourless oil droplets in their cone and rod photoreceptors, respectively (Kerr, 1902) (Walls, 1942, Pfeiffer, 1968, Ali and Anctil, 1973), whereas *N. forsteri* only possess colourless oil droplets within some

⁷ Active predominantly during twilight (dawn and dusk).

types of cone photoreceptors and coloured oil droplets and/or coloured pigments in other cone types (Bailes et al., 2006a). The presence of coloured oil droplets (and the potential for tetrachromatic colour vision) in the lungfish retina is surprising because these characteristics are typically found only in strongly diurnal animals such as birds and reptiles. *N. forsteri* is thought to have a predominantly nocturnal lifestyle, often living in turbid, and therefore relatively dim, underwater light environments. Colourless oil droplets are found in marsupial mammals which are generally nocturnal, crepuscular or cathemeral⁸ and are thought to improve light gathering ability and thus, overall sensitivity. Coloured oil droplets act as miniature spectral filters within each photoreceptor and, by narrowing the spectral sensitivity function of the photoreceptors, are thought to improve colour discrimination by reducing overlap with adjacent spectral classes of cone (Vorobyev et al., 1998). This potentially enables the animal to perceive a far greater number of distinct colour “hues” in the visual spectrum than they would without the coloured oil droplets (Govardovskii, 1983, Hart et al., 2006).

The lungfish eye is something of a conundrum. The large size of the photoreceptors suggests that lungfish eyes are adapted for maximising the amount of light detected by the retina. *N. forsteri* also possesses a tapetum lucidum, a reflective layer behind the retina that enables light to pass through the photoreceptors a second time, thereby increasing photon capture (i.e. sensitivity). This structure is typically seen in crepuscular, nocturnal species or animals that live in dim habitats and is consistent with the lungfish environment. However, the complex visual apparatus of the lungfish eye, in particular the presence of coloured oil droplets and the impressive complement of cone photoreceptors for colour vision, suggests that colour vision is more important to

⁸Active sporadically at random intervals during the day or night dependant on food availability, predation pressures and other environmental cues such as temperature.

lungfishes than previously thought,⁹ or indicated by their relatively low light environment.

1.5 INPERCEPTION: ARTISTIC INQUIRY INTO THE AUSTRALIAN LUNGFISH *UMWELT*

The difficulty for humans to perceive the visual *Umwelten* of aquatic animals is inherent in the subjectivity of visual perception. Coupled with the need to perceive an environment through water instead of air, with visual machinery that is optimised for a particular habitat, the quest of seeing through the eyes of a lungfish becomes even more problematic. Philosopher Thomas Nagel articulated that the “conscious experience” of a non-human animal (in his example, the bat) exists “only if there is something it is like to *be* that organism – something it is like *for* that organism,” but due to the “subjective character of experience” the “something it is like” can never be completely revealed (Nagel, 1974). He recognized that the restriction resides in our own minds, which is inadequate to understand another perceptual world because of its subjectivity. However, he called for attempts to bridge this subjective character of experience, claiming that “to deny the reality or logical significance of what we can never describe or understand is the crudest form of cognitive dissonance” (Nagel, 1974). Therefore, there is always merit in the gesture of trying to pierce *Umwelten* despite the apparent impossibility of doing so.

Nagel speculated that the only way to move forward is to take imagination out of interpretation and form a more objective approach that will not give the entire picture,

⁹Early lungfish studies suggested that these fish were almost blind, and possessed degenerate eyes DEAN, B. Notes on the living specimens of the Australian lungfish, *Ceratodus forsteri*, in the Zoological Society's collection. Proceedings of the Zoological Society of London, 1906. Wiley Online Library, 168-178..

but may provide a description of ‘the subjective character of experiences in a form comprehensible to beings incapable of having those experiences’ (Nagel, 1974) .

‘*Inperception*’ (2012), a collaborative artistic interpretation and translation of scientific data onto a large mixed media mural, extended Nagel’s argument to aquatic organisms, and considers the additional complication of vision in water rather than air.

Previously, contemporary artists Olly and Suzi explored the interaction of the human and aquatic non-human *Umwelten* in the installation, ‘*Shark Bite*’ (1997). Along with photographer Greg Williams, the duo tracked and painted Great White sharks off the coast of South Africa. Once the human artists had painted the animal’s portrait, the animal was encouraged to provide its “voice”, or point of view, by interacting physically with its portrait. One shark responded dramatically by biting its portrait; a performative signature. For Olly and Suzi, this signature was a powerful message that documented “the passing of animals and habitats that are here now but may not be for much longer” (Olly and Suzi, 2002). This can also illustrate the dissonance between crossing the *Umwelten* of humans and aquatic animals, illustrated by the antagonistic reaction/interaction between human outreach and non-human response. Although ‘*Shark Bite*’ (1997) documented a human/aquatic non-human interaction, it did not bridge the gap between *Umwelten*, demonstrating the futility of the pursuit in a spectacular gesture.

In an attempt to understand the perceptual *Umwelten* of Australian lungfishes, three artists engaged in the ‘*Inperception*’ project (2012), a collaborative interpretation and translation of scientific data onto a large mixed media mural. Unlike ‘*Shark Bite*’ (1997), ‘*Inperception*’ (2012) endeavoured to tease out the nuances of the futility of crossing *Umwelten* by focusing on human attempts to understand the *Umwelten* of lungfishes, banal aquatic organisms. Audrey Appudurai, and two other contemporary

artists, Shannon Williamson, and Devon Ward, responded to scientific data generated as part of the broader research into the visual perception of the Australian lungfish and Uexküll's theory of *Umwelten* as "soap bubbles". The artists were provided with scientific research papers which included data in the form of words, anatomical photographs, diagrams and figures about *N. forsteri*¹⁰. Williamson is a painter who usually works with watercolour, charcoal and paper, Ward is a graphic designer and photographer, and Appudurai is an artist who primarily uses pencils, pens, and paper. Appudurai provided the participants with a large canvas, acrylic, watercolour paints, charcoal, oil pastels and graphite as materials to use. The artists then translated the scientific data onto a large canvas that was suspended around the water pools housing Australia lungfishes (Fig. 1.1 and 1.2). These lungfishes were being used for behavioural and physiological experiments. Each artist worked separately on sections of the canvas. Williamson also completed a written reflection on her attempt to interpret the *Umwelt* of the non-human other.

This artistic inquiry humbly aspired towards an informed subjectivity. It was an attempt to create a shared experience which allowed for a scientifically informed glimpse into a non-human vision while also acknowledging the limitations of human perceptions and knowledge systems. '*Inperception*' (2012) aimed to bridge the disparate disciplines of biological science and visual art, analysing data generated through anatomical and behavioural studies to provide alternate interpretations of the visual perception of lungfishes.

¹⁰ For resources given to artists see Bailes et al. (2006) and Hart et al. (2008).

Fig. 1.1 '*Inperception*' (2012) (Audrey Appudurai et al., 2013).

Fig. 1.2. The '*Inperception*' (2012) mural hanging near the lungfishes' housing tanks during the behavioural experiment (Audrey Appudurai et al., 2013).

Through informed subjectivity, '*Inperception*' (2012) was interested in the following research question: What might be produced when three humans are provoked to

consider how an Australian lungfish might see its world? It was an exploration of the overlap between various human *Umwelten* and the visual *Umwelten* of Australian lungfishes. Ultimately, the artistic responses showed the difficulty in navigating and piercing individual human *Umwelten*, which made the pursuit of doing the same to an aquatic animal even more problematic. Although there are numerous reflections in the mural, we will be focusing on three different pieces from each contributor in this analysis.

Devon Ward's contribution (on the far left of the mural) is shown in Fig. 1.3. He used predominantly green, blue and yellow circular dots of varying size, with some red punctuating the piece. Small, densely placed circles to the left of the canvas became bigger and more separated as the eye moves to the right (Fig. 3). The abstract dots are evocative of the visual 'machinery' of the lungfish's eye, similar to the retinal wholemound photograph (Fig. 1.4) presented to each artist during the project brief.

Fig. 1.3. Devon Ward's contribution to the '*Inperception*' project (2012). (Audrey Appudurai et al., 2013).

Fig. 1.4. Retinal wholemount of morphological characteristics in the Australian lungfish photoreceptors showing the red oil droplets in the red cones (rc), the orange/yellow pigment of yellow cones (yc), and small clear profiles of the small cones (arrowhead), and the large rods (asterisks). Scale bar = 10µm. Reproduced with kind permission from Wiley-Liss Inc. (Bailes et al., 2006a).

Ward's contribution focuses on understanding the lungfish *Umwelt* from a more mechanistic point of view, which may have been influenced by Ward's interest in the mechanisms of sight due to his red-green colour blindness. Further, the colours and shapes of the dots are reminiscent of bubbles, creating a feeling of playfulness and being immersed in a river water environment. It resonates with the environment of the lungfish, and locates the lungfish *Umwelt* within the environment and the eye.

Shannon Williamson's response is stylistically more abstract expressionist, with recognisable lungfish-like forms represented in motion (Fig. 1.5). Circular motifs are present, with blue bodies suspended within a larger space and a brown substrate behind. Lungfishes are shown moving in time and space, indicated by the outlines below the fish's body; the figures become more solid towards the top of the canvas. A direct correlation between the scientific data provided is not apparent, unlike in Ward's work. The shades of brown used in the background are similar to the colour of the fibreglass tanks in which the fishes are housed (Fig. 1.2), which suggests that Williamson is more concerned with the *Umwelten* of the population of Australian lungfishes within the room, rather than their counterparts in the wild. This contrasts with Ward's contribution, which is more concerned with the river environment.

Fig. 1.5. Shannon Williamson's contribution to the '*Inperception*' project (2012). (Audrey Appudurai et al., 2013).

The presence of more than one body in Williamson's contribution suggests that the perception of an individual fish is affected by the relationships with other fishes; in fact, recognition of conspecifics and social interaction is an important aspect of the lungfish *Umwelt*. Lungfishes live in small groups in the wild and as social animals, recognition of other individuals of their group and species would be important. Like Ward, Williamson gives the impression of playfulness as well as serenity and calmness, indicated by the use of muted colours, tones and space. The space between individuals and the white circles are suggestive of air and breathing. There is also a sense of movement in the work, depicted by the transition between transparent and solid forms and the streaks down the right of the canvas. An element of observation is also apparent that is resonant with scientific experiments – we are outsiders observing the lungfishes in their community. Ultimately, Williamson's contribution suggests the importance of relationships between individuals, their housing and their environment.

Finally, I provided two separate responses, before and after the other artists. My initial response (Fig. 1.6) depicts a single figure of a large, salmon pink lungfish. The green and yellow figures (Fig. 1.7) were a response to the other artworks. My contribution is focussed on the individualism of each fish. This differs from Williamson's response, where each body is relatively uniform in colour with no distinguishing features and can either be interpreted as a different individuals or the same individual at different time points. My focus on individuals was influenced by being the primary caretaker of the fishes as each fish had a unique set of behaviours ("personality") and identifying features only apparent through time spent caring for them.

The pink lungfish was not a response to the same scientific data given to others, but to prior research that suggests the visual importance of a pink underbelly in mate selection. In this case, the pink colour is inverted, where the entire fish is presenting itself. This suggests that the whole body is sensual in those moments, and that perception is not restricted to certain parts. Although some aspects of the green and blue lungfishes were responses to artistic data, the colours are suggestive of a river environment rather than the brown fibreglass tanks of the laboratory. This response is also sensual; the bodies are touching, individuals are distinctive but together: I found lungfishes I cared for to be playful and romantic. Like the other artist's response, there is a feeling of air and breath; here illustrated by their heads facing upwards towards the surface and their supply of air and/or food. My interpretation of the lungfish *Umwelt* was not directly about sight, but a perception of spatial orientation and bodies, influenced by my knowledge as the primary caregiver of these fishes. Or perhaps she was driven by a desire for them to perceive us.

Fig. 1.6. Audrey Appudurai first response to the '*Inperception*' project (2012). (Audrey Appudurai et al., 2013)

Fig. 1.7. Audrey Appudurai’s second response to the ‘*Inperception*’ project (2012). (Audrey Appudurai et al., 2013).

All artists were present at the same time and gave their initial response concurrently, each choosing a space on the canvas, which did not initially overlap. However, as the sessions progressed, Williamson’s contribution diffused into Ward’s work and my contribution diffused into Williamson’s. Ward’s spots became further apart, giving way to Williamson’s work to the right of the mural. Recurring motifs permeate the mural in the forms of colours, circles and feelings of playfulness. As Williamson (2013) wrote, “being a collaborative project it was impossible to ponder and express our own versions of lungfish perception without taking into account the modes of visualisation that had been adopted by our peers.” The resultant “seeping” of one artist’s interpretation into another created a dialogue between the humans, and between human and aquatic non-humans to discover the informed subjective nature of perception that transcends individuals, species, and their shared environments.

Despite this, the mural lacks aesthetic cohesion. Each response is blurred at the edges, but clearly separate, illustrating isolation in *Umwelten* between individuals. Ward's contribution is disconnected from Williamson's by broad streaks of bright yellow. Although my fishes play at the edges of Williamson's pool, they are cut off by brown streaks. Williamson (2013) expanded on this dissonance, saying, "There were multiple perspectives that could be taken. With an undefined audience, the adaption of any specific mode was challenging". The chaos revealed the dynamic responses to the Australian lungfish *Umwelt* from individuals presented with the same scientific data, and the alternative agents that may be involved in an aquatic animal's perceptual experience. Additionally, it illustrated the frustration associated with trying to do so, even with informed subjectivity.

'*Inperception*' was a project that attempted to discover new information about the Australian lungfish's visual experience by revisiting Uexküll's concept of the "soap bubble", and utilizing scientific data "as a means for generating questions and considerations of a world beyond an exclusively human concept" (Williamson, pers. comm. 2013). The project showed that the *Umwelten* of lungfishes goes beyond the machinery of the eye. A dialogue exists between fish, between the fish and their human observers, and ultimately between the fish and their environment.

1.6 CONCLUSION

Although the *Umwelten* of lungfishes may never be completely unravelled to our satisfaction, attempts to demystify it is important and insightful research. Scientific data contributes to the growing knowledge of lungfish visual ecology, while artistic interpretations speculate on and communicate missing pieces of the puzzle. Both attempts reflect our own limitations in perceiving the “Other”. By combining these two approaches, the scientific and artistic, the lungfish *Umwelt* is revealed from different perspectives and new ways of exploration are recognised. Such interdisciplinary research is important to understanding of the fundamental similarities and differences in vision between human and non-human aquatic animals in order to connect us with the world and understand our place within it. It also helps us to appreciate the limitations involved in considerations of what lies outside our visual experience and, in doing so, increases awareness of organisms and environments under threat due to human impact.

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CHAPTER 2

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LUNGFISHES FROM A HISTORICAL PERSPECTIVE: HOW HUMANS SEE THE “OTHER”

.....

2.1 INTRODUCTION

Three hundred and ninety million years ago during the Devonian period, the Earth was devoid of four legged creatures. The land was colonised with primitive avascular plants and arthropods, and the Devonian seas were dominated by marine invertebrates and armoured fish. Within the freshwater basins of Gondwanaland lungfishes evolved, organisms that represent the transition between life in the water and atmospheric oxygen breathing life on land. These humble animals have contributed to shaping cultural groups across the world and throughout history. Human narratives about lungfishes reveal multiple aspects and concerns with this intriguing animal and act as mirrors which present biases (both personal and social) in our attempt to understand the “Other”.

For the Indigenous peoples of Africa and Australia, this creature has provided spiritual and physical sustenance, was a mythical harbourer of spirits that walked on land when children slept, and became a powerful clan totem that commanded respect. Its discovery by Western civilization in the 19th century created pandemonium for the taxonomic organisation and classification of animals, and added fuel to the controversy that surrounded Charles Darwin’s epochal release of ‘*On the Origin of Species*’. In the 21st century, these fishes are celebrated as national treasures. Sculptures are raised in their honour: they are seen as hallmarks of evolutionary success, and beacons to the conservation effort in sub-tropical Australia. In the United States of America, half a world away from where they are found endemically, one Australian lungfish is famous for being the oldest living animal in captivity. It is recognised as an ambassador to its species, solidifying international relations between countries. The fame has also bizarrely resulted in the belief that this long-lived individual may hold the secret to everlasting life. In Perth, Western Australia, within a laboratory in the most isolated capital city in the world, research conducted on the visual system of the Australian

species, has attempted to uncover the visual reality of these animals. The narrative of this research project includes an ironic, unexpected turn of events that illustrates the intimate connection between ingrained assumptions and understandings about Australian lungfishes.

This chapter delves into historical and contemporary stories concerning lungfishes. Indigenous stories are used to reveal alternative frames of knowledge, diverse attempts to understand and make meanings out of this curious animal which lives in water but possesses lungs. Stories from 19th century Europe illustrate how lungfishes, as “transitional animals”, contributed to the shift from a predominantly religious to a secular based taxonomy and world view. These stories confirm that science is an important tool and methodology to make sense of the “Other” animal but is always a reflection of the society within which it operates. Contemporary stories reveal other obsessions of Western societies as they reflect, through the lungfishes, national pride and human obsession with celebrity and desire for long life. My own personal narrative about a small group of Australian lungfishes reveals how ingrained assumptions result in looking but not seeing. In general, the chapter explores the rich human cultural archives of lungfish stories which reflect the human desire to make sense out of a transitional animal that, in turn, can help reveal anthropocentric limitations in the quest for the non-human *Umwelt*. In other words, these human narratives all show how personal and social biases influence how we choose to understand lungfishes and by inference, other non-human beings.

Perceptions of organisms, including humans, are constructed both through biological apparatuses as well as subjective experience and interpretation. Therefore, each organism constructs/forms their own subjective reality. As Thomas Nagel stipulated in ‘What Is It Like To Be A Bat?’ (Nagel, 1974) and Jakob von Uexküll suggested with his theory of the *Umwelt* (Uexküll, 2010), that when it comes to unravelling visual

experience, especially of non-human animals, it is usually expected that scientific methodology holds the key because objectivity is a fundamental aspect of its approach (Kitcher, 1993). However, the construction and outcomes of scientific research are always framed by humans within a certain perspective that forces a particular understanding of the world. Bruno Latour reminds us, “scientific activity is just one social arena in which knowledge is constructed” (Latour and Woolgar, 1986), and as Donna Haraway said, “biology is a discourse, not the living world itself” (Haraway, 1992). Therefore, science can provide an important aspect of the puzzle, but not all the pieces to see into the *Umwelten* of lungfishes. Other discourses based on human stories also offer new perspectives of the lungfish *Umwelt* and provide additional (or alternative) pieces of the puzzle together.

2.2 LUNGFISHES IN INDIGENOUS COMMUNITIES: THE DALA, THE MMAMBA, AND THE AMAZONIAN KILLER LUNGFISH

Before lungfishes captured international attention after their modern Western¹¹ discovery, their presence and unique position was well known to Indigenous communities. To the Gubbi Gubbi people¹² of Queensland, Australia, the Australian lungfish, or Dala in the Gubbi Gubbi tongue, are still sacred animals. The Dreamtime story of Dala, woven by Dr. Eve Fesl, a Gubbi Gubbi elder tells a story of a fish destined to be the ancestor to all vertebrates:

Many years ago, thousands of years ago, there were no animals on the Earth, and the Earth Mother and our ancestral spirits looked down and decided that they wanted to have animals on the Earth, which would be their children. So, they designated that

¹¹ By Western, I refer mainly to the Judeo-Christian version of perceiving/explaining life.

¹² Sometimes referred to as ‘the Dala’ people by other Aboriginal groups See: FESL, E. M. D. n.d. *Extinction is Forever! A bid to save "Dala", the Queensland lungfish, an endangered species in the Mary River across which a dam is proposed to be built*. [Online]. Brisbane. Available: http://www.savethemaryriver.com/_mgxroot/page_10786.html [Accessed 20 May 2014].

Dala would, first of all, get a lung, then it could get vertebrae and be able to walk, and that would be the forefather and foremother, really, of all the animals in the world. The breeding place would be near the edges of the river, so that the animals could come out and so we have Dala, the lungfish which only has one lung. It's the only place in the world you could find it, and this place where we stand is one of the most viable breeding places (Franklin, 2007).

Although Fesl was not aware of the scientific importance of the Australian lungfish when growing up, she understood that the Gubbi Gubbi people were their sacred custodians. She had been told about the cultural importance of Dala from a very young age, remembering a family meeting by the Mary River that involved lungfishes “swimming around...in the water below” and highlighted the value of such an encounter because it was “our sacred fish”(Franklin, 2007). Dala is even now a totem to the Gubbi Gubbi people. Fesl elaborates and says, “We were taught not to hurt it, to protect its habitat, where it feeds and swims” because Dala “are ancestor spirits” (Queensland, 2009).

This intimate tie that Fesl, and the Gubbi Gubbi people have to the lungfish is also linked to their relationship to the Mary River. Both Fesl's mother and uncle were born on the banks of the Mary River in traditional style, where, “when giving birth, the mother has to have her sisters by her side to assist her” (Franklin, 2007). In another anecdote Fesl speaks of her grandmother, and the stories told about Dala:

My grandmother wove shelter covers from bladey grass and used paperbark as a soft bed to lie on. At night the family would see the reflection of the stars in the water, and hear the ripple of the water and the occasional explosion of air as Dala, our sacred fish, chose to come to the surface to expel the breath from his lung. It was at these times, the children would be told the story of Dala. As they lay under the stars and beside the rainforest, with its night bird calls, the children listened to the story of the beginning of our culture (JerryinBrisbane, 2008).

The cultural importance of Dala, created by the close cultural relationships to the river landscape encompassing the Mary River, family, and community, influenced Fesl's understanding of the Australian lungfish. To Australian Aboriginal groups, oral narratives and the meanings they convey are "owned by the individual storytellers in accordance with their position within their social and spiritual universe" (Klapproth, 2004) and are "items of exchange in a cultural arena" (Muecke, 1983). In Fesl's case, these narratives are intertwined with anthropomorphic attitudes towards Dala. Fesl often mentions that "Dala will come up beside the canoe and you can stroke Dala. That's what a friendly creature it is" (Queensland, 2009) and when greeting them in her native tongue, does not forget to let them know that she is "part of the family" (Franklin, 2007). This does not necessarily suggest that Fesl is making Dala more human-like, but rather that non-human animals like the lungfish are important members in the human world. Gubbi Gubbi stories present an alternative position for the human living within the world. The human animal is integrated as part of the natural world and therefore must employ responsibility and respect to other animals.

The liminal nature of Dala, as a fish with a lung,¹³ is not a "problem" to cultural beliefs. Rather, this view of the world embraces the concept of fluidity in animals' forms and shapes. Dala was chosen as a totem and sacred animal because of its unusual nature. Fesl says, "Our people were very good at anatomy and physiology, and those things. They had to be to be able to survive, and so it's been perceived a long time ago that this was something special" (Franklin, 2007). The Gubbi Gubbi people were aware of Dala's ability to breathe atmospheric oxygen air long before the Western scientific community and unlike this community did not seek to disprove or condemn it (as will be illustrated in the stories from 19th century England, in a later part of this chapter).

¹³ The Australian lungfish, *N. forsteri*, is the only species that possesses one lung, all other lungfish species possess a pair of lungs.

The responsibility Fesl felt towards Dala played a major role in the cancellation of the Traveston Crossing Dam project in 2009. It is important to note that although this project did increase awareness of the Australian lungfish, there is debate whether the main motivations was to help the species directly, or rather use it to polarise opinions and/or to aid development lobbies and conservation groups in realising their own agendas (personal communication, Kind, 2015). The dam, proposed in 2006 after the worst drought Queensland had experienced in a century, was to be constructed approximately 16 kilometres south of Gympie (Meteorology, 2006, Franklin, 2007). The dam would have affected the surrounding built up areas north of Brisbane, and flooded the major breeding grounds of the Australian lungfish in the Mary River. This angered the local and international community. The importance of the Australian lungfish was articulated to the government through rigorous protests, petitions and media reports. At one such protest, then Greens leader, Senator Bob Brown said, “I’ve told the minister there is no way he should allow the major breeding ground for the Australian lungfish or the Mary River turtle, or the Mary River cod, to be obliterated” at a protest of “hundreds of farmers and local residents” (McGrath, 2008).

Fesl was a guest speaker at many of these rallies and at one, expressed how the government was attempting to buy support from the Indigenous communities to take away this sacred land. In the rebuttal to the proposal, on behalf of the Gubbi Gubbi people she said, “We would have nothing to do with it. We’re not going to sign away our history, our culture, the Mary Valley. No way.” (Crow_Boy, 2007). At the Climate Torch Relay 2008, Fesl told the Dreamtime story of Dala and finished with, “flowing through time, our duty is to care for Mummabulla (Mary River) and to care for Dala, and it is your duty too. She is the symbol of our past, our present, and our future” (JerryinBrisbane, 2008) to the cheers of supporters. Fesl campaigned through media reports and proposals to the Premier of Queensland about Dala, emphasising the

negative effects of Traveston Crossing on the ‘migration over long distances they need during spawning’ and asking, “Do people in Queensland, Australia and around the globe care enough to protect Dala, the Queensland lungfish and its habitat?” (Fesl, n.d.).

An Australian Broadcasting Corporation radio show titled ‘A Whisper From The Past’ was dedicated to the conservation effort (Franklin, 2007). Professor Jean Joss was also involved in the campaign against the dam, and stated that “the lungfish has been described as being the most important vertebrate because it sits at the transition between water and land” (Franklin, 2007) and “the Mary River dam will destroy its spawning and nursery habitat and threaten it with extinction; and this will be a scientific scandal that the scientific community will neither forgive nor forget, and which will permanently stain Australia's conservation record in the eyes of the world” (Joss, 2007a). In a petition by Joss, over 4,000 signatories were accumulated in its first week (Joss, 2007b) and the international scientific community was alerted through editorials published in *Nature* (Pearson, 2006, 2006). Finally in 2009, due to this growing pressure from popular media and members of the community, the project was refused approval by the federal Environment Minister, Peter Garrett, on the grounds of “Listed Threatened species and communities” under the Environment Protection Biodiversity Conservation Act 1999 (Garrett, 2009).

Similarly, *Protopterus aethiopicus*, one of the African lungfish species, is of special value to one of the biggest clans of Buganda, the largest sub-national kingdom within Uganda. More than fifty clans call Buganda home, and each clan can trace its lineage back to the historical waves of immigrations into the kingdom. The first of these brought the Nansangwa, or indigenous people, into Buganda. The Nansangwa are comprised of the six oldest clans in Buganda. One of these is Mmamba Gabunga, which translates to the native name for *P. aethiopicus*, also known as the marbled lungfish.

Like *N. forsteri* to the Gubbi Gubbi, *P. aethiopicus* is a totem to the Mmamba people, a

spiritual emblem to remind them of their ancestry and mythic past. In the Mmamba origin story, the clan's founder Mabiru, had close ties with the rivers, and gained the king, Kabaka¹⁴ Kintu's favour by offering his talents as an expert navigator and canoe maker, which resulted in Kabaka Kintu appointing many of Mabiru's clansmen as chiefs in his kingdom. The Mmamba remain one of the largest and most influential clan of Buganda, but do not eat their namesake due to its symbolism of their clan (2014a). Like the story of Dala, the Mmamba understand of the human as an integral part of the living environment.

While both employ some taxonomical systems in regard to the world around them, the Indigenous populations of Australia and Africa allow for the fluidity of nature in the living world; liminality and difference are accepted (and sometimes celebrated). The Western view appears to be more rigid in terms of order within a fixed hierarchy.

Therefore, it is not surprising that Western discovery of the lungfishes during colonization resulted in many mythologies featuring the lungfish as "monsters" due to their novel and bizaare nature. After the 1837 discovery of the South American lungfish by Johann Natterer, reports flooded in about a mythical beast, the *minhocao*¹⁵ of Brazil, and its possible ties to the lungfish. The tales of *minhocões* stem from Goyaz, a state in central Brazil, where in the deep lakes of Padre Aranda and Feia, *minhocões* torment the locals by dragging horses, mules and cattle into the water. Saint Hilaire, a scientific journalist visiting the area in the mid 19th century, investigated these reports by residents near the lake and described the creature thus:

The word *minhocao*, is an augmentative of *minhoca*, which, in Portuguese, signifies *earth-worm*; and, indeed, they state that the monster in question absolutely resembles these worms, with this difference, that it has a visible mouth; they also add, that it is

¹⁴ 'King' in the Mmamba language

¹⁵ Singular of *minhocões*

black, short, and of enormous size; that it does not rise to the surface of the water, but that it causes animals to disappear by seizing them by the belly (Saint Hilaire, 1847).

Further investigation led Saint Hilare to the Governor of Meiapoint, M. Joaquim Alvez de Oliveira. De Oliveira had heard of these monsters, and advised Saint Hilaire that fishermen reported the *minhocao* “was a true fish, provided with fins”. Saint Hilaire’s interest was further piqued, and turned his research to organisms that had recently been discovered and identified by Western science. This search led him to Professor Gervais, who directed Hilare to Richard Owen and Mikkel Bischoff’s papers on the newly discovered African species *Lepidosiren annectens* (Owen, 1840, Edwards, 1841) and Natterer and Fitzinger’s recent discovery of the South American *L.paradoxa* (Natterer, 1837, Bischoff, 1840). To Saint Hilaire, both these animals fit the description given by the fishermen, and *L. paradoxa* shared the habitat of the *minhocao*. He quoted Bischoff in his paper of the dentition of *L. annectens* and wrote, “These characters agree extremely well with those which we must of necessity admit in the *minhocao*, since it seizes very powerfully upon large animals, and drags them away to devour them”. Finally, he continued with great confidence that “it is, therefore, probably that the *minhocao* is an enormous species of *Lepidosiren*”. (Saint Hilaire, 1847). Saint Hilare’s story illustrates how the “different” can become monstrous and result in exaggerated tales; in this case, created by Western cultural biases surrounding the exotic and the unknown of newly colonised land.

More recently in the mid 20th century, the *buru*, an ancient animal of the Ziro valley of Arunachal Pradesh, India was thought to be a lungfish. At the end of World War II, explorer Charles Stonor and anthropologist J. P. Mills travelled to the valley and heard the legend of the *buru*, a now extinct group of creatures that were a blight to the Apatani people of the Ziro valley. According to Apatani elders, the valley’s marshes were populated by these creatures and when their people settled in the valley, livestock and

the residents were attacked by these large, reptilian-like water dwelling monsters. The Apatani people were so afraid of the *buru* that all marshes were drained of water, which eventually caused the extinction of *burus*; who either perished without water or burrowed deep into the underground springs of the valley. In 1948, Stonor accompanied news correspondent Ralph Izzard to the neighbouring Rilo valley, where locals insisted that *burus* still existed. After an extensive search, Izzard and Stonor failed to find evidence that *burus* were still at large.

Many cryptozoologists have tried to identify this mythical beast. The general contenders are reptiles, such as modern day dinosaurs, crocodiles or water-dwelling monitor lizards. However, cryptozoologist Karl Shuker believes that the *buru* was a now extinct species of lungfish; an Asian counterpart to the species inhabiting Africa, Australia and the South America. The lungfishes' aquatic lifestyle, behaviour, morphology and association with burrowing into the earth for long periods of time have convinced Shuker that the *buru* may have been a more primitive lungfish. More interestingly, a personal experience with an African species of lungfish may have cemented his beliefs:

One of the most popular exhibits of the ichthyological practicals during my days as a zoology student at university was a living specimen of an African lungfish *Protopterus*, which was sometimes placed on display in order that we could observe its behaviour. As it happened, for much of the time there was actually very little that we could observe, because it would spend most of the practical resting motionless at the bottom of its tank. Every so often, however, and usually when everyone's attention was diverted elsewhere, it would solemnly perform its pièce de resistance. All at once, without any prior warning, it would raise the front part of its large body upwards, until its head just touched the surface of the water. Sometimes it would then simply nudge the tip of its snout above the water, but if we were lucky (by now, everyone would have rushed up to its tank to watch its celebrated performance) it would actually raise its entire head, after which it would remain in this position for several minutes, ventilating (Shuker, 2012).

This experience, articulated in detail, left a lasting impression on Shuker. The locals of the Himalayan valley have recalled that the *buru* would occasionally raise its head out of the water to make a bellowing noise. Lungfishes, when respiring through their lungs do the same, and Shuker comments that “this scenario [of the *buru*’s bellowing] is one that has strong lungfish associations for me” even though others argue against the *buru* being a lungfish. Shuker’s personal and cultural background, that included a personal encounter with the captive animal, left a lasting impression that may have influenced why he chose to believe the lungfish to be the ancestor to a mythical beast. Although separated by time and context, this story is not unlike that of Saint Hilare and the *minhocao*. In both narratives, the “monstrous” nature of the *buru* and *minhocao* led Saint Hilare and Shuker, respectively, to associate them with the lungfish, a transitional animal – a fish that can breathe air – with no prior record of attacking locals and dragging animals into the water. The lungfish was suspect due to its inability to fit within a clear hierarchical taxonomy.

These stories also represent the clash between the Indigenous worldview and the Western colonialist one – the loved and respected animal became a dangerous beast: the human within and equal to the rest of the living world assumed dominion and control, and anything that did not fit within the order were deemed to be monstrous.

2.3 THE GREAT LUNGFISH CONTROVERSY: HOW THE DISCOVERY OF A FISH SHOOK THE WEST

In 1817, the young zoologist Johann Natterer was chosen to be part of one of the biggest scientific expeditions to leave Austrian soil (Barreto and Machado, 2001). In order to increase Austria's influence over South America, Prince Klemens Wenzel von Metternich, an enthusiastic student of natural history and one of the most influential statesmen of Europe, arranged the marriage between the Austrian Archduchess Maria Leopoldina and Don Pedro, who would eventually become the first Emperor of Brazil (Riedl-Dorn and Natterer, 1999). Fourteen scientists and artists were selected to accompany the Emperor's daughter to Rio de Janeiro and explore and document as much of the continent as they could. The expedition lasted four years, but due to political unrest in Brazil, the Austrian government was forced to officially call off the venture in 1821 (Taylor, 2013). Natterer however, chose to stay. During the next eighteen years, Natterer amassed a collection of over twelve thousand specimens from South America's natural landscape (2013). One of these was an animal he found so peculiar that he brought it to Leopold Fitzinger, curator of reptiles at the Imperial Museum of Vienna, for a closer inspection in 1837. Natterer had found two specimens, one in a swamp on the left bank of the Amazon and another in a pond in the river Madeira (Natterer, 1837, Gunther, 1871). The animals were long, slender, and had mottled brown patterns upon their backs. The head and bodies were decidedly eel-like. Their fins were extremely delicate for their size and descended from the creatures' bodies like vestigial appendages when they relaxed, motionless, on the river bed. By all accounts this animal was a fish at first glance. However, something caused Natterer to question this classification, so he handed over identification to Fitzinger. When Fitzinger examined the creatures he discovered, as Natterer had in the wilderness of the Amazon, they possessed what was undeniably a pair of lungs. In Fitzinger's day,

amphibians fell under the umbrella of “reptile”, but even classified amphibians such as particular salamander species (or “sirens”) that teetered upon the line of fish and reptile with their feather-like gills, had limbs with digits that resembled the fingers and toes of terrestrial reptiles. This creature had no such appendages. So bewildered was he by this unique animal, Fitzinger christened it *Lepidosiren paradoxa*: “lepidο” for the scales, “siren” for the eel-like salamanders it resembled, “paradoxa” for his confusion, and firmly placed the animal within the classification of reptile (Fig. 2.2) (Bischoff, 1840, Marshall, 1986, Zimmer, 1998).

Fig.2.1. Adult male *L. paradoxa*. (Jordan, 1907).

Although Charles Darwin’s “On the Origin of Species” had not yet been published, evolution as an explanation to new findings in natural history was already being discussed in the scientific community. Their personal stances upon the matter are unknown, but Fitzinger’s confusion and uneasiness about *L. paradoxa* is reflected in the naming of the South American lungfish as a “paradox”. It is important to note that even in his recognition of an animal that did not fit into pre-existing taxonomy, he still did not venture to change the boundaries and acknowledge this animal as being transitional. Fitzinger and Natterer were prisoners of 19th century Western scientific assumptions and the ideology of fixed species created by God’s will or plan. Nevertheless, the South

American lungfish was the first in a number of species that threatened to change the minds of many because of its transgressive nature.

Two years before Natterer's and Fitzinger's encounters with *L. paradoxa*, another similarly paradoxical animal was taken from the Gambian river, Senegambia, and presented to the Royal College of Surgeons by Thomas Weir (Gunther, 1871). Richard Owen, whose legacy would include being the first director of the Natural History Museum in London, took it upon himself to classify this new organism (Owen, 1840). His attempt to place this animal within the known taxonomic groups created a rift between what Owen believed and what he observed. What lay before him was evidently a fish, which he named *Lepidosiren annectens* in 1839¹⁶ (Fig. 2.3). Fish-like on the outside, this fish, like *L. paradoxa* of the Amazon, had lungs as well; a fact Owen could not ignore. The presence of an organ that could take in air from the atmosphere placed this creature out of the Class of Fishes, the taxonomic group he initially proposed. A fish with lungs was not a fish according to scientific convention of the time.

Fig. 2.2. *L. annectens* specimen examined by Owen (Owen, 1839)..

Owen famously opposed Charles Darwin. He did not believe in evolution by natural selection as Darwin explained it. To Owen, there was no such thing as transitional animals, that could belong to a number of phylogenies (Zimmer, 1998). The existence

¹⁶ This later changed to *Protopterus annectens*.

of such animals would further prove Darwin's theory of gradual change through time; that groups were not fixed and evolution occurred through natural selection. Owen was determined that this creature was not one of these animals. In other words, because of the lungfish, Owen (consciously or not) disregarded true scientific reporting in favour of his religious and cultural beliefs. As a result, Owen observations and notes about the lungfish's nose were congruent with these beliefs. He wished to prove that the lungfish was not using its lung to breathe, as only then could this animal truly be a fish. "The nostrils..." he wrote, "...appear as two small perforations leading to blind sacs." This was his proof: the nostrils did not lead to the lungs, rendering the lungs useless. He hypothesised that these organs were "swim- or air-bladder(s)"; hence the lungfish was a fish after all. His final statement after describing the various systems of animal in excruciating detail was as follows:

In the organ of smell we have, at least, a character which is absolute in reference to the distinction of Fishes from Reptiles. In every Fish it is a shut sac communicating only with the external surface; in every Reptile it is a canal with both an external and an internal opening.

According to this test, the *Lepidosiren* is a Fish: by its nose it is known not to be a Reptile: in other words, it may be said that the *Lepidosiren* is proved to be a Fish, not by its gills, not by its air-bladders, not by its spiral intestine, not by its unossified skeleton, not by its generative apparatus, nor its extremities, nor its skin, nor its eyes, not its ears, but simply by its nose (Owen, 1840).

However, Owen's conclusions were questioned in 1840 when M. Bishoff examined members of the same species and concluded that the blind sacs spoken of by Owen did in fact connect to the mouth, and thus this animal was a reptile (Bischoff, 1840, Edwards, 1841). Twenty years later, the Irish anatomist Robert M'Donnel examined another specimen of *L. annectens* and also found that the passageway from the lungfish's nose did not lead to the blind sacs as observed by Owen (Zimmer, 1998). He

concluded that this group of fishes were indeed the transitional creatures Owen fought to deny. He wrote, “I know of no animal more calculated leading to the adoption of the theory of Darwin than the *Lepidosiren*.” (Zimmer, 1998).

These narratives surrounding the Western discovery of the transitional lungfishes illustrate how religious ideologies fed into the classification and naming conventions of 19th century science. The Judo-Christian teleological influences stemmed from Carl Linnaeus (1707-1778) and his widely accepted system of plant and animal classification by binomial nomenclature introduced in the 18th century. Linnaeus was a devout and orthodox Christian who believed early in his career as a botanist, physician and zoologist, that as Man, he had been ordered by God to study nature and its laws (Ereshefsky, 2001, Gardiner, 2001). He then created what we know as the Linnaean system of ordered taxa that includes genus and species. Linnaeus believed that every species was decided by God’s original creation, writing “every genus is natural, created as such at the beginning – hence not to be rashly spilt up or stuck together by whim or according to anyone’s theory” (Translation by Ramsbottom, 1938). He also mentioned Noah’s ark as a reason for such wide diversity in the natural world and asserted that “it is not credible that the Creator should fill the world with animals only to destroy them all shortly afterwards through the deluge – with the exception of a single pair of each species preserved in the ark” (Translation by Ramsbottom, 1938). Although later in his career, Linnaeus adopted the theory of transformism¹⁷ and hybridization in speciation, he still did not alter his belief that the natural world consisted of systematically ordered works of Creation.¹⁸ Linnaeus’s fixism¹⁹ was widely adopted by the scientific community despite his creationist views because, for the first time, clear and consistent

¹⁷ For more information on the concepts of transformism, see Corsi, Pietro. 2005. ‘Before Darwin: Transformist concepts in European natural history.’ *Journal of the History of Biology*, 38, 67-83.

¹⁸ See Gardiner, B. G. 2001. ‘Linnaeus’s species concept and his views on evolution.’ *The Linnean*, 17, 24-36.

¹⁹ Fixism is a non-religious theory that all species alive today are identical to those in the past, and these organisms emerged already adapted to the environment without undergoing changes (Ereshefsky, 2001).

rules for classification were possible. For Fitzinger, these biases are evident in his classification of *L. paradoxa* as an anomaly that could still firmly fit within a pre-existing taxonomic Class, and more explicitly in Owen's incorrect identification of *L. annectens*.²⁰

On the other side of the world, Gerard Krefft, director of the Australian Museum, was the first scientist to see and name the Australian counterpart of lungfishes. The Queensland lungfish was offered to Krefft by William Forster, a squatter from Queensland who had acquired a position at the Australian Museum as a cook. Forster had been trying to alert Krefft to a new species of fish unknown to science for a number of years (Olsen, 2010, Semon, 1899). Known as *baramoonda* or *baramoondi* by the local indigenous populace (Fig. 2.4) (Sclater, 1870), the white colonisers of Queensland called it the Burnett, or Dawson salmon (Fig. 2.5) (for where it is found), but Krefft did not seem interested at first. Finally, Krefft decided it was time to get some proof. Krefft related the situation to a colleague in 1880:

During ten years, I dined with Mr Wm. Forster, the late Agent General for New South Wales...once a week or so, and every time William Forster tantalized me with his stories of 'fresh water salmon'. Well, when a thing of this kind is repeated too often, a man requires proof. Then the Honorable gentleman sent me a message about 11 years that THE fish had come to hand. I attended, and a messenger brought a box which I opened and found two fishes in it. Said Mr Forster, 'Well Krefft, what are these fish?' Said I, 'I cannot tell till you allow me to examine them.' Said Forster: 'Do so, you are welcome to them. I present them to you, if you will name them after me.' I replied: 'I will', took my knife out, exposed the teeth and told Forster: 'never saw anything to equal this in my life.' 'Are they new?' said Forster. 'No,' I said, 'They are as old as the mountains of Australia, and if you will let me alone we will make a fortune with these fishes.'...'Well take them away, do what you like with them, but make the discovery known in tomorrow's *Herald*.' (Monteith, 2001)

²⁰ It is interesting to note that lungfishes still remain in Owen's Class of choice, the Fishes.

True to his word, Krefft christened the fish *Ceratodus forsteri*²¹ in 1870 after Forster (Krefft, 1870, Marshall, 1986, Olsen, 2010).

Fig. 2.3. Australian lungfish lying on a cane fishing basket (Anon. 1895).

Fig. 2.4. Two fishermen with their Australian lungfish catch, Coomera River, Queensland. (Anon, 1917).

²¹ The name eventually changed to *Neoceratodus forsteri*.

The Australian discovery of its very own lungfish was momentous for the young country, because it was one of the first of new species to be announced in an Australian broadsheet (Krefft, 1840.). Krefft defied his conservative superiors, who had insisted he transport the specimens to England for a “proper” assessment, and in doing so, claimed the discovery for himself and Australia. This was one of the first public instances where regional science from British colonies attempted to extract itself from European authority and assert its independence (Olsen, 2010).²² The lungfish in this case, became a symbol of national pride, positioning Australia as a legitimate and important nation within the world. Krefft described this lungfish as an amphibian, allied to the ones discovered in Gambia and the Amazon (Krefft, 1840.). Unlike Owen, Krefft was an advocate of Darwinism and commented that “we cannot be surprised at fresh links connected the ancient fauna of the present day. It is in Australia in particular where zoological questions of great importance will yet be solved.” Scholars like Albert Günther who examined the Australian lungfish to verify Krefft’s claims were impressed (Günther, 1871), and entertained the possibility that this lungfish was indeed a living representation of the transitional animals of which Darwin spoke; those that first crawled out from the sea to colonize the land. James Hector, of the newly colonized New Zealand wrote:

The chief interest attaching to this (lung)fish arise from the circumstance that it is the living representative of an intermediate class of amphibious animals from which in early times sprung fishes on the one hand, and true reptiles on the other. Unlike any other fish, it has a lung, but also gills, thus possessing two distinct modes of purifying and oxygenating its blood (Hector, 1874).

In Krefft’s case, the cultural influences on his understandings of *N. forsteri* predominantly lay in his acceptance of Darwinian ideologies and a political desire for

²² See Monteith (2001) for a comprehensive review on the discovery of the Australian lungfish

Australia to be legitimised and independent from governing English authorities in matters of animal biology.

The origin of terrestrial vertebrates was a major and important focus in the history of scientific ideas. The discovery of lungfishes prompted excitement and heated debate on the existence of evolution by natural selection. Academics like William Cladwell flocked to Australia to study transitional animals like the lungfish and platypus (Cladwell, 1884). Another researcher, Richard Semon, spent two years in Australia attempting to collect and study the Australian lungfish's eggs (Semon, 1901). Carl Gegenbaur, who theorised that evidence of evolution could be found in the anatomy of organisms, constructed his model using the Australian lungfish as a template, a primitive creature with gills, a lung, and leaf-shaped fins that may have become fingers (Gegenbaur, 1878). Papers announcing this new species requested any new specimens to be sent to London and beseeched those in the lungfish's native land to resist temptation to hunt and eat this valuable species. P. C. Sclater placed such a note in *Nature*, writing:

In conclusion, I may express a hope that this short notice may have the effect of calling the attention of some of the colonists of Queensland to the wonderful nature of this relic of the Denovian epoch that is now swimming about beneath their noses, and that they will cease, for the present at least, to kill it and eat it as 'salmon'. Any specimens that may 'rise to their fly' should be carefully kept out of the way of the cook, preserved in alcohol and transmitted to the British Museum or some other scientific institution. When the existence of *Ceratodus forsteri* becomes more widely known, there will be no lack of applicants for examples of it (Sclater, 1870).

This time, the new Australian immigrants "colonised" the unique animals of the continent to establish their hold on the land. Despite the lungfishes' "discovery", long before, by the Indigenous population, it had to be 'rediscovered' to the rest of the

Western world by Western colonists. While the Indigenous people respected the lungfish within its environment, the colonialists captured and shipped it to be displayed in other countries (dead or alive); removing it from its environment and transforming it into a “commodity” of the human.

2.4 THE SECRET OF IMMORTALITY: CONTEMPORARY CULTURAL INFLUENCES OF *N.FORSTERI*

The Chicago World’s Fair of 1933 was named “A Century of Progress International Exposition” to commemorate the city’s centennial. The fair’s motto “Science Finds, Industry Applies, Man Conforms” celebrated technological innovation, but one particular exhibit had little to do with technology. Two Australian lungfishes, a male and female, had arrived from Sydney to the recently opened Shedd aquarium to be witnessed by Chicagoans for the first time. Walter Chute, the aquarium’s director, wanted rare, precious fish to attract visitors to the fair; a testament to the human fascination with the exotic. This act illustrated that despite all technological innovation and control, there were still things beyond our understanding, such as a fish with lungs. When Chute discovered the steamship collecting exotic fish from Hawaii was also headed to Australia, he promptly asked permission from Sydney’s Taronga Zoo for one of the continent’s own “mud-fish” to become a permanent resident of Shedd. Not long after, Shedd’s railroad car, The Nautilus, collected thirty containers of exotic fishes from Los Angeles to Chicago (2014b), including the fish Chute had requested, one of which was christened Granddad (Fig. 2.6). Although the age of this lungfish is unknown, as he came to Shedd as an adult (Fig. 2.7), the 80th anniversary of his captivity was celebrated on September 17th 2013, making him the oldest living animal in any aquarium in history since the death of George, the Galapagos tortoise (Hassler, 2013).

Fig. 2.5. Granddad arrives at the Shedd Aquarium (Anon, 1933).

Fig. 2.6. Granddad in his aquarium after arriving at Shedd (Anon, 1933).

Granddad has become a celebrity and national treasure in those eighty years. He has been praised as ‘an ambassador for the conservation of his 100 million year old species’ by the Consul-General of Australia, Hon. Roger Price (Hassler, 2013). The Shedd aquarium calls him “timeless”, and elaborates that “Granddad is like the Rolling Stones. Or blue jeans. Or the original Volkswagen Beetle” (2014b). His name also implies a familial fondness from the humans that care for him. The Shedd Facebook page is inundated with comments about Granddad, such as “He’s a true national treasure” (Konzerowsky, 2013), “I’m so glad to see Grandad’s still around” (Prus, 2013), “He’s so cute! Many years to come” (Cassady-Oliveira, 2013), and “My hero!” (Konzerowsky, 2015). His birthday celebration earned him even more well-wishers and reached an estimated audience of over four million on the day. He made the news, blog posts were published and comedian Jimmy Fallon mentioned him in his opening monologue (Michaels, 2013). A press release outlined the event and esteemed guests attended the celebration, including the Consul-General of Australia. Granddad and the four female lungfish in his tank were treated to an exclusive “ice cake” of shrimp, smelt, herbivore gel squares, yellow squash, green peas, grated carrot and sweet potato moulded into a two-tiered castle (Fig. 2.8). The cake covered in seaweed frosting and garnished with shredded greens, carrots and raspberries and placed into the tank (Hassler, 2013); an anthropomorphic gesture to honour the long-lived resident (Fig. 2.9).

Fig. 2.7. Granddad's 80th birthday cake, Shedd Aquarium (Brenna Hernandez, 2013).

Fig. 2.8. Granddad at his 80th anniversary celebration, Shedd Aquarium (Brenna Hernandez, 2013).

Bonnie McGrath illustrated the bond felt between human and lungfish when she wrote of Granddad in an article released about the lungfish's anniversary:

When I met Granddad in 1978, he was just about to celebrate his 45th anniversary as a South Loop resident. And I thought he was old then! He's been living in the South Loop a lot longer than I have. I've only been here 19 years. But we both came to live

here during the month of September, although I arrived 61 years later (McGrath, 2013).

Michelle Sattler of Shedd said, “A lot of people have a great relationship with [Granddad], and a lot invested in him. So, we hope to see him for years to come here at the aquarium,” and Ken Ramirez, Shedd’s executive vice president of animal care added “Granddad makes people happy. Everybody knows him” (Coffey, 2013).

After his milestone as the oldest fish in captivity, Granddad enthusiasts are pondering if he holds the secret to immortality. Robert H. Jordan reported, “Scientists are hoping that by studying [Granddad] and other lungfish, they may unlock the secrets to longevity, which can then be applied to us” (Jordan, 2013). Such stories are reveal the nature of human desires in contemporary Western society, illustrating our obsession with celebrity: we care more for one “famous” lungfish, than the environment that sustains the species. In addition, due to this celebrity, we create anthropocentric items of ritual celebration that have no meaning for the fish, like a birthday cake and Facebook comments, rather than choosing to release it back into the wild. They highlight our “need” for the “exotic” and the “Other” in order to feel “normal”, as well as our obsession with longevity and immortality. We see other animals and the living world as technology and tools that can be utilised for human desire.

Back on Australian soil, the Australian lungfish is a mascot of Queensland. Its influence and signature can be seen in a number of art forms scattered all over the state. In Bundaberg, “The Ceratodus”, a large sculpture of the lungfish made entirely of recycled material by John Olsen, is a landmark of the city’s riverside parklands. Electricity poles in the town of Gayndah (the town where the original specimens Krefft used to name the Australian lungfish came from) have lungfishes painted on them, and in Mundubbera, a mural covers the entirety of a water tower (Fig. 2.10). Painted in 1997, this mural

depicts the meeting of the Auburn, Boyne and Burnett rivers, and large lungfishes are seen swimming amongst other iconic flora and fauna of the area.

Fig. 2.9. Mundubbera water tower, Queensland (Mattinbgn, year unknown).

The signature of the lungfish is apparent all over the world; from contemporary sculptures and art that celebrate the lungfish and its legacy, to Granddad, whose presence has been in the hearts and minds of Chicago for nearly a hundred years. These narratives have shaped the way contemporary Western culture interprets and understands itself through the lungfish.

2.5 NINE BLIND LUNGFISHES: A PERSONAL NARRATIVE

The last four years of my life have concentrated on the visual perception of lungfishes. Although my research touches on several species, my encounters with lungfishes in the flesh have been with the Australian species, *N. forsteri* (Fig. 2.11). Part of my research into their visual perception involved testing the colour vision capabilities of *N. forsteri* (including the ultra-violet in juveniles) to confirm if they utilize their potential for colour vision. As discussed in the following chapters, my research shows that their eyes

resemble those typically found only in strongly diurnal terrestrial animals. The Australian species possess colour vision with coloured and colourless oil droplets within the light sensitive cells of their retinas (photoreceptors) (Bailes et al., 2006). These oil droplets improve light gathering ability and overall sensitivity, and improve colour discrimination, potentially enabling the animal to perceive a greater number of colour ‘hues’ than they would without them (Vorobyev et al., 1998). In addition, during their juvenile years, they have a photoreceptor type that is optimally sensitive to the ultra-violet (Bailes et al., 2006). Eyes such as these are more commonly found in diurnal birds or reptiles, not fish. Hence, this visual system does not seem to complement the behavioural patterns of the lungfish. *N. forsteri* lives a predominantly nocturnal lifestyle, and is found near the bottom of turbid, and therefore relatively poorly lit underwater environments. In many respects, their visual system makes them as ‘paradoxical’ as their lungs, as their visual machinery seems to be at odds with the environment they have evolved in.

Fig. 2.10. A juvenile Australian lungfish, *N. forsteri* Photo credit: SE Temple, SP Collin, NS Hart and RG Northcutt (2010).

After an arduous wait at the beginning of this project, nine juvenile Australian lungfishes travelled from Queensland to my laboratory in Western Australia. I

personally checked on their welfare every day, feeding them and making sure the water quality in their tanks was optimal. I noticed subtle differences between the personalities of each fish. It is difficult to remain indifferent when caring for an organism, human or otherwise, and I was no exception. I anthropomorphised my charges, grew fond of them, and looked forward to observing their responses to different coloured lights. After four months, a photographer visited our research group to document the various animals we were studying. I took advantage of this rare opportunity to photograph my own fish, preparing a freshly clean glass tank in a room bathed in light. I picked one fish and carefully brought it down to model for the camera. As the photographer was setting up the equipment, I noticed something peculiar. Amidst the bright lights and through a freshly cleaned glass, the head of this particular individual looked strange: the fish appeared to have no eyes. I was numb with shock. The shape and colour of the lungfish's head were normal, but in the area its eyes were not where they should have been (Fig. 2.12). There was no scarring, no indication that eyes had been removed or deformed in some way; they seemed simply to have not developed at all (Fig. 2.13).

Fig. 2.11. An abnormal juvenile *Neoceratodus forsteri* (Appudurai, 2013).

Along with more experienced researchers, I caught and personally checked the rest of the fish. They were all the same: not an eye in sight. No one had seen such a thing before. Before arriving, these fish had been born and raised in well-lit conditions, so this lack of development was not forced by darkness, such as might happen in a cave.

Fig. 2.12. Confirming the visual deformity in abnormal *Neoceratodus forsteri*: the eye is clearly absent (Appudurai, 2013).

Distraught, I could not believe the irony of the situation: how was I to research the visual system of lungfishes if my specimens had no eyes? I tried over and over again to understand how I had failed to notice the deformity when the fish first arrived, or when I had fed them and cleaned their tanks every day of the next four months. The only explanation I have is that I, and the others involved in my project, took it for granted that a fish has eyes. My understanding of lungfishes, garnered from basic gathered knowledge and assumptions of what a fish “should be” clouded my ability to until I was

faced with an undeniable “truth”. Like Owen, my expectation of a certain “truth” inhibited an understanding possible only when these expectations were cast aside.

2.6 THE CONNECTION BETWEEN HUMAN CULTURAL HISTORY AND THE LUNGFISH *UMWELT*

In this chapter, I have outlined different stories about lungfishes and the humans who invented and told these stories. To the Indigenous peoples of parts of Australia and Africa, the liminal nature of lungfishes have made them celebrated creatures of the living world, and illustrated an understanding of the embeddedness of the human within the natural environment. In the mythical stories of the *minhocao* and the *buru* spread by colonialists of the New World however, the differences of lungfishes as transitional animals made them monstrous. For Richard Owen in Victorian England, the fiercely embedded ideologies of a Linnaean creationist taxonomy combined with Owen’s own personal biases resulted in an incorrect description of *L. annectens*, a transitional creature that did not fit the established natural order of 19th century European science. In newly colonised Australia, the “rediscovered” lungfish was a commodity that shored up the position of “Australian” science. The narratives about Granddad illustrate a contemporary self-absorption and obsession with celebrity and immortality that positions lungfishes as commodities and tools for human advancement. Finally, my own story reveals how reality is obfuscated by preconceived assumptions of what a lungfish “should” be.

These human narratives show that many factors, aside from the evaluation of scientific data, influence our understanding of lungfishes. These inescapable biases are formed by ingrained beliefs, assumptions and ideologies which often subconsciously, force a particular understanding of what lungfishes are and/or should be. John Berger discusses

this intimate connection between what we “see” and what we “know”, saying, “The way we see things is affected by what we know or what we believe...we never look at just one thing; we are always looking at the relation between things and ourselves” (Berger, 2003). Such human cultural narratives of lungfishes show that these biases are prevalent across cultures and throughout history, and thereby influence how humans choose to understand liminal creatures like lungfishes because, “[narrative] accounts are not simply representations of the world; they are part of the world they describe” (Hammersley and Atkinson, 2007). In addition, these narratives also illustrate that non-human animals are capable of eliciting an unsettling response in humans, especially when they threaten the security of fundamental religious ideologies and definitions of life. Just as scientific research is one “social arena” in which knowledge about how a lungfish may visually perceive the world may be examined, cultural ideologies and assumptions influence how humans comprehend the visual experience of lungfishes. It is important therefore, to recognize these multiple discourses to obtain a complex understanding of lungfish *Umwelten*.

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CHAPTER 3

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**MORPHOLOGY,
CHARACTERISATION AND
DISTRIBUTION OF RETINAL
PHOTORECEPTORS IN THE
SOUTH AMERICAN
(*LEPIDOSIREN PARADOXA*) AND
SPOTTED AFRICAN
(*PROTOPTERUS DOLLOI*)
LUNGFISHES**

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3.1 INTRODUCTION

In this chapter, the use of anatomical techniques to investigate visual photoreception in the South American (*Lepidosiren paradoxa*) and spotted African (*Protopterus dolloi*) lungfishes is described. New findings concerning aspects of the morphology, density and topographic distribution of their retinal photoreceptors are presented that are essential in unravelling the visual mechanisms which influence how these lungfishes experience the world visually.

Of 70 lungfish species described through fossilised remains (Clack et al., 2011), six species remain in existence. The spotted African lungfish (*P. dolloi*), the marbled lungfish (*P. aethiopicus*), the West African lungfish (*P. annectens*) and the gilled lungfish (*P. amphibius*) are members of the family Protopteridae and are all found in Africa. The South American lungfish (*L. paradoxa*) within the family Lepidosirenidae inhabits the Amazon River and Parana-Paraguay River basins, and the Australian lungfish (*Neoceratodus forsteri*) resides in the rivers of Queensland, Australia.

The South American lungfish resides in the neotropics of South America and has the most extensive distribution of all lungfish species (Fonesca de Almeida-Val et al., 2011). Its range extends through Argentina, Bolivia, Colombia, Brazil, Paraguay, Venezuela and French Guiana, and although it is found in the Parana-Paraguay River system, it is preferentially located within the Amazon River basin (Fonesca de Almeida-Val et al., 2011). All African lungfishes are endemic to the river systems of a large part of the African continental landmass, and *P. dolloi* primarily inhabits the Congo River basin.

The species' used as model organisms in this research, the spotted African (*P. dolloi*) and South American (*L. paradoxa*) lungfishes have eel-like elongate bodies, filamentous pelvic and pectoral fins and a continuous diphyccercal tail formed by the

fusion of the dorsal, caudal and anal fins. All lungfishes possess paired pelvic and pectoral fins, although the paired fins of *L. paradoxa* and *P. dolloi* are thinner compared to other species. These paired fins have a minimal contribution to propulsion, but aid in stabilisation of the body, orientation and prey capture through tactile and chemosensory input (Johnels and Svensson, 1954, Curry-Lindahl, 1956, Thomson, 1969, Greenwood, 1986, Fonesca de Almeida-Val et al., 2011). It is not known at what depth in the shallow swamps of the Congo River the spotted African lungfish spends its time (Greenwood, 1986), but the South American lungfish tends to spend most of its time near the surface of the water (Fonesca de Almeida-Val et al., 2011). Both species swim using anguilliform²³ locomotion and may utilize their paired fins to navigate when moving over the bottom of the riverbed.

Both species are omnivorous carnivores, and are considered to be opportunistic feeders that consume a wide, generalist diet. For *Protopterus*, molluscs are the primary food source, but both the African and South American species also ingest small fish, crustaceans, aquatic insects, worms and vegetation (algae, weeds and terrestrial plant stems) (Greenwood, 1987, Fonesca de Almeida-Val et al., 2011, Mlewa et al., 2011).

All lungfish species, except for the gilled lungfish (*P. amphibius*)²⁴ are considered to be large. Greenwood (1986) reported *P. dolloi* to reach a length of up to 1.3 metres, and *L. paradoxa* to average 1.25 metres in total length (Arratia, 2003). In addition, there are no known natural predators of lungfishes that have been quantified outside of anecdotal accounts which may be a contributing factor, along with their diet, to their large adult body size.

²³ A type of fish locomotion using muscle contractions in the body to generate undulating body movements.

²⁴ Greenwood (1986) reported the largest known adult *P. amphibius* to be 44.3 centimetres.

The eyes of lungfishes, although relatively small compared to their body size, are well-developed and provide a clear image of the world. The optical image gives rise to a neural image at the level of the retina and more specifically, the photoreceptors.

Characterisation of the complement of photoreceptor types based on size, shape, and spectral sensitivity and their topographic distribution across the retina are important criteria and powerful predictors of retinal, and therefore visual function in animals.

Previous studies of *N. forsteri* show that at least some species of lungfishes possess a complex colour vision system that strongly resembles that of diurnal vertebrates such as birds and reptiles, which suggests that several of the ocular characteristics these animals possess may have evolved in shallow water before the transition of tetrapods onto land (Collin, 2010). One such characteristic is the presence of corneal surface microprojections that are important for clear vision in air, and which are present in both terrestrial vertebrates and in *N. forsteri*. As discussed previously, in *N. forsteri*, these form a thick carpet of microvilli extending over the dermal cornea, which stabilise the corneal tear film. As discussed in the General Introduction, some species of lungfishes may have evolved this characteristic in order to move onto land to aestivate and may require clear aerial vision (Collin and Collin, 2001).

Previous anatomical studies have shown that the eyes of lungfishes possess a number of different types of large photoreceptors, demonstrating that they have the retinal machinery to process colour (Walls, 1942, Pfeiffer, 1968, Ali and Anctil, 1973). The retina of *N. forsteri* – which is considered to be the most primitive of the extant lungfishes – is the only species that has been studied in detail (Marshall, 1986, Tokita et al., 2005, Bailes et al., 2006). Superficial examination of the other lungfish families has suggested that these species may have more complex ocular characteristics than *N. forsteri*. By describing the ocular mechanics of representatives of the other lungfish

families, this study therefore fills a neglected, yet crucial gap in knowledge of the evolution of terrestrial vision.

As previously discussed up to five spectrally distinct types of large retinal photoreceptors have been found in *N. forsteri*: one type of rod ($\lambda_{\max}$ 540nm) and four types of cones (UVS $\lambda_{\max}$ 366nm, SWS $\lambda_{\max}$ 479nm, MWS $\lambda_{\max}$ 558nm and LWS $\lambda_{\max}$ 623nm). UVS cones are only found in the retinas of juvenile *N. forsteri*, suggesting that sensitivity to ultraviolet light is lost during maturation (Bailes et al., 2006, Hart et al., 2008). The possession of four types of cones in juvenile lungfishes implies that they have the potential for tetrachromatic vision, which is perhaps unusual for an animal that has previously been considered predominantly nocturnal or crepuscular²⁵ (Dean, 1906, Dean, 1912, Grigg, 1965, Kemp, 1986). To date, the retinae of the South American and spotted African lungfishes have only been superficially characterised by using light microscopy and both species are thought to possess one type of rod and at least two types of cone, suggesting that these species may also have the capacity for colour vision. *P. dolloi* appears to possess both single and double cones, while *L. paradoxa* and *N. forsteri* possess only single cones (Ali and Anctil, 1973, Bailes et al., 2006).

P. dolloi and *L. paradoxa* possess oil droplets in both their cone and rod photoreceptors (Kerr, 1902, Walls, 1942, Pfeiffer, 1968, Ali and Anctil, 1973), whereas *N. forsteri* only possesses colourless oil droplets in their SWS cones, coloured yellow pigment in their MWS cones and red oil droplets in their LWS cones (Bailes et al., 2006). Colourless oil droplets are found in marsupial mammals which are generally nocturnal, crepuscular or cathemeral²⁶ and are thought to improve light gathering ability and thus, overall sensitivity. Coloured oil droplets act as miniature spectral filters within each photoreceptor and, by narrowing the spectral sensitivity function of the photoreceptors,

²⁵ Active predominantly during twilight (dawn and dusk).

²⁶ Active sporadically at random intervals during the day or night dependant on food availability, predation pressures and other environmental cues such as temperature.

are thought to improve colour discrimination by reducing overlap with adjacent spectral classes of cone (Hart et al., 2008, Vorobyev et al., 1998). This potentially enables the animal to perceive a far greater number of distinct colour ‘hues’ in the visual spectrum than would be possible without the coloured oil droplets (Govardovskii, 1983, Hart et al., 2008). The presence of coloured oil droplets in the lungfish retina is surprising because these characteristics are found only in strongly diurnal animals such as birds and reptiles, and the spotted African and South American lungfishes occupy swamps and lakes that consist of lentic (stagnant) water bodies associated with poor visibility, weedy vegetation, low oxygen content and seasonal drying (Greenwood, 1986, Mlewa et al., 2011).

In most animals with image-forming eyes, the densities of photoreceptor cells are not uniform across the retina (Collin, 1999). The topographic distribution of photoreceptors is instead species-specific, and usually reflects key features of their visual behaviour in respect to their physical environment, as well as determining the visual acuity or “spatial resolving power” of their eyes. Analysis of the topographic distribution of photoreceptors in the retina is a powerful way to gain insights as to the importance each species places on observing objects in specific regions of their visual field.

The area centralis and the horizontal streak are the two main topographical specialisations in the retina. The area centralis is a concentric increase in retinal cell density in a specific retinal location that allows for increased spatial sampling in the corresponding region of visual space (Fig. 3.1A). An area centralis is more commonly found in species that live in enclosed environments such as coral reefs and river systems (Fig. 1B). A horizontal streak is characterised by a band of increased cell density across the central meridian of the retina (Fig. 3.1C). This retinal arrangement facilitates increased spatial sampling of a large part of the visual image in panoramic environments such as open ocean or land, without the need for the extensive eye

movements that would be required to scan the same area of visual space with an area centralis (Fig. 1D).

Fig. 3.1. Topographic maps of ganglion cells in illustrating different specialisations and the species involved, in their natural habitat. **(A)** The area centralis in the coral cod, *Cephalopholis miniata* (<10.0 to 47.0×10^3 ganglion cells per mm^2). Adapted from (Collin and Pettigrew, 1988b). **(B)** The coral cod, *C. miniata* in its natural habitat amongst the marine reefs of the Indo-West and Central Pacific. Image taken by Erik Schlögl (Schlögl, 2002). **(C)** The horizontal streak in a small-spotted catshark, *Scyliorhinus canicula* (<0.5 to 2.4×10^3 ganglion cells per mm^2). Adapted from (Bozzano and Collin, 2000). **(D)** The small-spotted catshark, *S. canicula* in its natural habitat in the open marine environment of the Northeast Atlantic ocean. Image taken by Uwe Waller (Waller, 1998).

The areas that exhibit an increase in photoreceptor density in the *N. forsteri* retina vary as the fish matures (Bailes et al., 2006). The highest density of rods in the juvenile and sub-adult *N. forsteri* lie in the temporal retina, implying an increase in retinal sensitivity in the frontal visual field. As the fish matures, this becomes two areas of increased rod density in the more central retina in adults, forming a weak horizontal band across the retinal meridian. In general, the increased density of cone photoreceptors is predominantly in the dorso-temporal retina and the ventral-nasal retina in all growth stages of *N. forsteri*, indicating a downwardly directed visual axis (Bailes et al., 2006). However, the topographic specializations in *L. paradoxa* or *Protopterus* species are not yet known.

Both *Protopterus* spp. and *L. paradoxa* undergo aestivation during the dry season in their relevant habitats. Aestivation can be described as a 'light' state of dormancy, characterised by inactivity and a lowered metabolic rate that can be quickly reversed if the right conditions are met. Five out of the six species of lungfishes undergo aestivation, with the Australian species *N. forsteri*, being the only exception. This process enables these ancient fishes to avoid damage from high temperatures and desiccation during the dry season without leaving the swamps for permanent water, like some teleost fishes (Greenwood, 1986). In their respective burrows, both species of lungfishes remain until the onset of the wet season, which may be for up to eight months. It is not known if vision plays a significant role in their lifestyle during this aestivating phase, since normal metabolic function and activity is not triggered until the wet season begins.

Consequently, the visual system in lungfishes is something of a conundrum. As for *N. forsteri*, it appears the habitat and lifestyle of *P. dolloi* and *L. paradoxa* are also not heavily reliant on their visual system. Little is known about the visual behaviour of any lungfish species, and it has been stated a number of times that vision does not contribute heavily to their lifestyle, at least in prey capture and navigation (Carter and Beadle, 1930, Johnels and Svensson, 1954, Curry-Lindahl, 1956, Pfeiffer, 1968, Greenwood, 1986, Owen, 1840). The photic habitat of the swamps and rivers of the Amazon and Congo is limited, allowing only poor visibility, with a substantial amount of sediment and mineral deposits contributing to turbid, and therefore relatively dim, underwater light environments. Combined with their aestivating behaviour, where they remain dormant for most of the year, it would appear that vision does not play an essential role in survival. However, the complex visual apparatus of the lungfish eye, including a well developed lens and active accommodatory apparatus, the presence of coloured oil droplets and the presence of four spectral types of cone photoreceptor in *N. forsteri*

(Bailes et al., 2006, Bailes et al., 2007), suggests that colour vision is more important to lungfishes than previously thought²⁷.

This chapter endeavours to fill crucial knowledge gaps regarding the visual capabilities of *P. dolloi* and *L. paradoxo* through the characterisation of specific photoreceptor types based on their size, ultrastructure and topographic distribution. There have only been two published papers specifically focused on the retina of *P. dolloi* (Pfeiffer, 1968) and *L. paradoxo* (Ali and Anctil, 1973) using retinal tissue prepared in paraffin wax for light microscopy. This will be the first report on the retinal photoreceptors of *P. dolloi* and *L. paradoxo* examining resin embedded retinal tissue which significantly increases resolution, enabling the ultrastructure of the photoreceptors to be described at the level of the transmission electron microscope. The study reveals that based on morphology; there are at least three different photoreceptor types in juvenile *P. dolloi*, and one rod and one cone photoreceptor type in *L. paradoxo*. The topographical distribution of a cone photoreceptor type containing a red oil droplet is also presented for juvenile *P. dolloi*. This suggests that juvenile *P. dolloi* have the potential for dichromatic colour vision, and *L. paradoxo* may be the only lungfish species without the potential for colour vision. These findings provide novel data that contributes to our understanding of the visual experience of *P. dolloi* and *L. paradoxo*, and provide insight to the role vision plays in their behavioural ecology.

²⁷Early lungfish studies suggested that these fish were almost blind, and possessed degenerate eyes (Dean 170).

3.2 METHODS

3.2.1 Samples

All lungfish eye samples were donated by international collaborators who had obtained the animals through the international pet trade. Eyes from three juvenile *P. dolloi* (total length, TL 20.6 cm, 20.2 cm and 24. cm; eye codes Pd5L, Pd3R and Pd4R) were obtained from Dr. Guido Westhoff from the University Of Bonn, Germany. Two eyes from an adult *L. paradoxa* (TL 96 cm) were obtained from Prof. Glenn Northcutt from the University of California, San Diego (UCSD), USA. Following enucleation, the lens of *L. paradoxa* was removed. However due to the small size of the *P. dolloi* eyes, the lens was not removed and the eyes were immersion fixed *in toto*. All samples were preserved in Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4) prior to being transported to UWA.

3.2.2 Ocular measurements of *P. dolloi*

Total body length and the axial length of the eye were measured prior to fixation for all *P. dolloi* samples. The axial length of a vertebrate eye has a linear allometric relationship to body weight on a log-log plot (Howland et al., 2004). The relationship between axial length of *P. dolloi* eyes and weight were compared to the regression slope of *N. forsteri*, *P. dolloi*, and *L. paradoxa* by Bailes et al. (2007). The lens dimensions of Pd3R and Pd4R were measured after fixation in Karnovsky's. The ocular and lens dimensions of Pd5L were measured following fixation with Karnovsky's and osmium tetroxide, dehydration and embedding in araldite. Dimensions were measured on a digital photograph of a section representing the geometric centre (widest diameter) of the eye. Radii of curvature (r) were calculated using the formula:

$$r = \left(\frac{y^2}{2s} \right) + \left(\frac{s}{2} \right)$$

where r is the radius of curvature, y is half of the axial diameter and s is the sagittal diameter of the curved surface when assumed to be spherical (Bailes et al., 2007). From these measurements, the focal ratio (fr) was calculated using this formula:

$$fr = PND(0.5a)$$

where PND is the distance between the centre of the lens and the back of the retina at 90° from the plane of the cornea (posterior nodal distance), and a is the axial length of the lens (Bailes et al., 2007).

3.2.3 Light and electron microscopy

All eye samples were processed for both light and electron microscopy to examine the micro- and ultra-structural morphology of the photoreceptors within the retina of *P. dolloi* and *L. paradoxa*. Due to the fragility of the photoreceptors, the retina was not removed from the eye cup, but significant amount of connective tissue was removed prior to embedding in Procure-Araldite (ProSciTech). However, under some circumstances, the retina of *L. paradoxa* still peeled away from the underlying pigment epithelium, subsequently damaging the photoreceptors. The eyecup was then postfixed for one hour with 1% osmium tetroxide in 0.15 M phosphate buffer, dehydrated through an alcohol and propylene oxide series and infiltrated with Procure-Araldite (ProSciTech). For light microscopy, semi-thin (1 µm) sections were cut with a glass knife using a LKB Bromma Ultratome NOVA. Semi-thin sections were then deplastinated with a wash in a solution of sodium ethoxide, 70% alcohol and double distilled H₂O and stained with 4% Toluidine blue. Light micrographs were taken using

an Olympus camera (Model DP70) mounted on an Olympus compound light microscope (Model BX50F4). For transmission electron microscopy, ultrathin sections (110 nm) were cut using a diamond knife and mounted on a 200 mesh copper grid. Examination of ultrathin sections was done using a JEOL 2100 transmission electron microscope operating at 120 kV and photographed using an 11 megapixel Gatan Orius digital camera.

3.2.4 Assessment of photoreceptor dimensions and morphology

All measurements were conducted from digital images of light and electron micrographs using ImageJ 1.46r (National Institute of Health, USA). To allow for comparison between photoreceptor cells, the outer segment diameter was measured at the base, and the ellipsoidal diameter was measured at the widest point. Shrinkage of *L. paradoxa* eye tissue could not be determined due to unrecorded pre-fixation dimensions. Due to measurements of the *P. dolloi* eye tissue pre-fixation, shrinkage post-fixation was calculated to be 6.3% using the methodology outlined by Bailes et al. (2006). All measurements are quoted as mean $\pm$ standard deviation (SD) followed by sample size (n).

3.2.5 Preparation of retinal wholemount

In order to analyse the topographic distribution of the large red oil droplet containing cone photoreceptor type, one retina of a juvenile *P. dolloi* (24.3 cm in TL) was dissected and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PFA, pH 7.4). A retinal wholemount was prepared according to standard protocols (Stone, 1981, Coimbra et al., 2006). An incision was made in the pupil of the eye and the cornea

removed. A dorsal orientation slit was created by cutting towards the back of the eye cup far enough that an incision was made in the retina. The retina was carefully removed from the eye cup using No. 3 watchmaker's forceps and a fine natural-hair paint brush, and additional radial cuts were made in order to flatten the retina onto a subbed glass slide. The flattened retina was then rinsed in phosphate buffer and mounted onto a glass slide with the photoreceptor layer facing upwards in 100% glycerol. The cover slip was sealed with nail polish to prevent the retina from drying out. As the photoreceptor layer was facing upwards, the cones were visible in axial view and could be distinguished from rods (based on their size and tapered outer segment) by changing the fine focus on the microscope at a magnification of x1000 using a 1.40 numerical aperture (NA) oil immersion objective.

3.2.6 Stereological analysis and construction of a topographic map

The topographic distribution of the oil droplet containing photoreceptor in *P. dolloi* was assessed using the modified optical fractionator technique detailed in Coimbra et al. (2009). Using StereoInvestigator software (Microbrightfield Inc., USA) on a Windows 7 PC connected to a compound microscope (Olympus BX50) equipped with a motorised stage (MAC200, Ludle Electronic Products, USA) and digital video camera (MicroFIRE, OPTRONICS), the outline of the retinal wholemount was digitised using a x4 objective (NA 0.13). Using an x100 oil immersion objective (1.40 NA), all red cone photoreceptors in the retinal wholemount were counted.

The retinal outline and cell count data were exported as an Extensible Markup Language (.xml) format file and analysed using the open source statistical program R v2.15.1 (R Foundation for Statistical Computing 2012) modified with additional

packages and a custom script according to Garza-Gisholt et al. (2014) to construct the retinal topographical map.

3.3 RESULTS

3.3.1 Eye morphology and ocular measurements of *P. dolloi*

Each juvenile *P. dolloi* used in this study was normal and healthy with minor variations in body and head shape (Fig. 3.2). Eyes of juvenile *P. dolloi* are dorsally positioned, contain a dermal and scleral cornea (Fig. 3.2B, D), and are antero-posteriorly flattened (Fig. 3.3A). The dermal and scleral cornea appears to be non-pigmented, and the conjunctiva and iris are heavily pigmented with melanin granules. The sclera is also pigmented with clusters of melanin granules that form freckles around the periphery of the eye, and contains a dorsal indentation towards the iris. The iris also possesses silver/gold-reflecting material that is partly obscured by the pigmented sclera (Fig. 3.3B).

Fig. 3.2. General characteristics of the body and head of *Protopterus dolloi*, showing minor variations between animals. Images by Guido Westoff. (A) Pd3 body post-euthanasia. Scale bar = 1 cm. (B) Head of Pd3 post-euthanasia with dorsally positioned eyes. Scale bar = 1 cm. (C) Pd4 body post-euthanasia. Scale bar = 1 cm. (D) Head of Pd4 post-euthanasia with dorsally positioned eyes. Scale bar = 1 cm.

Fig. 3.3. External appearance of the isolated eye of a juvenile *Protopterus dolloi*. (A) The eye is antero-posteriorly flattened. The optic nerve (on) is visible, as is the cornea (c) where the pupil is situated. Scale bar = 1 mm (B) Front view of a juvenile *P. dolloi* eye. The cornea is over the iris (ir) and pupil, where the lens sits underneath. The iris and sclera (scl) is heavily pigmented, with bigger spots of pigment on the sclera, and a silver/gold-reflecting material on the iris, and a dorsal indentation (arrowhead) of the sclera towards the iris is visible. Image by Guido Westoff. Scale bar = 500 μ m.

The ocular lens of *P. dolloi* is relatively large and is positioned more dorsally within the eye (Fig. 3.4), which is similar to the juvenile *N. forsteri*. The radii of curvature and ocular dimensions were measured in fixed tissue prepared for light and electron microscopy (Fig. 3.4), and therefore will have suffered from some degree of shrinkage (Table 3.1.) The lenses of juvenile *P. dolloi* were measured following Karnovsky's fixation with an average shrinkage of 6.3%. The lenses are spherical with a ratio of 0.991-0.994 (Table 3.2).

Fig. 3.4. Transverse section of the geometric centre of a juvenile *Protopterus dolloi* eye. The sclera (scl) surrounds the back of the eye to become the cornea (cor) anteriorly. The lens is spherical and relatively large in the eyecup and sits more dorsally, with the iris (ir) length being unequal on either side of the lens. The retinal pigment epithelium is darkly pigmented (rpe). All retinal (ret) cell layers are present.

Table 3.1. Ocular dimensions of *Protopterus dolloi*

	Radius of curvature (mm)		Dimensions			
	Cornea	Retina	Corneal thickness (mm)	Lens axial length <i>a</i> (mm)	Posterior nodal distance (PND; mm)	Focal ratio (PND/(0.5 <i>a</i>))
Pd5L	0.48	0.41	0.03	0.55	0.87	2.94

All dimensions are based on measurements taken from Karnovsky and osmium tetroxide fixed eyes, which have been dehydrated and embedded in resin and sectioned to reveal the geometric centre of the eye of a juvenile (20.6cm in TL) *P. dolloi*.

Table 3.2. Lens dimensions of *Protopterus dolloi*

	Lens equatorial diameter (mm)	Lens axial length (mm)	% difference in lens dimensions	Lens ratio
Pd3R	0.84	0.83	0.60	0.99
Pd4R	0.59	0.58	0.86	0.99

All dimensions are based on measurements taken from the enucleated lenses of two *P. dolloi* animals (Pd3R TL=20.2cm and Pd4R TL=24.3cm) that has been preserved using Karnovsky's fixative.

A comparison of the axial eye length between *P. dolloi* and the data of *N. forsteri*, *P. dolloi* and *L. paradoxa* by Bailes et al. (2007) show that the eyes of juvenile *P. dolloi* are relatively smaller than *N. forsteri*, but falling within the linear allometric relationship of axial eye length and weight of other members of *P. dolloi* (Fig. 3.5).

Fig. 3.5. Comparison of body weight and axial eye length between juvenile *Protopterus dolloi* and *Neoceratodus forsteri*, adult *P. dolloi*, and *Lepidosiren paradoxa*. The data have been transformed into a $\log_{10}$ - $\log_{10}$ plot. The juvenile *P. dolloi* (blue circles) follows the relative linear allometric relationship of axial eye length and body weight of other representatives of the same species. Data for *N. forsteri* and adult *P. dolloi* are taken from Bailes et al. (2007).

3.3.2 Photoreceptor characterisation and morphology of *L. paradoxa*

The photoreceptors of adult *L. paradoxa* are large and tightly packed. Based on morphological characteristics at both the light and electron microscope levels and dimensions of the photoreceptors, there is one rod and at least one cone photoreceptor type in adult *L. paradoxa* (Fig. 3.6A). Both rods and cones possess oil droplets. No double cones were observed. Oil droplets visible in the electron micrographs of photoreceptors appear to contain electron-dense material (Fig. 3.6B). All light micrograph images do not contain inclusions within the oil droplets (Fig. 3.6A). We believe that the electron dense inclusions in the electron micrographs are artefacts, as the tissue that was observed at the ultrastructural level was not stained and did not undergo a washing stage, as it did in the preparation of the sections for light microscopy. All photoreceptor dimensions were measured in the light-adapted retina and are presented in Table 3.3.

Table 3.3. Photoreceptor dimensions of adult *Lepidosiren paradoxa*¹

	Basal diameter of outer segment (OS) (μm)	Length of OS (μm)	Diameter of ellipsoid (μm)	Length of ellipsoid (μm)	Oil droplet (OD) (Present/Absent)	Size of OD (μm ²)
Rod	10.1 ± 0.8 (14)	8.0 ± 1.4 (13)	18.0 ± 1.8 (13)	27.7 ± 4.1 (10)	Present	268.7 ± 51.0 (12)
Cone	7.5 ± 0.7 (16)	13.6 ± 2.8 (15)	10.5 ± 1.8 (10)	22.7 ± 2.4 (6)	Present	82.8 ± 22.0 (7)

¹All values are the mean ± SD with number of cells sampled in parentheses. All measurements are from one individual 96cm in TL.

The rod photoreceptor is large, and possesses a tapered outer segment that is usually shorter in length than the outer segment of the cones ($8.0 \pm 1.4 \mu\text{m}$ [$n = 13$], with a basal diameter of $10.1 \pm 0.8 \mu\text{m}$, $n = 14$) (Fig. 3.6A). The ellipsoid contains a larger oil

droplet than the cones ($268.7 \pm 51.0 \mu\text{m}^2$ [n = 12] in rods, compared to $82. \pm 22.0 \mu\text{m}^2$ [n = 7] in cones), with the majority of mitochondria and the paraboloid located closer to the nucleus (Fig. 3.6A). It is more difficult to differentiate rods and cones in *L. paradoxa* in comparison to *N. forsteri* and *P. dolloi*. However, rods can be identified by incisures within a more cylindrical outer segment.

Like all other species of lungfishes described, the cones are large ($13.6 \pm 2.8 \mu\text{m}$ outer segment length [n = 15], and $10.5 \pm 1.5 \mu\text{m}$ ellipsoid diameter [n = 10] in a 96 cm individual) and contain one oil droplet ($82.8 \pm 21.9 \mu\text{m}^2$ [n = 7]) within the ellipsoid. There are no additional oil droplets embedded within the mitochondria in the ellipsoid region in the cones (Fig. 3.6B). The mitochondria are distributed more centrally within the cone ellipsoid, close to the oil droplet (Fig. 3.6B) with a paraboloid that consists of an aggregation of granules (Fig. 3.6B).

Fig. 3.6. Morphological characteristics of rod and cone photoreceptors in adult *Lepidosiren paradoxa*. (A) Light micrograph of retina in transverse section showing the rod (r) and cone (c) photoreceptor. The rod outer segment (os) is cylindrical, as opposed to the conical, tapered cone outer segment. The oil droplet (od) of the rod is much larger than in the cone. Both photoreceptor types contain a paraboloid (p) and nucleus (n) below the oil droplet. Scale bar = 25 μm (B) Electron micrograph of a rod. The oil droplet has inclusions that are considered to be artefacts because of their absence in light micrograph (Fig. 3.8A). The majority of mitochondria (m) is situated in between the oil droplet (od) and paraboloid (p) and is located centrally, containing electron dense mitochondria of varying shapes and sizes. The nucleus (n) is below the paraboloid, and the cylindrical outer segment (os) is partially obscured. Scale bar = 5 μm .

3.3.3 Morphology of retinal photoreceptors in *P. dolloi*

Based on morphology, intracellular characteristics and size, juvenile *P. dolloi* possess one type of rod and at least two types of cones (one with a red oil droplet [red cone] and one lacking an oil droplet [clear cone]).²⁸ Like all other lungfish species described, most of the cell types within the retina of a juvenile *P. dolloi* ($147.2 \pm 17.9 \mu\text{m}$ thick in a 20.6 cm TL individual, $n = 6$, Fig. 3.4) are large. No double cones are observed. All photoreceptors are assessed in a light adapted retina. The dimensions of photoreceptor types are summarized in Table 3.4.

²⁸ There is one example of an additional cone type that contained a yellow pigment within the oil droplet, but there are only 36 cells of this type counted in the retina, so it is unknown if this represents a population of a different, rare cone type or oxidation of the red cones after enucleation.

Table 3.4. Photoreceptor dimensions and characteristics of juvenile *Protopterus dolloi*¹

	Basal diameter of outer segment (OS) (μm)	Length of OS (μm)	Diameter of ellipsoid (μm)	Length of ellipsoid (μm)	Oil droplet (OD) (Present/Absent)	Size of OD (μm ²)
Rod	9.2 ± 1.0 (32)	16.0 ± 2.1 (32)	11.7 ± 1.3 (34)	22.2 ± 2.7 (33)	Present	118.4 ± 23.6* (33)
Red cone	4.7 ± 0.8 (15)	6.4 ± 0.7 (14)	7.4 ± 1.8 (14)	12.7 ± 2.0 (12)	Present	39.0 ± 10.3 (13)
Clear cone	3.8 ± 1.0 (13)	5.6 ± 1.4 (13)	9.1 ± 1.0 (15)	12.3 ± 0.7 (14)	Absent	N/A

¹All values are the mean ± SD with number of cells sampled in parentheses. All measurements are from one individual 20.6cm in TL. *measurements were taken of largest oil droplet in the rod.

The rods are considered large (16.0 ± 2.1 μm outer segment length [$n = 32$], and 11.7 ± 1.3 μm ellipsoid diameter [$n = 34$], in a 20.6 cm TL individual) and contain one large oil droplet situated within the sclerad section of the ellipsoid of the inner segment amongst the mitochondria (118.4 ± 23.6 μm² oil droplet area, $n = 33$), and unlike *L. paradoxa*, a number of smaller oil droplets (between 0-5, $n = 12$) closer to the nucleus (Fig. 3.7A). The outer segment is cylindrical in shape, and consists of scalloped discs surrounded by a plasma membrane (Fig. 3.7B). The presence of rods is confirmed by the presence of incisures in the outer segment discs (Fig. 3.7C). There are also unknown inclusions within the rod outer segment (Fig. 3.7A). The ellipsoid of the rod is similar to that of *L. paradoxa* and consists of electron-dense, tightly packed mitochondria of various shapes and sizes that become smaller towards the nucleus (outer nuclear layer), and lack discernible internal structure apart from a few remaining cristae. A large paraboloid containing an aggregation of granules lies between the mitochondria and nucleus of the rod, but unlike *L. paradoxa*, there is a space (that may be an artefact) between the mitochondria and nucleus (Fig. 3.7A).

Fig. 3.7. Transmission electron micrographs detailing the ultrastructure of a juvenile *Protopterus dolloi* rod photoreceptor. **(A)** The rod (rd) is large with one large oil droplet (od) situated within the mitochondria (m) of the ellipsoid, with smaller oil droplets (*) in the mitochondria, closer to the paraboloid (p). The nucleus (n) is elongated, and the outer segment (os) is cylindrical in shape, containing unknown inclusions (arrowhead). Scale bar = 10 μm . **(B)** The outer segment consists of scalloped discs (arrowhead) surrounded by a plasma membrane. Scale bar = 0.5 μm . **(C)** The rods are confirmed by the presence of incisures (arrowhead), which appear as gaps in the discs of the outer segment. Scale bar = 0.25 μm .

Two morphologically different cone types were identified in *P. dolloi* in retinal wholemount, and using light and electron microscopy based on inclusions within the inner segment of the photoreceptors; a cone with a red oil droplet (red cone) (Fig. 3.8A), and a cone with no oil droplet (clear cone) (Fig. 3.8C).

The red cone contains a large red oil droplet within the ellipsoid of the inner segment (Fig. 3.8B). The outer segment is conical in shape, with a basal diameter of $4.7 \pm 0.8 \mu\text{m}$ ($n = 15$) and length of $6.4 \pm 0.7 \mu\text{m}$ ($n = 14$). The diameter of the ellipsoid is $7.4 \pm 1.8 \mu\text{m}$ ($n = 14$) and is filled with a single red oil droplet ($39.0 \pm 10.3 \mu\text{m}^2$, $n = 13$) in size (Table 3.3). The mitochondria within the ellipsoid have a similar organisation to the mitochondria in the rods, where larger mitochondria are concentrated towards the oil droplet and decrease in size closer to the nucleus (Fig. 3.8B). In this individual, eleven

smaller cones with a small red oil droplet were counted along the nasal edge of the retina. These may be red cones that were damaged by tissue fixation and/or processing or a developmental stage of the red cone receptor type as they resemble the large red-oil droplet bearing cones in all other characteristics.

The cone without an oil droplet has a tapered, conical outer segment with a basal diameter of $3.8 \pm 1.0 \mu\text{m}$ ($n = 13$) and a length of $5.6 \pm 1.4 \mu\text{m}$ ($n = 13$) in an individual of 20.6 cm in TL. The ellipsoid is $9.1 \pm 1.0 \mu\text{m}$ ($n = 15$) in diameter and is filled with mitochondria of varying sizes, the largest of which are positioned closest to the outer segment (Fig. 3.8C and 3.9A). Like the rods, the paraboloid of the clear cone consists of granules that sit between the mitochondria and nucleus (Fig. 3.9B).

Fig. 3.8. Morphological characteristics of cone photoreceptors in juvenile *Protopterus dolloi*. (A) Retinal wholemount of an unstained *P. dolloi* retina showing the red cones (arrowhead) at the level of the ellipsoid. Scale bar = 20 μm . (B) Electron micrograph showing the ultrastructure of a red cone (rc). The outer segment (os) is tapered with an oil droplet (od) located in the ellipsoid region above the majority of mitochondria (m), with the nucleus (n) more vitread. Scale bar = 5 μm . (C) Electron micrograph of the outer segment (os) and mitochondria (m) containing the ellipsoid of the clear cone. Scale bar = 2 μm .

Fig. 3.9. Morphological characteristics of photoreceptors in juvenile *Protopterus dolloi*. (A) Light micrograph showing the size of a red cone (rc) in comparison to a rod (rd). Scale bar = 6.25 μm . (B) Light micrograph of a clear cone (cc) in amongst rods. Note the lack of oil droplets. Scale bar = 12.5 μm .

3.3.4 Topographic distribution of the red-oil droplet bearing photoreceptor type in *P. dolloi*

The photoreceptors of the juvenile *P. dolloi* are tightly packed across the retina. There does not appear to be a geometrically regular photoreceptor mosaic, but no nearest neighbour analyses were undertaken. However, the red cones often lie adjacent to each other, in the middle of five other photoreceptors, usually rods (Fig. 3.8A). The juvenile *P. dolloi* red cone distribution shows regional variation, with a dorso-nasal area centralis in the retina with a peak density of $1.4 \times 10^3 \text{ cells mm}^{-2}$ and a total red cone population of 837×10^3 cells in an individual of 20.2 cm in TL (Fig. 3.10).

Fig. 3.10. Topographic density map of the red cone photoreceptors (mm^{-2}) in a juvenile *Protopterus dolloi* 20.2 cm in TL. Note that the greatest density of red cones is located centrally in the dorso-nasal region of the retina.

3.4 DISCUSSION

This chapter reveals the presence and ultrastructure of one rod and two cone photoreceptor types in juvenile *P. dolloi*, the topographical distribution of the red cone in a juvenile *P. dolloi*, and the characterisation of one rod and one cone type in adult *L. paradoxa*. Like the photoreceptors of *N. forsteri*, the photoreceptors of *P. dolloi* and *L. paradoxa* are large in absolute terms and their large inner segment cross sectional area and outer segment length will aid in maximising photon capture in dim light. This has been confirmed behaviourally in the cichlid (*Haplochromis sauvagei*) where an increase in photic sensitivity is associated with increased photoreceptor size (Van der Meer, 1994).

3.4.1 The eye of *P. dolloi*

The overall shape of the eyes in a juvenile *P. dolloi* is spherical, which is similar to the eyes of juvenile *N. forsteri*. However, the eye of *N. forsteri* is spherical in juveniles and

becomes more ellipsoidal in adults (Bailes et al., 2007). This ontogenetic change is also seen in some amphibian species that undergo metamorphosis such as in the toad (*Bufo americanus*) and newt (*Notophthalmus viridescens*) (Sivak et al., 1985, Sivak, 1988, Mathis et al., 1988). The optics of the growing *P. dolloi* eye have not been described, so it is unknown if the same ontogenetic changes are observed in this species, although this may be predicted given the plasticity that occurs in *N. forsteri*, the most basal species of lungfish, and in amphibians.

The spherical lens of juvenile *P. dolloi* is typical of those seen in most teleosts because it allows for greater refraction of light in the absence of any refractive power in the cornea (Walls, 1942, Sivak, 1990, Sivak et al., 1999). In aquatic animals, the lens is usually the only effective refractive element of the eye because the similarity of the refractive index between the cornea and the surrounding water negates any focusing ability. Most terrestrial species possess aspherical lenses because of the greater difference in refractive index between the air and the cornea to form a sharp image (Walls, 1942, Sivak, 1990, Charman, 1991, Land, 1991, Collin and Collin, 2001).

However, the lens of *N. forsteri* is spherical in juveniles and becomes more elliptical in shape as it grows, despite spending its entire life in water (Bailes et al., 2007). The same ontogenetic change may occur in *P. dolloi*, and because aspherical lenses are also seen in other aquatic animals such as lampreys (Collin and Fritzsche, 1993, Collin et al., 1999), sharks (Gilbert, 1963) and some species of teleosts (Pettigrew and Collin, 1995, Douglas et al., 2002), it appears that lens shape may depend on visual needs on a species specific basis.

3.4.2 Photoreceptor types in *L. paradoxa*

This study showed *L. paradoxa* possesses one rod and one cone photoreceptor type. This is in accordance with the description by Ali and Anctil (1973) using light microscopy and Zeiss et al (2011) using immunohistochemistry that identified the presence of LWS cones but no SWS cones. However, no immunostaining for MWS cones was undertaken (Zeiss et al., 2011). During aestivation, *L. paradoxa* remains dormant and does not feed during the dry, winter months that can potentially last for eight months a year. This is unlike the lifestyle of *P. dolloi*, which make frequent journeys to the top of their burrows during aestivation to breathe, and *N. forsteri* that do not aestivate at all (see the General Introduction for a review). This differing lifestyle may have resulted in less dependency on colour vision, as *L. paradoxa* is frequently described as the least fish-like, with the least developed retina (Ali and Anctil, 1973), but precisely what the role of colour vision is in all lungfish species is still a matter of speculation.

The morphology of the oil droplet bearing rod and cone photoreceptors in *L. paradoxa* closely resembles those of *P. dolloi*, and follows what was described in an ultrastructural study by Ali and Anctil (1973). However, Zeiss et al. (2011) described the rods of *L. paradoxa* as containing an ellipsoid without an oil droplet. This finding is in contrast with Ali and Anctil's (1973) report on rods despite specimens being processed in the same way. In processing the *L. paradoxa* retina for this study, unknown inclusions within the oil droplets of rods and cones appeared in the transmission electron micrographs that disappeared when sections were deplastinated and stained for light microscopy. This was the same method used to process *P. dolloi* specimens, but without the same artefact being produced. This may be an artefact due to the same phenomenon occurring in both rod and cone photoreceptors, or may indicate a difference in the composition of the ellipsoid and/or the oil droplet in the photoreceptors

in comparison to *P. dolloi*. In order to determine if the inclusions within the inner segment of *L. paradoxa* photoreceptors are oil droplets, the retina can be stained with Oil Red O to show the presence of neutral fats (triglycerides), as was carried out with *N. forsteri* (Bailes et al., 2006), but due to the similarity of the photoreceptors in *L. paradoxa* to those in *P. dolloi* and *P. aethiopicus*, and the presence of the inclusions in both rods and cones, it is presumed that this is an artefact.

Of all lungfishes, *N. forsteri* is the only known species that does not possess oil droplets in their rod photoreceptors. Many species of vertebrates contain colourless oil droplets, such as the turtle *Pseudemys scripta* (Kolb and Jones, 1987), the green frog *Rana clamitans* (Hailman, 1976), and the strawberry poison frog *Dendrobates pumilio* (Siddiqi et al., 2004). It is speculated that retinal oil droplets were originally coloured, but where species have subsequently become nocturnal these pigments were lost (Hart et al., 2006). This is presumably because the benefits of spectral tuning conferred by coloured oil droplets are outweighed by the reduction in absolute sensitivity that would make vision in dim light more difficult. Chickens (*Gallus gallus domesticus*) reared in dim light develop less dense pigmentation in their coloured oil droplets compared to those reared in bright light, presumably to maintain absolute sensitivity at the expense of spectral tuning (Hart et al., 2006), so it may be possible that adopting nocturnality provided the selective pressure to lose coloured pigments within oil droplets. These clear/colourless oil droplets can either contain an ultraviolet-absorbing pigment that will absorb scattered short wavelengths and sharpen contrast detection as it does in *Pseudemys* and *Rana*, or can be truly colourless without any wavelength absorbing characteristics as in *Dendrobates*. All oil droplets can potentially act as micro-lenses to increase photon capture (Sivak et al., 1999); although more research is needed to determine what role they play in *P. dolloi* and *L. paradoxa*.

3.4.3 Photoreceptor types in *P. dolloi*

The photoreceptors of the juvenile *P. dolloi* contain at least one type of rod and two types of cones (red oil droplet containing cones and clear, no oil droplet containing cones) that suggest it has the potential for dichromatic colour vision. The photoreceptor arrangement did not show regular mosaics, like *N. forsteri* and *L. paradoxa* (Ali and Anctil, 1973, Bailes et al., 2006). The description of oil droplet containing rods, and cones with and without oil droplets is in accordance to Pfeiffer (1968). However, our investigation did not reveal double cones. Pfeiffer (1968) described the retina of *P. dolloi* in paraffin wax processed tissue, and reported single cones with oil droplets, and double cones with only one member containing an oil droplet. Our investigation of *P. dolloi* photoreceptors did not show a clear cone next to a cone of a different type that was not another clear cone, rod, or red cone. In fact, there were many occasions where two clear cones were situated next to each other. Ali and Anctil's (1973) description of *L. paradoxa* photoreceptors from tissue processed in paraffin wax did note the presence of pairs of visual cells that may have been unequal double cones. However, they concluded that these instances were random associations of a single cone and rod (Ali and Anctil, 1973). The superior processing of our tissue in resin for greater resolution may account for artefacts that resulted in Pfeiffer's (1968) report of double cones, but without a reference image of *P. dolloi* double cones, this is difficult to confirm. Double cones are present in many teleost fishes (Stell and Hárosi, 1976, Collin, 1997, Collin and Shand, 2003, Pignatelli et al., 2010), and most terrestrial vertebrates such as birds (Hart, 2001), amphibians (Mariani, 1986) and diurnal reptiles (Detwiler and Laurens, 1920, Sillman et al., 1997), and some marsupials and monotremes (Young and Pettigrew, 1991, Ahnelt and Kolb, 2000, Ebrey and Koutalos, 2001). The function of double cones is still under consideration, but there is evidence that they aid in the detection of motion and discrimination in fine spatial detail rather than chromatic visual

tasks in birds (for review see Hart and Hunt, 2007) but are involved in colour discrimination in reef teleosts such as *Rhinecanthus aculeatus* (Pignatelli et al., 2010). In dipnoans, *N. forsteri* and *L. paradoxa* do not possess double cones, while double cones are unconfirmed in *Protopterus annectens* and *Protopterus amphibius*. The only species with described double cones is *Protopterus aethiopicus*. *P. aethiopicus* and *P. annectens* are the “youngest” members of the extant lungfishes in the Lepidosirenidae family, and are more closely related to each other than *L. paradoxa*, *P. dolloi* or *P. amphibius*. The confirmed lack of double cones in *N. forsteri*, *L. paradoxa*, and now *P. dolloi*, suggests that they may have evolved after the evolutionary separation from *P. dolloi*. However, until the photoreceptors in *P. annectens* and *P. amphibius* have been described in greater detail, the reason is uncertain because visual specialisations or degeneracy in species is greatly dependent on environmental pressures.

The red cones of *P. dolloi* are also present in *N. forsteri*. Coloured oil droplets tend to act as long-pass cut-off filters, selectively transmitting longer wavelengths of light and blocking shorter wavelengths. The spectral location of the cut-off varies, but in general their effect is to narrow the spectral sensitivity function of the cones while simultaneously increasing colour discrimination (by increasing contrast between adjacent spectral photoreceptor types) (Vorobyev, 2003). Without decreasing the total amount of ambient light entering the eye through a coloured lens, as in humans, coloured oil droplets allow an animal to utilize almost all the light available for vision without sacrificing clarity (Bowmaker, 1980). In *N. forsteri*, the red cone oil droplet absorbs all wavelengths below about 560 nm and, along with the yellow pigmented cone type, improves the animals’ ability to discriminate colours by a factor of approximately 1.3 which may aid their discrimination of foliage, prey items, conspecifics and potential predators (Hart et al., 2008). However, this assumes that we

understand the visual tasks that have driven the evolution of the eye in *N. forsteri*. Until the spectral absorption characteristics of the visual pigments and oil droplets of *P. dolloi* are measured, it is impossible to assess how much the inclusion of red oil droplets in one cone type improves colour discrimination. Nevertheless, the presence of red cones in *P. dolloi* suggests that greater discrimination of objects at long wavelengths is an adaptation required for animals living in freshwater rivers and swampland where substantial sediment and mineral deposits reflect light in the red part of the visible spectrum (Hart et al., 2008).

The position of the area centralis of the red cones in the retina of *P. dolloi* suggests the sampling of light with greater resolving power in the dorso-nasal region of the retina. This equates to a visual field dorsal and lateral to the lungfish, and may be useful in predator avoidance. In the African lungfish *P. aethiopicus* (Curry-Lindahl, 1956, Greenwood, 1987), an acute zone directed forward and into eccentric visual space may aid navigation between tight spaces in the dense vegetation of the swamp (Collin and Pettigrew, 1988a). This is different to the topographic distribution of the cones in *N. forsteri* that indicates a downwardly directed visual axis in juveniles (Bailes et al., 2006). Further study is required to investigate if this increased resolving power in the dorso-nasal region of the visual field is also reflected in other photoreceptor types and within the ganglion cell population thereby providing a better indication of how retinal structures reflects their visual ecology.

3.5 CONCLUSION

This study has revealed that the retina of a juvenile *P. dolloi* contains one rod and two cone photoreceptor types, with increased retinal sensitivity in the dorso-nasal region of the retina based on the topographical distribution of the red cones. It has also

characterised of one rod and one cone type in adult *L. paradoxa*. The large size of the photoreceptors and the presence of oil droplets in *P. dolloi* and *L. paradoxa* closely resembles the situation in *N. forsteri*, despite the lack of aestivation in the Australian species, and suggests a visual system that is adapted for high sensitivity, while the presence of one cone photoreceptor type infers that *L. paradoxa* is the only lungfish species described without the potential for colour vision. However, further study is needed to establish the importance of colour discrimination, and the selective pressures involved in the specializations of the retina of *P. dolloi* and *L. paradoxa* in order to establish the role vision plays in these species' behavioural ecology.

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CHAPTER 4

**ELUCIDATING THE VISUAL
PERCEPTION OF
NEOCERATODUS FORSTERI
WITHOUT VISIBLE EYES
USING BEHAVIOURAL,
CIRCADIAN,
ELECTROPHYSIOLOGICAL
AND ANATOMICAL
APPROACHES**

4.1 INTRODUCTION

This chapter investigates the visual perception of a unique group of juvenile Australian lungfishes (*Neoceratodus forsteri*) that do not possess externally visible eyes. Using behavioural, circadian, electrophysiological and anatomical approaches comparing abnormal *N. forsteri* (no visible eyes) and normal *N. forsteri* (visible eyes), new findings are presented that begin to uncover how a unique group of fishes experience the world without the ability to form images.

The external appearance of the head region typically containing the image-forming eyes of the abnormal *N. forsteri* was the same in all individuals; there was no scarring, no indication of damage or deformity except that the eyes appeared not have developed. Abnormal visual systems can arise from fishes being reared in complete darkness (Pankhurst, 1992), however, to our knowledge the abnormal lungfishes were developed in a normal light-dark cycle. Rather than discarding this anomalous resource, it was considered a unique opportunity to explore the visual *Umwelt* of a curious group of lungfishes without external eyes. In order to provide adequate comparison to normal lungfishes, three normal juvenile *N. forsteri* were acquired and four experiments were designed to compare the visual capabilities of the two groups. This chapter is organised into four separate experiments that investigated the visual *Umwelten* of abnormal lungfishes in three complementary ways.

1. The first experiment investigated if, despite the absence of external eyes, the abnormal *N. forsteri* were able to behaviourally discriminate between areas of relative light and relative dark.

The behavioural patterns of abnormal and normal *N. forsteri* were compared to determine whether the abnormal *N. forsteri* moved randomly between areas of light and dark, and whether these movements differ from the movements of the normal

N. forsteri. *N. forsteri* are typically nocturnal and seek shelter where available. In their natural habitat of the Mary, Burnett and Brisbane River systems of south-east Queensland, Australia (Kemp, 1986, Kind, 2002, Kind, 2011), *N. forsteri* typically shelter by day and exhibit a crepuscular and nocturnal lifestyle, where the majority of foraging occurs during the night (Dean, 1906, Grigg, 1965b). Observations that wild *N. forsteri* spawn at night (Grigg, 1965a) and anecdotal evidence from local fishermen show that they are most often heard gulping atmospheric air at night, suggesting nocturnal activity (Illidge, 1894, Spencer, 1892, Grigg, 1965b). When resting, *N. forsteri* typically prefer woodpiles, macrophyte beds and are often found amongst overhanging vegetation in shallow pools (Brooks and Kind, 2002). The observation that tagged individuals in Mary River prefer a complex shallow-water habitat with surface shading and avoid open, deep water regions (Kind, 2002), suggests that lungfishes will actively seek shelter and spend more time in the dark section of the tank in this experiment. It is possible that the abnormal fishes do not possess light sensitive tissue capable of perceiving differences in ambient light over short periods of time. Therefore, it is hypothesised that the abnormal lungfishes will not display the same behaviour as normal *N. forsteri* and will be unable to discriminate between areas of relative light and relative dark.

2. The second experiment was designed to observe and compare the circadian rhythms of normal and abnormal *N. forsteri* measured over a 72 hour period.

The physiological network that contributes to the creation of the circadian rhythm is controlled by a tightly interconnected system of regulatory molecules, cells, circuits and tissues at several layers of organisation (Kronfeld-Schor et al., 2013). Among the neural and endocrine centres that autoregulate the entrainment of the circadian clock in fishes is the pineal gland and the retina (Falcón et al., 2010). Factors such as thermoregulation,

food availability, and predation can affect the entrainment of circadian rhythms, but light remains the main synchronizer of the circadian clock (Falcón et al., 2007, Ekström and Meissl, 1997). The pineal gland possesses melanopsin in its photoreceptors, a non-image forming visual pigment that detects changes in illumination and responds by releasing melatonin (Falcón et al., 1992, Cahill and Besharse, 1995, Davies et al., 2012a, Davies et al., 2012b). Pineal melatonin provides a hormonal signal that affects the entire organism to regulate circadian patterns and plays a central role in the entrainment of fishes to changing light environments (Benyassi et al., 2000), while retinal melatonin is used mostly for local paracrine functions, and may attenuate pineal melatonin production by enabling the animal of light adapt in advance to actual changes in illumination (Zhdanova and Reeb, 2005, Li and Dowling, 2000).

The pineal gland develops early in fish embryos. Zebrafishes develop the pineal gland 19 hours post-fertilisation, and, at this stage, the pineal is already responsive to light and secretes melatonin (Kazimi and Cahill, 1999, Danilova et al., 2004). If the animals are exposed to a light-dark cycle during this stage of development, the rate of melatonin secretion follows a distinct circadian pattern that fluctuates over a diel cycle (Danilova et al., 2004). However, zebrafish embryos kept in constant darkness produce melatonin at a low level throughout life, and the circadian rhythm never develops (Kazimi and Cahill, 1999). Since the eyes and the pineal organ are both light sensitive organs and are involved in the regulation of circadian rhythms, this experiment aimed to establish whether the pineal is also atypical in abnormal *N. forsteri*. It is hypothesized that the circadian rhythm of abnormal *N. forsteri* will be more arrhythmic than normal *N. forsteri*.

3. The third experiment investigated the electrical response of the eye in normal *N. forsteri* and the region of the head that would typically contain the eye and brain in

abnormal *N. forsteri* to light stimuli using electroretinography (ERG) and visually evoked potentials (VEPs).

Electrophysiological experiments were conducted to determine whether the eye (normal condition), and the head region where the eye should be (abnormal condition), show a functional light response. ERG measures the voltage produced across the eye in response to light stimulation. ERG waveforms vary in amplitude and pattern between vertebrate species but consists of basic features common to all species that have been studied (Fig. 4.1). The ERG response to a flash of light can be separated into several distinct phases; the a-wave, which reflects the response generated by the outer segment of the photoreceptors, and the b-wave, which reflects the photocurrent created by the horizontal, bipolar and Müller cells (Brown, 1968, Asi and Perlman, 1992, Tian and Slaughter, 1995, Pinto et al., 2007). Oscillatory potentials can also be observed before the peak of the b-wave (Fig. 4.1D). ERG oscillatory potentials are generated by local networks of bipolar, amacrine and ganglion cells in the inner retina and are considered to be sensitive to disturbances in retinal circulation (Speros and Price, 1981, Asi and Perlman, 1992).

ERGs could not be recorded from any of the abnormal lungfishes. Therefore, it was decided to examine VEPs from the normal and abnormal lungfishes as well. VEPs are electrical potentials recorded from an electrode placed subcutaneously on the skin overlying the optic tecta of the *N. forsteri* brain, and are used to measure the functional integrity of the retina and visual pathway to the brain (Creel et al., 1974, Creel, 2012). Due to the inability of recording ERGs from abnormal lungfishes, it is hypothesised that it will not be possible to record VEPs in the abnormal *N. forsteri* as well.

Fig. 4.1. Electroretinograms (ERG) of various animal species, with the a-, b-, c- and d-wave separated. Calibration bars are denoted separately for each ERG. Adapted from Perlman (1995) (A) The ERG response of the red-eared terrapin (*Pseudemys scripta elegans*) elicited following a 900 ms light stimulus. (B) ERG response from the bullfrog (*Rana catesbeiana*) elicited by a 40 s light stimulus (C) The ERG response of a rabbit (*Oryctolagus cuniculus*) to a 20 s flash of white light. (D) ERG of a human (*Homo sapiens*) with oscillatory potentials on the ascending limb of the b-wave.

4. Finally, the head regions that typically contain the eyes of the abnormal lungfishes were investigated histologically to determine if there was any eye tissue embedded in the head.

The absence of external eyes is a phenotypic development that can arise from an interplay of genetic abnormalities and environmental factors. If the eyes of the abnormal lungfishes are not visible, then there is an assumption that they are not present, but can be buried or vestigial. Organisms that have failed to develop functional eyes often still possess some vestiges of ocular tissue upon histological examination. Congenital

defects manifested as primary anophthalmia (the absence of one or both eyes) in viable births still have remnants of ocular structures, such as an abnormally-sized lens, an underdeveloped optic tract, optic nerve and optic chiasma in cattle (Leipold and Huston, 1968), and nodules containing condensed scleral tissue and choroidal pigment particles in humans (Recordon and Griffiths, 1938, Verma and FitzPatrick, 2007).

The vestigial eyes of organisms adapted to subterranean habitats still contain remnants of ocular tissue. The eyes of the blind cave fish, *Astyanax mexicanus*, initially develop in the embryo, but subsequently degenerate in the larval stage and become buried inside the orbit, covered by epidermis and connective tissue (Cahn, 1958, Strickler et al., 2007, Wilkens, 2007). Similarly, the troglodytic moles *Notoryctes* (marsupial mole), *Chrysochloris* (golden mole), *Eremitalpa* (Chrysochloridae; Insectivora) and *Heterocephalus glaber* (naked-mole rat) all contain cells representing the lens (Sweet, 1906, Vaughn, 1978, Nikitina et al., 2004). Therefore, histological examination of the head region that would typically contain the eyes in abnormal *N. forsteri* is necessary to determine if there is vestigial ocular tissue present beneath the epidermis that would mediate some level of light detection.

Since eye tissue has previously been found in clinically described “eyeless” individuals (anophthalmia) and blind organisms, it is hypothesised that abnormal *N. forsteri* will possess ocular tissue beneath the skin.

4.2 GENERAL METHODS

4.2.1 Animals

The two groups of juvenile *N. forsteri*, abnormal and normal were sourced from different suppliers from Queensland (Qld), Australia. Three abnormal juvenile

N. forsteri (25 – 29.5 cm in total length [TL]; fish codes E1, E2 and E3) were chosen randomly from a cohort of nine fishes kindly donated by Dr. Michael Hutchison of the Queensland Department of Agriculture, Fisheries and Forestry (DAFF) (Bribie Island) and Dr. David Roberts of The University of Queensland/Seqwater, Qld. These fishes were bred in captivity at Professor Jean Joss' facility at Macquarie University, NSW, before being moved to DAFF and subsequently transported to UWA under a General Fisheries Permit authorised by the Qld DAFF (Permit number: 151483).

Three normal juvenile *N. forsteri* (26.4 – 29.4 cm in TL; fish codes N1, N2 and N3) were supplied by Jindalee International Pty Ltd (JITL). These fishes were bred in captivity through the Wildlife Trade Operation (GN22) and Aquaculture Program (2011/108755) under the provisions of the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) approved to JITL by the Department of Sustainability, Environment, Water, Population and Communities, Australia. All fishes could be identified individually by variations in tail length and shape, and individual markings on the head and/or body.

All fishes were housed indoors in grey fibreglass tanks: abnormal fishes in 190 x 190 cm tanks and normal fishes in a 70 x 160 cm tank. All fishes were maintained in tanks containing recirculating freshwater to a depth of 40 cm at a constant temperature of 22°C. The fishes were fed every second day on a diet of native fish pellets (52% protein 3 mm sinking pellets, Fishwerx Hobbyist Supplies). Each tank was illuminated using overhead fluorescent strip lighting on a 12h:12h light:dark cycle. Two experimental tanks (70 cm x 160 cm x 38 cm) containing one fish each was used for all behavioural experiments.

4.2.2 *Anaesthesia and Euthanasia*

Anaesthesia for Experiment 3 and euthanasia protocols for Experiment 4 were approved by the UWA Animal Ethics Committee (RA/3/100/1220) and follow a standard protocol developed by Fritsche et al. (1993). For Experiment 3, lungfishes were initially anaesthetised in dechlorinated tank water containing 100 mg/L tricaine methane sulfonate (MS222) buffered with 100 mg/L sodium bicarbonate (NaHCO_3) and subsequently maintained by directing a continuous stream of oxygenated tank water containing 75 mg/L MS222 buffered with 75 mg/L NaHCO_3 . Anaesthesia was reversed by immersion in, and active ventilation with, freshly oxygenated tank water without MS222. For Experiment 4, abnormal lungfishes were euthanased using an overdose of MS222 (500 mg/L buffered with 500 mg/L NaHCO_3).

4.3 EXPERIMENT 1: LIGHT AVOIDANCE BEHAVIOUR IN ABNORMAL *N. FORSTERI*

4.3.1 *Methods*

4.3.1a Apparatus

The experiment was run simultaneously in two experimental tanks. Each tank housed one fish, either normal or abnormal. Prior to the experiment, the tank was bisected by a light barrier in order to create a bright and a dark tank half (Fig. 4.2). The barrier (5 x 40 cm) was submerged in the water low enough to prevent light from entering the dark sector but high enough to allow the fish to pass freely between the two halves (the fish had approximately 20 cm of space to pass through into the other sections). A removable sheet of black plastic was placed over the top of the dark section of the tank. The presence and movement patterns of each individual were recorded in the bright

section of the tank with a Sony Handycam HDR-CX550 that was suspended on a tripod above the light sector (Fig. 4.2). Before each trial began, the pump in each tank was turned off and removed to prevent surface reflections from interfering with the clarity of the video images. The pumps were replaced when the trial was completed (6 hours).

4.3.1b Design

The activity patterns of three normal and three abnormal lungfishes were recorded four times each for a period of six hours over the course of four days – one period per day. Each six hour period took place between 0600 – 1200 hrs or 1200 – 1800 hrs (randomly allocated with 0.5 probability, with the restriction that each fish was observed twice during each time period). The four trials were conducted over eight days, with a rest and feeding day in between each experimental day. For each fish, the side of the tank shaded for the first six hour period (left or right) was chosen randomly (with 0.5 probability with the same restrictions as above), and swapped to the opposite side of the tank for the second period of six hours, and so on, to control for possible preferences within the tank. The fish (abnormal or normal) that inhabited the tank for each trial was randomly allocated (0.5 probability with above restrictions) and alternated to the other fish type for the subsequent trials.

Fig. 4.2. Top view of experimental tank for Experiment 1. The dark section was made dark by the addition of removable black plastic. The light and dark sections were separated by a barrier that allowed the fish to swim freely between sections but inhibited light from entering the dark section. A video camera (vc) recorded the presence of a fish in the light section.

4.3.1c Analysis

Video footage was transferred to a Windows 7 PC and converted to .avi using open-source software ffmpeg 2.5.3 and down-sampled to eight frames/s to reduce the processing time. The resulting movies were opened in Matlab 2012a (Mathworks Inc.), where custom-version software (Jan Hemmi, The University of Western Australia, modified from Hemmi, 2005 and How et al., 2008) was used to identify the presence of the fish in each section (either in light or dark) and the duration of time spent within each section. The presence of the fish in the dark section was inferred from its absence in the light section. Each video was manually checked to ensure data integrity. Due to the non-normal distribution of the underlying data, the statistical analysis was based on randomised permutations (Good, 2005). This non-parametric approach was used to avoid assumptions about normality for the underlying distribution of the amount of time each fish spent in a particular section per visit. The statistical analysis was performed in

Matlab 2012a (Mathworks Inc.) In short, the percentage of relative time spent in light or dark for each individual was calculated. Each visit, as a block of time in either the light or dark section, was permuted 10 000 times for each individual lungfish. That means each visit was randomly assigned to either the light or the dark section, but the overall number of visits to each section was held constant. After each permutation, a new percentage score was calculated. The score of the statistic measurement of each individual's observed data was then compared to all the scores from the permuted data sets for the same individual. Statistical significance was determined at $p = < 0.05$.

4.3.2 Results

Normal fishes spent more than 90% of their time in the dark sector of the experimental tank during each six hour trial period (93.2 ± 4.72 %) despite the experiment taking place during the day. However, the abnormal fish spent less time in the dark and showed more variability in time spent in the dark sector at each trial period (E1 = 60.77 ± 22.03 % [n = 4]; E2 = 57.85 ± 9.51 % [n = 4]; E3 = 77.83 ± 15.81 % [n = 4]) (Raw data is presented in Appendix Table 1).

Randomisation tests show that all normal *N. forsteri* are significantly non-random ($p \leq 0.001$) in their choice between sections. None of the 10 000 randomised permutations produced a bias towards the dark as strongly as observed for all normal fishes.

Abnormal fishes E1 and E2 are not statistically different from random ($p = 0.956$, 0.278 , respectively), but E3 is statistically non-random ($p \leq 0.001$), like the normal lungfishes. The numbers of crossings between sections for each fish is presented in Table 4.1, and shows that for the abnormal fish, the failure to achieve significance is not based on lack of choice behaviour. These results indicate that despite E3's absence of external eyes, it is still able to distinguish between sections of light and dark non-

randomly, and all abnormal lungfishes may not possess the same level of visual disability.

Table 4.1. *The number of crossings between the light and dark sections for normal (N) and abnormal (E) Neoceratodus forsteri*

Fish	# of crossings
N1	58
N2	69
N3	128
E1	77
E2	215
E3	156

4.4 EXPERIMENT 2: CIRCADIAN RHYTHM OF ABNORMAL *N. FORSTERI*

4.4.1 *Methods*

4.4.1a Experimental design

The circadian rhythm experiment was conducted in total darkness for each fish over 72 hours. The two experimental tanks were confined to a light proof room with a double containment entry/exit to prevent light from entering the room when accessed (Fig. 4.3). The filter and pump remained on for the duration of the experiment. A semi-permeable barrier was placed near the filter and pump (reducing the size of the tank by one quarter) to prevent the fish from accessing the shelter of the filter/pump. A plastic tube was placed vertically into the tank underneath the pump to prevent rippling water at the surface, but still allowing fresh water to diffuse through the tank. This reduced interference of surface waves with the video analysis. As in Experiment 1, the fish were recorded using a Sony Handycam HDR-CX550 suspended on a tripod over the tank. To increase contrast between the fish and the bottom of the tank, the gravel was pushed to the inaccessible filter/pump side of the tank, and one 48 LED infrared illuminator was

used in conjunction with the video camera's 'low lux' setting (Fig. 4.4). Infrared light was used to provide illumination for the camera as *N. forsteri* are unable to detect light at that wavelength (Hart et al., 2008). As in Experiment 1, the pairs of fishes used per 72 hour periods were randomised (with 0.5 probability). For example, if Tank 1 contained a normal fish for the first 72 hours, the subsequent experiment contained an abnormal fish. Each fish was moved to an experimental tank 48 hours prior to the experiment to acclimatize, and was fed the day prior to commencement of the experiment and on the morning following the experiment to limit feeding as a variable affecting activity.

Fig. 4.3. Circadian experiment room layout. The two tanks used in the experiment were placed in a light-proof room. A containment room with a light-proof entry and exit points was constructed to inhibit light entering the experimental room.

Fig. 4.4. Top view of a circadian experimental tank. Lungfish activity was recorded on a video camera (vc) that was suspended over the experimental arena. Additional light was provided by a 48 LED infrared illuminator (ir) placed near the video camera. The animal was prevented from resting near the filter by a semi-permeable barrier and water ripples were reduced by a plastic tube (t) inserted underneath the pump (p). Video contrast was increased by moving the gravel away from the recording arena.

4.4.1b Analysis

The video footage was transferred to the computer and converted to .avi using open-source software ffmpeg 2.5.3 and down-sampled to 8 frames/s to reduce the amount of data to process. The resulting videos were analysed in Matlab 2012a (Mathworks Inc.) with custom-version software (Jan Hemmi, University of Western Australia, modified from Hemmi, 2005 and How et al., 2008). The software automatically tracked the fish's position, which was subsequently used to calculate activity in the tank. The fish was distinguished from the background by calculating the difference between the current frame and an average background image. A background image devoid of the fish's presence was obtained by averaging 200 randomly selected images across the entire experiment. In cases where the infrared lighting changed slightly during an experiment, a local background image was calculated from 100 frames averaged over a set amount of frames when lighting remained constant. The fish's centre of gravity was calculated

for each frame and used to determine activity. The difference in the centre of gravity between the current frame and background was filtered and smoothed and the movement of the fish was determined by taking the difference between the current centre of gravity and the previous estimate of difference in movement (Fig. 4.5). The threshold for activity was movement exceeding 3.9 cm/sec. A rest period was defined as any movement below 1 cm/sec for > 5 mins. All videos were manually checked for outliers, and to make sure that periods of high activity and little or no activity were accurate. A custom-made script for Matlab (Mathworks Inc.) was used to visualise the data.

Fig. 4.5. A still image extracted from video footage using the custom-made software (Jan Hemmi, University of Western Australia) developed in Matlab (Mathworks Inc.). The animal's outline was visualised by calculating the difference between the current frame and the background image.

4.4.2 Results

The circadian rhythm of abnormal and normal *N. forsteri* was observed to investigate if the pineal gland was still functional in the abnormal group of fishes without visible image-forming eyes. The normal lungfishes did not exhibit a classical circadian rhythm, but exhibited two large periods of rest between approximately 24 and 35 hours, and between 41 and 63 hours, with reduced activity (Fig. 4.6A – C). The abnormal lungfish E2 also displayed two large periods of rest with reduced activity between 22 and 29 hours, and between 47 and 67 hours (Fig. 4.6E). E3's larger periods of rest occurred between 32 and 37 hours, and between 52 and 65 hours (Fig. 4.6F). E1 shows the most uncharacteristic activity pattern by being active for a continuous period between approximately 0 and 54 hours, resting only between 54 and 59 hours (Fig. 4.6D). General observations of all lungfishes showed that even during periods of activity, these animals liked to take 'naps' that generally remained under one hour, and during the large periods of rest, the lungfishes showed small bursts of activity that lasted a few minutes at a time (Fig 4.6). However, there is a difference in the distance each group of lungfishes travelled during the circadian experiment, where the abnormal lungfishes travelled at least 2.5 times more than the normal *N. forsteri* (unpaired $t_{(4)} = 3.71$, $p \leq 0.05$) (Table 4.2).

Table 4.2. The total distance travelled for normal (N) and abnormal (E) *Neoceratodus forsteri* for the duration of a circadian experiment.

Fish	Distance travelled (km)	Fish	Distance travelled (km)
N1	0.50	E1	4.50
N2	1.70	E2	2.50
N3	0.81	E3	4.60
Mean	1.00	Mean	3.87
Std. Dev	0.62	Std. Dev	1.18

Fig. 4.6. The circadian rhythm of normal (N1 – N3; **A – C**, respectively) and abnormal (E1 – E3; **D – F**, respectively) *Neoceratodus forsteri*. Activity was measured as the distance travelled between video frames at a speed of 3.9 cm/s over a period of 72 hours. Black arrows indicate periods of rest (prolonged activity less than 1 cm/sec) in each individual.

4.5 EXPERIMENT 3: ELECTORETINOGRAPHY AND VISUALLY EVOKED POTENTIALS OF ABNORMAL *N. FORSTERI*

4.5.1 Methods

4.5.1a Apparatus

ERGs and VEPs were measured in anaesthetised N1 and N2, and all abnormal lungfishes (see general methods of the present chapter for protocol). Both normal and abnormal lungfishes were placed in a container of tank water and secured to an inclined ramp to raise the head of the animal out of the water while keeping the rest of the body submerged. Oxygenated tank water containing the maintenance dose of anaesthesia (75 mg/L MS222 buffered with 75 mg/L NaHCO₃) was pumped from a reservoir to the container through a small delivery tube inserted into the mouth of the animal to deliver a continuous flow of water over the gills. Wet paper towels were placed on the animal's body to prevent dehydration of any exposed areas of the head and trunk. The exposed head was also dampened periodically using a pipette filled with water.

The electrophysiology apparatus consisted of a 175 W xenon arc lamp (Spectral Products) light source producing spectrally broad band 'white light' illumination that was transmitted to a mechanical shutter using a quartz fibre-optic and collimating lens. Located immediately after the shutter was a computer controlled neutral density (ND) filter wheel for attenuating the intensity of light (ND 0 – 5.8²⁹ in ND 0.2 steps). The filtered light was presented to the eye using another quartz fibre-optic cable with a collimating lens (Ocean Optics 74-UV). The collimating lens delivered the light stimulus to the eye at a distance of 8 mm from the surface of the cornea. For the single-flash ERG recordings in the normal lungfishes, an active platinum wire electrode was placed on the surface of the left cornea towards the centre of the eye, ensuring it did not

²⁹ No neutral density filters (0 ND), 1.01 x 10¹⁶ photons cm².sec⁻¹.

block the beam emanating from the fibre-optic light source. A silver/silver chloride (Ag/Ag-Cl) wire reference electrode was inserted subcutaneously on the top of the head 5 mm posterior to the midline of the eyes. Ag/Ag-Cl wire active electrodes were used instead of platinum electrodes in abnormal lungfishes due to the absence of external eyes. In this case, the recording electrode was placed subcutaneously under the skin where the left eye should have been.

For visually evoked potentials (VEPs), the active electrode was placed subcutaneously on the top of the head 20 mm from the midline of the eyes, over the optic tecta.

Differential AC voltages between the active and reference electrodes were amplified 1000 x using a WPI DAM-50 amplifier (World Precision Instruments), and hardware high-pass and low-pass filtered at 1 Hz and 1 kHz for all normal lungfishes. Differential AC voltages for abnormal lungfishes were amplified 10000 times with hardware high-pass and low-pass filters at 0.1 Hz and 1 kHz, respectively. The amplified voltage was digitised with a National Instruments USB 6353 multifunction data acquisition device, and stored on a laptop computer running Windows and using custom built software (Nathan Hart, University of Western Australia). Amplified signals were viewed in real time using a Tectronix digital oscilloscope.

The animal and recording amplifier were placed within a Faraday cage to reduce pick-up noise from mains electricity and other sources. The front of the cage was covered with a black cloth during the experiment to exclude dim ambient room light (e.g. the computer screen). Once the animal was placed in the container after dark adaptation, dim red head torches were used to make adjustments to the apparatus or to monitor the animal. Lungfishes were allowed to dark adapt for 30 minutes and light adapt for 15 minutes prior to ERG and VEP recordings.

Single flash (50 ms duration) ERGs were recorded at 8 different light intensities over a 5.8 log unit range. The time interval between successive stimulus presentation was increased as the stimuli increased in brightness (e.g. 5 – 60 s intervals at ND 5.8 – ND 0, respectively) to give the retina adequate time to return to a dark-adapted state. The number of replicates of each stimulus was also reduced with increasing intensity (e.g. 10 – 3 reps at ND 5.8 – ND 0, respectively) due to the improvement in signal-to-noise ratio of the responses. Light-adapted VEPs were conducted on all fishes that underwent ERG recordings, and dark-adapted VEPs were recorded from N1 and all abnormal lungfishes. Single flash VEPs were only recorded at the highest light intensity (no ND filters, 1.01×10^{16} photons $\text{cm}^2 \cdot \text{sec}^{-1}$) to maximize the potential response from the abnormal lungfishes. ERG and VEP recordings were conducted on the left eye of all fishes. To confirm that the VEPs were from the optic tecta and not the auditory structures of the brain or from the lateral line that detects sound or vibration from the mechanical shutter, single flash VEPs were recorded with the light source blocked, but the shutter opened and closed as normal. To confirm that VEPs from the abnormal fishes were generated in response to direct stimulation of the head region where the eye should be, rather than stimulation of other light-sensitive structures by scattered or off axis light, single flash VEPs were recorded from various parts of the fish, such as the flank of the animal. The VEPs were confirmed to be caused by stimulation to the retina rather than the pineal organ by assessing the time-to-peak potential. The pineal organ photoreceptors are specialised for the detection of slow, gradual changes in the ambient light environment and cannot discriminate between rapidly changing light (Meissl and Yanez, 1994). The rainbow trout (*Salmo gairdneri*) (Meissl and Ekström, 1988), swamp turtle (*Pseudemys scripta elegans*) (Baylor and Hodgkin, 1974), and toad (*Bufo marinus*) (Cervetto et al., 1977) pineal photoreceptor response is 5 – 6 times longer than the retinal rod photoreceptor response (Ekström and Meissl, 1997). A comparison

between the time-to-peak potential between ERG recordings and VEP recordings confirmed that stimulation was recorded from the retina and not the pineal organ.

4.5.1b Analysis

Replicated ERG and VEP recordings were averaged offline and smoothed using an unweighted running average using custom software (Nathan Hart, The University of Western Australia), and the implicit time (time to the peak of the b-wave) and the b-wave amplitude (the voltage difference between the trough of the ERG a-wave and the peak of the b-wave) were calculated. Individual replicates containing measurement artefacts such as voltage spikes, excessive baseline drift and muscle twitching from the animal were excluded prior to the analysis.

4.5.2 Results

4.5.2a Electroretinograms of normal and abnormal *N. forsteri*

The normal *N. forsteri* displayed typical ERG responses in dark- and light- adapted eyes. Their ERG responses to single flashes over a 5.8 – 0 (dark-adapted) or 5.0 – 0 (light-adapted) log unit range of light intensities show the characteristic vertebrate ERG wave form comprising the a- and b-waves, indicating the eyes were functioning normally in the presence of light stimuli (Fig. 4.7 and 4.8). The ERG responses of all normal lungfishes contained oscillatory potentials (OPs) in the rising phase of the b-wave. In dark-adapted N1, two or three OPs were observed with ND 4.0 and ND 1.0 – 3.0, respectively (Fig. 4.8A), whereas N2 showed two and three OPs with ND 3.0 – 4.0, and ND 0.0 – 2.0, respectively (Fig. 4.8B). In the light-adapted eye, N1 displayed one and two OPs with ND 1.0 – 2.0, and ND 0, respectively (Fig. 4.8A), and one and two

OPs were observed with ND 0.0 – 2.0, and ND 1.0, respectively in N2 (Fig. 4.8B). N1 and N2 displayed similar implicit times at each light intensity (Fig. 4.9). The b-wave amplitudes of normal *N. forsteri* were clinically normal (Perlman, 1995) the voltage measured in the dark- and light- adapted eye meet at 0.1 stimulus intensity (Fig. 4.10). No ERG responses were recorded from any abnormal lungfishes.

Fig. 4.7. Typical ERG responses to single flashes over a 5.8 log unit range in dark-adapted (A) and light-adapted (B) normal *Neoceratodus forsteri* (animal code N1). Each averaged ERG is composed of responses to 100 flashes.

Fig. 4.8. Typical ERG responses to single flashes over a 5.8 log unit range in dark-adapted (A) and light-adapted (B) normal *Neoceratodus forsteri* (animal code: N2). Each averaged ERG is composed of responses to 100 flashes.

Fig. 4.9. Single flash ERG implicit times over a range of 5.0 or -5.8 log units in dark- and light- adapted normal *Neoceratodus forsteri*; N1 (A) and N2 (B).

Fig. 4.10. B-wave amplitudes from single flash ERGs over a 5.0 or -5.8 log intensity units in dark and light adapted normal *Neoceratodus forsteri*; N1 (A) and N2 (B).

4.5.2b Visually evoked potentials of normal and abnormal *N. forsteri*

All normal and abnormal fishes exhibited a VEP under both dark- and light-adaptation at the light intensity of ND 0.0 (Fig. 4.11), suggesting that light is detected and transmitted to the optic tecta in both groups. N1 shows the highest response under dark adaptation in comparison to N2. The implicit time of N1 and N2 VEPs was 169.3 ms

and 193.5 ms, respectively, and the implicit time of N1 and N2 ERGs of the same light intensity was 235.7 ms and 251.1 ms, respectively. All abnormal lungfishes displayed a markedly lower VEP voltage compared to the normal lungfishes (Table 4.3), potentially due to the limited amount of light penetrating the skin where the eyes should be.

Interestingly, E2, a fish that demonstrated statistically random movement between sectors of relative light and relative dark exhibited the highest VEP response of all abnormal lungfishes (Fig. 4.11). Normalisation of the VEPs for each lungfish showed that, despite the differences in amplitude, they show a similar response duration (Fig. 4.12).

Fig. 4.11. Visually evoked potentials (VEPs) in normal and abnormal *Neoceratodus forsteri* in the dark- (A) and light- (B) adapted retina with ND 0.0. Each averaged evoked potential is composed of responses to 100 flashes.

Fig. 4.12. Normalised visually evoked potentials (VEPs) of normal (N) and abnormal (E) *Neoceratodus forsteri* in dark-adapted (A) and light-adapted (B) retina with ND 0.0 illustrating the VEP waveform. Each averaged evoked potential is composed of responses to 100 flashes.

Table 4.3. Maximum voltage response from VEPs in normal and abnormal *Neoceratodus forsteri*

	VEP Maximum (μ V)	
	Dark-Adapted	Light-Adapted
N1	3.11	2.91
N2	-	5.01
E1	0.337	0.394
E2	1.42	1.54
E3	0.768	0.844

4.6 EXPERIMENT 4: INVESTIGATION OF THE PRESENCE OR ABSENCE OF IMAGE-FORMING EYES IN ABNORMAL *N. FORSTERI*

4.6.1 Methods

4.6.1a Sample preparation

All abnormal juvenile *N. forsteri* were euthanased using an overdose of MS222 (500 mg/L buffered with 500 mg/L NaHCO₃). The general body and head morphology, including total body length of abnormal *N. forsteri* was observed and measured prior to dissection.

The eyes of juvenile *N. forsteri* are dorsally positioned on the head, and become lateral as the fishes mature into adulthood (Bailes et al., 2007). When comparing the eye position between abnormal and normal juvenile *N. forsteri* prior to euthanasia, it appeared that the site where the eyes should be on abnormal fishes was slightly indented with a lighter pigmentation to the rest of the head. This position was used to determine where the eyes may lie (if discovered beneath the surface of the skin) (see Fig. 4.14). Based on the centre of this indentation on the lightly pigmented area on the dorso-lateral side of the abnormal *N. forsteri* head, a patch of skin approximately 2 cm² in area was carefully removed. The underlying muscle and connective tissue to a depth of approximately 4 mm was removed before discovering subcutaneous eyes in all

abnormal lungfishes. The enucleated eyes of abnormal and normal *N. forsteri* were preserved in Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4). The lens of E1 and E2 was removed prior to fixation. The eyes of E3 were fixed with the lens still *in situ*. The normal *N. forsteri* eyes were used as a comparison for the gross morphology of the eye between abnormal and normal fishes.

4.6.1b General morphology, ocular dimensions and light microscopy

Following the discovery and localisation of the eyes of all abnormal *N. forsteri*, left eyes were enucleated and processed for light microscopy to examine and measure the gross morphology of ocular tissue and photoreceptor cells. The eyes were postfixed for one hour with 1% osmium tetroxide in 0.15M phosphate buffer, dehydrated through an alcohol and propylene oxide series and infiltrated with Procure-Araldite (ProSciTech). Semi-thin (1 μ m) sections were cut using a diamond knife on a LKB Bomma Ultratome NOVA and stained with 4 % Toluidine blue. Ocular tissue sections were examined on an Olympus compound light microscopy (Model BX50F4) fitted with an Olympus camera (Model DP70).

The lens of *N. forsteri* is known to increase with body length in a linear relationship (Bailes et al., 2007). In order to determine if the lens in the eyes of abnormal *N. forsteri* also followed the same relationship as the eyes in normal *N. forsteri*, the lens axial length and body length of E3 was compared to the regression slope of *N. forsteri*, previously published by Bailes et al. (2007).

4.6.1c Assessment of photoreceptor dimensions and morphology

All photoreceptor measurements were performed from digital images of light micrographs using ImageJ 1.46r (National Institute of Health, USA). To allow for comparison between photoreceptor cells, the inner segment (IS) diameter was measured at the widest point, and the outer segment (OS) diameter was measured at the base. The photoreceptor dimensions of abnormal lungfishes were also compared to normal *N. forsteri* using an unpaired t-test with data from Bailes et al. (2006) of an individual 31.5 cm in total length. The measurements of abnormal and normal lungfishes are from different data sets and are statistically not directly comparable. However, large differences between groups can still be taken as an indication of additional deformities in the abnormal *N. forsteri* eye. All measurements are quoted as mean $\pm$ standard deviation (SD) followed by sample size (n).

4.6.2 Results

4.6.2a General morphology and ocular measurements

All abnormal lungfishes are dark brown/green in colour and share a similar head and body shape. The tails of E1 and E3 are pointed (Fig. 4.13A, C), typical of normal *N. forsteri*, but the dorsal and ventral regions of the caudal peduncle of E2 are fused, resulting in a blunt and squared tail (Fig. 4.13B). All abnormal lungfishes possessed an excess amount of skin around the mouth and gills. The skin where the eyes should have been located generally contained a small indentation and a lighter pigmentation (Fig. 4.14).

Fig. 4.13. General characteristics of the body of abnormal *Neoceratodus forsteri* showing variations between animals. (E1) Abnormal fish 1 body post euthanasia. Scale bar = 2 cm. (E2) Abnormal fish 2 body post euthanasia. The tail of E2 was blunt and squared, with the tips of the caudal peduncle fused. Scale bar = 2 cm. (E3) Body of abnormal fish 3 post euthanasia. Scale bar = 2 cm.

Fig. 4.14. General characteristics of the abnormal *Neoceratodus forsteri* head, illustrating the small indentation and lighter pigmentation over the skin where the left eye should be (arrowhead) compared to a normal juvenile *N. forsteri* (N). (E1) Abnormal fish 1 head post euthanasia. Scale bar = 1 cm. (E2) Abnormal fish 2 head post euthanasia. Scale bar = 1 cm. (E3) Abnormal fish 3 head post euthanasia. Scale bar = 1 cm. (N) Normal live juvenile (25 cm in TL) *N. forsteri* with externally visible eyes. Scale bar = 1 cm. Adapted and reproduced with kind permission from Shaun P. Collin (Hart et al., 2008).

Dissection of the head region where the eyes would be expected to lie revealed the presence of subcutaneous eyes embedded in muscle and connective tissue approximately 4.2 mm below the surface in all abnormal lungfishes (Fig. 4.15), as opposed to normal *N. forsteri* with exposed eyes. There was no evidence of scar tissue observed during the dissection. When sufficient amount of muscle was removed, the eye is easily enucleated, although a thin layer of connective tissue still remained over the dorsal (anterior) regions of the eye, adherent to the cornea. An optic nerve was present, and the retina supported by a sclera eyecup as in normal-eyed individuals (Fig. 4.16). After removing most of the connective tissue dorsal to the eyes, the iris and cornea could be observed; although the cornea was not completely clear due to the presence of adherent patches of opaque connective tissue (Fig. 4.16A). Out of all

enucleated eyes, E3 appeared the most ‘normal’, with a more easily identifiable iris and pupillary aperture, and the least amount of connective tissue adherent to the cornea (Fig. 4.16B). The enucleated lens of E1 was rostrocaudally flattened with an axial diameter of 1.42 mm and an equatorial lens diameter of 2.25 mm (Fig. 4.17A). Upon examination of the fundus of the abnormal lungfish eye, the retina was present with axons joining the optic nerve, which exited the eyecup at the back of the eye (Fig. 4.17B). The ocular lens of E2 and E3 was spherical. Following examination of the ocular tissue cut in transverse section, the retinal cell layers appeared normal and intact but with the thickness of the scleral tissue found to vary around the eye: scleral posterior thickness of $214.72 \pm 27.47 \mu\text{m}$ ($n = 40$) compared to scleral anterior thickness (“cornea”) of $145.11 \pm 14.26 \mu\text{m}$ ($n = 40$) (Fig. 4.18). A comparison of lens axial diameter and total body size between E3 and data of normal *N. forsteri* (Bailes et al., 2007) showed that the E3 lens, although slightly smaller than the closest normal *N. forsteri* of similar size, did follow the relative linear allometric relationship of lens axial diameter and total body size (Fig. 4.19). The optic nerve of abnormal *N. forsteri* was also embedded in connective tissue as was the case for normal individuals. The diameter of the abnormal *N. forsteri* optic nerve was $363.97 \mu\text{m}$ at the widest point (in an individual 25 cm in TL) compared to $543.63 \mu\text{m}$ in a subadult normal *N. forsteri* (in an individual 39.5 cm in TL) (Bailes et al., 2006b). The fascicles surrounding the axons in the optic nerve of the abnormal eyes were present, although they were not as clearly defined as in the optic nerve in the normal *N. forsteri*. No myelinated axons were observed. Although axons and the nuclei of glial cells were observed, the optic nerve was not organised and contained many spaces situated between the axons and astroglial cells (Fig. 4.20).

Fig. 4.15. The subcutaneous eye of abnormal *Neoceratodus forsteri*, E3. The eye was embedded within muscle approximately 4.2 mm below the skin and surrounded by connective tissue. The entire eye is covered in scleral tissue, and darkly pigmented with no clear cornea present in this individual. Scale bar = 1 cm.

Fig. 4.16. External appearance of the enucleated eyes of two abnormal *Neoceratodus forsteri*. The diameter of each eye was approximately 5 mm. (A) The eye of E2 was embedded in more connective tissue than E3. Scleral tissue (scl) surrounded the entire eye in the absence of a clear cornea. An aperture (a) was present and appeared dark, indicating the presence of a pigmented retinal pigment epithelium underlying the retina. Scale bar = 2.5 mm. (B) The eye of E3 appeared to be the most representative of a normal juvenile *N. forsteri*. The cornea is relatively clear, revealing a clearly defined iris (ir) and the pupillary aperture (a). Scale bar = 2.5 mm.

Fig. 4.17. The internal appearance of the isolated eye of an abnormal *Neoceratodus forsteri*. (A) All abnormal lungfish eyes possessed a lens, but the lens (ln) of E1 was rostrocaudally flattened. Scale bar = 2.5 mm (B) The retina (r) was present with axons (arrowhead) converging at the optic nerve head (on) at the back of the eye. Scale bar = 2.5 mm.

Fig. 4.18. Transverse section of an abnormal *Neoceratodus forsteri* eye. The retina (ret) is intact, with a visible iris (ir) and spherical lens. However, unlike normal *N. forsteri*, there is an absence of a true cornea, where the sclera (scl) extends over the front of the eye. Note: this does not represent the geocentric centre of the eye.

Fig. 4.19. Comparison of total body length and lens axial diameter between normal *Neoceratodus forsteri* and abnormal *N. forsteri*. The abnormal *N. forsteri* (red circle) follows the relative linear allometric relationship of lens axial diameter and total body length of a normal *N. forsteri* of similar size. Data for normal *N. forsteri* is taken from Bailes et al. (2007).

Fig. 4.20. Transverse section of the optic nerve of an abnormal *Neoceratodus forsteri* (individual 25 cm in TL). (A) The optic nerve is embedded in a connective tissue sheath. The nerve is arranged into fascicles, although it is not as distinct as those observed in normal *N. forsteri*. Scale bar = 250 μ m. (B) The optic nerve tissue appears to be fasciculated (separated during histological preparation) with numerous nuclei of glial cells present (arrowhead). Scale bar = 50 μ m.

4.6.2b Photoreceptor dimensions and morphology

The eyes of all abnormal *N. forsteri* possess the requisite number of retinal cell layers (Fig. 4.21A) and the complement of photoreceptor types typical of the species; one rod, and three morphological types of cones (red, yellow and colourless, based on the appearance of the intracellular inclusions within the ellipsoid) with no differences in general morphology (Fig. 4.21B, 4.21C) (Bailes et al., 2006a). A comparison of dimensions between the outer segment (OS) length and inner segment (IS) diameter of photoreceptors of abnormal (two individuals TL = 25 cm, 29.5 cm) and normal *N. forsteri* (TL = 31.5 cm) showed that the IS diameter in all photoreceptor types was not statistically different. However, the OS length of rods was significantly smaller ($31.73 \pm 2.39 \mu\text{m}$ in abnormal, $34.64 \pm 2.45 \mu\text{m}$ in normal, $n = 10$), the red cone OS length, significantly larger ($7.67 \pm 0.90 \mu\text{m}$ in abnormal, $6.46 \pm 1.31 \mu\text{m}$ in normal, $n = 10$) and the clear cone OS length, significantly larger ($7.45 \pm 0.84 \mu\text{m}$ in abnormal, $5.78 \pm 1.54 \mu\text{m}$, $n = 10$) in abnormal compared to normal lungfishes ($p < 0.05$). However, these differences may be a reflection of comparing two different datasets generated by different protocols. The measurements and t-test statistics are summarized in Table 4.4.

Fig. 4.21. Morphological characteristics of the retina and photoreceptors in abnormal *Neoceratodus forsteri*. (A) Light micrograph of the retina of the abnormal *N. forsteri* showing the normal retina with all retinal cell layers. Moving from the top to the bottom of the micrograph lie the photoreceptors, outer nuclear layer (onl), outer plexiform layer (opl), inner nuclear layer (inl), inner plexiform layer (ipl) and retinal ganglion cell layer (rgc). Scale bar = 25 μ m. (B) Light micrograph showing the rods (rd) with cylindrical outer segments (os), a yellow cone (yc) with the yellow pigment situated above the paraboloid (p) (based on previous nomenclature published by Bailes et al. 2006), and a red oil droplet (od) containing cone (rc) surrounded by mitochondria (m) in the paraboloid. (C) Light micrograph depicting the clear cones (cc) with multiple oil droplets (arrowheads).

Table 4.4. Comparison of photoreceptor dimensions in abnormal and normal *Neoceratodus forsteri*¹

		Abnormal N. forsteri	Normal N. forsteri	t-stat	p-value
OS length (µm)	Rod	31.73 ± 2.39	34.64 ± 2.45	2.69	0.02*
	Red cone	7.67 ± 0.90	6.46 ± 1.31	2.41	0.03*
	Yellow cone	8.02 ± 1.04	8.03 ± 2.43	0.01	0.99
	Clear cone	7.45 ± 0.84	5.78 ± 1.54	3.02	0.01*
IS diameter (µm)	Rod	10.79 ± 1.79	7.96 ± 1.43	1.24	0.23
	Red cone	9.68 ± 1.2	6.16 ± 1.28	1.81	0.09
	Yellow cone	6.12 ± 0.65	10.744 ± 1.42	1.08	0.94
	Clear cone	7.16 ± 0.92	10.12 ± 1.13	1.49	0.15

¹All values are the mean ± SD. The number of individual cells measured for all fishes (n) = 10. Measurements from abnormal lungfishes were taken from light micrographs in individuals 25 and 29.5 cm in TL. Data for normal lungfishes was taken from Bailes et al. (2006) in an individual 31.5 cm in TL from electron micrographs. All measurements are raw data that have not been corrected for shrinkage. The two groups of lungfishes were compared using an unpaired t-test with df = 18 (* for p < 0.05).

4.7 GENERAL DISCUSSION

This chapter revealed the presence of subcutaneous eyes in a group of abnormal *N. forsteri*, supporting the hypothesis that vestigial ocular tissue is present beneath the epidermis. Contrary to the initial hypothesis, the recording of VEPs from abnormal lungfishes was successful, and showed that all eyes possessed a retina that was sensitive to light despite being embedded underneath muscle and connective tissue. However, these tests of functional visual integrity did not translate into a consistent behavioural phototaxis in all abnormal lungfishes, which partially supports the hypothesis that abnormal lungfishes will not display the same behaviour as normal lungfishes in response to differences in ambient light. Lastly, the hypothesis that the circadian rhythm of abnormal lungfishes would not be the same as normal *N. forsteri* was partially supported by the large differences in distance travelled by abnormal lungfishes and E1 remaining consistently active for 54 hours.

4.7.1 The abnormal *N. forsteri* eye

The enucleated eyes of abnormal *N. forsteri* contained most of the structures typical of a vertebrate eye (Walls, 1942) and what has been described for the species (Bailes et al., 2006a, Bailes et al., 2006b, Bailes et al., 2007). However, the most striking difference in the eyes of abnormal *N. forsteri* is the absence of a cornea and the sclera extending over the anterior pole of the eye that not always provide a clear window for light to enter the pupil. The vertebrate cornea typically consists of a transparent multi-layered epithelium over a basement membrane, a thick stroma, Descemet's membrane and an endothelium (Hay, 1980), with some variations in some of these structures that may include additional layers between species (reviewed in Collin and Collin, 2001). None of the differentiated layers typical of a cornea were observed in the abnormal *N. forsteri* eye. Instead, networks of opaque, dense connective tissue consisting of collagenous and elastic fibres of the sclera (Rada et al., 2006) extended over the anterior chamber of the eye in abnormal *N. forsteri*. Additionally, the absence of a limbus (also known as scleral roll or scleral spur) that marks the edge of the sclera and the beginning of the cornea (Walls, 1942) further suggests the lack of corneal tissue development. The cornea is one of the last structures to develop in the eye, so development may have been arrested at the point when the lens vesicle meets the surface ectoderm (Chow and Lang, 2001).

The lens is present in all abnormal *N. forsteri* and follows the linear allometric relationship of lens axial diameter and total body length (Bailes et al., 2007), but the aspherical lens of E1 suggests the lens development in at least one abnormal *N. forsteri* was also disrupted at this point in eye morphogenesis, and that there is variation in the eye phenotype between abnormal individuals.

The optic nerve of abnormal *N. forsteri* resembles the optic nerve of normal subadult *N. forsteri* in that the arrangement of fascicles is widely spaced and the tissue is not as tightly packed as in adult *N. forsteri* (Bailes et al., 2006b). However, unlike normal *N. forsteri*, there were spaces in between the axons of the abnormal optic nerve, although this may have been a result of histological processing. The diameter of the optic nerve was also smaller in abnormal *N. forsteri* compared with a subadult *N. forsteri* (Bailes et al., 2006b). This may be due to the subcutaneous location of the eyes in the abnormal fishes limiting photon capture, thereby affecting the development of axons and/or the hypertrophy of the connective tissue surrounding the eye and optic nerve, and subsequently the optic nerve profile. In order to investigate the integrity of the optic nerve further, the spatial resolving power and degree of retinal summation could be explored by examining the number, morphology and distribution of retinal ganglion cells and their axons. Interestingly, there were no myelinated axons observed in the abnormal optic nerve. A total of 18% of the optic nerve axons in the subadult *N. forsteri* (58 cm in TL) were found to be myelinated, increasing to 74% in the adult (113 cm in TL) (Bailes et al., 2006b). The abnormal optic nerve observed was from a juvenile 25 cm in TL, so this lack of axon myelination may be another phenotype of the abnormal *N. forsteri*. In order to confirm whether the degree of myelination varies during development in lungfishes, the optic nerve of normal juvenile *N. forsteri* needs to be examined and compared to the adult number of myelinated fibres. Myelination of axons greatly increases the conduction velocity from the eye to the visual centres of the brain (Hursh, 1939, Ritchie, 1984, Tasaki, 2012) and the absence of myelinated axons suggests that visual response in abnormal *N. forsteri* is slower than in normal subadult *N. forsteri* (Bailes et al., 2006b).

The photoreceptors of abnormal lungfishes were compared to normal *N. forsteri* to determine if there were large differences between the size of the photoreceptors in the

two groups. In general, the retina of abnormal *N. forsteri* contained all cell layers and photoreceptor types typical of the species (Bailes et al., 2006a). Although there were some significant differences in the outer segment length of the rod, red and clear photoreceptor types, it is important to note that the comparisons made between normal and abnormal lungfishes were from different datasets generated from slightly different protocols, and the differences, although significant, are small. The rod OS length in *Protopterus dolloi* (16.0 ± 0.37 SEM μm , $n = 32$) and *Lepidosiren paradoxa* (8.0 ± 0.39 SEM μm , $n = 13$) (Chapter 3) are notably smaller than the rod OS length of normal *N. forsteri* (34.64 ± 0.77 SEM μm), and clear differences like this were not found between normal and abnormal *N. forsteri* in this study.

4.7.2 Visual perception of abnormal *N. forsteri*

Despite the subcutaneous location of the eye, the absence of a cornea in some individuals, and other differences in the size of some of the retinal photoreceptor types and differences in the optic nerve structure, all abnormal lungfishes exhibited a VEP. Although the amplitude of the VEPs varied between individuals, this finding demonstrates that the retina of abnormal *N. forsteri* is still photoreceptive, that the optic nerve is relaying this information to the brain, and (presumably) the optic tectum is receiving this input. The differences between the amplitude measured in normal and abnormal lungfish VEPs are probably affected by the amount of light stimuli reaching the retina as the subcutaneous eyes of abnormal lungfishes were buried deep beneath the skin. Despite the fact that VEPs were measured from all three abnormal lungfishes, only E3 demonstrated statistically non-random behaviour in the two regions of the tank (light and dark). This suggests that either the sclera over the anterior of the eye allowed enough ambient light to pass through and elicit a behavioural response in E3, the light

intensity in Experiment 1 was not bright enough to elicit a behavioural response in E1 and E2, or the VEPs generated by E1 and E2 were not from the optic tecta. VEPs are generally produced by the optic tecta, but in human studies, VEPs have been reported to also be generated by sub-cortical or extrageniculate sources in functionally blind individuals rather than the visual cortex (Kupersmith and Nelson, 1986, Mackie and McCulloch, 1995, Clarke et al., 1997). More investigation into the brain of abnormal lungfishes may provide greater information on how this organ may be affected.

Sensory plasticity has been described in a number of species. Adverse environmental conditions in early development that results in a deficit in one sense can result in adaptations that heighten the capacity in another to enable organisms to respond effectively to environmental change (see the “compensatory plasticity hypothesis” by Rauschecker and Kniepert, 1994). Guppies (*Poecilia reticulata*) have been shown to exhibit sensory plasticity in vision and chemoreception in response to differing light intensities. Guppies reared in low light environments had a significantly stronger response to chemical food cues and a weaker response to visual cues in isolation, compared to those reared in higher light conditions (Chapman et al., 2010). Terrestrial snakes (*Notechis scutatus*) reared in an aquatic environment have shown improved locomotor performance in water compared to snakes reared on land (Aubret et al., 2007, Aubret and Shine, 2008). These examples suggest that sensory plasticity causes a stronger response to chemical food cues in guppies, and an increase in locomotor response in terrestrial snakes reared in aquatic environments.

Other senses reported to be important to lungfishes are the lateral line (Lippolis et al., 2009) and electroreception (Watt et al., 1999), which provide enhanced sensory capacity at low light conditions. A 72 hour observation of lungfishes in total darkness showed that the distance abnormal *N. forsteri* travelled when active was more than 2.5 times

that of the normal *N. forsteri*. The increase in distance travelled in abnormal fishes and, in the case of E1 and E2, the inability to discriminate between light and dark sections of the holding tank, may be a form of behavioural plasticity that, in the absence of external eyes, has contributed to a greater dependence on the lateral line and electroreception systems.

The lateral line system is a mechanoreceptive sense that consists of a series of hair cell bundles (neuromasts) that occur either on the surface of the skin (superficial) or within canals lying beneath the skin, where water enters the canals via small pores over the head and trunk. This system mediates the ability to detect minute hydrodynamic disturbances and acts as a specially-adapted mechanoreceptive system for aquatic life (Bleckmann, 1986, Montgomery et al., 1997). The lateral line can aid in obstacle, wave and chemical detection (Dijkgraaf, 1963, Hashimoto et al., 1970, Müller and Schwartz, 1982, Kawamura and Yamashita, 1983) and in schooling fish, helps maintain the swimming speed and direction of travel (Partridge and Pitcher, 1980). Studies on the blind cave-fish *Astyanax fasciatus* (Bleckmann, 1986) and the nocturnal torrent fish *Cheimarrichthys fosteri* (Montgomery and Milton, 1993) demonstrate that this can provide tactile reference between the substrate and oncoming water current. The lateral line of *N. forsteri* is used in predator detection and avoidance (Lippolis et al., 2009) so, in the absence of vision, this system may also be utilized in general to compensate in navigation.

Organisms with electroreception possess ampullary receptors, modulated by movement, that enable the detection and measurement of weak bioelectric fields that surround prey animals (Kalmijn, 1971, Bullock et al., 1993, Manger and Pettigrew, 1995). All lungfishes possess passive electroreception and, during foraging, juvenile *N. forsteri* utilize this sense to prey on animals buried in the substrate with greater accuracy after

assessing the general whereabouts of the organism using olfaction (Watt et al., 1999).

The importance of vision in the lifestyle of *N. forsteri* is unknown, but the reduced distance travelled by normal *N. forsteri* compared with abnormal *N. forsteri*, suggests that other senses (i.e. lateral line and electroreception) affect how far the fishes travel.

However, E3, the only abnormal fish that could behaviourally discriminate between light and dark, exhibited the greatest distance travelled over a 72 hour period, suggesting there may be other reasons associated with this increased activity, such as the absence of spatial vision. Early exposure to low light levels during development has been shown to result in a severe impairment of spatial tasks that include locating food (Axelrod, 1959). Further investigation and comparison into the regions of the brain responsible for these other senses may provide additional information on how the abnormal lungfishes are compensating for the lack of image forming vision or the absence of light intensity information.

Some of the behavioural differences, such as the continuous activity of abnormal *N. forsteri* E1 for 54 hours in complete darkness, and the statistically random movement of E1 and E2 between relative light and dark sections of an aquarium, may also be due to potential differences in the pineal organ. The general morphology of the pineal gland in lungfishes has been described as an intermediate between teleost fishes and amphibians (Holmgren, 1959), and the developmental changes over the first 12 months in *N. forsteri* resembles that of pre-metamorphic amphibians (Joss et al., 1997a). Unlike teleosts, lungfishes do not possess a parapineal organ (Holmgren, 1959), and unlike amphibians, the proportion of photoreceptors decreases and the proportion of photoreceptors lacking outer segments increases in a developing *N. forsteri* pineal organ (Joss et al., 1997a). Some of the functions common to both the amphibian and teleost pineal organ are setting the circadian locomotor rhythms, entrainment to environmental

light cycles and free-running rhythms (Adler, 1976, Ekstrzm and Meissl, 1997).

Removal of the pineal organ terminated the circadian free-running locomotor activity rhythm and caused arrhythmicity in stinging catfish (*Heteropneustes fossilis*) (Garg and Sundararaj, 1986). The abnormal *N. forsteri* E1 was continuously active for 54 hours, and this arrhythmicity may have been due to an abnormal pineal organ. The teleost pineal organ can also affect swimming activity and phototactic behaviour. White suckers (*Catostomus commersoni*) with shielded pineal regions spent significantly more time in the illuminated portion of the aquarium (in a two choice light:dark based system) when kept at a constant temperature (Kavaliers, 1982). The behavioural differences observed between normal *N. forsteri* and abnormal *N. forsteri* (E1 and E2) in the light and dark portions of the aquarium may also be due to differences in the pineal organ. The pineal organ of dipnoan fishes has not been extensively researched, and it is unknown if the pineal photoreceptors of lungfishes function in the same way as in teleosts and amphibians. However, the close histological and developmental resemblance of the pineal organ in lungfishes, teleosts and amphibians suggests that a comparative study between the pineal organs of normal and abnormal *N. forsteri* may provide insights into some of the differences seen in the behavioural and circadian experiments, such as the 54 hours of continuous activity of E1 in complete darkness and the statistically random behaviour in the two regions of the aquarium (light and dark) in E1 and E2.

4.7.3 Possible causes for the abnormal morphological deformities in N. forsteri

A clear cause for the morphological deformities in the abnormal lungfishes cannot be determined from the experiments conducted in this project. However, some speculation regarding their ocular abnormality can be explored.

The integrated roles of the environment and/or genetics can cause various diseases and deformities. In fishes, the environment can adversely influence the development of the visual system through the absence of light affecting the development of both the image forming eyes and the pineal organ. The pineal organ of teleosts and amphibians is responsible for body lightening in the larval stages when raised in complete darkness; presumably due to melatonin contracting the melanophores in the skin (Bogenschütz, 1965) (Bagnara, 1965) (Joss et al., 1997b). All abnormal fishes possessed a darkly pigmented body and two out of the three abnormal *N. forsteri* exhibited a circadian response similar to normal *N. forsteri*. This suggests that these fishes perceived a normal light/dark diel cycle when developing because of the early development of the pineal gland and its dependence on a consistent light/dark cycle to properly regulate melatonin secretion, the circadian rhythm and prevent blanching of the skin (Joss et al., 1997b, Kazimi and Cahill, 1999, Danilova et al., 2004). However, morphological deformities in fishes can also occur in contaminated water (reviewed in Pereira et al., 2013) and as a result of poor nutrition (Monaghan, 2008). Morphological deformities have been observed in other fishes as a result of water quality and temperature, such as the tilapia *Oreochromis* spp. (Sun et al., 2009), the japanese eel *Anguilla japonica* and a number of species in Skellefteå and Bothnian Bay, Sweden (Bengtsson et al., 1985). Zebra fish larvae exposed to organic mercury had the greatest concentration of this heavy metal in the eye (Korbas et al., 2008, Korbas et al., 2010, Korbas et al., 2011, Korbas et al., 2013) and the accumulation may cause visual impairment, as it does in humans and other mammals (Warfvinge and Bruun, 1996, Erie et al., 2005). The water conditions in the development of the abnormal lungfish examined in this study are unknown and until they are confirmed, cannot be disregarded as a possible cause of their morphological deformities.

All the abnormal *N. forsteri* are from the same brood. The diversity of the genetic pool of captive lungfishes at Macquarie University is unknown, so the visual (and other potential deformities) may be due to congenital defects caused by inbreeding. Pax6 is a one of the best studied transcriptional regulatory genes essential for the normal development of the brain and eye. It is essential for normal eye development in *Drosophila* (Halder et al., 1995) and over- and under-expression of the gene through mutations can result in severe visual abnormalities (Schedl et al., 1996). Heterozygous mutations of Pax6 in mice result in the reduction of eye size and iris hypoplasia, and homozygous mutations result in the lack of eye cavities, nasal cavities, and brain abnormalities that result in death shortly after birth (Hogan et al., 1988, Ton et al., 1991, Schedl et al., 1996). Heterozygote mutations in humans result in aniridia, an umbrella term that describes visual defects of varying degrees such as iris hypoplasia, optic nerve hypoplasia corneal opacification and cataracts (Glaser et al., 1994). Therefore, molecular analyses of Pax6 may uncover the genetic basis of the congenital defects observed in abnormal *N. forsteri*.

4.8 CONCLUSION

This study compared aspects of the visual *Umwelten* between groups of normal *N. forsteri* and abnormal *N. forsteri* with ectopic eyes. Through behavioural, electrophysiological and histological experimentation, it has revealed the presence of subcutaneous eyes in all abnormal lungfishes that are capable of photo-transduction, but perhaps not spatial vision. The abnormal lungfish eye lacks a cornea, possesses an atypical optic nerve, but has a retina and a photoreceptor complement typical of *N. forsteri*. In addition, the measured functional integrity of the eye does not translate into

behavioural phototaxis in all abnormal lungfishes, suggesting there is variation between individuals. However, further study is needed to uncover other aspects of the abnormal lungfish visual *Umwelt*, and establish the genetic and/or environmental basis underlying their morphological deformities.

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CHAPTER 5

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ENTERING THE

SOAP-BUBBLE:

INTEGRATING SCIENCE

AND ART TO DESCRIBE

ENCOUNTERS WITH THE

LUNGFISH *UMWELT*

.....

5.1 INTRODUCTION

The visual perception of any organism is multifaceted, and can be explored and understood in a variety of ways. In scientific research, perception is investigated through a reductionist approach where the organism or species in question is “broken” into smaller parts and defined within a strict set of rules that govern scientific methodology. These rules/methodologies create a framework to obtain a general and mechanistic understanding of the visual perception of organisms that is detectable by means beyond the human, such as human-designed technologies, as discussed in previous chapters. However, understanding the visual experience of lungfishes is problematic because our interpretation of the knowledge obtained through scientific experimentation is always informed by our own visual and cognitive apparatus, and is thereby fundamentally anthropocentric. Nagel acknowledged the failings of human cognition in this respect, saying, “Yet if I try to imagine (what it is like to be a bat), I am restricted to the resources of my own mind, and those resources are inadequate to the task” (Nagel, 1974). Uexküll also touched on this futility, describing the soap-bubbles of the *Umwelt* as inaccessible worlds where “certain convictions are able to bar the entrance to those worlds so securely that not even one ray of all the splendour that spreads over them can penetrate it” (Uexküll, 2010).

No complete depiction of the visual perception of lungfishes is possible due to the embedded subjectivity of the organism and the individual, but science and art enable a multilingual philosophy that uses the strengths and weaknesses of either discipline in mutual respect, rather than relying solely on one while discounting the other (Anderson, 1995, Mullen, 1998). Scientific data contributes to the growing knowledge of lungfish visual ecology, and artistic research contributes and connects the knowledge gained that

uses “objectivity as a direction in which understanding can travel” (Nagel, 1974). This interdisciplinary platform may demystify lungfish perception.

This chapter contextualises several artworks and exhibitions generated through scientific and artistic research that attempt to extend and complicate issues and understandings surrounding the lungfish *Umwelt*. The artwork ‘*Inperception*’, examined in chapter 1, focused on human attempts to represent the lungfish *Umwelt* by inviting three artists to respond to scientific data on the visual perception of the Australian lungfish (*Neoceratodus forsteri*) onto a large mural suspended around the lungfish pools. In retrospect, the project was anthropocentric because it was explicitly framed by human interpretations of scientific data generated by humans and their technologies, and translated through human drawings.

The artworks produced since this early attempt evolved to question the multiple anthropocentric obstructions that inhibit full immersion into the lungfish *Umwelt*, and reflect on the experience of working between science and art. These works are discussed in chronological order to illustrate this progression. The first set of works, ‘*Third Eye*’ (2013) and ‘*In Focus*’ (2013), explored the “machinery” of the lungfish eye through biological dissection, macro-photography and histology when viewed using light microscopy. Sculptural works collectively titled ‘*The Forms*’ (2014), featured in a group exhibition (2014), used the previous photography works as a departure point to explore the aesthetics of, and human engagement in, the production and presentation of scientific images. ‘*The Forms*’ also chronicles my personal experience of investigating the lungfishes’ *Umwelten* as labour fraught with uncertainty; I was both literally and figuratively, “in the dark”.

The final exhibition ‘*Cernentia: exploring the visual perception of lungfishes without eyes*’ (2015) extended beyond photography as visual “evidence” to question the use of

digital data (collected through behavioural studies and other technological apparatus) as an objective scientific tool, and revisited the sublime³⁰ of an individual's multi-sensorial experience; something that is hard to capture with words or using any other technology.

Collectively, these artworks conceptualise and represent my journey with lungfishes, and offer new encounters with the lungfish *Umwelt* that allows for speculation outside of the mechanistic and reductionist realm of scientific research, while acknowledging the anthropocentric lens through which the non-human animal *Umwelt* is explored.

5.2 'THIRD EYE' AND 'IN FOCUS': ENCOUNTERS THROUGH DISSECTION AND LIGHT MICROSCOPY

'Third Eye' (Fig. 5.1) and 'In Focus' (Fig. 5.2) are black and white photographs taken through a dissecting light microscope³¹. They document my first experience at removing a lens less than 0.5 mm in diameter from the enucleated eye of a juvenile *Protopterus dolloi* in order to prepare the retina for microscopic examination (chapter 3). The documentation began as a response to the difficulty and finesse involved in the "craft" of biological dissection and turned into an interest in the gap that exists between scientific representation and representing the *Umwelt*. Here, I contextualise the artworks through a discussion about scientific photography and truth, abstraction through dissection, and ask whether the use of these techniques (photography and dissection) alone are enough to discover hidden truths of the lungfish *Umwelt*.

³⁰ Here, I subscribe to the Kantian definition of the sublime that "assumes an unbridgeable gulf between an idea and its representation". See MULLEN, L. 1998. *Truth in photography: Perception, myth and reality in the postmodern world*. University of Florida. and describes the "feeling about the rupture between knowledge and desire (that) ultimately gives rise to despair". See NAHMOD, S. H. 1987. Artistic Expression and Aesthetic Theory: The Beautiful, the Sublime and the First Amendment. *Wisconsin Law Review*, 221-263.. In other words, the "sublime" can be described as the restless feeling of a need to achieve understanding.

³¹ 'Third Eye' and 'In Focus' have been published in the following: *In Focus*. 2012. Trove Creative Arts Journal, Volume 4: Issue 2; *In Focus*. 2013. Postscript Magazine, UWA Press.

Fig. 5.1 *Third Eye*. (Audrey Appudurai, 2013).

Fig. 5.2. *In Focus*. (Audrey Appudurai, 2013).

Scientific imaging is profoundly connected to the history of photography. Not long after its invention, photography was ordained as an objective witness of phenomena (Marien, 2011). When the technological advances of cameras moved beyond the stiff poses required for long-exposure cameras, the resulting immediate, realistic images shifted the way humans were able to perceive and document the world. The camera could deliver objective renderings of reality; better than human eyes and free from emotions and the senses. In relation to its use in science, the camera was capable of capturing a moment in time that our eyes could not see. For example, Muybridge's '*Animal Locomotion*' (1830 – 1904) (Fig. 5.3) exposed, for the first time, that all the hooves of a galloping horse leave the ground in a sequence of motion. Photographs became credible witnesses of reality (Mullen, 1998), as Ralph Waldo Emerson claimed, "Photography is distinguished by its immediacy, its authenticity, and the remarkable fact that its eye sees more than the human eye. The camera shows everything."

(Manchester et al., 1989). To Susan Sontag, “photographs furnish evidence” and become “tools of power” to be used in order to make “reality atomic, manageable and opaque” (Sontag, 1977).

Fig. 5.3. *Animal locomotion - 16 frames of racehorse "Annie G." galloping. Plate 626. (Eadweard Muybridge, c1887)*

Photography’s reputation as an objective machine with neutral vision that could rid evidence of subjectivity and idealization (Marien, 2011) is partly why photography became a tool of power in science. As articulated by Daniel Rubinstein:

photography occupies a privileged place....for it is an image, the truthfulness of which is underwritten by the scientific procedure than created it. By situating visual representation within a framework of empirical knowledge supported by automatism on the one hand, and by logical rational use of light, optics and chemistry on the other, photography has been framed as an offshoot of objectivity and empiricism (Rubinstein and Sluis, 2013).

Unresolved images of biological samples in early photography were seen as more truthful than the highly detailed drawings of the same specimens. When Alfred Donné (1845) published microscopic-daguerreotypes of human cells, he declared them independent from interpretation because of the “rigorous fidelity of photomicrography to the original subject”: “the plates show exactly the microscope appearance and the truth of the details” (Jardim and Peres, 2014). When photographer Berenice Abbott ventured into the realm of scientific photography, she made mathematics and theoretical physics visible to the eye through dramatic, minimalist black and white photography (Fig. 5.4). Abbott believed that photography was a scientific endeavour, and this methodological, unsentimental approach is evident in her practice; whether it is portraits of art-world figures in Paris, documentations of the New York cityscape, or illustrating the decrease of energy in a moving object.

Fig. 5.4. *A Bouncing Ball in Diminishing Arcs* (Berenice Abbott, 1958)

The reliance on photography as objective truth is evident in my own work as a biologist. Photography is the primary tool in communicating evidence and experimental findings. For example, I have used light and electron micrographs to describe the structure of the retinal photoreceptor types of *Protopterus dolloi* and *Lepidosiren paradoxa* (chapter 3), and videography and histology to observe and describe the behaviour and visual apparatus of abnormal *Neoceratodus forsteri* (chapter 4). In ‘*Third Eye*’ and ‘*In Focus*’ the photographs capture details of *P. dolloi* that our eyes cannot resolve by themselves, such as the pattern of “freckles” on the periphery of the eye and the fibres forming the crystalline lens, thus creating an avenue to view the tiny eyes of a juvenile lungfish that is only attainable through the mediation of dissection, microscopy and photography.

However, it is well established that photography is a construction made by humans and their technological apparatuses. No photograph is without agency, and photography manipulates reality to reveal truths that are apparent to the critical eye (Mullen, 1998). Mullen (1998) says, “a photograph, although professing to depict truth, actually involves manipulation of both object and message. To say that photographers depict the truth is not correct; what they actually depict are slices of life selected and framed by the photographer”.

The manipulation of scientific imaging predominantly lies in the framing of the photograph to focus on a specific subject matter, and an alteration of the images to enhance the quality, for examples see the micrographs presented in chapters 3 and 4. However, this close association of photography and truth, and science and truth can lead to constructed images that have no basis in reality, but are taken as such. For example, when Eduardo Kac’s ‘*GFP Bunny*’ (2000) was revealed by the artist in popular media, the world was confronted with the dystopian and utopian potentials of transgenics. Kac’s artwork was a *gfp* rabbit he called Alba; a laboratory rabbit with green fluorescent protein (*gfp*) inserted into its DNA. Kac produced an image of a vividly glowing rabbit under ultraviolet light. The rabbit was never seen in the flesh, and the only “evidence” of its existence was through carefully selected images released by the artist. Transgenic *gfp* mice used in scientific research do not glow in the way Alba did in the photographs (Philipkoski, 2002); only living cells express *gfp*, which does not include the keratin within fur (Chalfie et al., 1994, Prasher, 1995). Kac’s ‘*GFP Bunny*’ photograph had been constructed to enhance the fluorescent properties of *gfp*, and in doing so, created an exaggerated spectacle that exploited the idealization of truth presented ‘scientifically’ (Philipkoski, 2002). Similarly, the disseminated image of Laura Cinti’s ‘*Cactus Project*’ (2001) persuaded people to believe that she had created a cactus capable of growing human hair due to the clever construction and framing of the object to create a

convincing narrative. These examples (and there are many more) illustrate the importance of acknowledging the aesthetic choices used in ‘*Third Eye*’ and ‘*In Focus*’ in order to discuss the role of photography in exploring the *Umwelten* of lungfishes.

‘*Third Eye*’ and ‘*In Focus*’ both illustrate the disembodied eye and lens of *P. dolloi* floating in aqueous space. ‘*Third Eye*’ (Fig. 5.1) documents the eye before the lens was removed, and is presented as a triptych with changing lighting, while ‘*In Focus*’ (Fig. 5.2) is a diptych of the *P. dolloi* lens in changing light. Both works are an experiment with scale, and aside from documentation of a scientific methodology (and “craft”), attempt to bridge the gap between human and non-human animal perceptions by manipulating scale, presenting a magnified “universal” vertebrate eye and its properties that are only visible through the mediation of technology.

Ocularcentrism is a term that recognises a perceptual and epistemological bias of vision over other human senses. Plato and Aristotle argued that sight was directly associated with reason and the traces of this are still present in Western culture; to ‘see’ is to ‘understand’, to agree is to ‘see eye to eye’ (Jay, 1993). In the context of my research, these works are naturally ocularcentric because of the focus on visual perception, and specifically, a comparative investigation of the ocular morphology of different species. Although perception is never uni-sensory and involves many sensorial apparatuses, my research parameters at the time did not extend, especially for *L. paradoxa* and *P. dolloi*, to the other senses and how they contribute to their *Umwelten*. This eventually changed when I was faced with exploring the visual perception of *N. forsteri* without visible eyes, but ‘*Third Eye*’ and ‘*In Focus*’ signified the period when I was focused (obsessed?) with the eyes and the visual perception of lungfishes.

As a documentation of the dissection process used in scientific research, ‘*Third Eye*’ and ‘*In Focus*’ are visual apparatuses that are abstracted from context. The floating eyes are large and all-consuming; they comprise almost the entire photograph. There is an element of surveillance: as I observe the eye, it observes me. Despite being preserved, they are “real”, and a trace of the animal that once used them to see. Without context we do not know what kind of eye this is (if an eye at all); it appears to be universal. In ‘*In Focus*’, the abstraction of the eye from the body creates images with open meanings that go beyond the ocular focus. Here, the lens appears to take the form of a celestial body in space. The bubble next to the lens alludes to the aquatic nature of this particular “space”, and the changing light between the images pertains to the changing of time, making it appear as though the bubble is orbiting the lens, like a planet around a star.

The concept of the *Umwelt* does not lend itself well to the carefully (human) constructed, two-dimensional nature of photography. The *Umwelt* of any organism is deeply entwined with that organism’s relationship to and within its environment, and scientifically themed photographic images illustrate the limitations of photography to represent the *Umwelt*. Lennart Nilsson’s ‘*Drama of Life Before Birth*’ (1965) photo essay and National Geographic’s ‘*Extraordinary Animals in the Womb*’ (2012) depicts unborn human and non-human fetuses, respectively, within their *Umwelt* of the uterus. The media and public read these images as “real”, but each photograph was carefully constructed either by modelling with ultrasounds or with dead fetuses, and many details were manipulated to remove the fetus from its placental *Umwelt*. Blood is either absent, or coloured a pleasant red, unlike the dark red of blood within the body. In all the images, the body of the mother is absent, and especially in Nilsson’s work, so is the uterine wall. The animals are floating free, either in what appears to be celestial space or nestled comfortably within the placenta. In all these constructed images, the

foetus is abstracted from its environment, and the *Umwelt* is superfluous to the “truth” of the object.

Similarly, ‘*Third Eye*’ and ‘*In Focus*’ do not exhibit the all encompassing nature of the lungfish *Umwelt*. Firstly, the spatial aspect of the eyes and lens is lost because the image is two-dimensional. The photographs are in black and white, and although this is traditionally associated with documentary photography because it “avoids distraction” from the subject matter, it does obscure the pre-photographic “reality” of the subject. Most importantly, these photographs are a reductionist fragmentation of the organism and its *Umwelt*; the animal and its human experimenter are missing, and the visual organ and its structures are disembodied and floating in space. The photographs are a result of a scientific dissection process that takes the eye out of context, and abstracts body parts into singular forms open to be analysed in many ways.

Sontag describes photographic images as “clouds of fantasy and pellets of information”, and elaborates that, ‘the ultimate wisdom of the photographic image is to say: ‘There is the surface. Now think – or rather feel, intuit – what is beyond it, and what the reality must be like if it looks this way’” (Sontag, 1977). The human acts of concealment and revealing in photography is precisely why, when used alone, it is inadequate to describe the *Umwelten* of lungfishes because the anthropocentric fantasy of what we believe permeates through the images. With this in mind, ‘*Third Eye*’ and ‘*In Focus*’ were an exercise in experimentation with photography to explore the *Umwelt*. When used alone, they are limited in their ability to describe and convey the *Umwelten* of lungfishes. However, they were an entry into the gap between scientific representation and representations of the lungfish *Umwelt*.

5.3 ‘THE FORMS’: ENCOUNTERS THROUGH ELECTRON MICROSCOPY, SCULPTURE AND PERFORMANCE

‘*The Forms*’ (Fig. 5.5 and Fig. 5.6) are a series of sculptures that poetically explore light and electron-mediated optical media in relation to the micro-/nano-scopic study of the South American (*Lepidosiren paradoxa*) and Spotted Africa (*Protopterus dolloi*) lungfishes³². ‘*Third Eye*’ and ‘*In Focus*’ were used as departure points that explored human engagement and presence in the production and presentation of scientific imaging. The works were created through a combination of transmission electron microscopy (TEM), camera-less photography (photograms) on silk, and physical manipulation of the image to create three-dimensional sculptures that consciously exposed the human presence in all forms of scientific exploration into the lungfish *Umwelt*. They were also experimentations with scientific and artistic craftsmanship and materialities.

On a more personal level, the process of creating the sculptures developed into a performance that reflected my experience working with *L. paradoxa*, *P. dolloi*, and abnormal *N. forsteri*. ‘*The Forms*’ is contextualised by drawing on the struggle of working between science and art, the challenge of using electron microscopy to compare the visual morphology of *L. paradoxa* and *P. dolloi* (chapter 3), and the discovery of Australian lungfish (*Neoceratodus forsteri*) without visible eyes (chapter 4). ‘*The Forms*’ was an attempt to extend the representation of the lungfish *Umwelt* beyond the mechanism of scientific imaging by acknowledging anthropocentric limitations and using performance to embody the lungfish and my lived experiences.

³² ‘*The Forms*’ have been exhibited in the following group exhibitions: *Material/Immaterial*. 2014. ARTLAAB, UWA, Australia; *An Introduction to Neolife*. 2014. The University Club, UWA, Australia.

Fig. 5.5. *The Forms*, installation. From left to right: *Form III: the art of capturing perception*, *Form IV: the data itself*. ARTLAAB, The University of Western Australia (Audrey Appudurai, 2014).

Fig. 5.6. *The Forms*, installation. Clockwise from front: *Form I: subjective conception*, *Form III: the art of capturing perception*, *Form IV: the data itself*, *Form II: things which we see are not by themselves what we see*. ARTLAAB, The University of Western Australia (Audrey Appudurai, 2014).

The micrographs were collected while acquiring data to characterize and describe the retinal photoreceptors of *L. paradoxa* and *P. dolloi* (presented in chapter 3). TEM is frequently used in vision studies to comparatively investigate the ultra-structure of retinal cells in many organisms because the combination of high resolution and high magnification enables the description and differentiation of biological cells in the greatest detail. EM is one of the few photographic techniques that are conducted in the absence of light, within a high vacuum environment (Williams and Carter, 1996).

The electron micrographs captured for '*The Forms*' are considered a form of digital imaging. Although the construction of an electron image is not digital because the image is formed by electrons interacting directly with the specimen, the only way to see and subsequently analyse the data is through digital capture. Digital photography differs from traditional analogue photography in the sense that the images are algorithmic, and composed of fixed information in the form of discrete pixels with precise gradients of spatial and tonal resolution (Rubinstein and Sluis, 2013, Mullen, 1998). Digital imaging is often considered "less authentic" compared to traditional photography because of the ease and readily accessible ways to manipulate the images, but it is argued to have solved the ethical qualms surrounding photography because it explicitly acknowledges the manipulative nature inherent in all forms of the practice, forcing an understanding that "truth" is rarely represented in a still image. Fred Ritchin, in the context of truth integrity in photography and journalism says, "Saying, 'the camera never lies' is as foolish as asserting that the computer always does. Just because words can be fictional does not require the outlawing of news articles; similarly with photographs" (Ritchin, 1999). Batchen expands by saying, "Digitization abandons even the rhetoric of truth that has been an important part of photography's cultural success...newspapers have of course always manipulated their images in one way or another. The much-heralded advent of digital imaging simply means having to admit it" (quoted in Mullen, 1998).

However, another argument may push in favour of digital objectivity – digital data is represented as the ultimate form of abstraction (digital code) because it avoids the “biases” of looking at a photographic image – the data gathered is presumably objective beyond the human eye. However, the data is still a construction made by technology created by humans for humans.

The digital images in ‘*The Forms*’ were manipulated by mirroring the micrographs to create interesting patterns from some of the unknown sub-cellular structures in the retina of *L. paradoxa* and *P. dolloi* (Fig. 5.7). The pattern making reveals a desire to understand and extrapolate ideas from forms that would otherwise be meaningless.

Fig. 5.7. Mirrored patterns created from electron micrographs of unknown structures within the *Lepidosiren paradoxa* and *Protopterus dolloi* retina. Clockwise from top left: *Form I: subjective conception*, *Form II: things which we see are not by themselves what we see*, *Form III: the art of capturing perception*, *Form IV: the data itself*,. (Audrey Appudurai, 2014).

Nina Sellars explores how perceptions of images and phenomenological experiences with light and anatomical representations are articulated and engaged with outside of a scientific context in ‘*Anatomy of Optics and Light*’ (2009) (Fig. 5.8) and ‘*Lumen*’ (2011) ‘*Lucida*’ (2012) (Fig. 5.9) (Sellars, 2012) .

Fig. 5.8. *Anatomy of Optics and Light* installation (Nina Sellars, 2009)

Fig. 5.9. *Lucida* installation detail (Nina Sellars, 2012)

Both of Sellars' works present representations of the anatomical body in a diagrammatical or an abstract language, either through the creation of drawn images depicting a schematic of the sacral plexus or "plausible, fictional anatomies that reference cytology" (Sellars, 2012). For Sellars, the structures presented and the technical processes involved in contemporary biological imaging is secondary to the phenomenological engagement and discourses surrounding the anatomical body in culture and science (Sellars, 2010). Similarly, the act of creating patterns with electron micrographs for '*The Forms*' to abstract anatomical information was a way to focus on how we engage with the scientific representation (analogue or digital) rather than a need to understand or describe the structures presented. As '*Anatomy of Optics and Light*' simplified the anatomical sacral plexus as a schematic network, '*Lumen*' and '*Lucida*' went a step further and removed scientific context entirely. Sellars explains:

'*Lumen*' is an appropriated phenomenological experience, which has been removed from a scientific context and stripped of its original protagonists,

My working methodology has led me to focus not on *things* as objects but on our *engagement* with things, to a point where the original things are no longer needed. References to people, cadavers, anatomical images, medical equipment, all start to disappear from my work. Instead, what becomes more important is the attempt to elicit and unfold that initial, first-hand phenomenological experience so that it exists and resonates as if it were the original one (Sellars, 2012)

However, unlike '*Lumen*' and '*Lucida*', '*The Forms*' is not a purely phenomenological experience because the anatomical information is still present; and although the images are mirrored, all the information necessary to identify and describe the ultrastructure of the *L. paradoxa* and *P. dolloi* retinal photoreceptor cells is contained in the forms.

An interest in the differences and similarities between digital and analogue scientific imaging, and how they can influence our engagement with scientific representation motivated me to combine both forms of photography and explicitly add physical

manipulation in the creation of these sculptures to question the level of human manipulation (unseen/seen unconscious/conscious) at each stage of the process; the digital image acquisition and manipulation, the creation of the photograms, and the physical manipulation of the final image into a three-dimensional sculpture.

'The Forms' juxtaposes TEM with photograms; two different optical-media that have been important in scientific observation. Photograms are made by exposing objects or negatives onto light-sensitive material and are developed and fixed. Photograms were first used in science to create a copy or silhouette of an object that could expose finer details without having to draw them. Anna Atkins in 1843 used the cyanotype, a type of photogram that uses light-sensitive paper that is exposed via sunlight, to render delicate algae specimens that she was unable to recreate through her drawings skills (Atkins, 1843-1853). The algae specimens were placed onto the sensitized paper, and left to expose in sunlight to create a photographic image that was more like a direct copy than a representation (Fig. 5.10).

Fig. 5.10. *Dicelyola dicholoma* in the young state, and in fruit. (Anna Atkins 1843 – 1853).

Photographer Berenice Abbott used photograms to illustrate physical phenomena, such as the interference pattern of energy as it pulses through water. Here, the interference pattern of waves could only be visible by a step-wise exposure of projected shadows formed by oscillating waves in water (Fig. 5.11).

Fig. 5.11. *Interference of waves* (Berenice Abbott, 1958 – 1961).

Photograms are interesting because the images are more than a representation; they possess the essence of the object (algae specimen) or phenomena (interference pattern) being photographed. In *'The Forms'*, the essence was not of the retinal cells themselves, but my (the human) presence in the process of creating the photographic image. The material needed to be prepared with silver gelatine in a darkroom, and any imperfections, which cannot be corrected, are visible in the resulting images; the human could not be erased (Fig. 5.12).

Fig. 5.12. *Form V*, detail. ARTLAAB, The University of Western Australia (Audrey Appudurai, 2014).

The white silk was used as the support to expose the negative patterns and create the sculptures (Fig. 5.13). The production of silk is analogous to the transformation of biological tissue for TEM (see methods section in chapter 3). Silk is produced by unwinding the delicate filaments of a silkworm's cocoon. The reeled filaments are then put through a number of washing, drying and treatment stages before the thread is woven into material. Similarly, the retina needs to be fixed, dehydrated, infiltrated with resin and cut into ultrathin (110 nm) sections before it can be observed using a transmission electron microscope. These processes change the origins of these materials as parts of organisms: they became materials of craft and data acquisition.

Fig. 5.13. *Form II: things which we see are not by themselves what we see.* ARTLAAB, The University of Western Australia (Audrey Appudurai, 2014).

The physical manipulation of the photograms into sculptures gave the images physical and tactile dimensions, allowing them to “escape” the photographic document. The construction of three-dimensional sculptures from images brought back dimensionality and tangibility that was lost in the two-dimensional photographs of ‘*Third Eye*’ and ‘*In Focus*’. The physical manipulation of the silk illustrates the human agency in the process and presentation of scientific imaging. In addition, sculptures allow for the micrographs to be observed from different angles, changing the information that is perceived (Fig. 5.14).

Fig. 5.14. *Form IV: the data itself* at different angles. (Audrey Appudurai, 2014).

The process of creating the photograms and sculptures of ‘*The Forms*’ developed into a personal performance that reflected my attempt to experience how the abnormal *N. forsteri* lacking external eyes (chapter 4) sense their environment. The performance was conducted entirely in the confines of a darkroom, and represented a hybridisation of the metaphorical and literal darkness of uncovering the *Umwelten* of lungfishes. I drew particularly on the struggle of working in between science and art, and the difficulty in creating a discourse between the two cultures in order to describe the visual world of lungfishes. Although the works directly related to the histological examination of *L. paradoxa* and *P. dolloi* retinal photoreceptors, I had recently acquired the abnormal *N. forsteri*, and wished to conceptualise my journey into the lungfish *Umwelt* through an attempt to embody the lungfishes without visible eyes. I lost my sight in that room, and had to rely primarily on touch and memory to navigate and complete the process of creating the sculptures.

‘*The Forms*’ are a poetic exploration into human engagement with the lungfish *Umwelt*. By explicitly acknowledging the anthropocentric limitations within scientific imaging, and combining it with a performance that changed my perception (dark room)

in order to complete the artworks, *The Forms* extended *Third Eye* and *In Focus* to provide a more immersive representation of the lungfish *Umwelt*.

5.4 ‘CERNENTIA’: MUTLI-SENSORIAL ENCOUNTERS WITH LUNGFISHES LACKING VISIBLE EYES

The exhibition *‘Cernentia: exploring the visual perception of lungfishes without eyes’* (2015) contained artworks that invited the audience to experience the “world” of a group of *N. forsteri* born without external eyes (hereafter referred to as “abnormal”)³³. Using data generated from the scientific investigation into abnormal *N. forsteri* (chapter 4), historical references about the study of visual perception and lungfishes (chapter 1 and 2), and artistic practice, *‘Cernentia’* revisited the sublime of an individual’s multi-sensorial experience, and revealed the limitations that prevent us from complete understanding of subjective experience, be it human or non-human. The exhibition contained three artworks, *‘Parts per Animal’* (2015), *‘Untitled’* (2015) and *‘Bloop’* (2015).

‘Parts per Animal’ (Fig. 5.15) was a collection of tissues used in the histological investigation of the abnormal *N. forsteri* visual system (see chapter 4). Relics of the three abnormal lungfishes studied were displayed in individual fixative-filled glass jars and slide boxes in a traditional vitrine observable from all sides. The specimens included three enucleated subcutaneous eyes, patches of skin and muscle tissue that covered the eyes of *N. forsteri*, a brain, and transverse sections of the eyes presented on stained microscope slides (Fig. 5.16). Part documentation, part aesthetic and affective object, *‘Parts per Animal’* were physical traces of the “Other” that illustrated 1) the

³³ *Cernentia: exploring the visual perception of lungfishes without eyes* was presented at Kidogo Arthouse, Fremantle, Western Australia.

utilitarian ideals in scientific methodology and presentation, 2) the reductionist, disembodied approach of scientific investigation, 3) how public institutions like museums, and art galleries shape our understanding of other animals such as lungfishes, and 4) my homage to the individuals that participated in my research.

Fig. 5.15. *Parts Per Animal* (Audrey Appudurai, 2015). Photo credit: Julian Frichot.

Fig. 5.16. *Parts Per Animal* detail (Audrey Appudurai, 2015).

The museology aesthetic was inspired by my investigation of cultural narratives surrounding the Western discovery of lungfishes (see chapter 2). Animal specimens for museum and scientific presentation are produced by cultural practices and undergo many transformations. Schumutzer says that, “specimens are not a mere image of nature, but artificial things designed and constructed by naturalists to answer various scientific needs” (Schmutzer, 2012). With particular reference to Johann Natterer’s South American “paradox” (*L. paradoxa*) (Bischoff, 1840) and the incorrect examination/identification of *P. annectens* by Richard Owen (Edwards, 1841, Zimmer, 1998), ‘*Parts per Animal*’ presented in-between individuals of in-between species to question the museum specimen as a representation of a species, and how natural objects are transformed into scientific objects (Klemun, 2012). The identification of *L. paradoxa* and *P. annectens* was conducted on preserved animals that did not resemble the animal in its natural state, yet were taken to represent their species. The specimens

used to identify *L. paradoxa* did not possess any internal organs, having been removed by Natterer, which contributed to Fitzinger's frustration at classifying the animal as a fish or a reptile (Fitzinger, 1837, Schmutzer, 2012). The specimens Owen used to classify *P. annectens* were desiccated, which may have contributed to his incorrect documentation of the nostrils not being connected to the lungs (Bischoff, 1840, Owen, 1840, Edwards, 1841). Although methods of preservation and documentation have improved considerably since the 19th century, it is still important to recognize that specimens used for histological examinations are far removed from the original organism and its environment. '*Parts per Animal*' explicitly acknowledges the processes involved in order to make sense of the 'Other' and understand the perception of lungfishes.

Other artists that incorporate scientific presentations of non-human animals in their art practice include Brandon Ballengée, Mark Dion and Janet Laurence. As a biologist and artist, Ballengée's main interest is the impact of human-caused phenomena, such as pollution and climate change, on the natural world and its inhabitants (Ballengée, 2015a). His work '*Collapse*' (2012) responded to the 2010 BP Deepwater Horizon oil disaster that subjected the Gulf of Mexico's food web to the largest accidental marine oil spill of the petroleum industry's history (Roberston and Krauss, 2010). A seven tiered pyramid consisted of 26,162 preserved specimens representing 370 species of aquatic organisms in gallon jars dominated the room. Some of the jars were empty, representing species in decline, forgotten or already extinct and other containers presented deformed organisms affected by pollution (Cotter, 2012). According to Ballengée, the scientific presentations of organisms were used as "a sculptural expression of the complexity of the issue, and also the complexity of the food chain itself" (Beitiks, 2012). In '*Collapse*', the dead organisms, preserved whole and suspended in clear buffer or fixative were a sterile reminder of the slow destruction of

our environment (Ballengée, 2015b). ‘*Parts per Animal*’ does not touch on ecological crises directly, but can be seen as a “sculptural expression” of ways of understanding other organisms, in particular a group of captive *N. forsteri* whose deformities may have been caused by humans.

Mark Dion applies archaeological and scientific methods of collecting, documenting, ordering and presenting objects to explore cultural representations of nature (Dion, 2015). His arrangements of natural and man-made objects displayed in curiosity cabinets (see ‘*Marine Invertebrates*’ [2013]) explore the distinctions between scientific methods and subjective, irrational influences. The carefully designed sets containing artefacts involved in knowledge acquisition and dissemination of the natural world (such as ‘*Department of Marine animal Identification of the City of New York [Chinatown Division]*’ [1992] and ‘*Tate Thames Dig*’ [1999]) reveals how public institutions shape our understandings, and questions the power structures behind the Linnaean taxonomic system (see Chapter 2) (Fiske and Bottinelli, 2002). Dion believes “nature is one of the most sophisticated arenas for the production of ideology” (Stonard, 2000) and through art, he questions the authoritative role of the scientific voice that underlies environmental politics and public policy in contemporary society (Dion). Like Dion, ‘*Parts per Animal*’ examines the role that animal specimens, as cultural artefacts, play in the construction of knowledge, thereby shaping our understanding of lungfish perception and the natural world.

Janet Laurence’s ‘*Fugitive*’ (2013) was an installation that includes life/non-life human/non-human objects, such as taxidermy and preserved non-human animals, to create “a place of healing, hope, breeding and care for our native animals” (Gibson and Laurence, 2015). The immobile specimens are surrounded by photographs and the sounds of their natural habitat (*Umwelt*), which give voice to non-human organisms

under threat. Laurence's work is grounded in posthumanism through the act of care. She believes the current "ecological crisis demands us to shift our focus from the human-centred perspective to a broader multi-species, environmental approach" and "participates in discourses of agency and realities outside a singular human experience by considering the point of view of animals, even in death" by archiving nature artistically through the museology aesthetic (Gibson and Laurence, 2015). Similarly, '*Cernentia*' and this thesis consider the point of view of lungfishes through their subjective experience. Care is certainly part of the embodied experience, as seen in '*The Forms*' and '*Untitled*' (discussed below), but '*Parts per Animal*' does not do this alone. The sounds and the sights of the lungfishes' environment, their *Umwelt*, were encompassed within the other works in the gallery, '*Untitled*' and '*Bloop*'. It is important to note that '*Cernentia*' did not contain any elements of the *N. forsteri* native habitat. I did not want to give the illusion that these fishes were ever from the wild; the rivers of Queensland were never their habitat. Nevertheless, I wanted to stress that their *Umwelt*, the aquariums at the University of Western Australia, is still an important feature in understanding their perception.

'*Parts per Animal*' cannot fully describe or embody the *Umwelt* of the abnormal *N. forsteri* born without external eyes. It was more a trace and sculptural expression of how humans attempt to understand the subjective experience of non-human animals. The *Umwelt* of the lungfishes was lost when the animal was broken apart and its visual system examined in isolation. Nonetheless, it is still important to openly acknowledge that this is one "aspect of knowing" the abnormal *N. forsteri* *Umwelt*, complete with its research opportunities and limitations.

'*Untitled*' was an artwork that evolved from the investigation of the circadian rhythm of the *N. forsteri* born without visible eyes' over a period of 72 hours in complete darkness

(see experiment 3, chapter 4) and my own experience observing their behaviour in real time. It was presented in two parts: 1) upon a wall as a triptych of video footage of the three abnormal *N. forsteri* and, 2) throughout the gallery as marks made in UV paint upon the floor that invites the audience to follow in my tracks (Fig. 5.17 and 5.18, respectively).

Fig. 5.17. *Untitled* triptych of experimental footage detail. (Audrey Appudurai. 2015). Photo credit: Julian Frichot.

Fig. 5.18. *Untitled* detail. Performance still of ultra-violet paint on gallery floor. (Audrey Appudurai, 2015). Photo credit: Julian Frichot.

The video footage is a bird's eye surveillance of the fishes in complete darkness over 72 hours to measure their activity patterns. The movies were recorded in low lux and are silent, showing the slow, undulating movements of each fish as it circulated within its minimal, experimental environment. The surveillance-style videos of '*Untitled*' taken as

part of scientific behavioural studies (chapter 4) illustrate the relationship and power dynamic between experimenter and subject prevalent in scientific investigations of the “Other”. On a more personal note, as I watched these fishes through a computer interface, I became intrigued by the way they moved around the perimeter of their tanks, sometimes without stopping. The fishes’ endless “going around in circles” prompted a metaphorical interpretation of the inability to pierce the *Umwelt*; any effort to perceive subjective experience was met by anthropocentric limitations (chapter 2). Therefore, the futility of “watching” from a distance as a scientific experimenter led to a second attempt of understanding through performance: the tracks of UV paint.

Prior to the opening of the exhibition, I traced the fishes’ movements during the circadian experiment (experiment 2, chapter 4). On hands and knees and in complete darkness, I felt my way around the floor of the gallery space and marked the abnormal lungfishes’ direction and positions of rest with fingers dipped in UV paint. During the exhibition, two black-light LED torches were placed near the video footage, and audience members were invited to follow my, and the lungfishes’ tracks around the gallery (Fig. 5.19).

Fig. 5.19. *Untitled* installation. (Audrey Appudurai. 2015) Photo credit: Julian Frichot.

Without the black-lights to aid them, my tracings and the abnormal *N. forsteri* tracks were not perceivable to the human eye. Unlike humans however, juvenile *N. forsteri* have the potential to perceive ultra-violet light (Hart et al., 2008). In reference to some

of the differences between the human and lungfish visual system, I chose an indiscernible medium to create my markings; one only visible to the human visitors with technological intervention (black-light torches). I created marks around the gallery space with my fingers, one of the most sensitive parts of the human body based on the divisions of the primary somatosensory cortex (Marieb and Hoehn, 2007). The results of chapter 4 suggested that, in the absence of external eyes, the abnormal *N. forsteri* may use their lateral line to compensate for navigation (chapter 4). I focused on my/our tactile senses to navigate around the gallery space to allude to this, and because it is comparable to the lateral line of fishes (Dijkgraaf, 1963, Bleckmann 1986, Montgomery et al., 1997). Inviting the audience to follow the trace and to enact this performance extended the isolated performance of '*The Forms*', allowing others to take part. This pilgrimage, following me following the lungfishes, formed a dialogue between the audience, experimenter/artist, and subject/non-human animal that created a temporary subjective experience exclusive to each individual taking part, and illustrated 1) the physical limitations of our senses (our inability to perceive ultra-violet light), and 2) how technology (black-light LED torches) offers us a fleeting, inadequate glimpse into the *Umwelten* of non-human animals.

Richard Long and Shannon Williamson also utilize mapping and traces of themselves in their art practice. Long is a British land artist who redefined traditional approaches to sculpture. One of his most famous works, '*A Line Made by Walking*' (1967) produced alterations in a meadow by a repetitive journey back and forth across grass. Long's walking artworks redefined the line between an action through performance, and sculpture (Burgon, 2012). Long's mud paintings are often site-specific, impermanent examples of his attempt to bring his experiences back into public institutions like museums and galleries (O'Hagan, 2009). Most of Long's works that include mud as his primary medium (such as '*Waterfall Line*' [2000]) involve direct contact with the

artwork by using his fingers and hands as tools to create marks upon the walls, floor, or paper. Long believes that using mud and his hands add another form of physicality to his work, as “the wateriness of the mud, and water is the main subject and content of these works, they show its nature” (Brettell and Friis-Hansen, 1996). Long’s interventions in the landscape, either within the field or in a gallery space are “transient and humble” (Burgon, 2012), and in most cases, reveal the impermanency of nature and our environment (O’Hagan, 2009).

The performative aspect of ‘*Untitled*’ is a site-specific, impermanent work that attempts to embody the subjective experience of non-human animals. However, ‘*Untitled*’ is not land art, but rather refers to the aquatic environment of abnormal *N. forsteri*. Richard Long stressed that “the meaning of his work lay in the visibility of his actions rather than the representation of a particular landscape” (Costa) and “represents a trace of a corporeal presence and bodily action” (Burgon, 2012). Like, Long’s mud drawings, I am not attempting to recreate or represent the lungfishes’ aquaria within the gallery space, but to render visible their embodiment through marks that audience members can track. It was important for me to create an intimate dialogue with the abnormal *N. forsteri* through performance and touch, and make that experience temporarily accessible to others. Tactility, in some form, is a sense shared by all organisms and does not require verbal language or the possession of eyes.

Shannon Williamson utilizes mapping and physical traces to navigate personal narratives (Williamson and George, 2013). Like Richard Long’s mud drawings, and ‘*Untitled*’, Williamson’s 2013 work ‘*No More On Broken Bones*’ and ‘*Flight Path*’ were site-specific impermanent drawings/sculptures on the floor and walls of a gallery space. These works responded to Williamson’s personal experience with displacement and sudden loss after the 2010 New Zealand earthquakes, with a particular focus on the

anatomy and migration patterns of sea birds that frequent the southern island of New Zealand and Australia (Mullen, 2013).

In the 2014 series titled '*Night Shift*', Williamson revisited mapping to trace the intimate and personal accounts of bodily experience as a shift worker in a long-term relationship (Williamson, 2014). '*Cartography of one sided sleep (work ii, series i)*' overlaid Williamson's estranged circadian rhythm after working a number of night shifts as a sleep technician (Williamson, 2014). '*Untitled*' continues with the theme of circadian rhythm mapping, except the "estranged circadian rhythm" is that of non-human animals lacking external eyes. Like Williamson, I navigated personal geographical and psychological terrain (Mullen, 2013) with the performative aspect of '*Untitled*', but in a way that juxtaposed the two-dimensional, objective view of experimentation seen in the video footage. A map activated through audience participation attempted to relieve some of the frustration caused by passively watching these animals within an experimental context.

The final artwork in '*Cernentia*', '*Bloop*' (in collaboration with Devon Ward), was an immersive sound and light installation contained within a headset; a symbolic gesture that attempts to embody the *Umwelt* (see chapter 1) of abnormal *N. forsteri* during experimentation (see chapter 4) (Fig. 5.20).

Fig. 5.20. *Bloop* (Audrey Appudurai in collaboration with Devon Ward, 2015) Photo credit: Julian Frichot.

The visual/sonic piece was derived from the electrophysiological investigation of normal and abnormal *N. forsteri* outlined in experiment 3, chapter 4. In that experiment, the functional integrity of the retina and visual pathway to the brain was measured through the recording of visually evoked potentials (VEPs) in abnormal *N. forsteri* (Creel et al., 1974, Creel, 2012). The subjective experience of an individual during

scientific experimentation differs from the assumed *Umwelt* of an animal within its “native” environment. However, in the case of the abnormal *N. forsteri*, the “scientific arena” is the dominant (if not the only) environment, or *Umwelt*, they know. In ‘Bloop’, it was important to artistically intervene within these experiments and consider what it might be like for *N. forsteri* to be within a mechanistic, “reductionist *Umwelt*” of the “scientific arena”. This is by no means the true *Umwelt* of these fishes, simply because I am deconstructing the *Umwelt*, choosing which senses are obstructed and stimulated, and define the sensations received with scientific data from technological apparatus. In a way, the audience is presented with a paradoxical experience; a humble attempt to perceive a “simplified (and reductionist) *Umwelt*”.

In order to render the light/electrical response of the abnormal lungfishes’ retina and brains into sound and light, the averaged data generated from measuring their VEPs was converted into a low frequency acoustic pulse that played intermittently over a three minute time course. This acoustic pulse was layered over the underwater sound of the lungfishes’ aquariums to create a hypnotic ambience permeated by the deep, pulsing rumbles of the VEPs. The VEP data was converted into light pulses activated in time to the acoustic VEP pulses heard through closed headphones. The light was emitted by red LEDs attached to the eyepieces of heavy duty welding goggles that deprived the wearer of any stimulus besides the feedback loop of the abnormal *N. forsteri* retina and brain’s response in sound and light. Closed headphones that emitted the sound were attached to repurposed welding goggles that emitted the light. The electrical hardware containing the light and sound information was programmed into an arduino board, and placed in full view, attached above the eyes of the goggles to explicitly illustrate how technology enables the extension of human perception; it is never without an interface of human-derived technological apparatuses. The LEDs were placed in front of the lenses that were blacked out to prevent ambient light from entering (Fig. 5.21).

Fig. 5.21. *Bloop* installation detail. (Audrey Appudurai in collaboration with Devon Ward, 2015). Photo credit: Julian Frichot.

‘*Bloop*’ was inspired by Uexküll’s own experiments to describe the *Umwelten* “soap bubble” of other non-human animals. Uexküll used experiments to illustrate how a limpet may navigate “home”, how time might pass for a fast moving fish versus a snail, and how the environment looks through the eyes of a housefly or mollusc by modifying

a camera or speculating through different drawings and watercolour (Uexküll, 2010).

‘*Bloop*’ endeavours to create an *Umwelt* that is not defined by verbal, written, or drawn description, which are mainly associated with human expression, but through immersive experience. This experience is characterised (and obscured) by the individual participant’s sensory systems (their own *Umwelt*), and addressed the objective/subjective dilemma of visual perception by incorporating human speculation with scientific data of the abnormal *N. forsteri* visual and neurological machinery.

The artist Vaughn Bell and designer Kenichi Okada present artistic examples of individual augmented *Umwelten*, and created new experiences that changed relationships between humans and their environment. Bell’s ‘*Personal Home Biosphere*’ (2004) and ‘*Personal Portable*’ (2008) are biospheres (closed ecological systems) that individuals place their heads into to experience with eyes, ears, and nose a miniature natural world amid an urban environment. Other than “playing with the idea of what we really yearn for when we’re in an urban location”, Bell says there is satire in her works because “you could wear (the biospheres) over your head and it has this green horizon that was this layer of mosses right in front of your nose. You wouldn’t have to smell the exhaust fumes from the cars and everything would be muffled, so it’s a really personal piece of nature, but also absurd” (Mitchell, 2008). Bell’s biospheres are about creating meaningful relationships between humans and plants that are also deeply personal because of the isolated, individual experience within each miniature ecosystem (Bell). In other words, the new *Umwelt* within Bell’s biospheres creates a new experience that can change your relationship with the “Other”. Like Bell, I am interested in creating new, personal relationships and experiences with ‘*Bloop*’ by altering the *Umwelten* of the audience. However, I was not interested in the literal borders created with Bell’s biospheres that cut the individual off from the surrounding environment. ‘*Bloop*’ encountered lungfish perception by playing with the subtleties of

sensation and the *Umwelt*; the person was still present within their environment, but some of the senses were selectively obstructed or stimulated.

Okada is a designer who created '*Animal Superpowers*' (2007), a series of children's toys that transformed an individual's "self" to reflect the *Umwelten* of other non-human animals (Okada). '*Ant*', a helmet containing a screen that projects footage of a video microscope attached to the wearer's hands, mimicked the way ants utilize feelers in order to navigate and sense their microscopic *Umwelten*. '*Giraffe*' enabled the wearer to feel and sound taller than they are by using a periscope, mirrors and a voice changer to alter their perception. '*Animal Superpowers*' modified what Uexküll would describe as the "effect space"; the knowledge of where our limbs, body and self are at all times (Uexküll, 2010). By changing vision using mirrors and altering our sense of scale, '*Ant*' and '*Giraffe*' created a disorientating experience that changed the wearer's relationship with the environment. '*Bloop*' also modified how the wearer received sensory information to create new experiences of non-human animals through a technological interface. However, unlike '*Animal Superpowers*', the experience is not constructed as child's play or a naïve experience, but rather individual-to-individual, or person-to-person, human-and-lungfish.

The artworks of '*Cernentia*' represented my journey and the challenges faced when investigating a group of abnormal *N. forsteri* without external eyes. '*Parts Per Animal*', '*Untitled*', and '*Bloop*' revisited the sublime of subjective sensory perception and created new encounters with the *Umwelten* of other animals through scientific and speculative investigation (Fig. 5.22). These works explicitly revealed the anthropocentric limitations that prevents us from complete comprehension of subjective experience, either through an examination of how knowledge is collected and understood about lungfishes ('*Parts Per Animal*'), embodying the lungfish experience

through sensory deprivation and mapping (*'Untitled'*), and creating an immersive abnormal *N. forsteri* experience mediated directly through technological apparatuses (*'Bloop'*). I was not trying to create the *N. forsteri Umwelt* in the literal sense; my works were more poetic and humble gestures that explored alternate experiences.

Fig. 5.21. *Parts Per Animal* and *Untitled* (Audrey Appudurai, 2015). Photo credit: Julian Frichot.

5.5 CONCLUSION

This chapter contextualised artworks that poetically and sensorially extended and complicated understandings and limitations surrounding the *Umwelten* of lungfishes. Generated through scientific and artistic research, the works revealed that understanding the subjective experience of other animals is multifaceted, and can be explored and interpreted in a number of ways. ‘*Third Eye*’, ‘*In Focus*’, ‘*The Forms*’, and the artworks from ‘*Cernentia: exploring the visual perception of lungfishes without eyes*’ all reflected on the often challenging experience of working between science and art, and the different obstructions that inhibit full immersion into the *Umwelten* of non-human animals through photography, scientific documentation, sculpture and embodied performance.

Understanding the *Umwelten* of lungfishes is futile because of our (human) visual and cognitive apparatus and individual embedded subjectivity. However, art allows for speculation outside the mechanistic realm of scientific research, thus creating a discourse that generates new encounters and relationships between human and non-human “Others”.

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II

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GENERAL DISCUSSION

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ii.1 INTRODUCTION

The main aim of this thesis was to provide an interdisciplinary platform to better understand the visual perception of dipnoan fishes through science and art. This study was divided into two objectives that utilized cross-disciplinary approaches to 1) investigate scientifically the visual machinery of three different species of lungfish and, 2) provide artistically a larger context, including philosophical and cultural background to provide a sensorial speculation of lungfish perception that also highlights exploration and limitations in the human attempt to unravel the lungfish visual experience.

ii.2 THE VISUAL PERCEPTION OF *PROTOPTERUS DOLLOI*, *LEPIDOSIREN PARADOXA* AND ABNORMAL *NEOCERATODUS FORSTERI*

The first objective of this thesis was to investigate visual photoreception, predominantly at the ocular and retinal levels through anatomical, electrophysiological and behavioural methodologies (chapter 1) of representative species from the three dipnoan families; *Protopterus dolloi*, *Lepidosiren paradoxa* (chapter 3), and normal (with external eyes) and abnormal (lacking external eyes) *Neoceratodus forsteri* (chapter 4). Findings from these studies confirmed that the overall eye shape and the photoreceptors of all three species are adapted for high sensitivity, and aid in maximising photon capture in dim light environments (chapters 1 and 3). Due to the range of environments and lifestyles of all lungfish species, findings suggest that “variety” is an important descriptor of the lungfish visual system. In addition, it confirms that the Order Ceratodontiformes, which contains *N. forsteri*, is the only dipnoan Order lacking oil droplet-bearing rod photoreceptors, and this adaptation may have evolved after the separation of the Order Lepidosireniformes, of which *L. paradoxa* and *Protopterus* spp. are a part (chapter 3).

In chapter 3, it was revealed that adult *L. paradoxa* possess one oil droplet-containing rod and one oil droplet-containing cone photoreceptor type, and may be the only lungfish species without the potential for colour vision. The visual system of animals deeply reflects each species' ecology and lifestyle, and *L. paradoxa* has been described as the least fish-like, with the least differentiated retina out of all the lungfish species (Ali and Anctil, 1973). Although the reason for this lack of colour vision is still unknown, it may be due to its lifestyle as this species is the only dipnoan to remain dormant within its aestivation cocoon for up to eight months a year (Carter and Beadle, 1930, Fonesca de Almeida-Val et al., 2011).

The juvenile *P. dolloi* eye contains at least one type of rod and two types of cones (a red oil droplet-containing cone and a clear, no oil droplet-containing cone), suggesting that it has the potential for dichromatic colour vision. The presence of red oil droplet-containing cones suggests that *P. dolloi* is able to discriminate objects at long wavelengths because of its freshwater, swampland habitat that often contains sediment and mineral deposits that reflect light in the red part of the spectrum (Hart et al., 2008). Coloured oil droplets allow an organism to tune the spectral sensitivity of its retinal photoreceptors without sacrificing clarity (Bowmaker, 1980, Vorobyev, 2003), and may improve *P. dolloi*'s ability to discriminate foliage, prey items, conspecifics and predators (Hart et al., 2008). Based on the topographic distribution of the red cones, juvenile *P. dolloi* have increased visual acuity in the dorso-nasal region of the retina that receives information from the ventro-lateral region of its visual field, which may be useful in predator avoidance. Contrary to previous studies, double cones were not observed in the juvenile *P. dolloi* eye (Pfeiffer, 1968). *N. forsteri* and *L. paradoxa* also do not possess double cones (Ali and Anctil, 1973, Bailes et al., 2006a); *P. aethiopicus* is the only species confirmed to have this specialised photoreceptor types (Walls, 1942). *P. annectens* and *P. aethiopicus* are the most recently evolved lungfishes, and are more

closely related to each other compared to the other members of the family Lepidosirenidae (*L. paradoxa*, *P. dolloi*, and *P. amphibious*). The confirmed lack of double cones in *L. paradoxa*, *N. forsteri* and now *P. dolloi* suggests that this specialisation may have evolved after the separation of *L. paradoxa* and *N. forsteri* from *P. dolloi*.

The study focussing on the abnormal *N. forsteri* (chapter 4) confirmed histologically the presence of subcutaneous eyes embedded beneath muscle and connective tissue that contained most of the structures typical of eyes of normal *N. forsteri*. The successful recordings of VEPs in all abnormal *N. forsteri* demonstrates that the retina is still photoreceptive, and that the optic tectum received this input despite the eyes being buried under skin and the absence of a clear cornea. However, only individual E3 demonstrated statistically non-random, behavioural responses to light and dark, suggesting that the sclera over the anterior of the eye allowed enough ambient light for phototaxis.

Some of the behavioural differences observed in abnormal and normal *N. forsteri* may be due to sensory plasticity and potential differences in the sensitivity of the pineal organ. A 72 hour observation of *N. forsteri* in complete darkness revealed that the abnormal lungfishes travelled more than 2.5 times further than normal lungfishes. This increase in distance travelled may be a form of sensory plasticity (Rauschecker and Kniepert, 1994) that, in the absence of external eyes, resulted in a greater dependence on other senses such as electroreception (Watt et al., 1999) and the lateral line (Lippolis et al., 2009), where movement increases the levels of afferent input, the localisation of food and enhanced navigational abilities. Although the importance of vision in the lifestyle of lungfishes is unknown, the increase in the distance travelled in abnormal *N. forsteri* compared to normal *N. forsteri* suggests that these senses play a role in navigation, prey capture and predator avoidance.

ii.3 ENCOUNTERS WITH THE LUNGFISH *UMWELT*

The second objective of this thesis focused on the wider contextual underpinnings of the human exploration into non-human visual experience. This objective drew primarily from the conceptual ideas about subjective experience of Thomas Nagel and Jakob von Uexküll, and combined these with scientific research into the visual perception of lungfishes.

Investigation into historical (scientific and non-scientific) attempts at uncovering non-human animal perceptual experiences, and human narratives about lungfishes revealed that any attempt to conceptualise a reality that is different from our own is affected by the limitations of technology, our anthropocentric perception of reality, and cultural frames of reference (chapters 1 and 2). Through the collaborative art project ‘*Imperception*’ (2012) that endeavoured to interpret the *N. forsteri Umwelt* through contemporary scientific knowledge, it was revealed that, despite aspiring towards informed subjectivity, the work was built upon anthropocentric foundations because it was 1) informed by scientific data generated by humans and their technologies, 2) framed by human interpretations of scientific data and, 3) translated through the human expression of drawings (chapter 1). In addition, human narratives about lungfishes across cultures and throughout history showed that, despite our attempts at objectivity through the evaluation of scientific data, inescapable cultural factors influence how we understand lungfishes, and highlight the idea that scientific research can be seen as one ‘social arena’ in which the visual experience of lungfishes can be examined (chapter 2) (Latour and Woolgar, 1986, Haraway, 1992, Cole, 1998). Any attempt at demystifying the lungfish *Umwelt* through scientific and artistic research, while recognizing anthropocentric limitations, is important and insightful because it establishes a more informed discourse that connects us with the world and enables us to better understand our place within it.

The multifaceted nature of the lungfish visual experience outlined in chapters 1 - 4 led to the creation of artworks that attempted to extend, “embody” and complicate issues and knowledge surrounding the lungfish *Umwelt*, and reflect on the experience of working between science and art (chapter 5).

‘*Third Eye*’ (2013) and ‘*In Focus*’ (2013) explored the scientific methodologies of chapter 3, and the conversation that exists between scientific representation and representing the *Umwelt* through biological dissection, macro-photography and histology viewed using light microscopy. Despite the close relationship between science and photography, photography (a human- made, and interpreted technology) still may be seen as anthropocentric, and the abstraction of the lungfish through dissection showed that, when used alone, photography is a very limited tool with regard to the ability to convey the *Umwelten* of lungfishes.

In light of ‘*Third Eye*’ and ‘*In Focus*’, ‘*The Forms*’ was a more poetic exploration into the human engagement with the lungfish *Umwelt* through light- and electron-microscopy (chapter 3), sculpture and performance. ‘*The Forms*’ was an attempt to extend the representation of the lungfish *Umwelt* beyond the mechanisms of scientific imaging and photography by acknowledging anthropocentric limitations (chapters 1 and 2) and using performance to embody the lungfish, particularly the abnormal *N. forsteri* of chapter 4. ‘*The Forms*’ extended ‘*Third Eye*’ and ‘*In Focus*’ to provide a more immersive representation of the lungfish *Umwelt* by explicitly acknowledging the anthropocentric limitations within scientific imaging, and combining it with a performance that changed my environment in order to complete the artworks.

The final exhibition ‘*Cernentia: exploring the visual perception of lungfishes without eyes*’ (2015) contained artworks generated from the scientific investigation of abnormal *N. forsteri* lacking external eyes (chapter 4), and historical references about visual

perception and lungfishes (chapters 1 and 2). The three artworks, '*Parts per animal*' (2015), '*Untitled*' (2015) and '*Bloop*' (2015) invited the audience to experience the sublime of an individual's multi-sensorial experience and, at the same time, reveal the limitations that prevent us from complete embodiment.

'*Parts Per Animal*' was a collection of tissues used in the histological investigation of the abnormal *N. forsteri* visual system (see experiment 4, chapter 4) presented in a wood and glass vitrine. The museology/scientific aesthetic was inspired by my research into cultural narratives about the Western discovery of lungfishes (see chapter 2), and also acted as a homage to the individuals that participated in this study. '*Untitled*' was a triptych of surveillance footage observing the three abnormal *N. forsteri* over 72 hours in complete darkness (see experiment 2, chapter 4), and included an invisible trace of their movements in the gallery space. The video footage illustrated the power dynamic between experimenter and subject and the apparent "transparency" of the minimal *Umwelt* of experimental conditions. However, like '*The Forms*' there was a performance element in order to embody the lungfishes and their *Umwelt*. I traced and marked the mapped movements of the fishes in the tanks within the gallery space using invisible UV paint in darkness, and during the exhibition, the audience were invited to follow the fishes, and my footsteps using a black light torch. '*Bloop*' provided an immersive experience of the abnormal *N. forsteri* undergoing electrophysiology examination (see experiment 3, chapter 4) through sound- and light- mediated technology over the eyes and ears. Sounds of the lungfishes' aquaria were punctuated with the deep rumblings of their brain's response to light stimuli with red LEDs flickering in time to their visually evoked potentials. '*Bloop*' was an attempt to provide a multi-sensory experience of the abnormal *N. forsteri* perception that incorporated data generated by the animal and its environment. However, it simultaneously questions the use of digital data as the new objective scientific tool because it explicitly shows the

technological interface needed to achieve it, illustrating the ‘sublime’ of non-human subjective experience.

ii.4 CONCLUSIONS AND FURTHER DIRECTIONS

Ultimately, this thesis focused on the human exploration of non-human animal visual experiences through scientific methodologies (chapters 3 and 4), history (chapters 1 and 2), and the creation of artworks (chapters 1 and 5), while highlighting the anthropocentric limitations that prevent complete embodiment (chapter 2).

From a scientific perspective, these results have contributed to our understanding of the visual perception of lungfishes by confirming that lungfishes possess a visual system adapted for high sensitivity (Bailes et al., 2006a, Bailes et al., 2006b, Ali and Anctil, 1973, Walls, 1942), and reveals that the ectopic eyes of abnormal *N. forsteri* still provide some level of light detection, but do not have the ability to form images.

However, a lot remains to be investigated about the visual perception of this evolutionarily important group of fishes. The most important is to establish the role vision plays in these species’ behavioural ecology. The relatively small eyes of lungfishes are well-developed, and strongly resemble those of diurnal vertebrates such as birds and reptiles (Bailes et al., 2006a). Yet, little is known about the visual behaviour of any lungfish species besides their proclivity for nocturnal activity.

Curiously, it has been stated a number of times that vision does not contribute greatly to their lifestyle, at least in prey capture and navigation (Carter and Beadle, 1930, Johnels and Svensson, 1954, Curry-Lindahl, 1956, Pfeiffer, 1968, Greenwood, 1986).

L. paradoxa may be the only lungfish species described without the potential for colour vision. This supports the descriptions of Ali and Anctil (1973) and Zeiss et al (2011).

However, immunohistochemical examination of the retina of *L. paradoxa* only targeted LWS and SWS cones, and not MWS cones (Zeiss et al., 2011). The intracellular inclusions within rod photoreceptors of *L. paradoxa* are still unconfirmed. Our observation of oil droplets within the rods in this species follows the description by Ali and Anctil (1973), although Zeiss et al. (2011) described the inner segment of *L. paradoxa* rods containing an ellipsoid lacking an oil droplet. In order to determine if the inclusion within the *L. paradoxa* rod photoreceptors are oil droplets, the retina could be stained with Oil Red O to show the presence of triglycerides, as was conducted in *N. forsteri* (Bailes et al., 2006a).

Characterisation of the complement of photoreceptor types of the remaining African species *P. annectens*, *P. aethiopicus* and *P. amphibius* in greater detail will help establish the selective pressures underlying the specialisation of the retina of *N. forsteri*, *L. paradoxa* and *P. dolloi*, such as the lack of double cones, and if other species, such as *L. paradoxa*, do not have the potential for colour vision because of their long periods of aestivation. The topographic distribution of the red cone photoreceptors in *P. dolloi* suggests that there is an area of increased retinal sensitivity in the dorso-lateral visual field of this species, which may be useful in predator avoidance. This is different to the downwardly-directed visual axis of all the cone photoreceptor types in *N. forsteri* (Bailes et al., 2006a). Further study to investigate the areas of increased resolving power of other photoreceptor types and the ganglion cell population in *P. dolloi* will provide a better indication of how retinal structure and cell arrangement reflects this species' visual ecology.

The most important questions still remaining about the abnormal *N. forsteri* lacking external eyes is to investigate how the brain and other senses are affected, and establish the genetic and/or environmental factors underlying their morphological deformities. Interestingly, there were no myelinated axons observed in the optic nerve of the

abnormal *N. forsteri* (25 cm in TL), suggesting that the conduction velocity of retinal response from the eye to the visual centres of the brain is slower than in normal *N. forsteri* (Hursh, 1939, Tasaki, 2012). Eighteen percent of optic nerve axons in the subadult *N. forsteri* (58 cm in TL) are myelinated, increasing to 74% in the adult (113 cm in TL) (Bailes et al., 2006b). The lack of myelination in the optic nerve axons in the 25 cm long individual examined in this species may be another phenotype of the abnormal fish, but in order to confirm whether the degree of myelination varies during development in lungfishes, the optic nerve of normal juvenile *N. forsteri* needs to be examined and compared to the number of myelinated fibres in adults.

A comparative study of the brains of normal and abnormal *N. forsteri* may explain some of the differences observed in the behavioural and circadian experiments, and provide insights into how the brain may be affected in cases of anophthalmia and/or environmental induction of vestigial eyes in lungfishes. For example, the pineal organ of teleosts can affect swimming activity and phototactic behaviour. White suckers (*Catostomus commersoni*) with shielded pineal regions spend more time in the illuminated portions of an aquarium (in a two choice light:dark based system) (Kavaliers, 1982) and pinealectomy in stinging catfishes (*Heteropneustes fossilis*) results in circadian arrhythmicity (Garg and Sundararaj, 1986). Moreover, the proposed sensory plasticity in abnormal *N. forsteri* may be further explored by comparing the optic tecta and areas of the brain that receive electroreceptive and lateral line information in normal *N. forsteri*.

Finally, all abnormal lungfishes originated from the same brood and their deformity may be due to congenital defects caused by inbreeding. Molecular investigation into important transcriptional regulatory genes involved in normal eye development, such as Pax6, may uncover the genetic basis of the congenital abnormalities observed in the abnormal *N. forsteri* (Glaser et al., 1994, Halder et al., 1995, Schedl et al., 1996).

These studies attempted to combine scientific and artistic methodologies to create a robust platform to interrogate ideas exploring the subjective visual experience of lungfishes. Following upon C. P. Snow and John Brockman's views about a "third culture", where disparate cultures could meet, Snow's third culture was a place where non-scientists ("literary intellectuals") would engage and close the communication gap with scientists to further the progress of knowledge and to benefit society (Snow, 1998). Brockman's third culture replaces literary intellectuals entirely with scientists that render "visible the deeper meanings of our lives, redefining who and what we are" (Brockman, 1991) by directly communicating to the general public through books and media without the intervening assistance of practitioners of the arts (Brockman and Gratzner, 1995). In a way, this thesis is the point where the scientific and non-scientific cultures meet. However, it is not the third culture Snow predicted, nor is it the one Brockman uses to describe the scientific outreach to the public forum. Here, an interdisciplinary platform is created where science and the arts meet on equal grounds to investigate the multifaceted nature of lungfish visual perception. This is not always a seamless integration, and there are many points along this journey when the different disciplines challenged each other's understandings, language, and methodologies. As a scientist, I am subscribing to Brockman's view of innovation in communicating with the general public, but not in a way that displaces the non-scientific intellectuals. As an artist, science is an important and fascinating tool/methodology to gain knowledge and to reflect on vital questions surrounding subjective experience and the ability to bring it outside of specialised research communities, but it is still a place for speculation on how our own (human) perception has a direct effect on how we understand the natural world. Investigation into the subjective nature of visual experience through the sciences and the arts is a vast area of research, and it is important to assess if this new interdisciplinary venture was successful. It provided a deeper understanding of, and created new

encounters with, the visual perception of lungfishes that lies outside of disciplinary boundaries, and created new narratives in the quest for informed knowledge about non-human animal visual perception. Scientific data progresses the knowledge of lungfish visual ecology and artistic research contributes and connects the knowledge gained, using “objectivity as a direction in which understanding can travel” (Nagel, 1974). From a posthuman perspective that rethinks the stature placed on humanity, the connections made between humans and lungfishes through evolutionary relationships, embodiment through art practice, and human cultural narratives, reinforced ideas that we are part of the environment and interdependent on it (Wolfe, 2010). This study achieved posthumanism because it also aspires to understand the world from multiple, heterogenous perspectives and manifests itself in consideration with the different identities (Wolfe, 2010). In doing so, it allows us (humans) to understand more about the organisms we share the world with, and our place within it. The creation and exhibiting of artworks enabled this research to extend outside the academic community, and encourage more open ended and speculative understanding of cultural ideas about subjective experience to a wider and diverse audience. Despite lungfishes being humans’ closest evolutionary link to aquatic animals, there are still large gaps in our knowledge about lungfishes and their visual ecology. Pragmatically, artworks about the visual perception of lungfishes create curiosity about non-human animals. Additionally, the artworks address and provoke aesthetic, cultural and philosophical questions concerned with new knowledge about the visual ecology of lungfishes, as well as shifting understandings of how humans traverse the terrain of visual perception, especially in regards to lungfishes.

However, this interdisciplinary investigation about the subjective visual experience is not without its challenges. At a fundamental level, aspects of Snow’s “two cultures” are still apparent in academia today. Scientists and “literary intellectuals” (including artists)

still have difficulty in communicating because of their highly specialist language, and have vastly different, often opposing approaches when addressing common questions, such as visual perception (Snow, 1998). There was considerable difficulty in creating a platform to present ideas to the scientific and artistic community in a robust, relevant and accessible way. This was especially evident in the challenge of contextualising artworks and exhibitions to the scientific community, and describing scientific concepts to non-scientists. In fact, as a reader of this thesis, you are currently engaged in this experimental pursuit. This challenge in working between two cultures is more clearly seen in the style and language choices of chapters 1 – 5. The changes in writing style between chapters showed, more explicitly, how the visual perception was examined through different “lenses”. However, some chapters were also purposefully written to follow discipline conventions to respect and maintain discipline autonomy. For example, all scientific chapters followed the “scientific style” of communicating that include utilitarian structure and objective language (chapters 3 and 4), and chapter 5 was purposefully non-didactic to conform to the style of the arts. Chapter 1 and perhaps chapter 4 attempted to bring aspects of both disciplines together. There is limited language that is accessible to both artists and scientists on equal grounds, and it is often a requirement to be educated in both cultures in order to traverse this terrain. Members of Brockman’s third culture have succeeded somewhat in creating a bridge between science and popular culture in the form of written and oral works (Brockman, 1991, Brockman and Gratzner, 1995), but there is still much to be learned and improved upon to combine multiple disciplines in order to further human knowledge. Nevertheless, this interdisciplinary endeavour created a platform that encouraged members of one culture to understand disciplines of another culture. The scientists and artists that have either contributed to or collaborated with aspects of this project are now more comfortable to discuss art and science, respectively. These researchers, including myself, have grown

to accept the limitations of their specialty discipline. Preconceived notions of what “art” and “science” are, some of which are unchanged since Snow’s discussion in “The Two Cultures”, has shifted for academics and artists, resulting in the beginning of what could be bridging the gap.

Utilizing an interdisciplinary, posthumanist approach to subjective topics like the visual perception of non-humans is still useful despite its challenges. This study explored the visual experience of some aquatic, non-human animals. A similar interdisciplinary framework can also be applied for the pursuit of understanding visual perception of other non-humans. This would be particularly useful in species with vastly different visual systems to humans. For example, some bioluminescent deep-sea fishes have retinæ composed of only rod photoreceptors that are sensitive to dim light and viewing bioluminescence (Locket, 1977, Marshall, 1979). On the other hand, reptiles such as the chameleon (*Chamaeleo*) (Bowmaker et al., 2005) and gecko (*Gonatodes albogularis*) (Ellingson et al., 1995) that have evolved in high light environments have retinæ composed of only cone photoreceptors. A similar research framework can also be applied to the *Umwelt* of animals that do not utilize vision, such as strictly subterranean mammals like troglodytic moles such as *Notoryctes* (the marsupial mole), and *Chrysochloris* (the golden mole), and *Heterocephalus glaber* (naked-mole rat) (Sweet, 1906, Vaughn, 1978, Nikitina et al., 2004) and the blind cave fish *Astyanax mexicanus* (Cahn, 1958). In addition to scientific experiments that uncover the *Umwelten* of these animals, there is much room to explore other understandings of these animals through art-making, cultural studies and philosophy to highlight human limitations and extend perception across species.

ii.5 REFERENCES

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III APPENDIX

Table 1. *Percentage of time spent in the dark sector for normal and abnormal Neoceratodus forsteri*

Fish	% time spent in dark	Fish	% time spent in dark	Fish	% time spent in dark
N1	98.43	N2	96.14	N3	84.99
N1	98.12	N2	93.62	N3	90.82
N1	92.37	N2	91.25	N3	86.64
N1	89.62	N2	98.81	N3	97.62
Average	94.64	Average	94.96	Average	90.02
Std deviation	4.35	Std deviation	3.25	Std deviation	5.63
Fish	% time spent in dark	Fish	% time spent in dark	Fish	% time spent in dark
E1	57.72	E2	59.46	E3	73.42
E1	37.39	E2	46.42	E3	95.80
E1	96.75	E2	69.47	E3	58.49
E1	51.22	E2	56.05	E3	83.61
Average	60.77	Average	57.85	Average	77.83
Std deviation	22.03	Std deviation	9.51	Std deviation	15.81