Photoluminescence in fur

Thesis submitted by

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Acknowledgements

This topic began with a Facebook post from the president of the Queensland Mycological Society, Wayne Boatwright, wondering if fungi fluoresced. I bought an ultraviolet torch and ventured into the forest at night with my bushwalking buddy, Lori Lorenz. After ten nights, I discovered that the fur of mammals also glowed. Already writing about fluorescent fungi for *The Queensland Mycologist*, the editor, Dr David Holdom, allowed the addition of mammals, and one of the reviewers, Dr Patrick Leonard, encouraged me to go back to university to pursue the questions surrounding fluorescent fur. By their seemingly small words and deeds, these people planted the seed for me to research this phenomenon full-time.

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Statement of contribution of others

Figures

Dr Tasmin Rymer produced the timeline figure for the literature review.

Photographs

Dr David Wilson took the photograph of the RP-HPLC plate wells glowing pink.

All the other photographs in this thesis were taken with a Panasonic Lumix TZ-80 camera. I did not use a yellow filter, nor were any photographs brightened or enhanced by any post-processing.

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Research contributing to this thesis was supervised by Dr Tasmin Rymer, Dr David Wilson and Prof Kristofer Helgen.

Copyright

Permits and ethics approval

The collection of roadkill animals was conducted within the conditions stipulated by Queensland Department of Environment and Science Research Permit number WA0034269, under the *Nature Conservation (Animals) Regulation 2020*. The Department of Main Roads and Transport, Cairns Regional Council, Mareeba Shire Council, Cassowary Coast Regional Council, Tablelands Regional Council and Douglas Shire Council gave permission to collect roadkills. Specimens with intact skulls and data have been lodged with the Queensland Museum.

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Publications arising from this thesis

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Abstract

Photoluminescence (encompassing both fluorescence and phosphorescence) is the absorption and re-emission of light, usually converting photons from lower to higher wavelengths. Since this phenomenon occurs vividly in some, but not all, mammals, the question emerges of whether fur photoluminescence is optically meaningful for those species that possess it. Despite sporadic accounts of photoluminescent mammal species in the literature, there have been no dedicated studies of the prevalence of this trait in any region of Australia. The photoluminescent characteristics of fur have never been examined for most mammal species worldwide. Only a handful of fur luminophores (fluorophores and/or phosphors) have been identified to date, with more suspected to be present in fur. The nature of photoluminescence in fur is also little understood, but has been noted as brighter in live and recently dead animals, with recent museum-based studies flagging, but not accounting for, the chemical changes that fur undergoes in different conditions. Since its detailed documentation in European rabbits (*Oryctolagus cuniculus*) more than 100 years ago, most studies have assumed that photoluminescence is a dormant by-product of some unknown physiological function. However, potential visual functions have recently been hypothesised because of a resurgence of interest coupled with colour photographs of mammals photoluminescing.

In this thesis, I studied photoluminescence in Australian mammals from the Wet Tropics of Far North Queensland. I addressed gaps in the literature associated with prevalence, the luminophores responsible, retention of photochemical properties, and the function of photoluminescence in the field. Firstly, I investigated how prevalent the phenomenon of photoluminescence is among mammals of the Wet Tropics, Australia, using fresh roadkill animals and frozen specimens from three collections. Although only a subset of Wet Tropics mammal diversity was studied here, I present the most comprehensive account to date of the occurrence of fur photoluminescence across taxa using fresh roadkill animals. Ninety-five per cent of mammals displayed at least a subtle photoluminescence in the fur at some wavelengths. Forty-two per cent of marsupial species and 29% of placental species displayed noticeably bright photoluminescence. Both monotreme species exhibited subtle photoluminescence. There appeared to be no pattern associated with specific diet or lifestyle factors based on species life history characteristics. My findings suggest that photoluminescence is more common than previously known, and that the biochemical basis of fur photoluminescence may be common among mammals.

Secondly, I collected fur samples from seven of these Wet Tropics mammal species to extract and identify the luminophores contributing to photoluminescence. I used high-performance liquid chromatography and liquid chromatography/electrospray ionisation mass spectrometry to identify these luminophores. For two species of bandicoot (the long-nosed bandicoot (*Perameles nasuta*) and the northern brown bandicoot (*Isoodon macrourus*)), the northern quoll (*Dasyurus hallucatus*) and the platypus (*Ornithorhynchus anatinus*), the work presented here is the first attempt to isolate luminophores from the fur in these genera. I found evidence that supported the presence of coproporphyrin and protoporphyrin, and molecules matching the monoisotopic masses of uroporphyrin and heptacarboxylporphyrin, in the species studied here. These porphyrins had already been identified in the pelage of other mammal species, and exist in a range of organisms from bacteria to birds. Several other photoluminescent molecules extracted from the fur remain to be identified.

Thirdly, I investigated the lability of pink fur photoluminescence in response to light exposure, to ascertain whether observed intraspecies differences can be taken at face value, or whether they may be confounded by environmental conditions. I also tested the effects of wet preservation on both pink and blue fur photoluminescence. I conducted photobleaching experiments using northern brown bandicoot and long-nosed bandicoot pelts and found that pink photoluminescence noticeably fades in as little as two minutes of full sun exposure. These experiments have important implications for researchers working with porphyrin-based photoluminescence. Wet preservation in ethanol nearly extinguished the photoluminescence of both laboratory (Norway) rat (*Rattus norvegicus*) and bandicoot fur, but initial fixation in formalin partially preserved photoluminescence at a low level. These findings flag the probability of false negatives in studies based solely on museum specimens.

Finally, I investigated the plausibility of a visual function for fur photoluminescence by placing photoluminescent and non-photoluminescent models in the field and assessing the behavioural responses of wild animals to these models over a six-month period. I used remote cameras to observe behaviour under both full moon and new moon cycles to determine whether photoluminescence could be triggered by natural nocturnal lighting conditions. I found that wild nocturnal animals did not show a preference for either model, suggesting either that natural moonlight was not sufficient to stimulate photoluminescence, that wild nocturnal vertebrates were unable to detect photoluminescence in natural conditions, or that these animals do not use this visual property of fur when making behavioural decisions.

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Chapter 1

General introduction

1.1 Introduction

The recent increase in media reports and articles on photoluminescence in biology suggests that photoluminescence in mammals is a rare and exciting new phenomenon (Kohler et al. 2019; Giaimo 2020; Main 2020; Olson et al. 2021). However, research from the last 111 years (1911–2022) indicates that the fur of most mammals is likely photoluminescent to some degree, at least at the microscopic level, due to presence of the protein keratin (Rebell et al. 1956; Pine et al. 1985; Toussaint et al. 2023). Photoluminescent pelage occurs in numerous mammal taxa, from rats and bats (Udall et al. 1964), sheep and humans (Millington 2020), to tree-kangaroos (Nicholls and Rienits 1971) and flying squirrels (Kohler et al. 2019). The luminophores described so far from fur are either tryptophan metabolites (Rebell et al. 1957; Nicholls and Rienits 1971) or porphyrins (Hamchand et al. 2021; Olson et al. 2021), although other classes of luminophore may yet be described.

Photoluminescence is an optical property of some chromophores (Kricka 2003; Valeur and Berberan-Santos 2011; Tomalia et al. 2019), although they may appear colourless in white light. The luminophore absorbs light and usually converts the photons to a higher wavelength in the process of re-emitting them (Johnsen 2012). Lower-wavelength excitation light (often blue to blue/green to elicit visible-wavelength photoluminescence in nature, Marshall and Johnsen 2017) must be present at sufficient strength to activate photoluminescence, otherwise the property remains unobserved. The photoluminescent properties of internal biological tissues and fluids are never observed unless they leave the body. The term ‘photoluminescence’ encompasses both ‘phosphorescence’ (the glowing of an object after a light source is switched off, Valeur 2001) and ‘fluorescence’ (the glowing of an object only when a light source is pointed at it, Stokes 1852). Mammalian pelage and some of

its luminophores are variously capable of both fluorescing and phosphorescing (Wilson and Beccari 1775; Millson 1943, cited in Collins 1992; Konev 1967; Gouterman and Khalil 1974); in the absence of knowing the atomic state of the luminophore (fluorophore and/or phosphor), I use the word 'photoluminescence'.

With the first published colour photographs of fur photoluminescence came the first hypotheses on a visual function for photoluminescent fur. Kohler et al. (2019) hypothesised that the bright pink bellies of flying squirrels (Humboldt's flying squirrel (*Glaucomys oregonensis*), northern flying squirrel (*G. sabrinus*) and southern flying squirrel (*G. volans*)) could be: 1) adaptive to a nocturnal lifestyle in their low-light environment; 2) adaptive to snow cover; 3) a method of intraspecific communication as they glide between trees; or 4) an antipredator strategy, either mimicking lichen or owls (Strigiformes). The latter hypothesis could be applied more broadly across different animals and habitats outside of the flying squirrels' environment. Owls in different regions also photoluminesce bright pink (Roulin et al. 2008; Blythman et al. 2016), and their cone-based vision (Meyknecht et al. 1941, cited in Potier et al. 2020; Martin 1974; Potier et al. 2020) may make them more likely to see the colour pink than the colour-blind flying squirrels themselves (Carvalho et al. 2006).

Some of these hypotheses have since also been suggested as potential visual functions for the photoluminescence of platypuses (*Ornithorhynchus anatinus*, Anich et al. 2021), springhares (South African springhare (*Pedetes capensis*) and East African springhare (*P. surdaster*), Olson et al. 2021) and pocket gophers (southeastern pocket gopher (*Geomys pinetis*), plains pocket gopher (*G. bursarius*), desert pocket gopher (*G. arenarius*), northern pocket gopher (*Thomomys talpoides*) and yellow-faced pocket gopher (*Cratogeomys castanops*), Pynne et al. 2021). Photoluminescence could be an indicator of individual body condition, as already correlated in Eurasian eagle owls (*Bubo bubo*, Galván et al. 2018) and red-necked nightjars (*Caprimulgus ruficollis*, Camacho et al. 2019). A photoluminescent indicator of condition could have important applications for captive animals, especially if luminophores are diet-related or a sign of disease (e.g. congenital porphyria in canefield rats (*Rattus sordidus*), Rivera and Leung 2008).

1.2 Motivation for the thesis

My project aimed to fill some of the knowledge gaps relating to prevalence, photochemistry, causation, excitation, and visual detection of photoluminescence in fur. The study of photoluminescence has offered novel insights into marine species (Michiels et al. 2008; Gerlach et al. 2014; Sparks et al. 2014; De Brauwer et al. 2017), and a visual role for photoluminescence in terrestrial ecosystems has also begun to receive attention (Arnold et al. 2002; Lim et al. 2007; Douglas III et al. 2021; Czarnecki et al. 2022). Photoluminescence has been demonstrated as being ecologically significant in scorpions (Scorpiones, Kloock 2005; Kloock et al. 2010), and some authors have suggested that photoluminescence may be visually important for terrestrial animals in twilight environments (Taboada et al. 2017; Kohler et al. 2019). However, some recent reviews of biological photoluminescence have excluded mammals (Lagorio et al. 2015; Macel et al. 2020) or skipped much of the existing research (Jeng 2019; Croce 2021). Advances in laboratory equipment and technology mean that a greater understanding of the underlying chemistry of photoluminescence is now possible. Establishing which luminophores contribute to vivid photoluminescence will allow researchers in this field to trace the biosynthetic pathways of these molecules, thereby determining potential physiological functions.

Vivid photoluminescence occurs in some, but not all, mammals. Thus, the overarching scientific interest to studying mammalian photoluminescence is whether it is an important trait for those species that have vivid photoluminescence. Knowing how it varies between individuals or species could reveal patterns associated with habitat, lifestyle or diet, which could be important for developing hypotheses on ecological significance. Part of the question of importance to the animal is whether the optical properties of photoluminescence are visible, or whether the luminophores are never triggered by natural light over and above the level of reflectance. The marine environment and its organisms seem to be uniquely placed to use photoluminescence in a visual context (Michiels et al. 2008), but the optics of crepuscular-nocturnal terrestrial environments are not so compelling (Marshall and Johnsen 2017). Whether the luminophores in fur can be excited by natural light, and seen by nocturnal-crepuscular animals, has not been tested. Because we have so little understanding of how photoluminescence may affect or be an indicator of ecological interactions, physiological health or poor diet, this thesis serves as a foundation from which future research on terrestrial photoluminescence can be investigated.

1.3 Chapter layout

Chapter 1 introduces the topic of photoluminescence in mammal fur and outlines the content of the thesis. Chapter 2 is a literature review on mammalian pelage fluorescence and phosphorescence studies from Stübel's (1911) early work, up to and including studies from 2022. Chapter 3 documents the prevalence of fur photoluminescence in roadkill mammals of the Wet Tropics, Queensland, Australia. Chapter 4 presents analyses of fur chemistry in two species of bandicoots and other representatives of photoluminescent marsupials, as well as a monotreme and a placental mammal. In Chapter 5, I investigate the lability of fur photoluminescence, testing its resilience to light and wet preservation post-mortem. Chapter 6 details a field experiment to test whether nocturnal vertebrates react more frequently to photoluminescent fur than non-photoluminescent fur. Chapter 7 summarises the contribution this thesis makes to the current understanding of photoluminescence in the fur of mammals.

1.4 Thesis outline

This thesis comprises the general introductory chapter (Chapter 1), followed by a literature review on photoluminescence in fur (Chapter 2). This chapter (Photoluminescence in mammal fur: 111 years of research) is in press for publication in *Journal of Mammalogy.* Chapters 3 to 6 each address an experimental topic and comprise the main body the thesis. Chapter 3 focuses on the prevalence of photoluminescent fur in mammals of the Wet Tropics. Chapter 4 investigates the chemical composition of fur photoluminescence. Chapter 5 studies photobleaching and provides a commentary on specimen preservation and its effects on photoluminescence. Chapter 6 describes a field experiment using photoluminescent and non-photoluminescent models to test whether photoluminescence can be detected in natural lighting, giving a foundation for the plausibility of hypotheses on visual function. The final chapter, Chapter 7, consists of a general discussion. As Chapter 2 is in press for publication, chapters have been formatted as separate manuscripts for later publication. These chapters may contain repetition in background information and methodology. The pages, tables and figures are numbered sequentially throughout the thesis.

Chapter 2

Photoluminescence in mammal fur: 111 years of research

Manuscript in press for publication in *Journal of Mammalogy*

2.1 Abstract

Photoluminescence in the pelage of mammals, a topic that has gained considerable recent research interest, was first documented in the 1700s and reported sporadically in the literature over the last century. The first detailed species accounts were of rabbits and humans, published 111 years ago (1911–2022). Recent studies have largely overlooked this earlier research into photoluminescent mammalian taxa and their luminophores. Here I provide a comprehensive update on existing research on photoluminescence in mammal fur, with the intention of drawing attention to earlier pioneering research in this field. I provide an overview on appropriate terminology, explain the physics of photoluminescence, and explore pigmentation and the ubiquitous photoluminescence of animal tissues, before touching on the emerging debate regarding visual function. I then provide a chronological account of research into mammalian fur photoluminescence, from the earliest discoveries and identification of luminophores to the most recent studies. While all mammal fur is likely to have a general low-level photoluminescence due to the presence of the protein keratin, fur glows luminously under ultraviolet-violet light if it contains significant concentrations of tryptophan metabolites or porphyrins. Finally, I briefly discuss issues associated with preserved museum specimens in studies of photoluminescence. The study of mammal fur photoluminescence has a substantial history, which provides a broad foundation on which future studies can be grounded.

2.2 Introduction

Photoluminescence in biology results from photons hitting an organic object and causing a change in the energy levels of the electrons within certain molecules, resulting in the re-emission of light at a higher wavelength as the electrons return to their ground energy level (Murthy and Virk 2014; Visser and Rolinski 2014). In the oceans, photoluminescence is widespread in corals (Mazel and Fuchs 2003), fish (Sparks et al. 2014) and other organisms (Shimomura et al. 1962; Mazel et al. 2004). On land, photoluminescence occurs in some fungi (Soop 2005), bacteria (Hurley et al. 2019) and ubiquitously in the chlorophyll of plants (Krause and Weis 1991). Photoluminescence has also been recorded in terrestrial invertebrates (Kloock 2005), amphibians (Lamb and Davis 2020), reptiles (Prötzel et al. 2021), birds (Derrien and Turchini 1925) and mammals (Bolliger 1944; Kohler et al. 2019).

Recent reviews on biological photoluminescence have focused on terrestrial plants, invertebrates, birds and marine organisms (Lagorio et al. 2015; Macel et al. 2020), and it appears that what is known about external photoluminescence in mammals is very limited. Only Jeng (2019) and Croce (2021) mention mammals, and only as far back as 1985. Photoluminescence in the pelage of mammals is most well-known from opossums in the Americas (Pine et al. 1985). However, the discovery of mammalian photoluminescence predates the work on opossums, with historical publications documenting photoluminescence in a range of species and the isolation of some of the luminophores involved. Specifically, the term ‘luminophore’ (Kricka 2003) encompasses groups of atoms that luminesce, whether they are specifically fluorophores (coloured compounds called chromophores that fluoresce, Tomalia et al. 2019) or phosphors (chromophores that phosphoresce, Valeur and Berberan-Santos 2011), or whether they may exist in both states of excitation. Although most natural substances contain a photoluminescent component, they vary in brightness, and it is the conspicuously bright photoluminescent compounds that are generally thought of as being luminophores (Tomalia et al. 2019).

Photoluminescence is commonly perceived to only occur when ultraviolet light excites a surface to give off a visible colour. However, photoluminescence can also be excited and emitted entirely in the ultraviolet (Millington 2020), entirely in the visible (Lamb and Davis 2020), in the infrared (Huang et al. 2006) or even in the X-ray wavelengths of the electromagnetic radiation spectrum (Rakovan 2021). Most documented biological photoluminescence is triggered by visible blue or blue/green light (Lagoria et al. 2015; Johnsen 2012; Marshall and Johnsen 2017). Although much photoluminescence in mammal

fur has been identified using ultraviolet light, the precise range of excitation wavelengths is unknown for many of the historical observations. Although the extent of brightly photoluminescent fur across mammalian taxa has not been comprehensively documented, the phenomenon has been sporadically recorded across 14 of 28 extant mammal orders (Table 2.1). Figure 2.1 is a timeline of discovery, dividing these orders into the mammal families in which species with luminescent pelage have been found.

Table 2.1. Mammalian orders in which photoluminescent fur has been documented.

Order	Citations
Monotremata	Reinhold 2020; Anich et al. 2021
Didelphimorphia	Pine and Abravaya 1978; Pine et al. 1985; Toussaint et al. 2023
Dasyuromorphia	Reinhold 2020
Peramelemorphia	Reinhold 2020; Reinhold 2021
Diprotodontia	Bolliger 1944; Nicholls and Rienits 1971; Reinhold 2021
Primates	Stübel 1911; Daly et al. 2009; Millington 2020
Lagomorpha	Stübel 1911; Tumlison and Tumlison 2021
Rodentia	Rebell et al. 1956; Kohler et al. 2019; Olson et al. 2021
Eulipotyphla	Derrien and Turchini 1925; Hamchand et al. 2021
Artiodactyla	Hirst 1927; Smith et al. 1994; Millington 2020
Chiroptera	Udall et al. 1964; Reinhold 2022
Perissodactyla	Posudin 2007
Pholidota (scales)	Jeng 2019
Carnivora	Latham 1953; Millington 2020; Tumlison and Tumlison 2021

In this review, I convey the historical extent of research on photoluminescence in mammals, filling the gap left by recent reviews (e.g. Lagorio et al. 2015; Jeng 2019; Macel et al. 2020). I first describe photoluminescence and explain the distinction between fluorescence and phosphorescence, and how photoluminescence differs from bioluminescence, then discuss how the physics of photoluminescence might operate in the context of terrestrial illumination. Next, I introduce the chemistry of mammal fur regarding the pigments that give colour to fur in white light and the presence of luminophores that effect photoluminescence. Although a possible visual function of luminophores in fur has not been specifically studied, I also briefly discuss the hypotheses recently put forward in this emerging debate. I then document the history of mammal photoluminescence research, from the earliest discoveries to the present day, attributing photoluminescence predominantly to the two groups of luminophores that are so far known from fur, namely tryptophan metabolites and porphyrin derivatives (Box 2.1). Finally, I add a cautionary note about the over-reliance on museum specimens for the documentation of photoluminescence in fur. This review brings together a wealth of historical knowledge that remains relevant in the context of today's discoveries.

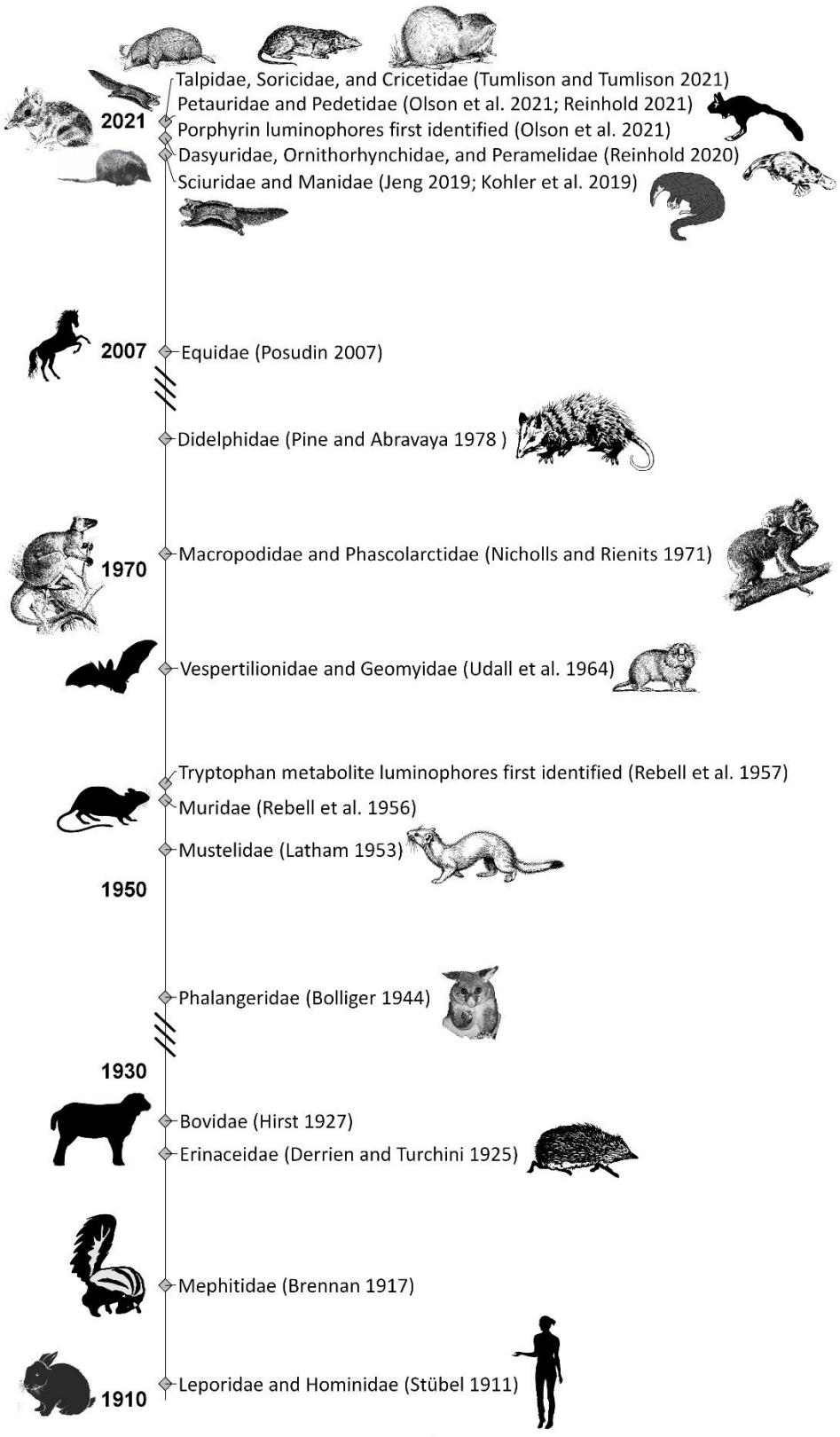


Figure 2.1. Discovery timeline of luminescence in the pelage of mammal families.

Antechinus image: David Wilson; bandicoot image: Linda Reinhold; springhare image: Revolutionrock1976; https://commons.wikimedia.org/wiki/File:Pedetes_capensis_(South_African_Springhare).jpg; https://creativecommons.org/licenses/by-sa/4.0/deed.en

2.2.1 The physics of luminescence

2.2.1.1 Terminology of photoluminescence relating to fur: fluorescence versus phosphorescence

Luminescence is the blanket term for cold light (Wiedemann 1888), which can be emitted via either chemiluminescence or photoluminescence. In biology, the generation of true glow-in-the-dark light only occurs by chemiluminescence (bioluminescence), a chemical reaction catalysed by an enzyme (e.g. luciferase) or photoprotein (Abercrombie et al. 1992). Photoluminescence, including phosphorescence and fluorescence, is the re-emission of light from matter after excitation by absorption of an external light source (Valeur and Berberan-Santos 2011).

Phosphorescence is a process whereby the electrons of the phosphorescent molecule temporarily reside in an intermediate state before returning to the ground energy level (Valeur and Berberan-Santos 2011). Phosphorescent objects initially need light to glow, and are often defined simply by the length of time the glow lasts after the light source is turned off (Harvey 1957; Johnsen 2012). In phosphorescence, the duration of light emission is typically $> 10^{-8}$ s (i.e. tens of nanoseconds to seconds) (Murthy and Virk 2014; Visser and Rolinski 2014).

Fluorescence occurs when electrons in fluorescent molecules temporarily jump to an electronically excited higher energy state before they decay back to their original ground state. The outgoing photon is usually emitted at a longer wavelength than the incoming wavelength (Herman et al. 2015). In fluorescence, the duration of light emission is usually $< 10^{-8}$ s (nanoseconds), so appears to cease as soon as the excitation light source is stopped (Murthy and Virk 2014).

However, both long-lived fluorescence and short-lived phosphorescence can last for several hundred nanoseconds, meaning the length of light emission alone is not always enough to define the difference between fluorescence and phosphorescence (Valeur and Berberan-Santos 2011). It was not until 1929 that the ‘fluorescence’ observed in some animals by Stokes (1852) was separated from ‘phosphorescence’ at the atomic level (Perrin 1929, cited in Valeur and Berberan-Santos 2011). Even though fluorescence and short-lived phosphorescence differ in their atomic processes, there is little practical difference between them, and in such cases where the processes have not been differentiated, the distinction between the two is sometimes viewed as arbitrary (Harvey 1957). However, the interchangeable use of these two terms in biology can cause confusion.

Interestingly, Stokes (1852) coined the term *fluorescence* to describe the biological property that he identified and reported in white feathers, shells, quills, bristles, skin, nails, horn, bone and most unpigmented organic materials. However, *phosphorescence* of such materials had been recorded earlier by de Mairan (1715) (cited in Harvey 1957) and Wilson and Beccari (1775). Giese and Leighton (1937) also found some of these materials—feathers, shells, skin, nails, horn and bone—to *phosphoresce* for 2–25 s. This phosphorescence was attributed to the aromatic amino acids tryptophan and tyrosine in proteins (Warren 1982).

Historically, 'phosphorescence' was applied to cold light in general (Harvey 1957); however, the newer distinction of 'fluorescence' now seems to have taken its place as the default term unless 'phosphorescence' is demonstrated. In describing any light-induced glowing where the atomic state of the luminophores is unknown, it would be more prudent to use the encompassing term 'photoluminescence'. In a biological context, the prefix 'bio-' may be added to any of these words, but would be applicable to the cells of live animals more than to the fur of specimens examined in museum studies. However, 'bio-' would also imply that the photoluminescence is coming from the organism, whereas photoluminescence is initiated by photons external to the organism. For clarity, 'bio-' should be reserved for the chemiluminescent process of 'bioluminescence' (Johnsen 2012; Toussaint et al. 2023).

2.2.1.2 Practicalities of seeing photoluminescence: excitation and visibility

It is a popular misconception that ultraviolet vision is a prerequisite for seeing the kind of photoluminescence that emits at visible wavelengths. However, the process of photoluminescence can transfer photons from the invisible into the visible spectrum (Stokes 1852). Therefore, seeing fluorescence and phosphorescence only requires that the *emitted* wavelengths are within the visible wavelength range of the observer. Consequently, the ability to see photoluminescence is not special. It is not ultraviolet *vision* that is needed to make this phenomenon visible, but rather an external light source, such as ultraviolet light.

When a photoluminescent object absorbs ultraviolet light in an environment that is otherwise dark and re-emits light in the visible spectrum, the object will look as if it is glowing. This light appears to come from the object itself because the incident light source is invisible to the naked eye (Baird 2015). The emitted wavelength may also be unusual in the ambient light spectrum illuminating the surroundings, resulting in increased contrast of the object. In the oceans, this means turning the ubiquitous blue light into rare red light (Johnsen 2012; Marshall and Johnsen 2017), a conversion effected by diurnal reef fish (Michiels et al. 2008). A blue glow may blend in to ambient lighting, whereas a red glow would stand out.

In terrestrial environments, photoluminescence (and bioluminescence) may provide the only visible colours during otherwise monochromatic twilight (Pohland 2007). The colour contrast of a photoluminescent object against its background would make the light emission more noticeable. Even if an animal is colour-blind, it may still have the potential to detect the brightness of photoluminescence or its increased contrast against the background. Humans frequently exploit this phenomenon by using photoluminescence to deliberately make objects such as traffic signs appear brighter, particularly in low light conditions (Schnell et al. 2001; Baird 2015). Photoluminescence can also result in a brighter overall appearance without a change in colour (Marshall and Johnsen 2017), intensifying the saturation of colours. When photoluminescence occurs in the strong and multiple excitation wavelengths of sunlight, it can add to the appearance of brightness of the visible light reflecting off an object (Baird 2015). Sunlight delivers excitation wavelengths of 330–500 nm at enough intensity to trigger most natural photoluminescence (Marshall and Johnsen 2017). However, the intense ambient light of sunlight may also act to overpower more subtle photoluminescence (Viitala et al. 1995). Without the overpowering yellow rays of the sun, moonlight (Kloock 2005) and the lower wavelength light of twilight (Taboada et al. 2017) have the potential to excite such subtle photoluminescence.

While excitation of photoluminescence in the oceans is widely accepted (Michiels et al. 2008), how photoluminescence is placed in a terrestrial environment is less well understood. While humans regularly employ photoluminescent pigments to make objects ‘hi-vis’ (Schnell et al. 2001; Baird 2015), our knowledge of how natural photoluminescence may be seen in a terrestrial landscape is relatively limited. Forest structure, low sun angles and some weather conditions can create terrestrial environments where overpowering middle wavelengths are lessened (Endler 1993). In closed forest shade during the day, light is greenish due to being shone through or reflected from leaves. However, in woodland shade, light also does not come directly from the sun, but holes in the canopy instead let in blueish light from the sky (Endler 1993). Although some habitat and environmental conditions may promote more of the lower wavelengths of light relative to others (Silberglied 1979), no studies have measured whether these levels of ultraviolet, purple or blue light actually trigger the excitation of natural photoluminescence at a level that can be detected by animals.

2.2.2 Pigmentation and the ubiquitous background photoluminescence of mammal fur

In mammals, the colouration of fur is largely driven by pigments (chromophores, Hubbard and Kropf 1965) that are insoluble in water and absorb light of different wavelengths. However, for some coloured molecules dissolved in solution in animals, the term 'pigment' is not applicable, so 'biochrome' or 'zoochrome' is preferred (Fox 1944; Needham 2012). Fur in general absorbs ultraviolet radiation due to a characteristic of keratin, a structural protein in hair (Dawson et al. 2014). How light or dark a coat is can change with climate, season, or through the lifetime of an animal (Pawelek and Körner 1982; Mills and Patterson 2009). The dominant colouration in mammal fur is from melanin, a group of natural zoochromes that animals synthesise in the fur follicles by oxidising the amino acid tyrosine, and incorporate into the shaft as the fur grows (Pawelek and Körner 1982; Hudon 2005). Melanin largely limits colour patterns to brown, tan, grey, black, white (absence of melanin), red and yellow (Newman et al. 2005; Penteriani and Delgado 2017). Eumelanin gives fur its characteristic black or brown colouration, while pheomelanin provides yellow and red (Pawelek and Körner 1982). When black sheep's wool is exposed to light, the black eumelanin is converted into red or yellow pheomelanin (Sumner et al. 1994, cited in Smith 1995). Other zoochromes include cinnabarinic acid, which contributes to the red colour of fur in red kangaroos (*Macropus rufus*) (Nicholls and Rienits 1971), and an organic iron pigment, trichosiderin, which is involved in the colouration of human red hair (Flesch and Rothman 1945; Barnicot 1956).

Most zoochromes are also fluorescent and/or phosphorescent in the free state, but *in vivo* quenching means that few cause visibly strong photoluminescence in animals (Needham 2012). Even eumelanin photoluminesces with ultraviolet, visible and infrared wavelengths (Kozikowski et al. 1984; Mosca et al. 1999; del Rosal et al. 2016). For example, the melanin-rich black fur of a black and white domestic cat (*Felis catus*) photoluminesces in the infrared with greater intensity than the white fur (Huang et al. 2006). However, fur photoluminescence by ultraviolet excitation shows the opposite pattern in striped possums (*Dactylopsila trivirgata*), with the white stripes, and not the black, emitting visible photoluminescence (Reinhold 2021). The fur lacking melanin displays the brighter photoluminescence when exposed to ultraviolet light. The presence of melanin quenches luminophores and their visible photoluminescence (Rebell et al. 1957; Rebell 1966; Daly et al. 2009), so fur containing more melanin absorbs more ultraviolet light, yet re-emits less (Posudin 2007).

In addition to masking, melanin can also have a photoprotective effect on photoluminescence (Daly et al. 2009). Fur is susceptible to photobleaching, correlated with the degradation of tryptophan (Lennox and Rowlands 1969), which results in decreased

photoluminescence (Smith 1995). Tryptophan-based photoluminescence gradually degrades under light exposure over months (Schäfer et al. 1997; Posudin 2007; Longo et al. 2013). Porphyrin molecules are significantly more prone to photodegradation, and photobleaching of porphyrins can occur within minutes of sunlight exposure (Galván et al. 2016). This extreme lability means that porphyrin photoluminescence will quickly degrade in the fur of animals exposed to sunlight (Toussaint et al. 2023).

Keratin is a high-sulphur, fibrous structural protein comprising a filament-matrix structure embedded in an amorphous keratin matrix (Wang et al. 2016). Forming as a pleated sheet, β-keratin is found in feathers, beaks and claws. Forming as a helix, α-keratin is found in fur, wool, hair, quills, nails and horns (Wang et al. 2016). Wool fibres are composed of 82% high-cystine (a sulphur-containing disulphide-bonded dimer of the semi-essential amino acid cysteine) keratinous proteins, 17% low-cystine non-keratinous material and 1% lipids and polysaccharides (Rippon 2013). Keratin not only functions as a scaffold for luminophores, but the disulphide bonds in the high-cystine content partially quench the tryptophan fluorescence and phosphorescence in wool (Smith 1995). Dietary supplementation of cystine increases the cystine content in the wool (Reis and Schinckel 1963), which would hence result in reduced photoluminescence. Wool keratin itself photoluminesces cyan upon maximal excitation by 430 nm violet-blue light (Melhuish and Smith 1993, cited in Smith et al. 1994).

Pine et al. (1985) reported a yellow-green photoluminescence, which they surmised to be from keratin, coming from all opossum, rodent and human hair under a fluorescence microscope, even if photoluminescence was not induced in the whole pelt with ultraviolet light. Photoluminescence in keratin is caused by the photoluminescent amino acids tryptophan, tyrosine and phenylalanine involved in its protein structure (Smith et al. 1980; Longworth 1983, cited in Millington 2020). These are also the only three aromatic amino acids known to cause both fluorescence and phosphorescence of proteins in a free state (Konev 1967; Pailthorpe and Nicholls 1972). This background fluorescence and/or phosphorescence of the amino acids in keratin means that fur, in general, photoluminesces to some extent unless it is masked by melanin.

Mammals can also display photoluminescence not just of their own production, but as a host for other organisms. Photoluminescence in fungi is a widespread phenomenon, and photoluminescence in human and other mammal tissues can also be caused by (and used to diagnose) fungal infection (Margarot and Devèze 1925, cited in Chattaway and Barlow 1954; Rao et al. 2008). An important example is orange-yellow photoluminescence in the micro-lesioned wings of Holarctic bats with white-nose syndrome, a disease caused by the fungal

pathogen *Pseudogymnoascus destructans*, because this photoluminescence can help to rapidly diagnose bats infected with this pathogen (Turner et al. 2014). The fungal luminophore was identified as lumichrome, a degradation product of riboflavin (Flieger et al. 2016). Bacterial infection by *Staphylococcus aureus* and *Pseudomonas* spp. on human skin is also diagnosable by detection of photoluminescence (Hurley et al. 2019), with *Propionibacterium* spp. producing coproporphyrin III (Cornelius and Ludwig 1967).

Photoluminescence in animal tissues is so ubiquitous it is the norm, not the exception (Stübel 1911). Natural photoluminescence is a characteristic of biological substances such as enamel, chitin, collagen, elastin, lipofuscins, reticulin fibres and urine (Stübel 1911; Kellie et al. 2004; Viegas et al. 2007). A substantive literature surrounds photoluminescence in bone of humans and other mammals affected by pathologies of congenital erythropoietic porphyria, which apparently occurs normally in eastern fox squirrels (*Sciurus niger*) without ill effects (Turner 1937; Wolff et al. 2005; Rivera and Leung 2008; Neves and Galván 2020).

The molecular trends associated with potential luminophores is a difficult topic to address beyond the basic general description that molecules in which electrons can be elevated to an excited electronic state may fluoresce or phosphoresce if the excited electrons can return to the electronic ground state via radiative decay by spontaneous emission. Sumita et al. (2022) highlight that several intricately intertwined factors, including reactions with oxygen molecules, molecular collisions, intra/intermolecular electron transfer, and aggregation, may deactivate the molecule as it travels in the excited state. This makes it difficult to correlate luminescence with molecular structure and, therefore, there are no clear guidelines for creating or predicting luminescent molecules. In biological luminophores, molecules containing planar conjugated systems, such as aromatic rings, have the potential to luminesce and are regularly observed due to the common occurrence of $\pi^* \leftarrow \pi$ (bonding pi (π) to antibonding pi (π^*) molecular orbital) transitions. However, the potential luminescence of these molecules cannot be easily predicted.

An intrinsic photoluminescence (both fluorescence and phosphorescence) has been described in a range of abiotic and biological molecules, including amino acids (tryptophan, tyrosine, phenylalanine), and peptides and proteins containing these amino acids (Tomalia et al. 2019). This generic blue glow is so pervasive that it can confuse the recognition of synthetic luminophores used in medical imaging (Tomalia et al. 2019). Furthermore, Toussaint et al. (2023) recorded blue photoluminescence in the pelage of all 23 mammal specimens from 18 species they examined with emission spectroscopy, but they suspected it was at least partially due to keratins.

The ubiquitous background photoluminescence of mammal fur would seem to obscure a distinction between a 'photoluminescent' and a 'non-photoluminescent' mammal, as even mammals regarded as non-photoluminescent may still display this phenomenon when examined microscopically (e.g. Pine et al. 1985). Hirst (1927) saw the difference in photoluminescence of textile fibres as a matter of degree, making only a qualitative distinction between most fibres that yielded ordinary photoluminescence, and those that glowed with brilliant colour. Those fibres that contain enough of a concentration of luminophores to be regarded as photoluminescent or not are ill-defined. Therefore, future studies could include spectroscopy to quantitatively measure the intensity of photoluminescence. However, how photoluminescent an animal is under particular excitations would only be relevant if the phenomenon is visually significant in nature. It is the cases of the stunningly bright human-visible photoluminescence that have recently attracted speculation on visual function (Kohler et al. 2019).

2.2.3 *Does fur photoluminescence have a visual function?*

Stübel (1911) doubted that photoluminescence could be biologically significant given that it is so common in both external and internal tissues. A visual function for photoluminescent fur has not been tested, nor have studies explored whether natural twilight or moonlight can excite the luminophores in fur. However, several hypotheses have been proposed, namely that photoluminescence is: 1) adaptive in nocturnal-crepuscular (especially snowy) light environments (Kohler et al. 2019); 2) used in intraspecific communication (Kohler et al. 2019; Pynne et al. 2021); or 3) an antipredator strategy (Kohler et al. 2019; Anich et al. 2021; Olson et al. 2021; Pynne et al. 2021). Several studies have focused on intraspecific communication in other animals, including: budgerigars (*Melopsittacus undulatus*) (Arnold et al. 2002); ornate jumping spiders (*Cosmophasis umbratica*) (Lim et al. 2007); fairy wrasse (*Cirrhilabrus solorensis*) (Gerlach 2014) and crested auklets (*Aethia cristatella*) (Douglas III et al. 2021). However, these have used either artificial ultraviolet lighting or artificial photoluminescent paint. Studies using natural photoluminescence under natural lighting are rare and results are mixed. One study found that flying insects avoided photoluminescent scorpions (*Vaejovis* sp.) on a full moon (Kloock 2005), whereas another found that house crickets (*Acheta domesticus*) did not react differently to photoluminescent scorpions (*Centruroides granosus*) under a half moon (Gálvez et al. 2020). Until field experiments using real fur and natural lighting are conducted, whether a visual function exists or not remains speculative.

2.3 Photoluminescence in fur: an historical account

Some of the earliest documentations of photoluminescence in hair were those of de Mairan (1715) (cited in Harvey 1957), Wilson and Beccari (1775) and later Stokes (1852). Hair, fur and wool phosphoresced after excitation by sunlight (Wilson and Beccari 1775). The first mammals for which photoluminescent pelage properties were comprehensively described by species were European rabbits (*Oryctolagus cuniculus*) and humans (*Homo sapiens*) (Stübel 1911; Fig. 2.1). Black rabbit fur did not photoluminesce, but unpigmented fur photoluminesced intense light yellow under excitation by 300–400 nm light. Similarly, in humans, pigmented hair did not photoluminesce, but white hair photoluminesced bright blue and white. Only tissues containing pigment or haemoglobin and its derivatives had their photoluminescence suppressed (Stübel 1911).

In 1917, a trapper reported 'phosphorescence' (bioluminescence in this context) from large black, and black and white skunks (Mephitidae) in deserted mine tunnels in Arizona, North America (Brennan 1917; Fig. 2.1). Red luminescence emanated from the head, turning blue down the rest of the body and tail (Brennan 1917).

Red photoluminescence, believed to be caused by porphyrins (Box 2.1), was then recorded in the quills, but not in the soft fur, of young European hedgehogs (*Erinaceus europaeus*) (Derrien and Turchini 1925; Fig. 2.1). In the same year (1925), a bright green photoluminescence was noted in the hair cortex of some humans and cats (and later in guinea pigs (*Cavia porcellus*)), that was ultimately due to a dermatophyte (keratin-eating) fungal infection rather than intrinsic to the fur itself (Margarot and Devèze 1925, cited in Chattaway and Barlow 1954; Stockdale et al. 1965).

Interest then turned to textile fibres, with ordinary sheep's wool noted as having a bright blue photoluminescence (Hirst 1927, cited in Collins 1992; Fig. 2.1). The wool of Australian merino sheep photoluminesced yellow with blueish-white tips. Sheep's wool phosphorescence with an afterglow lasting 12 s was described in 1943 (Millson 1943, cited in Collins 1992). Fluorescence of wool was yellowish white, whereas phosphorescence was colourless.

Photoluminescence in the fur of a marsupial, the Australian common brushtail possum (*Trichosurus vulpecula*), was first described by Bolliger (1944; Fig. 2.1). An otherwise colourless substance gave a brilliant sky-blue photoluminescence to the fur shafts (Bolliger 1944). An extraction of this substance photoluminesced in daylight, visible to the human eye. The sky-blue photoluminescence also exuded from the sweat gland walls and coated the skin all over the animal except for the soles (i.e. the palmar and plantar surfaces) of the paws

(Bolliger 1944). Male possums also had vivid salmon red photoluminescence of the fur between the head and middle of the rump, whereas this salmon red photoluminescence was localised to small tufts around the shoulders in females. Newly regrown fur on the dorsal surface or flanks of possums photoluminesced vivid red or purple, whereas fur on the ventral surface photoluminesced pink. Bolliger (1944) also reported fur photoluminescence to be common in other mammals, but with lesser intensity.

In the 1950s, vivid lavender photoluminescence was proposed as a taxonomic character to distinguish the brown summer coats of least weasels (*Mustela nivalis*), recorded as photoluminescent, from two other sympatrically occurring weasel species, ermines (*M. erminea*) and long-tailed weasels (*Neogale frenata*) that were observed not to photoluminesce (Latham 1953; Fig. 2.1). Brilliant photoluminescence was also observed in the fur of albino laboratory (brown/Norway) rats (*Rattus norvegicus*) (Rebell et al. 1956; Fig. 2.1). The photoluminescence was confined within the fur itself, with no similar photoluminescence emitted from the skin.

Using paper chromatography, the luminophores extracted from rat fur were identified as the tryptophan metabolites (Box 2.1) kynurenine, kynurenic acid and N-acetyl-kynurenine (Rebell et al. 1957; Rebell 1966). Washed fur from albino lab rats had 3.3 mg of kynurenine (including N-acetyl-kynurenine) per 1 g of fur, cinnamon rats had 3.4 mg per 1 g, and black rats had 4.6 mg per 1 g (Rebell 1966). Although the fur of cinnamon and black lab rats contained equivalent amounts of luminophores, the melanin in the fur of rats with black coats quenched the visible photoluminescence. Approximately 1000 times as much kynurenine was concentrated in the fur of photoluminescent rats than in non-photoluminescent white house mice (*Mus musculus*) or guinea pigs, which still yielded low concentrations of this luminophore (Rebell 1966). Extracts of guinea pig, rabbit and cat fur also displayed a weak blueish photoluminescence (Rebell et al. 1956).

Around the same time as Rebell's work on luminophores, Udall et al. (1964) reported on photoluminescence studies in a large number of museum specimens of both Old and New World rodents. Both black rats (*R. rattus*) and laboratory/Norway rats photoluminesced brilliant green-blue. However, members of related genera, such as Malayan spiny rats (*Maxomys rajah*) and African multimammate mice (*Mastomys* spp.) did not photoluminesce. North American pocket gophers of the genus *Geomys* photoluminesced (Fig. 2.1), whereas pocket gophers of the genus *Thomomys* did not. Udall et al. (1964) also found that photoluminescence was a useful taxonomic character for distinguishing similar species of African gerbils (Gerbillinae) that would otherwise be challenging to discriminate. Museum

specimens of one species of mouse-eared bat, genus *Myotis*, photoluminesced (Fig. 2.1), but three other species of *Myotis* did not. Live and freshly dead individuals of various Trinidadian bats also showed variation in photoluminescent colours and intensities (Udall et al. 1964).

Kynurenine was also identified as one of the luminophores in the photoluminescent blue fur of Goodfellow's tree-kangaroos (*Dendrolagus goodfellowi*) from New Guinea (Nicholls and Rienits 1971; Fig. 2.1). Tree-kangaroo fur contained an additional tryptophan metabolite, the purple photoluminescing 3-hydroxyanthranilic acid (Watanabe et al. 1972), which also produced photoluminescence in the fur of common brushtail possums (Nicholls and Rienits 1971). Photoluminescent pigments were exuded from follicles into the internal structure of the fur shafts, and similar secretions were also manufactured in a skin gland in the tree-kangaroos. Unpigmented fur of red kangaroos had a moderate blue photoluminescence (Nicholls and Rienits 1971). By this time, photoluminescence was also already known in the fur of other marsupials, such as koalas (*Phascolarctos cinereus*), although Nicholls and Rienits (1971) did not provide specific references for these studies. Nicholls and Rienits (1971) could not completely describe the photoluminescent compounds in the species they investigated, and highlighted that the extent of photoluminescence in marsupials was generally unknown. Australian research into the fur photoluminescence of wildlife species did not progress beyond this study until the 2020s (Reinhold 2020, 2021).

Photoluminescence in mammals was next identified in Brazilian long-nosed short-tailed opossums (*Monodelphis scalops*) (Pine and Abravaya 1978; Fig. 2.1), Virginia opossums (*Didelphis virginiana*) (Meisner 1983) and 21 other opossum species from the Americas (Pine et al. 1985). Opossum fur photoluminesced purple, lavender, blue, yellow-green, pink-orange, salmon, pink, rose and/or red under excitation by 366 nm light. In some specimens, all of the fur exhibited photoluminescence, but more brightly on the ventral surface of the animal, which often photoluminesced a different colour to that of the dorsal surface. In other specimens, the pattern of photoluminescence in fur involved spots or a stripe. Photoluminescence characteristics of some taxa were so consistent to genera and species that they could be used as taxonomic characters (Pine et al. 1985). Photoluminescence of specimens did not differ appreciably with age or season, but in one species, the grey four-eyed opossum (*Philander opossum*), females and adult males photoluminesced, whereas juvenile males did not.

Pine et al. (1985) also used thin-layer chromatography of extracts of photoluminescent blue pigment from the fur of the bare-tailed woolly opossum (*Caluromys philander*) to identify the luminophore as 3-hydroxyanthranilic acid, the same tryptophan metabolite found

in the fur of both common brushtail possums and Goodfellow's tree kangaroos (Nicholls and Rienits 1971). Fluorescence microscopy revealed that the photoluminescence emanated from inside the medulla and cortex of the hair shaft of big lutrine opossums (*Lutreolina crassicaudata*). Pine et al. (1985) examined Australian marsupials and monotremes, but found they did not photoluminesce to the same extent as opossums. Pine et al. (1985) also studied weasels, but in contrast to Latham (1953), ermines photoluminesced, whereas least weasels did not.

Photoluminescence in the hair of Ukranian sportive and Przewalski horses, and Scotch and Estonian ponies (*Equus ferus*), was examined with microfluorometry spectroscopy in 2007 (Posudin 2007; Fig. 2.1). The body hair of the horses photoluminesced with more than twice the intensity of their manes and tails. The photoluminescence of pony hair was less intense than that of horse hair, but their tails were the most photoluminescent. Photoluminescence intensity also depended on coat colour (Posudin 2007).

Humans and production fur animals have continued to be the focus of much of the research on mammal pelage photoluminescence throughout the last century and into this one. The wool of sheep was again recorded phosphorescing, this time blue-cyan when excited by 330–360 nm ultraviolet light, thought to be from N-formylkynurenine (Smith and Melhuish 1985). At least three luminophores are thought to produce phosphorescence in wool, two of which are derived directly or indirectly from tryptophan (Collins 1992). The tryptophan in wool also reacts with α-keto acids, producing β-carbolines that photoluminesce blue and yellow/green when excited by ultraviolet and blue wavelengths (Smith et al. 1994). However, even with the extensive amount of research conducted on sheep's wool, the luminophores have not all been identified with certainty, with even the contribution of N-formylkynurenine in doubt (Millington 2006). Millington (2020) warned that intrinsic blue photoluminescence (Niyangoda et al. 2017; Chen et al. 2018) occurring in wool keratin at similar excitation and emission wavelengths as N-formylkynurenine could not be ruled out.

Using spectrophotometry to match photoluminescence peaks, the tryptophan metabolites kynurenine, *N*-formylkynurenine and 3-hydroxykynurenine were identified as luminophores in human hair (Daly et al. 2009). Hair pigmented with melanin did not photoluminescence as strongly as unpigmented hair, agreeing with Stübel's (1911) initial observation that it was unpigmented human hair that displayed blue and white photoluminescence. However, levels of tryptophan itself are higher in darker human hair (Allegri et al. 1990), with masking by melanin confounding observable photoluminescence as an indicator of the chemical concentrations of luminophores (Rebell 1966). The 330 nm emission of tryptophan metabolite

photoluminescence in human hair was weaker than that of commercial fur animals, such as mink (*Neogale vison*), European rabbit, Cashmere goat (*Capra hircus*) and sheep (Millington 2020).

In 2019, there was a resurgence of interest in the fur photoluminescence of wildlife species with the publication of colour photographs of New World flying squirrels (*Glaucomys* spp.) (Kohler et al. 2019; Tumlison et al. 2019; Fig. 2.1). The squirrels photoluminesced bright pink under 395 nm illumination, mostly on the ventral body surface and tail. Although there was variation between individuals, intensity of photoluminescence could not be clearly divided on species, sex, month, year or latitude. Live animals in the wild photoluminesced comparably to museum specimens, although they were not compared using the same photographic qualitative scale (Kohler et al. 2019). Diurnal non-flying squirrels did not photoluminescence; however, a subsequent study elicited some photoluminescence from both grey squirrel (*Sciurus carolinensis*) and red squirrel (*Tamiasciurus hudsonicus*) fur extracts when excited at 350 nm (Hughes et al. 2022). Twenty unidentified potential luminophores were also found to be present in the fur of non-photoluminescent squirrels (hinting that non-photoluminescent animals carry the potential luminophores, but they are only observed in photoluminescent animals) and were inconsistently present in the fur of all photoluminescent flying squirrels (Hughes et al. 2022). Excitation spectroscopy identified only porphyrin S-411 in the fur of *Glaucomys* spp. (Toussaint et al. 2023). Pink photoluminescence was also found later in the red-cheeked flying squirrel (*Hylopetes spadiceus*) and the smoky flying squirrel (*Pteromyscus pulverulentus*) of south-east Asia (Toussaint et al. 2023).

In addition, photographs of light-blue photoluminescence in the fur of Coxing's white bellied rats (*Niviventer coninga*) and the scales of Chinese pangolins (*Manis pentadactyla*) (Fig. 2.1) came from Taiwan (Jeng 2019). In the following year, photoluminescence was identified in platypuses (*Ornithorhynchus anatinus*) (Reinhold 2020; Anich et al. 2021; Fig. 2.1), antechinus (*Antechinus* spp.), northern brown bandicoots (*Isoodon macrourus*) and long-nosed bandicoots (*Perameles nasuta*) in Australia (Reinhold 2020; Fig. 2.1). The list was soon extended to include striped possums, Krefft's gliders (*Petaurus notatus*), mosaic-tailed rats (*Melomys* spp.) and bush rats (*Rattus fuscipes*) (Reinhold 2021). Photoluminescence was absent from the skin and whiskers in these species. The live, wild ground-dwelling mammals from these studies photoluminesced either brindled bright pink or blueish white all over their fur, whereas in arboreal gliders, the mild blueish-white photoluminescence was confined to their ventral surfaces (Reinhold 2020, 2021).

Attention returned to Africa with the discovery of photoluminescence in the fur cuticle of two species of springhares (*Pedetes* spp.) (Olson et al. 2021; Fig. 2.1). Both live and museum animals displayed patchy orange-red photoluminescence, although this photoluminescence had a greater intensity in live animals. There was no sexual dichromatism of photoluminescence, and patterns were consistent over time for an individual. Thin-layer chromatography and high-performance liquid chromatography (HPLC) of photoluminescent red fur extracts of the South African Springhare (*P. capensis*) identified some of the luminophores as porphyrins (uroporphyrin I, uroporphyrin III, heptacarboxylporphyrin and coproporphyrin I; Olson et al. 2021).

Excitation spectroscopy also identified uroporphyrin I or uroporphyrin III in the pink-red fur of the Guyanan short-tailed opossum (*Monodelphis brevicaudata*) and Linnaeus' mouse opossum (*Marmosa murina*) (Toussaint et al. 2023). HPLC, ultraviolet-visible spectral and electrospray ionisation mass spectrometry analyses confirmed European hedgehog luminophores as coproporphyrin III, uroporphyrin III and protoporphyrin IX (Hamchand et al. 2021). Hamchand et al. (2021) suspected that Actinobacteria in the spine microbiome of European hedgehogs could be producing the porphyrin photoluminescence. However, the porphyrin photoluminescence in European hedgehog spines is distributed in the walls of the inner lumen, a pattern inconsistent with commensal bacteria (Toussaint et al. 2023).

One of the most recent studies reported fur photoluminescence under ultraviolet light excitation (385–395 nm) in several mammal species from Arkansas, North America (Tumlison and Tumlison 2021). Based on museum specimens, the study examined dry pelts, alcohol-preserved and an untreated frozen specimen of the Eastern mole (*Scalopus aquaticus*). All specimens of this species across preservation techniques photoluminesced similarly greenish. In dry pelts of southern short-tailed shrews (*Blarina carolinensis*), the tips of the fur photoluminesced greenish, whereas the underfur of muskrats (*Ondatra zibethica*) photoluminesced yellow-green (Tumlison and Tumlison 2021; Fig. 2.1). Two additional rabbit species, eastern cottontails (*Sylvilagus floridanus*) and swamp rabbits (*S. aquaticus*), displayed small amounts of cyan photoluminescence (Tumlison and Tumlison 2021). However, the fur of the mountain hare (*Lepus timidus*) did not photoluminesce (Toussaint et al. 2023).

Tumlison and Tumlison's (2021) documentation of mammal species that did not photoluminesce was particularly informative. While not settling the debate on which of least weasels and ermines is the photoluminescent species (Latham 1953; Pine et al. 1985), Tumlison and Tumlison (2021) established that, within long-tailed weasels with brown

summer pelage, one specimen photoluminesced greenish, whereas the other specimen did not photoluminesce. Therefore, intraspecies variation may account for the previous conflicting observations, but larger sample sizes in the previous studies should have detected such an anomaly. Toussaint et al.'s (2023) observations of lavender photoluminescence in ermines (white winter pelage) agreed with those of Pine et al. (1985). Latitudinal differences in seasonal coat phases may have played a role in the original discrepancy.

American mink fur did not photoluminesce (Tumlison and Tumlison 2021), whereas Millington's (2020) mink fur did; however, Millington (2020) examined white mink fur, whereas Tumlison and Tumlison's (2021) minks were brown. Raccoons (*Procyon lotor*), red foxes (*Vulpes vulpes*), grey foxes (*Urocyon cinereoargenteus*), coyotes (*Canis latrans*) and bobcats (*Lynx rufus*) also did not photoluminesce (Tumlison and Tumlison 2021). Furthermore, while laboratory/Norway rats photoluminesced, 11 other species of rodent did not. Wild-caught brown to greyish (Renn Tumlison, Henderson State University, Arkadelphia, Arkansas, personal communication, March 2022) house mice did not photoluminesce, giving broader substantiation to Rebell's (1966) observation of non-photoluminescence in captive-bred albino mice.

The absence of photoluminescence in the eight species of bat examined by Tumlison and Tumlison (2021) is not surprising given that Udall et al. (1964) found photoluminescence in only one of four *Myotis* species preserved as museum specimens. However, Udall et al. (1964) had found bats to be particularly photoluminescent when examining live and freshly dead mammals. Tumlison and Tumlison (2021) likewise found none of Brennan's (1917) live-animal luminescence in museum specimens of striped skunks (*Mephitis mephitis*) or spotted skunks (*Spilogale putorius*).

The observed lack of photoluminescence in the fur of Baird's pocket gopher (*Geomys breviceps*) (Tumlison and Tumlison 2021) sets this species apart from both Udall et al.'s (1964) and Pynne et al.'s (2021) records of photoluminescence in other *Geomys* species. Pynne et al. (2021) suggested that the orange-pink photoluminescence in the fur of five species of pocket gophers, including species of *Cratogeomys*, *Geomys* and *Thomomys*, could be a consequence of bacteria, sequestration from eating photoluminescent blue roots, or a result of orange-pink photoluminescent soil adhering to the fur. The anomaly of *Thomomys* spp. photoluminescence, as observed by Pynne et al. (2021) but not by Udall et al. (1964), indicates that the sporadic observations documented so far are not yet adequate to piece together a taxonomic pattern of photoluminescence in pocket gophers, nor in other mammalian taxa.

Box 2.1. Description of the two known luminophore groups for fur.

Tryptophan metabolites

Tryptophan is an essential amino acid, meaning that mammals are unable to synthesise it endogenously and must obtain it from their diet (Yao et al. 2011). Tryptophan is metabolised in tissues via well-defined steps under enzymatic control (Nicholls and Rienits 1971), producing a suite of molecules that photoluminesce in various colours (Pine et al. 1985). Tryptophan metabolism can be affected by steroid hormones or an excess of tryptophan in the diet, so the resulting luminophores have the potential to vary with sex, hormone cycles and diet (Pine et al. 1985). Various tryptophan metabolites in fur that give rise to visible photoluminescence have absorption peaks of ~320 nm (brushtail possums, Nicholls and Rienits 1971), 358 nm (laboratory rats, Rebell 1966) and 380 nm (mink fur, rabbit fur, cashmere wool, sheep's wool and human hair, Millington 2020). Some tryptophan metabolites present in pelage can emit *phosphorescence* as well as *fluorescence* (Leaver 1978; Smith and Melhuish 1985).

Porphyrins

Porphyrins are large heterocyclic organic molecules that act as precursors to haemoglobin and can be synthesised internally by biological organisms (Neves and Galván 2020). Porphyrins can produce red, pink, brown and green pigmentation in birds, and a reddish photoluminescence in general (Hudon 2005; Riedler et al. 2014). Additionally, porphyrins function as chlorophyll intermediates in plant photosynthesis (Lee et al. 2018), and dietary chlorophyll can break down into porphyrin-based compounds that transfer into red photoluminescence in the skin of mice (Weagle et al. 1988; Croce 2021).

Porphyrins have an absorption maxima around 400–405 nm (Goldoni 2002; Huang et al. 2010; Plavskii et al. 2018), only slightly longer than the 395 nm emission wavelength of the most common modern ultraviolet flashlights (Kohler et al. 2019; Pynne et al. 2021). Lesser absorption bands extend up to 700 nm in the visible spectrum (Goldoni 2002). This means that the optimal excitation wavelengths for porphyrin photoluminescence are higher than those for tryptophan metabolites. Porphyrins are also weakly *phosphorescent*, some with afterglows of 70 s and longer being recorded (Gouterman and Khalil 1974). The near-infrared phosphorescence of these porphyrins, however, is excited by wavelengths of 485–633 nm (cyan-yellow-orange) (Gouterman and Khalil 1974).

2.4 A cautionary note on photoluminescence in museum specimens

Many of the recent studies on mammalian photoluminescence have been based on preserved museum specimens (e.g., Kohler et al. 2019; Anich et al. 2021; Tumlison and Tumlison 2021; Toussaint et al. 2023). While an invaluable resource, museum specimen photoluminescence should be verified by fresh material where possible. In particular, photoluminescence can fade over time with or without exposure to light (Pine et al. 1985; Olson et al. 2021; Tumlison and Tumlison 2021), and porphyrins in museum specimens are often not detectable (Hill 2010). As a result, absence of, or quantitative comparisons between, porphyrin photoluminescence of specimens cannot be made with certainty.

In addition, while the loss of fur photoluminescence that occurs during various chemical preservation procedures has not been quantified, these procedures could drastically affect luminophores and their resulting photoluminescence (Tumlison and Tumlison 2021). Artificial photoluminescent stains, particularly bright greenish or yellowish, are also sometimes inadvertently added to museum specimens during taxidermy (Pohland 2007). X-ray fluorescence toxin testing by some museums routinely uncovers methyl bromide, and to a lesser extent, mercury (Kehoe and Becker 2017), both of which emit green photoluminescence (BOC Sciences 2022; Department of Physics, Imperial College 2022). If the photoluminescence of museum specimens is more vivid, or covers different areas, to that of fresh animals, contamination should be suspected and tested for.

2.5 Summary

Photoluminescence in fur was first observed in the 1700s, by de Mairan (1715) (cited in Harvey 1957) and Wilson and Beccari (1775). Since Stübel (1911), photoluminescence has been described in detail for numerous species from half of all mammal orders. Wool has been documented both fluorescing and phosphorescing (Collins 1992). The most comprehensive work on wildlife species was the description of photoluminescence in opossum fur in 1985 (Pine et al. 1985). Until 2019, vividly photoluminescent pelage was already known from mammals such as rabbits, possums, tree-kangaroos, opossums, weasels, rats, bats, humans and sheep. Since the publication of colour photographs of photoluminescent flying squirrels in 2019 (Kohler et al. 2019), the accessibility of the internet and availability of ultraviolet flashlights has led to an increasing number of mammal species being documented with photoluminescent fur.

Animal tissues in general, including keratin proteins (Stübel 1911), exhibit a low-level photoluminescence, but some chemical compounds present in fur generate an additional bright photoluminescence. Photoluminescence differs in degrees of brightness (Hirst 1927), making a definition of how bright a mammal's fur has to be (over and above that of the generic background glow) to be termed photoluminescent, or when luminescent molecules can be classified as luminophores, difficult. Luminophores can be incorporated into fur at the follicle (Nicholls and Rienits 1971; Pine et al. 1985) and can reside in different sections of the fur shaft (Pine et al. 1985; Olson et al. 2021). Two classes of luminophore are currently known to cause photoluminescence in mammal fur: tryptophan metabolites, and porphyrin and its derivatives. Tryptophan metabolites photoluminesce in a rainbow of colours (Pine et al. 1985), while porphyrins in mammal fur photoluminesce pink-orange-red (Olson et al. 2021). Both porphyrins and tryptophan metabolites already have important physiological functions within the body (Richard et al. 2009; Neves and Galván 2020). Few species of mammal have had the chemical composition of their fur analysed (Nicholls and Rienits 1971; Olson et al. 2021), so more luminophores may be active than are currently known.

The colours and patterns of photoluminescence mostly seem to be species-specific. Intraspecies variation is not correlated with any particular factor (e.g. flying squirrels, Kohler et al. 2019; springhares, Olson et al. 2021) apart from some species with sexual dichromatism (e.g. common brushtail possums, Bolliger 1944; grey and black four-eyed opossums, Pine et al. 1985). Why the fur of some species contains luminophores at orders of magnitude greater than others, and why equal amounts of luminophores are incorporated into fur whether or not the resulting photoluminescence will be quenched by melanin, is unknown (Rebell 1966). As to why so many species of mammal photoluminesce, and whether the incorporation of luminophores into fur serves a visual purpose (Kohler et al. 2019), is a by-product of some metabolic process (Toussaint et al. 2023), or is a largely dormant property incidental to functions of fur chemistry (Stübel 1911), awaits investigation.

What we have learned from 111 years of investigations into photoluminescent fur can be used as a basis for the next wave of research. Big picture research can look for patterns of which species have levels of luminophores in their fur over and above the background levels found in fur in general. Quantification of luminophores per gram of fur (Rebell 1966) will enable comparisons between species. Much more needs to be done at the molecular level to determine the causes of photoluminescence and the conditions in which potential luminophores are activated. More detailed research can concentrate on the metabolic pathways that incorporate the property of brilliant photoluminescence into fur.

The existence of a visual function for nocturnal-crepuscular mammals seems to be the overriding speculative conclusion of current research on fur photoluminescence (Kohler et al. 2019). Measurements of whether the photoluminescence of fur can be excited by natural twilight or moonlight could be carried out to establish the photophysical basis for experimentation on potential visual function. Visual function behavioural experiments would then be the most urgent focus of future research. If a visual function is demonstrated, then it opens up a whole new field of study into terrestrial photoluminescence. However, if visual function is not supported, then future research into the optical properties of fur photoluminescence seems obsolete.

A promising avenue of future enquiry may instead be to determine correlations between photoluminescence and individual condition. In some birds, pink photoluminescence (Camacho et al. 2019), or the concentration of coproporphyrin III in feathers (Galván et al. 2018), is correlated with body condition. Although apparently tied to species-specific metabolism, the photoluminescence of mammals may also be individually affected by diet or disease. An accumulation of porphyrins in mammals may not necessarily be from porphyria, but an indication of iron deficiency (Needham 2012). Chemical analysis of hair can be used to determine nutrient deficiencies in the body (Wołowiec et al. 2013; Jaworski et al. 2016). If a nutrient deficiency can be cursorily diagnosed by photoluminescence, it may become an efficient and non-invasive diagnostic tool to adjust the diets of animals in captivity. Ultraviolet light is already routinely used to diagnose bacteria (Hurley et al. 2019) and fungal infection (Chattaway and Barlow 1954; Turner et al. 2014). Tryptophan levels in human hair have already been correlated with sex and age (Allegri et al. 1990; Bertazzo et al. 2000), and spectroscopy of tryptophan photoluminescence could indicate deficiency of this important amino acid. However, quenching by melanin or other conditions of the fur microenvironment may mean that the concentration of luminophores occurring in fur cannot necessarily be measured with an ultraviolet torch or spectroscopically. If a relationship can be established for some luminophores in some hair types, then perhaps not just a shiny coat, but a photoluminescent coat, can be used as an indicator of health.

Chapter 3

Prevalence of photoluminescent fur in mammals of the Wet Tropics

3.1 Abstract

Photoluminescence, or 'biofluorescence', in an increasing variety of terrestrial animals has become a recent focus of media attention. However, systematic investigations into the overall occurrence of the phenomenon in a particular subset of mammals or habitats are rare, are usually based on museum specimens, and have not been attempted in Australia. Carcasses of 128 fresh, old or frozen wild mammals (mostly roadkill) from the Wet Tropics of North Queensland, Australia, were examined for the presence of photoluminescence in their fur. Two species of monotreme, 19 species of marsupial and 17 species of placental mammal were collected. Torches of different wavelengths were used to assess the presence of photoluminescence. All specimens were photographed. Both species of monotreme yielded a dull green colour change when viewed under 380–410 nm wavelengths, with the platypus (*Ornithorhynchus anatinus*) displaying some pink at 395–410 nm. All marsupials displayed some photoluminescence when viewed under 310–410 nm wavelengths, but less than half brightly so (to the extent that the glow stood out vividly compared to background photoluminescence). Marsupials that showed at least traces of pink photoluminescence excited at higher wavelengths (380–410 nm) generally also had blueish photoluminescence excited at lower wavelengths (310–380 nm). Two genera of placental mammals showed brilliant blue-white photoluminescence, with another two genera revealing traces of pink. These findings bring into context the sporadic observations of fur photoluminescence to date, and highlight the trait as near-ubiquitous in mammals, only differing in the degree of intensity and colouration.

3.2 Introduction

Photoluminescence has been recorded in the fur of half of all mammal orders. In terrestrial mammals, some studies have comprehensively surveyed fur photoluminescence in a single taxonomic group (Pine et al. 1985), or have taken a broad-ranging look across specimens in museums (Udall et al. 1964; Tumlison and Tumlison 2021; Toussaint et al. 2023). However, photoluminescence has often been identified in species opportunistically rather than systematically. This has led to speculation over the intensity and occurrence of photoluminescence in mammals more broadly. A targeted approach at investigating photoluminescence in mammals of various taxa from the same region will provide important insights into whether the perceived rarity of the trait is real, or an artefact of the majority of mammal species not having been surveyed.

Targeted studies may also indicate whether photoluminescent fur is species-specific, sexually dimorphic or varies with habitat or lifestyle characteristics. While photoluminescent fur has already been proposed as a useful taxonomic characteristic for closely related species (Latham 1953; Udall et al. 1964), changes in photoluminescence because of diet or body condition could provide an indicator of health or reproductive status (Galván et al. 2016; Galván et al. 2018; Camacho et al. 2019). High concentrations of porphyrins in the fur could signal a way of excreting them and preventing toxic levels from building up in the body (Toussaint et al. 2023). Some captive animals develop porphyria as a disease, expressing the excess molecules in their skeletons and urine, but wild vertebrates seem to be able to regulate porphyrin accumulation without ill effect (Neves and Galván 2020). If vividly photoluminescent fur, or the lack thereof, is found to indicate habitat quality, nutrient deficiency, or a build-up of toxins, it could be used for providing recommendations for the management of these animals in captivity, or could allow for the development of hypotheses on an ecological role for photoluminescence in wild mammals.

Some attempts have been made at fitting patterns of photoluminescent mammal life history characteristics into selected datasets. For example, Gray and Karlsson (2022) found that all of the photoluminescent mammals in their analysis were nocturnal. However, they excluded known diurnal photoluminescent species, such as sheep (*Ovis aries*, Smith et al. 1994). Gray and Karlsson (2022) also concluded that the dominant photoluminescent colours in the fur of mammals are pink and purple. However, they excluded many species that are known to photoluminesce in different colours, such as rats (*Rattus* spp., Udall et al. 1964).

Using fresh or frozen material is essential for documenting natural photoluminescent colours. Some luminophores may otherwise degrade from exposure to light (i.e. porphyrins, Hill 2010) or by undergoing museum preservation processes (Tumlison and Tumlison 2021). Artificial photoluminescent stains are also sometimes inadvertently added to dry specimens during taxidermy (Pohland 2007). Therefore, a reliance on specimens with an unknown history of treatments may not yield reliable results. Some recent studies with sample sizes of 18 (Toussaint et al. 2023) to 37 (Tumlison and Tumlison 2021) species of mammal have been based almost entirely on museum specimens.

Photoluminescence in Australian mammals was largely overlooked until recently (Reinhold 2020, 2021). The photoluminescent pelage of one Australian marsupial (common brushtail possum, *Trichosurus vulpecula*) was described in detail last century (Bolliger 1944; Nicholls and Rienits 1971). However, it is seldom cited in the current literature. Bolliger (1944) had stated that long-nosed bandicoots (*Perameles nasuta*) were non-photoluminescent, and examples of Australian marsupials and monotremes examined by Pine et al. (1985) did not show a similar intensity of photoluminescence to didelphid marsupials. The literature is inconsistent regarding Australian mammals, and particularly lacking in examination of fresh specimens that have not been exposed to light, fumigated or undergone other taxidermy treatments. Therefore, I investigated how widespread and varied the phenomenon of photoluminescent fur is in a subset of mammal taxa from the Wet Tropics region of Far North Queensland, Australia. The study presented here represents the largest documented cross-taxa examination of photoluminescence in fresh and frozen mammal material to date.

3.3 Methods

A total of 53 individual mammals were sourced as roadkill between August 2021 and November 2022 from within a two-hour drive of the city of Cairns in Far North Queensland. Nine dedicated night and pre-dawn (8:30pm–8:30am) collecting trips, each lasting between 2 and 6.5 hours, and covering from 56 to 256 km, were conducted. Pre-dawn gave the greatest length of time for roadkills to accumulate, and to access them before the sun caused photobleaching. Animals were also sampled opportunistically (dawn, day, or night) in the Wet Tropics region over this same period. At the site of each roadkill, I recorded the GPS coordinates, broad-scale habitat, time, weather, species, sex and which way up (dorsal, ventral, lateral) the animal was facing. When roadkill animals were found at night, they were

often moved to a safe place off the road and then examined on site. If animals were found nearing and in daylight hours, whole carcasses were usually taken and transported wrapped in labelled aluminium foil and plastic bags in an insulated cold box (onto a Styrofoam layer over freezer gel packs) until they could be taken to a dark laboratory on the James Cook University (JCU) Nguma-bada campus or a garage at my residence to be examined and photographed.

A total of 75 individual mammals were also examined from local freezer stores at the Atherton Queensland Parks and Wildlife office (QPWS, n = 15), the JCU Nguma-bada campus (n = 52) and the Tolga Bat Hospital (n = 8). Rangers, researchers, landholders, and other members of the public bring carcasses (from car strikes, dog attacks or barbed wire fences) into these institutions. Most of the animals from the JCU freezers did not have location data, but were assumed to be from the Wet Tropics region. Specimens that had intact skulls, date and location data were lodged with the Queensland Museum. There were no data on length of time of exposure to sunlight, or which side up they had been facing, prior to collection. Therefore, the amount of post-mortem luminophore degradation is unknown for these specimens. From all collection methods combined, a total of 128 individual mammals from 38 species wild to the Wet Tropics were examined for their external photoluminescence.

Animals were identified to species using field guides (Covacevich and Easton 1974; Churchill 1998; Menkhorst and Knight 2011). Frozen specimens were thawed before examination (n = 29) except for those that were required for other research (n = 46). The pelage of each animal was examined for photoluminescence at several wavelengths using a range of ultraviolet, violet and blue torches (Appendix A). I qualitatively described the reaction of the fur to light of each wavelength. Intensely vivid photoluminescence was classed as ‘bright’, and photoluminescence that was almost imperceptible, or equivalent to the level expected by keratin alone, was classed as ‘subtle’ or ‘mid’, in relation to the brightest photoluminescence. There was a continuum between the two extremes of ‘none’ and ‘bright’. See Appendix B for a visual comparison of qualitative intensity classification.

Photoluminescence was usually less bright at 310 nm than at 365 nm excitation, thus descriptions from this band of wavelengths are dominated by the photoluminescence excited at 365 nm. Where photoluminescence was noticeable, brighter or elicited a different colour at 310 nm, it is detailed as such in Appendix C. The 470 nm blue light torch generally did not elicit photoluminescence. When animals photoluminesced in shades of pink or orange, these

were invariably brighter at the higher excitation wavelengths centred around 395 nm. Blue-coloured photoluminescence was generally brightest at 365 nm.

I recorded observations of photoluminescence directly from the carcasses, not from photographs. Due to the variable nature of the torch distance and battery power, photographs act only as a qualitative record to visually document these observations, and not a way to quantitatively compare photoluminescence between animals. All photographs were taken with a Panasonic Lumix TZ-80 camera and not enhanced by filtering or post-processing. Photography was conducted on whole animals, with the background varying from dirt/gravel or grass by roadsides, the plastic bags they had been frozen in for messier specimens in the laboratory, or blackout curtain material where possible. Whether the sides of the animals photographed were dorsal and ventral or left and right lateral depended on the position the animal had stiffened into during rigor mortis. A CrimeTech 8 cm L-shaped greyscale ruler was placed in the corner of each photograph for scale. The ruler also acted as a standard for light reflection. Unless specified, the appearance of purple in the photographs is from reflected violet torchlight. Depending on the brightness of the torch and the surface area of the animal, the camera lens was open for 5–30 seconds, as each ultraviolet torch scanned the animal. Torch beams were shone over the animal just out of sight of the camera lens, or further away for the brighter torches. Other camera settings were automatic. Close-up photos of photoluminescence amongst the fur were usually taken with automatic exposure times.

This study only aimed to determine the prevalence of photoluminescent fur across mammal taxa of the Wet Tropics. If the comparative intensity of photoluminescence is found to be important for visual signalling, or indicative of luminophore concentrations in the fur, future studies could use calibrated photography (Troscianko and Stevens 2015; van den Berg et al. 2020; Hakanson et al. 2022) to compare individuals. However, the use of a fixed broad-spectrum light source was impractical for the current study. Additionally, the three-dimensional nature of both the rounded body surface (Johnsen 2016), and the thousands of tapered cylindrical fur shafts that comprise the pelt of a mammal, make standardised comparison problematic. Brindling, and differing photoluminescence along the fur shafts, further complicates the uniformity of colour. In life, varying photoluminescence may be displayed as an animal bristles its fur or turns in relation to the viewer, but in death, how bristled the fur is may depend on the stuffing of a museum specimen or the flattening of a roadkill. Standardised photography also only measures colour change and saturation, not the light re-emitted by the ‘glow’, which is problematic for whitish photoluminescence.

3.4 Results

Thirty-eight species of mammal (Table 3.1) were identified from roadkill and freezer specimens. Both species of monotreme (Table 3.1) displayed a faint green photoluminescence in some of their fur (Appendix C). One of the platypuses (*Ornithorhynchus anatinus*), also had some mild pink photoluminescence (Fig. 3.1). The pink had not been apparent when viewing the animal as a whole, only on closer inspection of the fur once it had been clipped.

All marsupials displayed some level of photoluminescence in their fur when exposed to ultraviolet or violet excitation wavelengths (Appendix C). Forty-two per cent of marsupials demonstrated striking photoluminescence (either pink and/or blue-white). Only the northern quolls (*Dasyurus hallucatus*) and both bandicoot species (northern brown bandicoots (*Isoodon macrourus*) and long-nosed bandicoots) displayed stunningly bright pink fur (Fig. 3.2). The brushtail possums (*T. johnstonii* and *T. vulpecula)* and the bettongs (rufous bettongs (*Aepyprymnus rufescens*) and northern bettongs (*Bettongia tropica*)) also photoluminesced pink, but in a softer hue. Seventy-four per cent of marsupial species had at least some pink or orange photoluminescence in their fur when excited at higher wavelengths. Reddish or pink colouration was never observed in the teeth, exposed bones, or skin of the animals. Of the marsupials (Table 3.1), the antechinuses (*Antechinus* spp.), striped possums (*Dactylopsila trivirgata*) and Lumholtz's tree-kangaroos (*Dendrolagus lumholtzi*) showed a brilliant blue-white photoluminescence (Fig. 3.3). Although paler fur often yielded the brightest glow, fur that was a regular brown colour in white light also often yielded brilliant photoluminescence, only being muted where the coverage of melanin was darkest.

Out of 17 placental species, 29% had brightly photoluminescent fur (Table 3.1). Only the three *Rattus* species and the delicate mouse (*Pseudomys delicatulus*) showed all-over brilliant photoluminescence (Fig. 3.4), and the eastern tube-nosed fruit bat (*Nyctimene robinsoni*) had reasonably bright fur (Fig. 3.5). The fur photoluminescence of the other placental species tended to be subtle. Two lacked observable photoluminescence of the fur: the dingo (*Canis lupus dingo*) and Gould's wattled bat (*Chalinolobus gouldii*, Fig. 3.5). Twenty-three per cent of wild placental mammal species, confined to the introduced European rabbit (*Oryctolagus cuniculus*) and the native non-*Rattus* rat species, had traces of pink or orange photoluminescence in their fur, but none as bright as the marsupials. Appendix C records detailed observations for each taxon.

Table 3.1. Representation of the photoluminescent colours elicited from the fur of roadkill and frozen mammal specimens of the Wet Tropics. Combining photoluminescent excitation wavelengths of 310–410 nm. Vividness is whole-animal impression. Black represents absence of photoluminescence. Visual observations were made directly from the carcasses.

Order	**Family**	**Species**	**Vividness**	**Colour**				
				Pink-orange	Yellow	Green	Blue-white	Silver-grey
Monotremes								
Monotremata	Ornithorhynchidae	*Ornithorhynchus anatinus*	subtle					
	Tachyglossidae	*Tachyglossus aculeatus*	subtle					
Marsupials								
Dasyuromorphia	Dasyuridae	*Antechinus adustus*	**bright**					
		Dasyurus hallucatus	**bright**					
		Sminthopsis sp.	subtle					
Peramelemorphia	Peramelidae	*Isoodon macrourus*	**bright**					
		Perameles nasuta	**bright**					
Diprotodontia	Acrobatidae	*Acrobates pygmaeus*	subtle					
	Macropodidae	*Dendrolagus lumholtzi*	**bright**					
		Thylogale stigmatica	mid					
		Wallabia bicolor	subtle					
	Petauridae	*Dactylopsila trivirgata*	**bright**					
		Petaurus notatus	subtle–mid					
	Phalangeridae	*Trichosurus johnstonii*	mid–**bright**					
		Trichosurus vulpecula	mid–**bright**					
	Potoroidae	*Aepyprymnus rufescens*	subtle–mid					
		Bettongia tropica	subtle–mid					
	Pseudocheiridae	*Petauroides volans*	subtle					
		Pseudochirops archeri	subtle–mid					
		Pseudocheirus peregrinus	subtle–mid					
		Pseudochirulus herbertensis	subtle–mid					

Placentals				
Lagomorpha	Leporidae	*Oryctolagus cuniculus*	mid	
Rodentia	Muridae	*Mesembriomys gouldii*	subtle	
		Pogonomys mollipilosus	subtle	
		Pseudomys delicatulus	**bright**	
		Rattus fuscipes	**bright**	
		Rattus rattus	**bright**	
		Rattus tunneyi	**bright**	
		Uromys caudimaculatus	subtle	
Chiroptera	Pteropodidae	*Nyctimene robinsoni*	mid–**bright**	
		Pteropus conspicillatus	subtle	
		Pteropus scapulatus	subtle	
	Vespertilionidae	*Chalinolobus gouldii*	none	
		Miniopterus schreibersii	subtle	
		Mormopterus ridei	subtle	
		Rhinolophus megaphyllus	subtle	
		Vespadelus sp.	subtle	
Carnivora	Canidae	*Canis lupus dingo*	none	

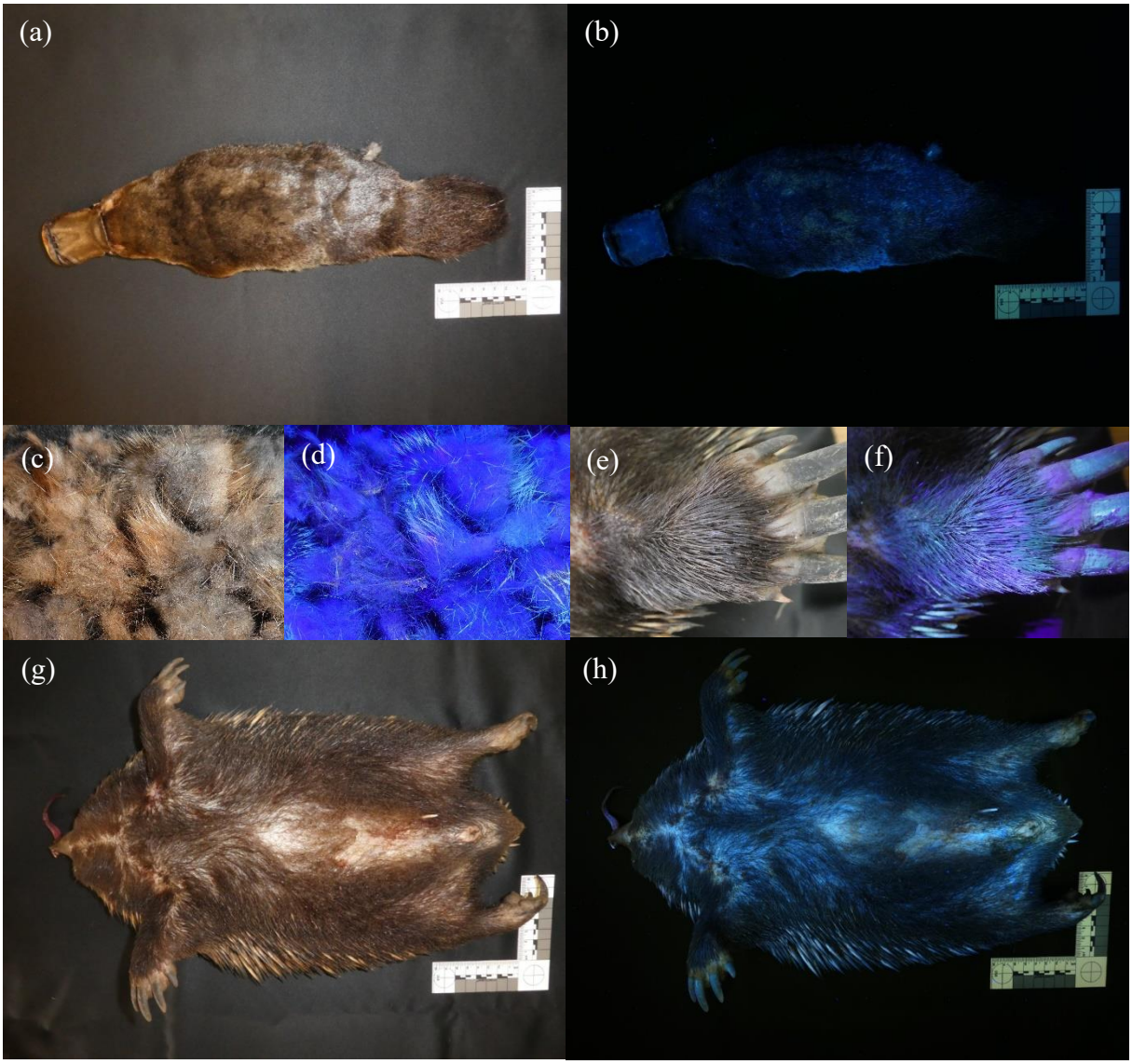


Figure 3.1. Photoluminescence of monotremes. (a) Female platypus (*Ornithorhynchus anatinus*) frozen-thawed, dorsal, with white flash and (b) displaying faint photoluminescence under 395–410 nm torchlight (20 s exposure). (c) Close-up of clipped fur, with white flash and (d) showing mild pink photoluminescence along with green/blue under 395–410 nm torchlight (6 s exposure). (e) Short-beaked echidna (*Tachyglossus aculeatus*) roadkill, close-up of highly keratinised fur on front paw, with white torchlight and (f) showing faint green photoluminescence under 395–410 nm torchlight (automatic exposure). The purple colour is reflection from the torch. (g) Ventral, with white flash and (h) displaying blue photoluminescence of skin near the cloaca under 365 nm torchlight (20 s exposure).

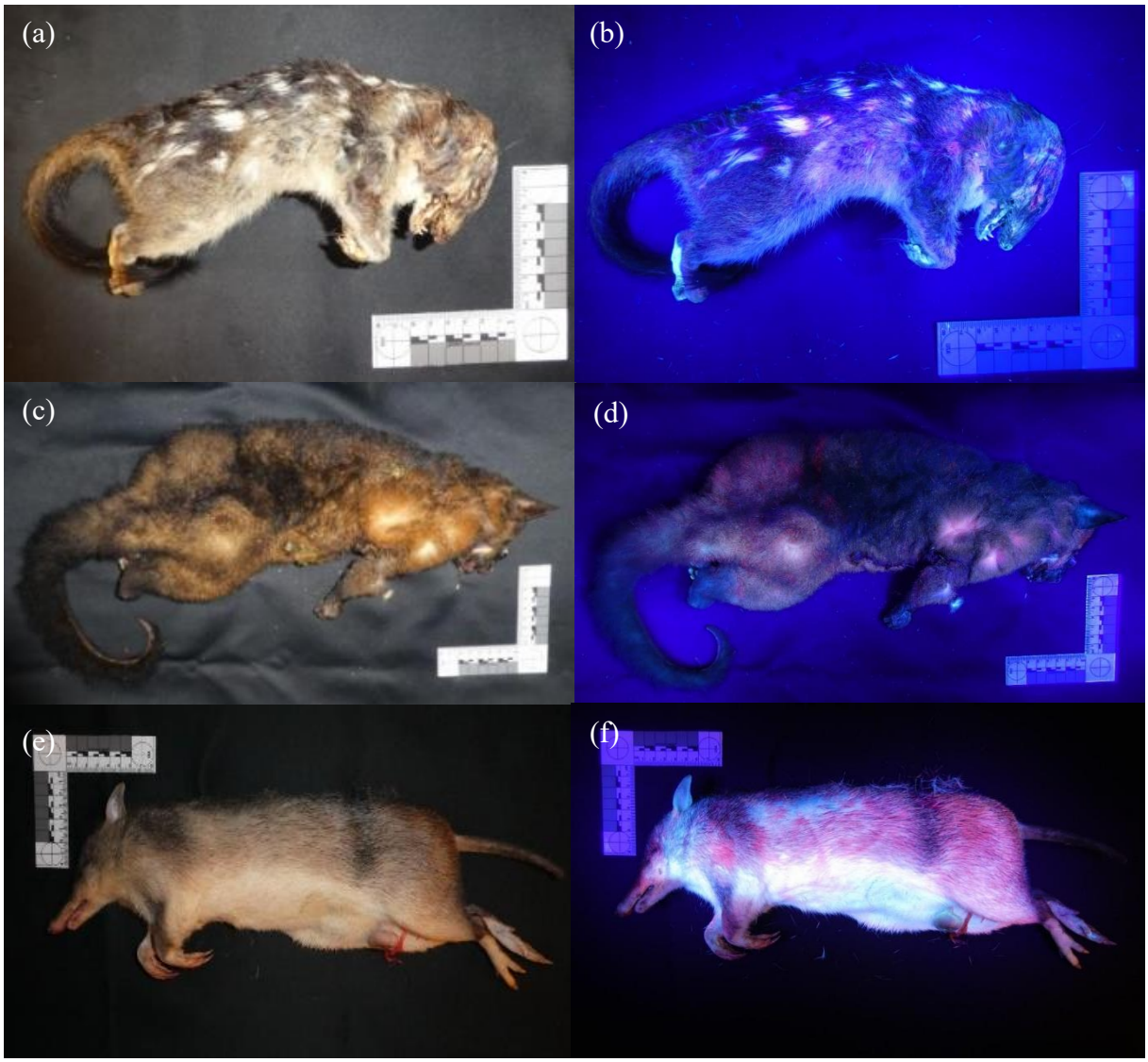


Figure 3.2. Pink photoluminescent marsupials. (a) Male northern quoll (*Dasyurus hallucatus*) frozen-thawed, right flank, with white flash and (b) under 395–410 nm torchlight (10 s exposure). (c) Female coppery brushtail possum (*Trichosurus johnstonii*) roadkill, dorsal/right flank, fur parted, with white flash and (d) under 395–410 nm torchlight (10 s exposure). (e) Male long-nosed bandicoot (*Perameles nasuta*) roadkill, left flank/ventral, with white flash and (f) under 395 nm torchlight (10 s exposure). Showing strong pink, white and blue photoluminescence on the same animal.

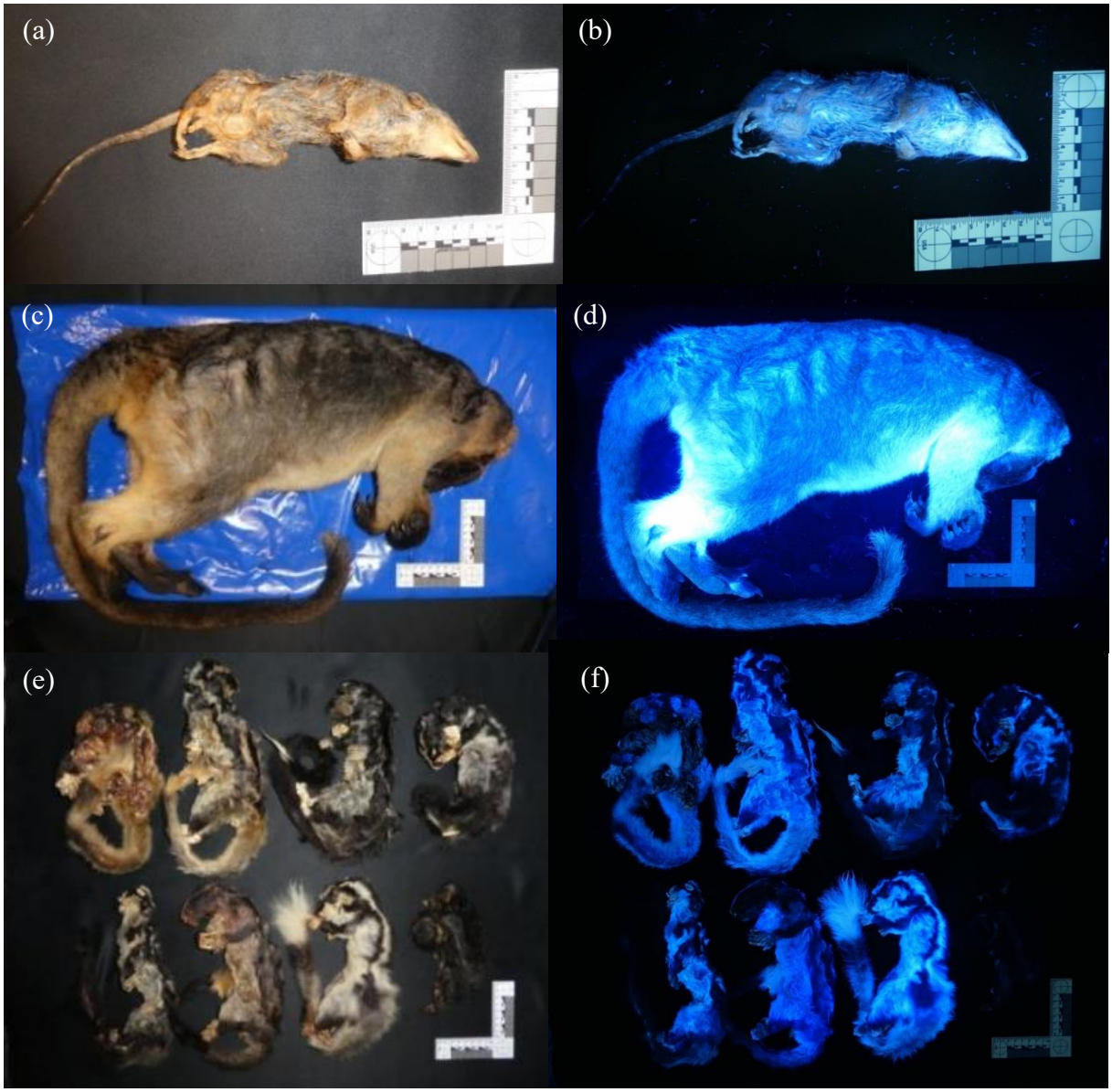


Figure 3.3 Blue-white photoluminescent marsupials. (a) Male antechinus (*Antechinus* sp.) frozen-thawed, ventral, with white flash and (b) under 365 nm torchlight (15 s exposure). (c) Male Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*) frozen-thawed, right flank, with white flash and (d) under 365 nm torchlight (20 s exposure). (e) Male striped possums (*Dactylopsila trivirgata*) × 8 frozen-thawed, left flanks, with white flash and (f) under 365 nm torchlight (30 s exposure).

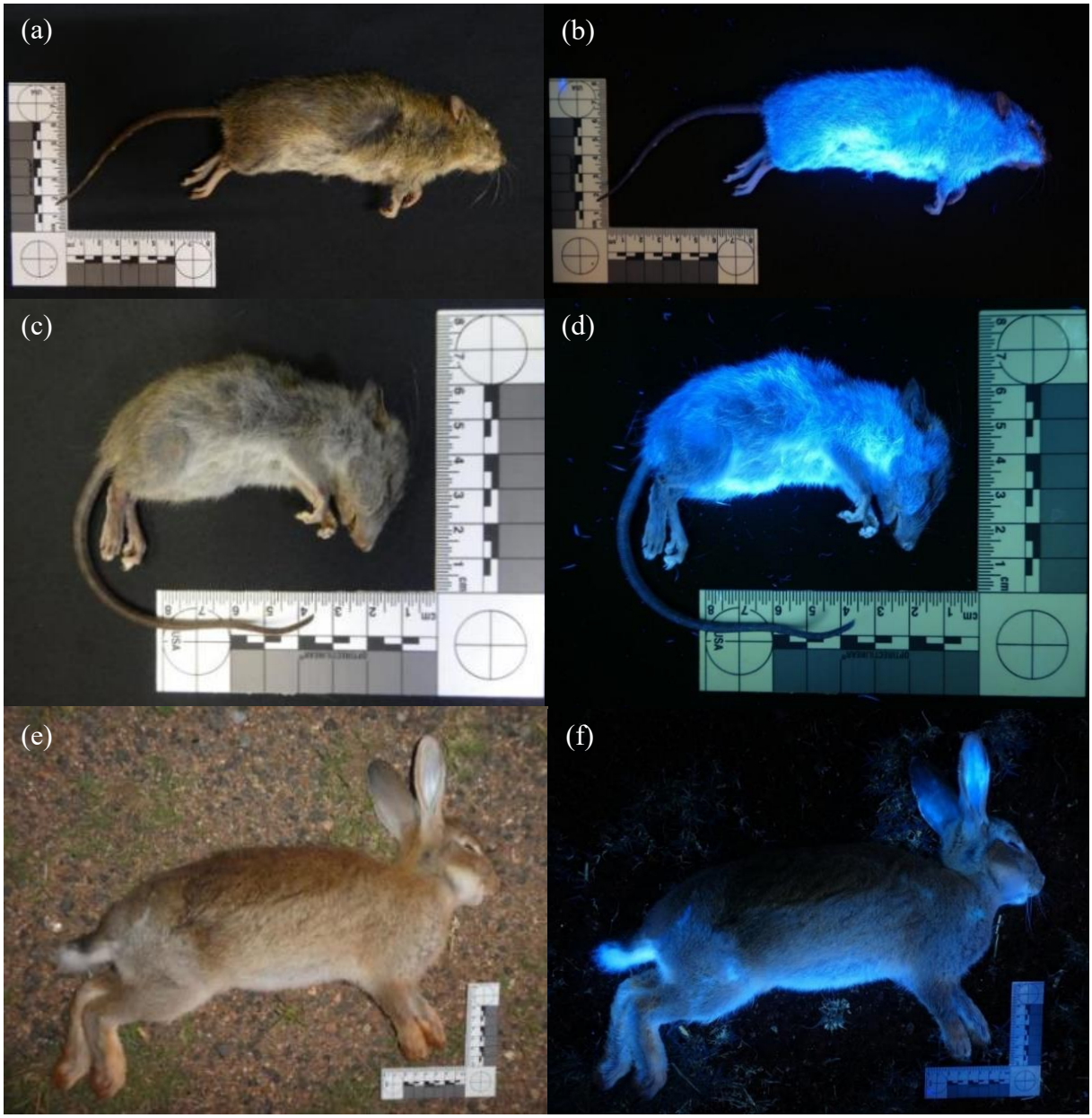


Figure 3.4. Photoluminescence of placentals. (a) Female pale field rat (*Rattus tunneyi*) roadkill, right flank, with white flash and (b) 365 nm torchlight (8 s exposure). (c) Female delicate mouse (*Pseudomys delicatulus*) frozen-thawed, right flank/ventral, with white flash and (d) 365 nm torchlight (10 s exposure). (e) Male European rabbit (*Oryctolagus cuniculus*) roadkill, right flank, with white flash and (f) displaying white photoluminescence on the underside of the tail and the skin inside the ear under 365 nm torchlight (10 s exposure).

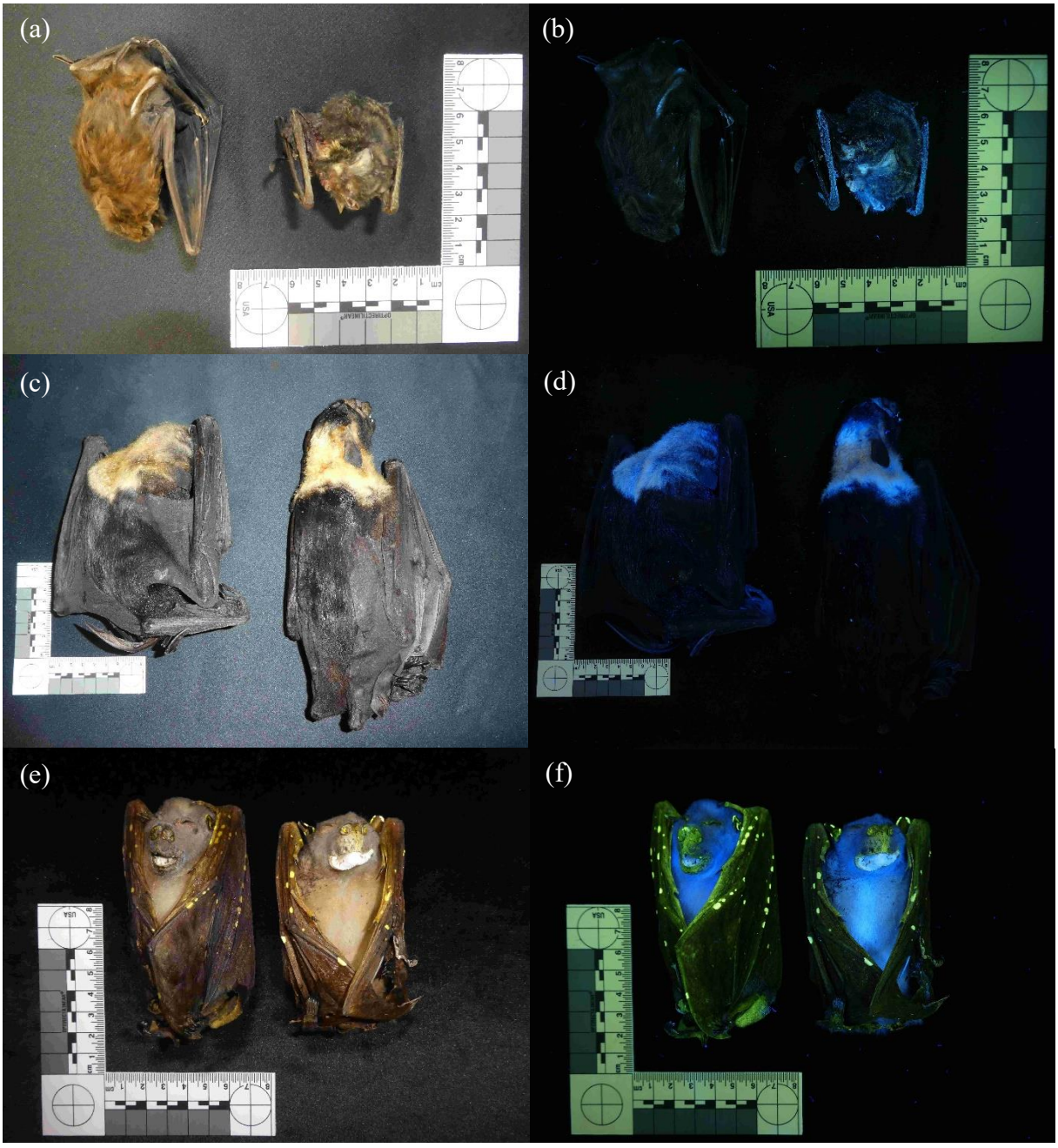


Figure 3.5. Degrees of photoluminescent intensity in bats. (a) Female Gould's wattled bat (*Chalinolobus gouldii*) (left) and a female forest bat (*Vespadelus* sp.) (right). Both frozen-thawed, dorsal, with white flash and (b) 365 nm torchlight (10 s exposure). Gould's wattled bat with no observable photoluminescence of the fur, and forest bat with subtle grey-white photoluminescence of the fur tips. (c) Female spectacled flying foxes (*Pteropus conspicillatus*). Both frozen-thawed, dorsal, with white flash and (d) 365 nm torchlight (20 s exposure). Showing mid-level blue-grey photoluminescence through the ginger collar, but only very subtle photoluminescence flecked through the rest of the fur. (e) Eastern tube-nosed fruit bats (*Nyctimene robinsoni*), male (left) and female (right). Both frozen, ventral, with white flash and (f) 365 nm torchlight (6 s exposure). Displaying mid–bright blue photoluminescence of the fur, and strikingly vivid yellow photoluminescence of the skin markings.

3.5 Discussion

The observations of wild mammal species described here give an insight into the prevalence of fur photoluminescence in the Wet Tropics. Of the 38 species, all but two showed at least some photoluminescence of the fur, suggesting that the trait is likely to be near-ubiquitous in Wet Tropics mammals. There was brightly vivid photoluminescence (either pink or blue) in 34% of species, although degradation of some specimens could mean that photoluminescence is also bright in other species in life, but was not observed in this study. The marsupials had a greater occurrence of, and brighter, pink photoluminescence in the fur than the placental mammals, while some species from both marsupials and placentals had bright blue-white photoluminescence. Monotremes were principally dull green, but other colours were present in platypus fur. The pale photoluminescence of echidna spines is likely to be from keratin.

My observations of some species differed to some in the literature. For example, the photoluminescence of the monotremes examined here and by Pine et al. (1985) and Reinhold (2020) was only subtle. However, both Anich et al. (2021) and Toussaint et al. (2023) reported conspicuous green/cyan photoluminescence in dry-preserved platypus specimens, although relative brightness would need to be compared under the same conditions. Additionally, the green photoluminescence I observed in the fur did not extend onto the skin. This is the only case where the photoluminescence reported in the literature for preserved museum specimens was more vivid than that of fresh specimens. Conversely, the mild pink photoluminescence I observed in platypus fur was not recorded by either Anich et al. (2021) or Toussaint et al. (2023). My observations of reddish photoluminescence in the fur of common brushtail possums agree with those of Bolliger (1944), but reddish colours were not recorded by Nicholls and Rienits (1971). Bolliger (1944) recorded long-nosed bandicoots as being non-photoluminescent, whereas I found fresh specimens of this species that were strikingly bright pink. Whether these differences are because of intraspecies variation, the use of different torches, preservation treatments or light exposure history is unknown.

Specimen degradation seemed to affect blue photoluminescence to some degree, although even some rotting roadkills displayed vivid photoluminescence (Appendix C). An antechinus that had been stored in a freezer since 1995 still glowed blue-white, confirming that freezing conserves photoluminescence. Exposure to sunlight seemed to affect pink photoluminescence, as observed by the pink only occurring on the dark-preserved side of some animals. Some northern quolls lacked any pink photoluminescence, whereas others displayed a pink as vivid

as that observed in bandicoots. Whether the lack of photoluminescence in some frozen specimens was true to life, or a consequence of being exposed to light, cannot be known.

Both species of bandicoot showed intraspecies variation in the coverage and intensity of photoluminescence, but always displayed pink (all but one bandicoot were collected as fresh roadkill, Appendix C). Brightly vivid pink occurred in both sexes of quolls and bandicoots, and in bandicoots was observed to be most intense and entire in virginal individuals of both sexes, although the vividness of pink varied within this size class. Some adult male bandicoots displayed uniform bright pink photoluminescence, but in others of the same size it was patchy, even though they were collected in the pre-dawn. This may indicate either different amounts of luminophores being excreted at the follicle, or different light exposure histories in life. The coverage and intensity of pink photoluminescence had not varied within the same rainforest population of live long-nosed bandicoots (Reinhold 2021). However, adult male roadkill long-nosed bandicoots displayed two photoluminescent colour morphs, with white and blue photoluminescence only prominent on the pale-flanked and/or banded individuals. Small sample sizes precluded statistical analyses on age or sexual dimorphism.

Although comprising only about 20% of the mammal species found in this bioregion (Department of Environment and Science, Queensland 2013), the range I collected shows taxonomic consistency of this phenomenon among genera. Bright pink photoluminescent fur occurs in diverse marsupial taxa, but was not observed in the local placental mammals. Bright blueish-white photoluminescence occurred in some species across both marsupial and placental genera. Whereas photoluminescence may be fairly consistently described within a species or genus, broader scale taxonomic patterns are not possible in this small dataset.

In the Wet Tropics, bright blueish-white photoluminescence can occur in the fur of small insectivorous largely diurnal semi-arboreal antechinuses, in ground-dwelling rodents with largely herbivorous diets, in larger tree-dwelling marsupials that are either insectivorous or folivorous, and in mostly frugivorous bats. Most marsupial species, and all placental species examined here, are nocturnal and/or crepuscular, but some may be exposed to sunlight as they shelter in vegetation during the day (Menkhorst and Knight 2011). Monotremes can be active day or night. Half of the bright blueish-white species were placentals, and half were marsupials. Bright pink photoluminescence, while seemingly confined to marsupials for this small sample size from the Wet Tropics bioregion, occurs consistently within varied taxa from ground-dwelling omnivorous bandicoots, to semi-arboreal carnivorous quolls, to the

mostly folivorous tree-dwelling brushtail possums. Pink photoluminescence is suspected to be common in the fur of distantly related mammals, both marsupial and placental, worldwide (Toussaint et al. 2023). No consistent external parameter appears to predict whether a taxon will have brightly photoluminescent fur or not.

3.6 Conclusion

Photoluminescent pelage occurs across a diverse array of mammal taxa, from sheep (Hirst 1927, cited in Collins 1992) to bats (Udall et al. 1964). Although the trait seems to be a general characteristic of fur at low to mid background levels, only about a third of Wet Tropics mammal species examined had brightly vivid photoluminescent fur. This vividly photoluminescent fur occurred across different phylogenies, habitats, diets and lifestyles. This study has expanded our understanding of photoluminescence in the pelage of mammals, finding the phenomenon to be both common and varied in intensity and colouration amongst the local mammal assemblage.

Chapter 4

Luminophores in the fur of seven Australian Wet Tropics mammals

4.1 Abstract

Bright photoluminescence in the fur of mammals has recently raised considerable scientific interest. The fur of many mammal species, including Australian long-nosed (*Perameles nasuta*) and northern brown (*Isoodon macrourus*) bandicoots, photoluminesces strongly, displaying pink, yellow, blue and white colours. I used reversed-phase high-performance liquid chromatography and electrospray ionisation mass spectrometry to isolate and identify the luminophores contributing to this photoluminescence. At least two classes of luminophore were observed in the bandicoot fur extracts, and four of the orange-pink photoluminescent molecules had monoisotopic masses matching those of protoporphyrin, coproporphyrin, uroporphyrin and heptacarboxylporphyrin. Fur extracts of three other species of marsupial, a placental and a monotreme also contained a luminophore matching the monoisotopic mass of protoporphyrin, whether or not pink photoluminescence was evident in their pelage as a whole. These findings are the first investigation into the luminophores in the fur of Australasian mammals since two tryptophan metabolites were identified more than 50 years ago, and provide the first chemical-based analysis of porphyrin luminophores in Australian mammal fur.

4.2 Introduction

Photoluminescence (fluorescence and/or phosphorescence) is the re-emission of photons from matter, usually at a higher wavelength than at which they were absorbed (Valeur and Berberan-Santos 2011). Photoluminescence is a characteristic common in many animals (e.g. amphibians, Stübel 1911; birds, Derrien and Turchini 1925), including mammals (Pine et al. 1985). The photoluminescence of fur has been documented in half of all mammal orders worldwide (see Chapter 2), and there has been a recent surge in interest in mammal fur photoluminescence (Giaimo 2020; Main 2020). While the fur chemistry of production fur animals has been extensively analysed (Millington 2020), particularly for sheep's (*Ovis aries*) wool (Rippon 2013), the fur composition of other animals has not been examined as thoroughly.

The colouration of fur in white light is typically a consequence of eumelanin (black or brown pigmentation), pheomelanin (red and yellow colouration) (Pawelek and Körner 1982), trichosiderin (Flesch and Rothman 1945; Barnicot 1956) and cinnabarinic acid (contributes to red colouration) (Nicholls and Rienits 1971). However, Bolliger (1944) noted that otherwise colourless components, termed 'fluorescent compounds' (Bolliger 1944) were incorporated into the fur structure of some mammals, which contributed to bright photoluminescence. Work on identifying such luminophores in mammal fur began in the 1950s (Rebell et al. 1956), finding that the photoluminescence of laboratory rats (*Rattus norvegicus*) was caused by the presence of the tryptophan metabolites kynurenine, and to a lesser extent kynurenic acid and *N*-acetyl-kynurenine (Rebell et al. 1957; Rebell 1966; Fig. 4.1).

Kynurenine, along with 3-hydroxyanthranilic acid (Fig. 4.1), was later extracted from the photoluminescent fur of Goodfellow's tree-kangaroos (*Dendrolagus goodfellowi*, Nicholls and Rienits 1971). Only 3-hydroxyanthranilic acid was extracted from the fur of Australian common brushtail possums (*Trichosurus vulpecula*), although the full suite of luminophores present was not identified (Nicholls and Rienits 1971). Pine et al. (1985) also found 3-hydroxyanthranilic acid in the fur of blue photoluminescent bare-tailed woolly opossums (*Caluromys philander*). While tryptophan metabolites primarily contribute to violet, blue, green and yellow photoluminescence, another tryptophan metabolite, 8-hydroxyquinaldic acid, photoluminesces red, but at a low ultraviolet excitation of ~254 nm (Roy et al. 1959). This low excitation wavelength distinguishes 8-hydroxyquinaldic acid from the ~400 nm and higher excitation wavelengths of red photoluminescent porphyrins (Goldoni 2002).

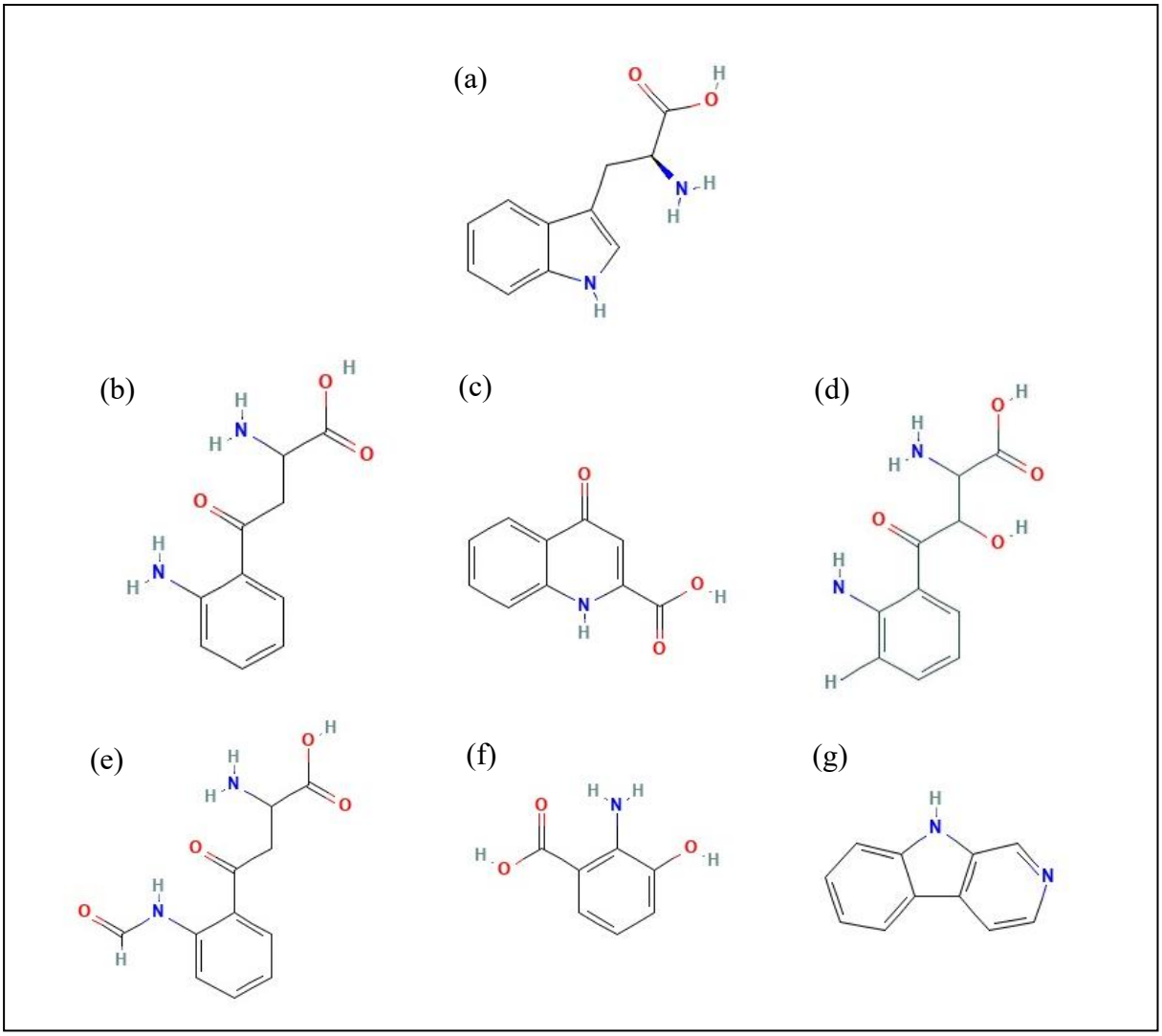


Figure 4.1. Molecular structure of tryptophan and the tryptophan metabolites identified in mammalian pelage (National Center for Biotechnology Information 2022). (a) Tryptophan. (b) Kynurenine. (c) Kynurenic acid. (d) *N*-acetyl-kynurenine. (e) *N*-formylkynurenine. (f) 3-Hydroxyanthranilic acid. (g) Beta-carbolene.

The presence of porphyrins in mammal pelage was first suspected due to a red photoluminescence in the spines of European hedgehogs (*Erinaceus europaeus*, Derrien and Turchini 1925). The luminophores were later confirmed as coproporphyrin III, uroporphyrin III and protoporphyrin IX (Hamchand et al. 2021; Fig. 4.2). Coproporphyrin I, uroporphyrin I, uroporphyrin III and heptacarboxylporphyrin (Fig. 4.2) were identified in the orange-red photoluminescent fur of South African springhares (*Pedetes capensis*, Olson et al. 2021), and uroporphyrin I or III (Fig. 4.2) was also detected by emission and excitation spectroscopy in Guyanan short-tailed opossums (*Monodelphis brevicaudata*) and Linnaeus' mouse opossums

(*Marmosa murina*) (Toussaint et al. 2023). In addition, porphyrin S-411 (an analogue of coproporphyrin) was identified by emission and excitation spectroscopy from southern flying squirrel (*Glaucomys volans*) and northern flying squirrel (*G. sabrinus*) fur (Toussaint et al. 2023).

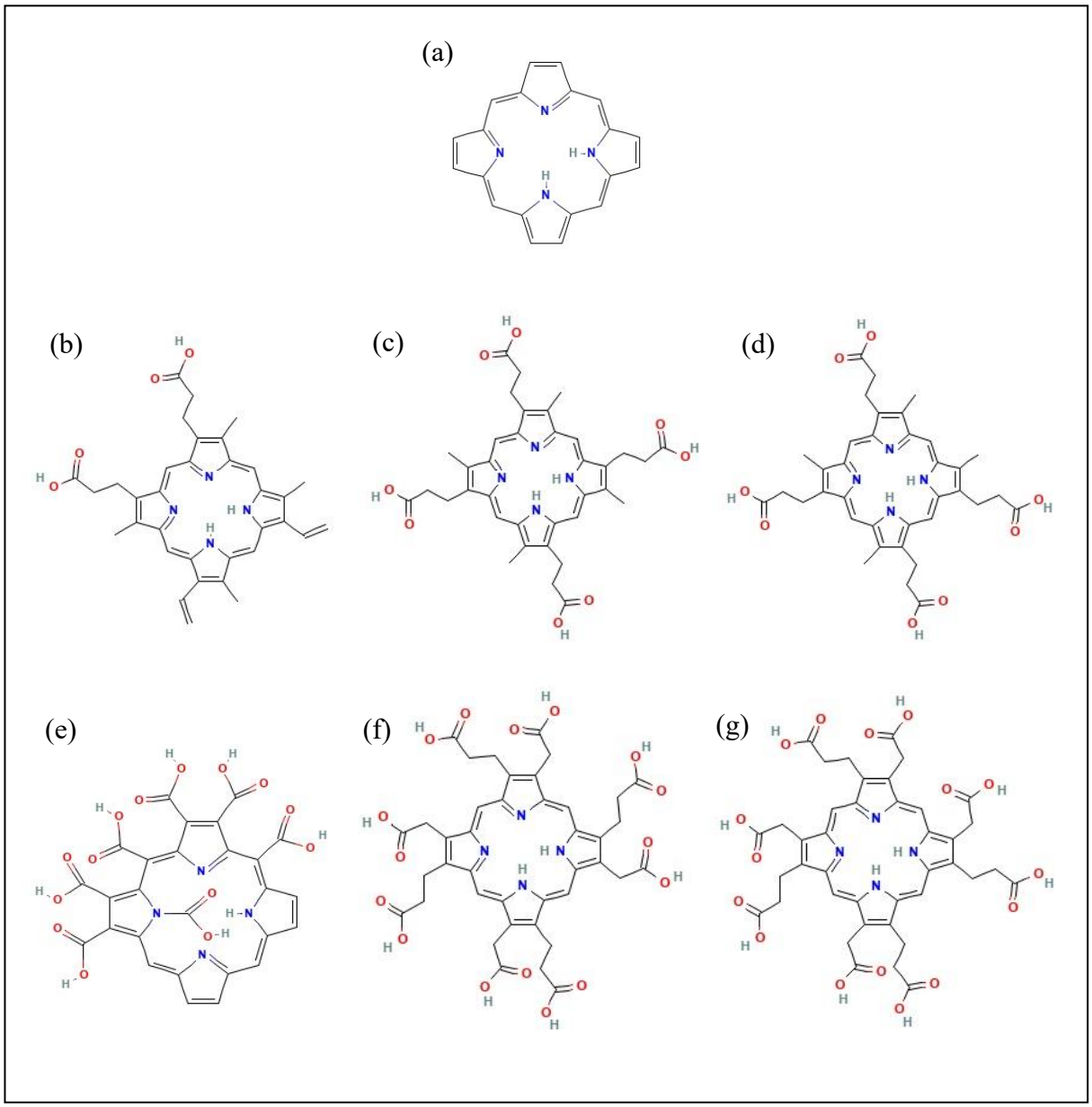


Figure 4.2. Molecular structure of porphyrin and the porphyrin derivates identified in mammalian pelage (National Center for Biotechnology Information 2022). (a) Porphyrin. (b) Protoporphyrin IX. (c) Coproporphyrin I. (d) Coproporphyrin III. (e) Heptacarboxylporphyrin (f) Uroporphyrin I. (g) Uroporphyrin III.

Recent studies have used emission and excitation fluorescence spectrophotometry, spectroscopy, and spectrofluorimetry to match absorption and emission maxima of fur luminophores to wavelengths of known molecules, but not all photoluminescent compounds have distinct, identifiable spectrographic signatures (Daly et al. 2009; Millington 2020; Toussaint et al. 2023). Other studies have used thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), electrospray ionisation mass spectrometry (ESI-MS) and microplate reader fluorescence analysis of extractions from fur to identify luminophores, with mixed success (Nicholls and Rienits 1971; Olson et al. 2021; Hughes et al. 2022). In general, much remains unknown about the luminophores contained in the fur of mammals.

In this study, I used a range of analytical techniques to attempt to identify some of the luminophores in several Australian marsupials, a placental and a monotreme from the Wet Tropics region of Far North Queensland. Fur samples of both long-nosed (*Perameles nasuta*) and northern brown (*Isoodon macrourus*) bandicoots (Reinhold 2020, 2021) were used initially, and I hypothesised that porphyrins were the cause of the photoluminescence observed in these samples because they photoluminesced bright pink in response to 395 nm light (Toussaint et al. 2023). In addition, northern brown bandicoots display varying colours as the animal turns and different parts of the fur shaft are angled to the viewer (Reinhold 2020). The fur of some individual long-nosed bandicoots also contains blue and white photoluminescence (see Chapter 3) leading me to hypothesise that additional luminophores are present in the fur.

To determine whether the luminophores present in bandicoot fur might be common across multiple species, I compared the results from the two bandicoots to five other species of Wet Tropics mammals: two marsupials that photoluminesce pink, the northern quoll (*Dasyurus hallucatus*) and the coppery brushtail possum (*Trichosurus johnstoni*); one marsupial that only photoluminesces blue, the Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*); one placental that also photoluminesces blue, the pale field rat (*Rattus tunneyi*); and one monotreme that photoluminesces silvery grey to dull green and mild pink, the platypus (*Ornithorhynchus anatinus*) (see Chapter 3).

4.3 Methods

4.3.1 Fur collection

Fur from a northern brown and a long-nosed bandicoot (both young males) was sourced from fresh roadkill animals collected at night around the Yungaburra/Lake Eacham area of the Atherton Tablelands in Far North Queensland, Australia. Each carcass was examined with 310 nm (Tao Yuan), 365 nm (OLight) and 395–410 nm (Dulex) torches, and photographed with a Panasonic Lumix TZ-80 camera with no filters or post-processing. Clean fur was shaved (blade 0 electric shaver) from the dorsal, flank and ventral surfaces of each animal and the fur was separately wrapped in aluminium foil and sealed in separate resealable plastic bags. Samples were then stored at -18°C until analysis. Fur samples of other species were variously sourced, either as fresh roadkill (female coppery brushtail possum and female pale field rat), or as frozen specimens at James Cook University (previously collected by other researchers—male northern quoll, male Lumholtz's tree-kangaroo and female platypus).

4.3.2 Emission spectroscopy

I used an Ocean Insight FLAME-S-UV-VIS-ES (200–850 nm) Miniature Spectrometer with OceanView Spectrometer Operating Software (Ocean Optics 2013) to determine the fluorescent emission wavelengths of bandicoot fur. I recorded the photoluminescence emission maxima from the pelts of an adult male northern brown bandicoot (brindled brown flank fur and pale buff ventral fur) and of a virginal female long-nosed bandicoot (brindled brown rump fur). Wavelength readings were taken from five areas of uniformly photoluminescent fur of dry pelts for each animal. The software was set to fluorescence mode with a continuous strobe period of 10,000 μs, acquisition delay of 20,000 μs, integration time of 100 ms and averaged over 10 scans. A Labsphere Spectralon® white diffuse reflectance standard was used for reference. The accompanying UV-VIS PX-2 Pulsed Xenon Light Source was not used because it gave strong reflectance peaks at 485 and 529 nm, around the cyan-green part of the spectrum. Therefore, an external Dulex 395–410 nm torch was used to elicit photoluminescence, with the spectrometer placed 1.5 cm away from the fur.

4.3.3 Fluorescence microscopy

More than 100 strands of fur from each of the studied species were set on microscope slides with mounting medium (Entellan^{TM} new) and 170 micron coverslips. Slides were examined both on a confocal microscope (ZEISS LSM 900 Aryiscan 2) at a wavelength of 405 nm, and a fluorescence microscope (Cri Nuance FX with Zeiss Axio Imager M1) under 305–390 nm excitation (DAPI). The broad excitation wavelength band of DAPI only allows emission wavelengths filtered between 420–470 nm (violet-indigo-blue). Brightfield images were taken in colour, and photoluminescence was photographed with the fluorescence microscope's black-and-white camera (i.e. false colours were not added) under 200× magnification.

4.3.4 Luminophore extraction

For each extraction, unwashed flank fur was weighed out into capped 15 mL polypropylene tubes, and 20% trifluoroacetic acid (TFA; Auspep) was added until the fur was covered with the solvent. The ratio of mass of fur to the volume of solvent was not the same for each species as quantitative differences between species were not being calculated. The amount of fur used was chosen to maximise the chances of identifying luminophores from each species. The masses of fur, volumes of solvent and extract used for each species are listed in Table 4.1. Tubes were kept wrapped in aluminium foil whenever possible to prevent photobleaching (Hamchand et al. 2021; see Chapter 5).

Table 4.1. Amounts of fur, solvent and extract used for the RP-HPLC of each species.

Species	Fur mass	Solvent volume	Extract volume
Perameles nasuta	258.38 mg	8 mL	5 mL
Isoodon macrourus	793.18 mg	12 mL	6 mL
Dasyurus hallucatus	758.37 mg	7 mL	4.5 mL
Trichosurus johnstonii	387.02 mg	7 mL	5 mL
Dendrolagus lumholtzi	1166.94 mg	10 mL	6 mL
Rattus tunneyi	400.85 mg	7 mL	5 mL
Ornithorhynchus anatinus	520.05 mg	7 mL	4.5 mL

The tubes of fur and solvent were then heated at 95.0°C in a heat block (major science Dry Bath Incubator) for 1 hour, during which they were agitated briefly four separate times (Grant-bio type PV-1 version V.3 GW Grant Instruments). If not used immediately, tubes were then stored in darkness at 4°C until analysis. Tubes were spun at 4000 rpm for five minutes (Rotina 420R centrifuge) to pellet the fur, then observed at 395–410 nm in a dark

room. Extract supernatant (see Table 4.1 for volumes) was transferred to clean separate tubes and syringe filtered (25 mm, 0.22µm PVDF filter, Whatman® UNIFLO®) into new 15 mL tubes prior to further use.

4.3.5 Reversed-phase high-performance liquid chromatography (RP-HPLC)

Filtered fur extract (Table 4.1) was loaded onto a reversed-phase column (Phenomenex Aeris peptide XB-C18, 250 x 10 mm, 5 µm, 100 Å) and run on an Agilent 1260 Infinity HPLC system (Agilent Technologies). A gradient from 0–80% Buffer B in 120 minutes [Buffer A, 0.05% TFA (Auspep)/H_2O; Buffer B, 90% acetonitrile (ACN; Sigma-Aldrich)/10% H_2O/0.045% TFA (Auspep)] was run at a flow rate of 3 mL/min. The absorbance was monitored at wavelengths of 214, 280, 330, 365, 380, 395 and 400 nm. Fractions were collected every 30 seconds into 2 mL 96-well deep-well plates (Axygen). Tray illumination was set to 'off' during collection of fractions. Plates containing fractions were observed under wavelengths of 310–410 nm and wells that showed the brightest photoluminescence, different colours and/or significant RP-HPLC peaks were transferred into individual 2 mL microcentrifuge tubes for further analysis. The most strongly coloured fractions were selected and photographed for each species. Photographs were taken with a Panasonic Lumix TZ-80 camera without any filters or post-processing.

4.3.6 Liquid chromatography/electrospray ionisation – mass spectrometry (LC/ESI-MS)

The RP-HPLC fractions that showed the brightest photoluminescence, different colours and/or significant absorbance peaks from each species (long-nosed bandicoot: n = 7 fractions; northern brown bandicoot: n = 7; northern quoll: n = 6; coppery brushtail possum: n = 7; Lumholtz's tree-kangaroo: n = 9; pale field rat: n = 8; platypus: n = 8) were loaded (50 µL) onto a reversed-phase column (Phenomenex Aeris peptide XB-C18, 150 x 2.1 mm, 3.6 µm, 100 Å) and analysed on a Shimadzu Prominence HPLC system coupled to a Shimadzu LCMS2020 mass spectrometer. A 1% gradient from 0–80% LCMS Buffer B in 80 minutes [LCMS Buffer A, 0.1% formic acid (FA; Sigma-Aldrich)/H_2O; LCMS Buffer B, 90% ACN (LCMS grade; Thermo Fisher Scientific)/10% H_2O/0.09% FA (Sigma-Aldrich)] was run at a flow rate of 0.25 mL/min. The absorbance was monitored at 330 nm and 400 nm. Mass spectra were collected in positive and negative ionisation mode over a scan range of *m/z* 160–2000 and *m/z* 200–2000, respectively, with a detector voltage of 1.35 kV, nebulizing gas flow

of 1.5 L/min, and drying gas flow of 3 L/min. Data were collected and analysed using Shimadzu LabSolutions v5.96 software. Coproporphyrin I (Sigma-Aldrich) and protoporphyrin IX (Sigma-Aldrich) standards (5 µL of ~1 mg/mL) in both methanol-d4 (Cambridge Isotope Laboratories) and dimethyl sulfoxide-d6 (Cambridge Isotope Laboratories) were run under the same conditions.

4.4 Results

4.4.1 Fur collection

Both bandicoot specimens showed vivid pink photoluminescence over the entirety of their pelts (Figs. 4.3 and 4.4). The ventral fur was a uniform pink, and the brindled flank fur shafts generally had a magenta photoluminescent base, a middle section that did not photoluminesce, and a yellowish photoluminescent tip (Fig. 4.5). Other strands were wholly pink. The effect that the brindling of fur photoluminescence had on the overall appearance of the pelt could only be fully observed when the fur was still in place on the skin. The way the colours lay together meant that from a few metres away, a live northern brown bandicoot appeared to photoluminesce orange or yellow depending on the angle to the observer, or deep pink when viewed in close proximity. When live long-nosed bandicoots were viewed from a greater distance with relatively faint excitation, the red wavelengths were lost and the animals instead appeared to glow whitish or yellowish (Reinhold 2020). Photographs of the other individuals/species from which fur was collected are in Chapter 3 (Figs. 3.1, 3.2, 3.3 and 3.4). Descriptions of whole-pelt photoluminescent characteristics of each species are given in Appendix C.

4.4.2 Emission spectroscopy

To quantify the emission wavelengths of bandicoot fur, pelt samples were irradiated with a 395–410 nm torch and the resulting spectra monitored with a spectrometer. Emission of photoluminescence from the northern brown bandicoot (both flank and ventral) fur had a main peak at 633 nm (red) and lesser peaks at 656, 666 and 705 nm (Fig. 4.6a and 4.6b). The long-nosed bandicoot rump fur had a slightly longer peak emission wavelength at 636 nm with lesser peaks at 624, 674 and 706 nm (Fig. 4.6c). A lower broad cyan peak was also emitted from the pelts of both species of bandicoot.

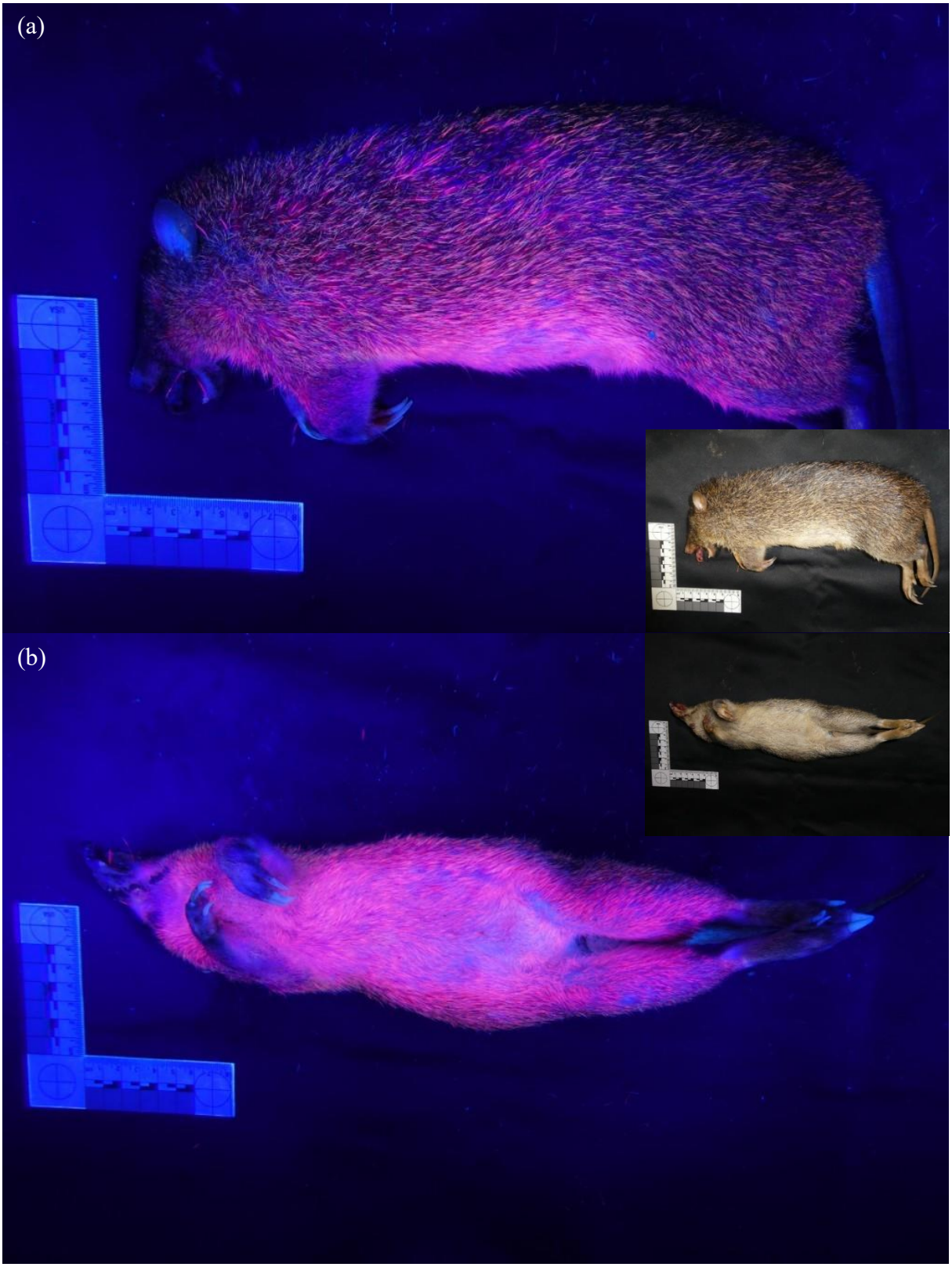


Figure 4.3. Pink photoluminescent male northern brown bandicoot (*Isoodon macrourus*) roadkill collected for extraction of fur luminophores. 7 h after collection. Under 395–410 nm torchlight (6 s exposure). (a) Left flank. (b) Ventral surface. Insets: with white flash.

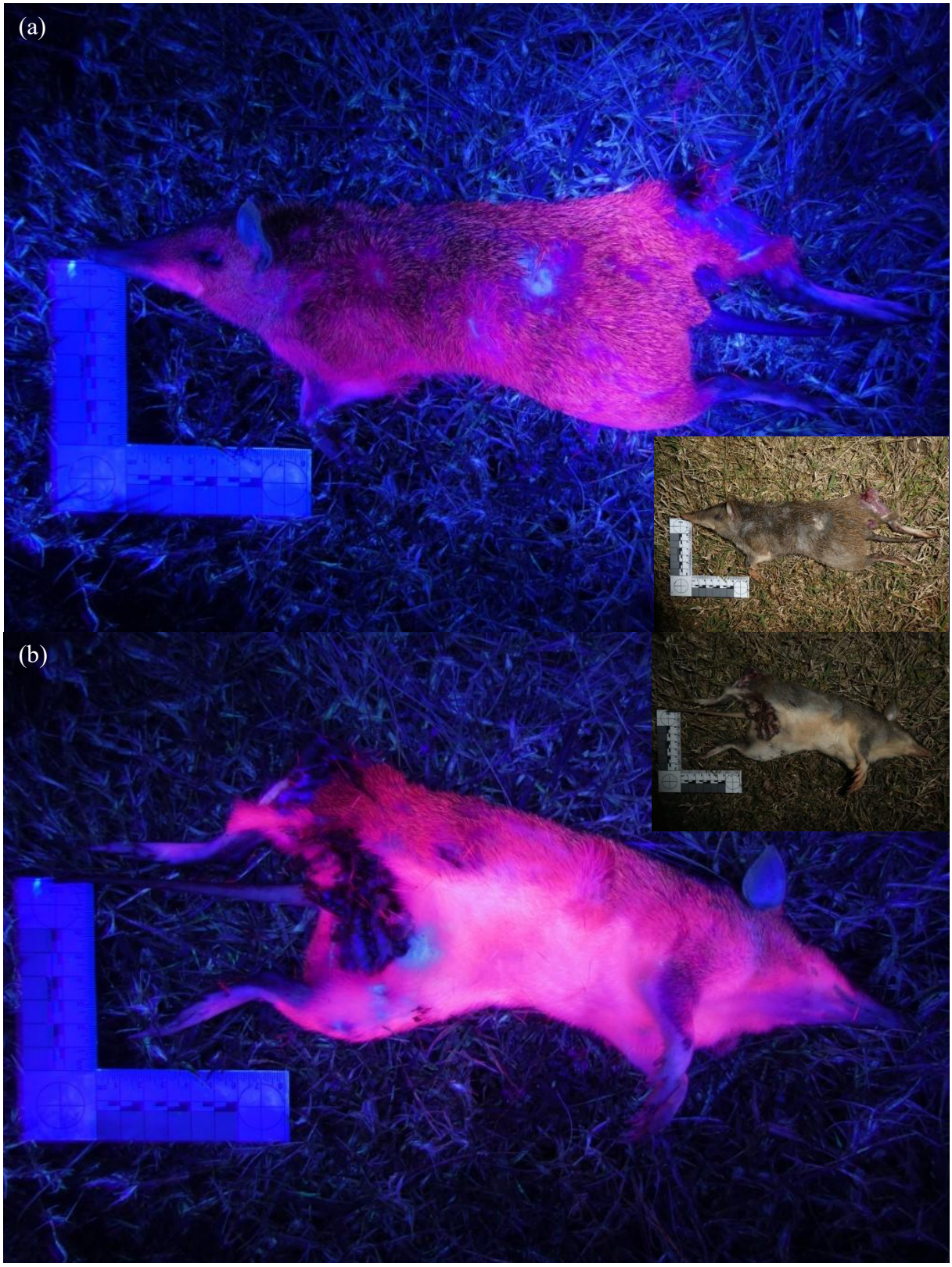


Figure 4.4. Pink photoluminescent male long-nosed bandicoot (*Perameles nasuta*) roadkill collected for extraction of fur luminophores. Immediately after collection. Under 395–410 nm torchlight (5 s exposure). (a) Dorsal/left flank. (b) Ventral/right flank. Insets: with white flash.

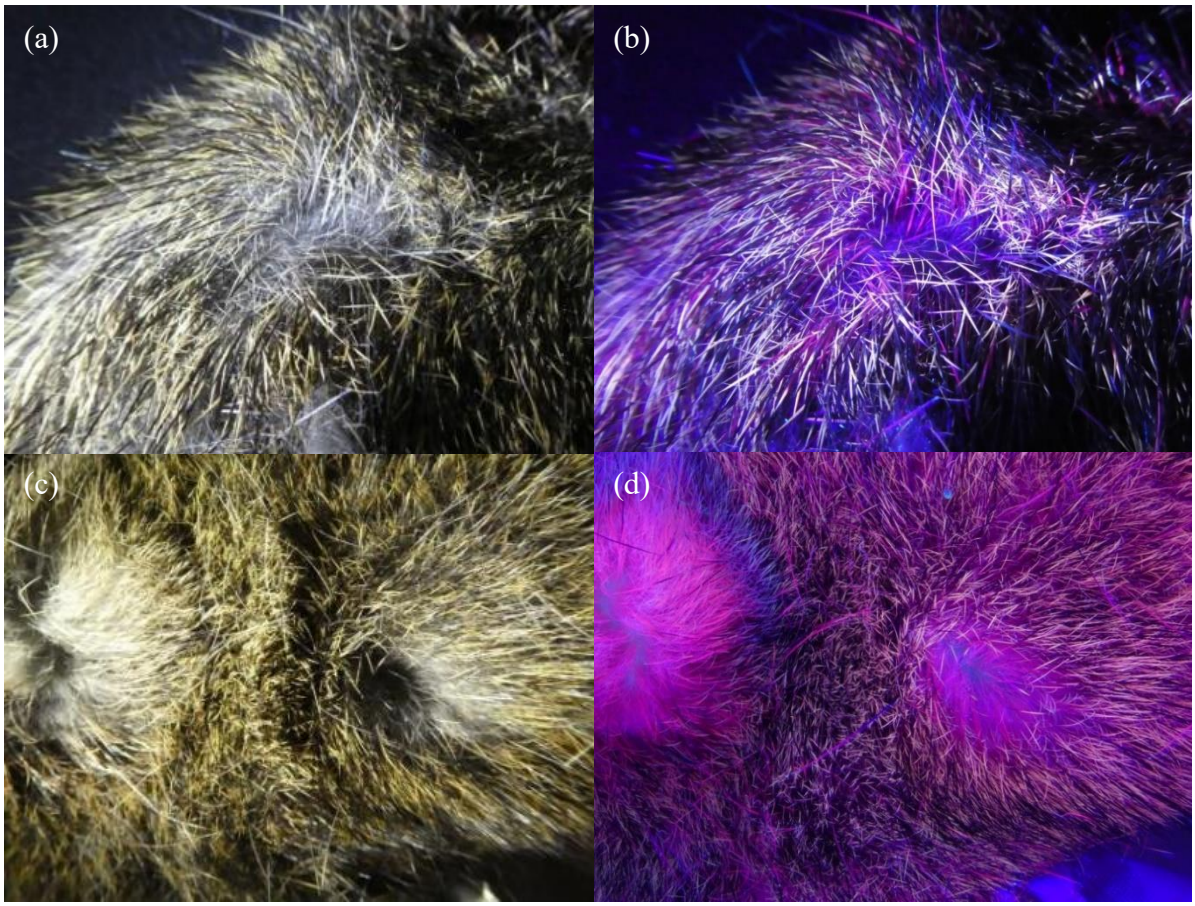


Figure 4.5. Close-up photographs of brindling and the distribution of photoluminescent colours in bandicoot fur. (a) Female northern brown bandicoot (*Isoodon macrourus*) roadkill, under white torchlight and (b) under 395–410 nm torchlight (automatic exposure). (c) Female long-nosed bandicoot (*Perameles nasuta*) roadkill under white light and (d) under 395–410 nm torchlight (automatic exposure).

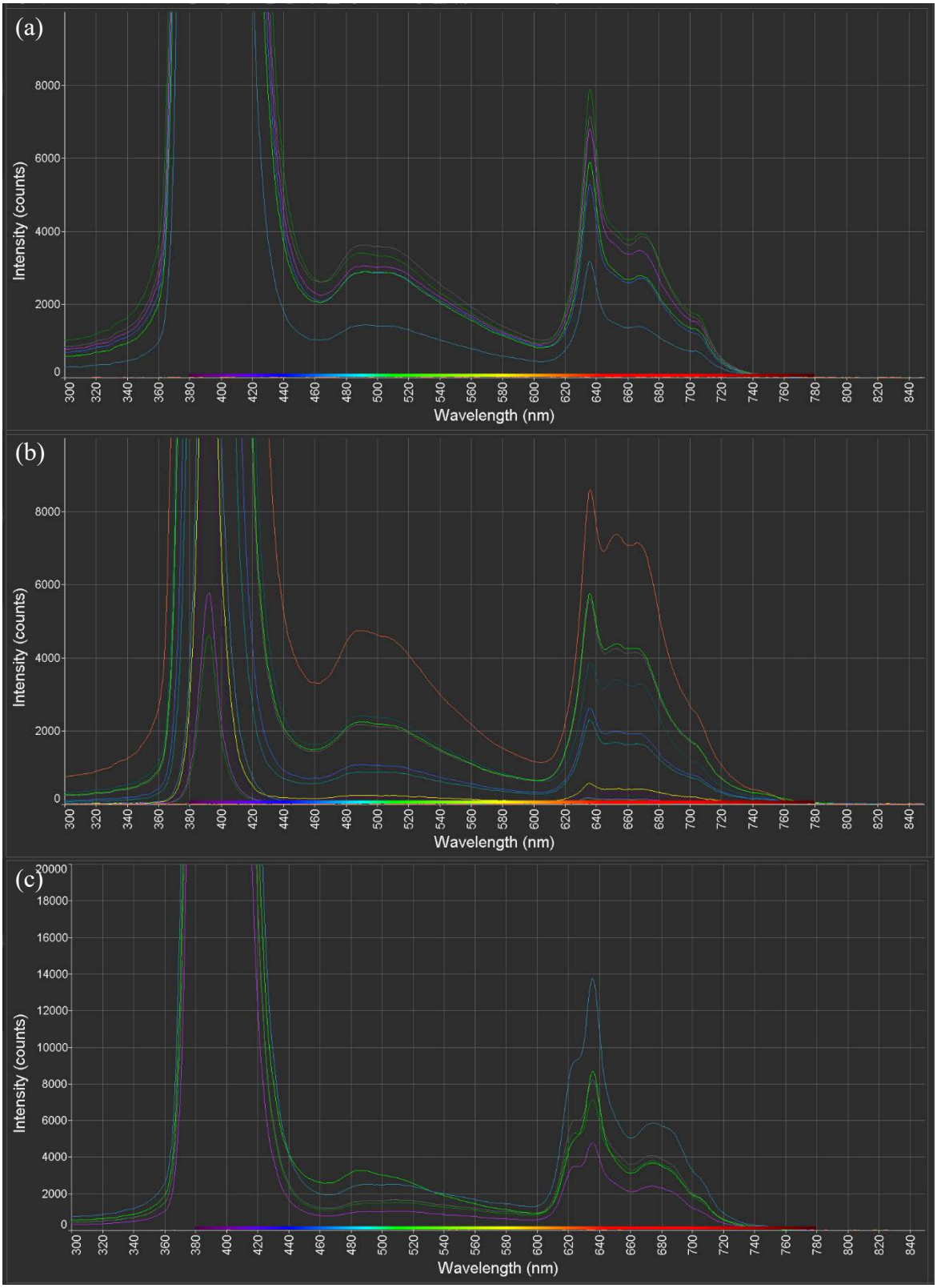


Figure 4.6. Spectrographs of dried bandicoot pelts. (a) Flank fur of male northern brown bandicoot (*Isoodon macrourus*). (b) Ventral fur of same northern brown bandicoot. (c) Rump fur of female long-nosed bandicoot (*Perameles nasuta*). The peak around 400 nm is the emission from the torch. Line colours indicate replicate scans for different areas of the pelt.

4.4.3 Fluorescence microscopy

All fur examined photoluminesced under low-wavelength excitation (305–390 nm). The photoluminescence had a ladder-like appearance in the fur structure due to its presence along the cuticle and across the medullary cells. This general photoluminescence was present regardless of whether the interspaces were filled with pigment (zoochromes). Fig. 4.7b shows the ladder-like photoluminescence in the long-nosed bandicoot. Fig. 4.7d displays a similar phenomenon in platypus fur.

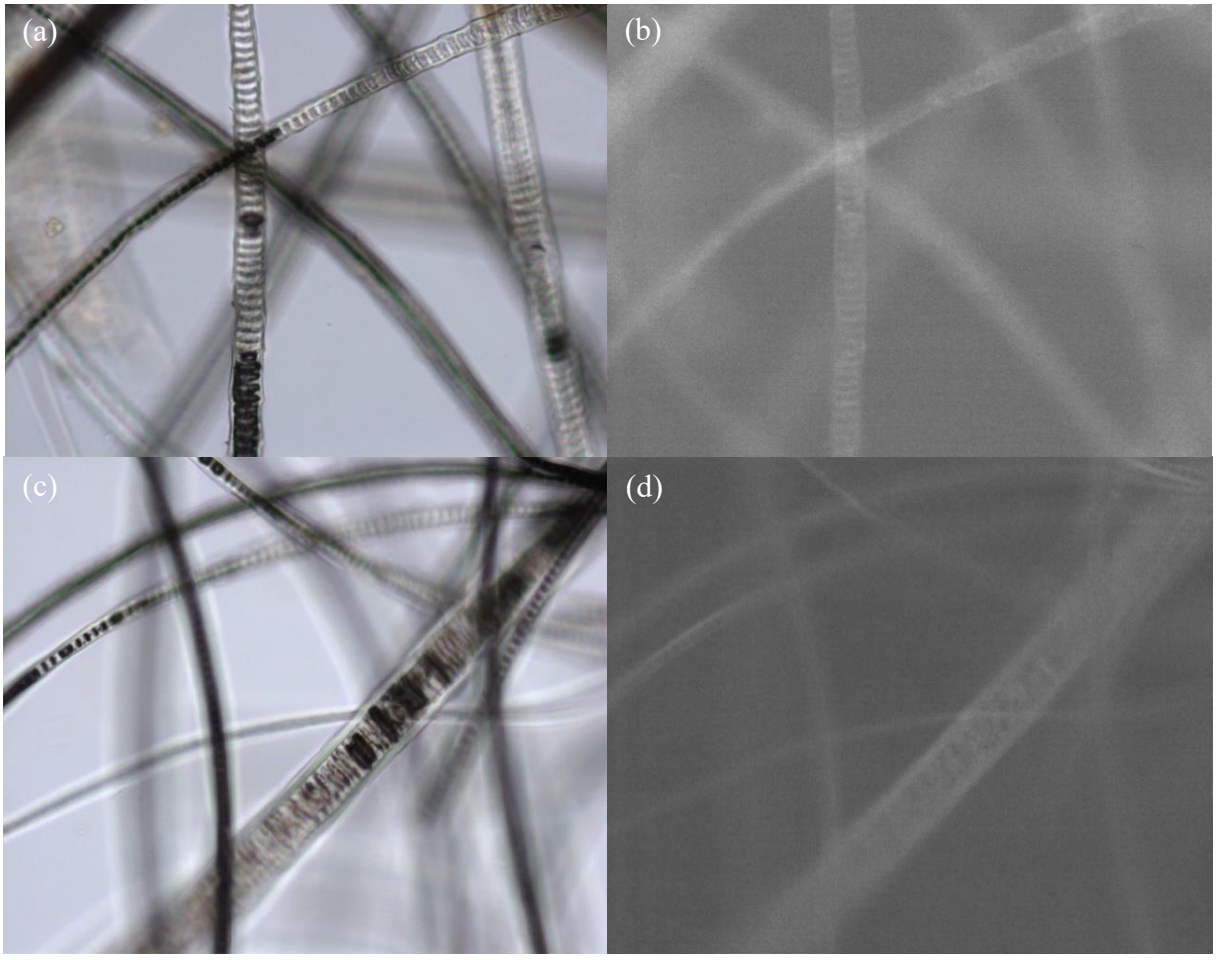


Figure 4.7. Fluorescence microscopy of selected fur demonstrating positioning of photoluminescence associated with keratin. (a) Male long-nosed bandicoot (*Perameles nasuta*) ventral fur in brightfield light and (b) with DAPI filter showing photoluminescence of the keratin structure regardless of pigment in the interspaces. (c) Female platypus (*Ornithorhynchus anatinus*) flank fur in brightfield light and (d) with DAPI filter showing photoluminescence of the keratin structure regardless of pigment in the interspaces. Fluorescence microscope, 200× magnification, 7.8 ms exposure time.

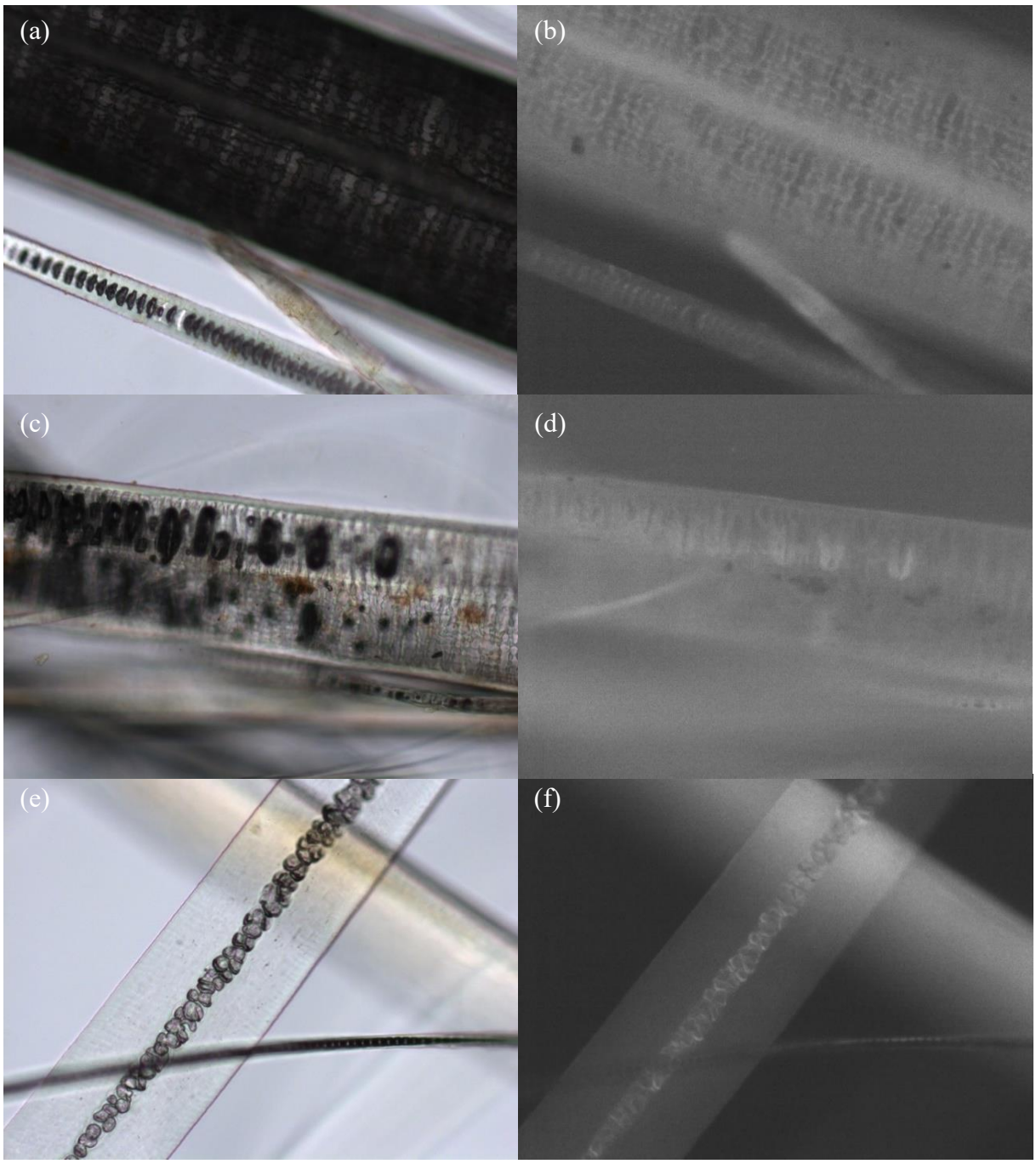


Figure 4.8. Fluorescence microscopy of selected fur demonstrating positioning of photoluminescence associated with pigment. (a) Male long-nosed bandicoot (*Perameles nasuta*) ventral fur (thick and thin) in brightfield light and (b) with DAPI filter showing photoluminescence associated with the pigment granules, which are brighter than the cuticle. The root sheath of the thinnest hair photoluminesces with similar brightness to the medulla of the thickest hair. (c) Male long-nosed bandicoot ventral fur (mid-thickness) in brightfield light and (d) with DAPI filter showing photoluminescence associated with the pigment granules, which are brighter than the cuticle. (e) Female platypus (*Ornithorhynchus anatinus*) flank fur in brightfield light and (f) with DAPI filter showing photoluminescence emanating from the pigment granules in the centre of the fur shaft. Fluorescence microscope, 200× magnification, 7.8 ms exposure time.

In some fur, the photoluminescence did not appear to result from the melanin pigment granules but appeared strongest in the interspaces between the melanin granules in the medulla. In other fur, the pigment granules photoluminesced brighter than the ladder-like photoluminescence that is presumed to be associated with the keratin structure. In thinner fur, the pigment granules were deposited discretely along the centre of the fur shaft, and in thicker fur the pigment granules were dispersed throughout the cortex. In Fig. 4.8b and 4.8d, this pigment-based photoluminescence can be seen in the ventral fur of the long-nosed bandicoot. The simple fur structure of the platypus allowed this pigment-based photoluminescence to be discriminated most clearly (Fig. 4.8f). The root sheaths of fur photoluminesced similarly to the fur shafts.

4.4.4 Luminophore extraction

Shaved fur samples were soaked in a 20% TFA solution to extract the luminophores, and photoluminescence was evident in the extract (Fig. 4.9). The fur of all species still exhibited varying amounts of photoluminescence after extraction. After separating the fur and extract layers by centrifugation, a layer of coloured liquid was visible above the fur in white light for all species, and in 395–410 nm light these layers photoluminesced. When examined with 310 nm light, the fur extracts of the northern brown bandicoot and the Lumholtz's tree-kangaroo photoluminesced pale purple. This purple photoluminescence was evident in the tree-kangaroo fur extract up to 410 nm, but in the bandicoot fur extract it was eclipsed by hot pinkish orange photoluminescence in 365 nm and longer wavelength excitation light.

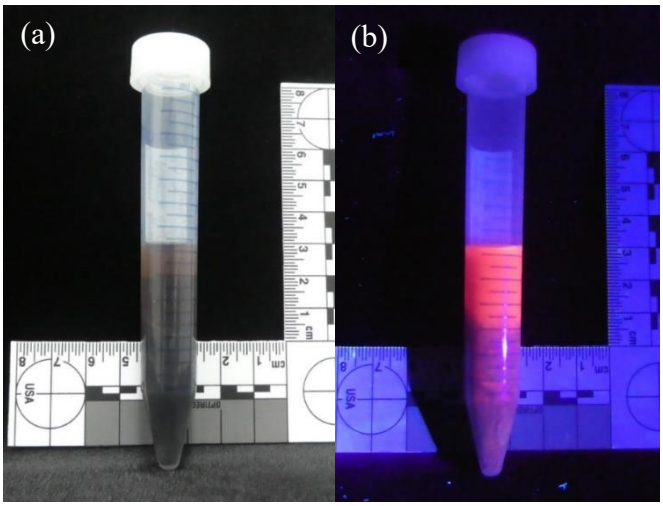


Figure 4.9. Female coppery brushtail possum (*Trichosurus johnstonii*) fur luminophore extraction in 20% TFA (a) in white light and (b) 395–410 nm ultraviolet-violet light.

4.4.5 RP-HPLC

Extracts from fur samples were run on RP-HPLC to separate and purify the luminophores. The retention times of the main peaks in the chromatograms (Fig. 4.10) at four different absorbance wavelengths for each species are listed in Table 4.2. Some peaks seemed to be common across several species, showing the same retention times. For example, a peak with a retention time ~43 minutes at 214 nm absorbance was present in the chromatograms of the long-nosed bandicoot, the northern quoll, the coppery brushtail possum, the Lumholtz's tree kangaroo and the pale field rat. Peaks at 37, 39, 47 and 78 minutes at 400 nm absorbance were present in both bandicoots and the possum, and only the ~47 and 78-minute peaks were present in the quoll. Numerous luminophores were present in each sample, evident from the RP-HPLC fractionation and presence of photoluminescent compounds distributed across the fractionation well-plates (Fig. 4.11). Fractions containing any colour under white light or photoluminescence under ultraviolet-violet wavelengths were removed from the plates and stored individually in micro-centrifuge tubes. Figures 4.12 and 4.13 show selected isolated fractions for each species in white light and 310–410 nm ultraviolet-violet light, respectively. The general pattern of photoluminescence across most species was a couple of early-eluting yellow fractions (optimal excitation at 365 nm), and later-eluting orange-pink or pink fractions (395–410 nm) (Appendix D). The tree-kangaroo's fractions were mostly blue or lavender blue. No other species yielded blue well fractions (Appendix D). However, once transferred to individual tubes, some greenish fractions appeared blueish to the eye, likely due to dichromatism (Needham 2012), and perhaps further blue-shifted by the camera. The coppery brushtail possum yielded the most colourful photoluminescent rainbow, and had a non-photoluminescent fraction that was distinctly purple in white light (Fig. 4.12e). Figure 4.14 displays the equivalent fractions from both species of bandicoot fur extracts together.

Table 4.2. Approximate retention times (min) of the highest intensity RP-HPLC peaks from the RP-HPLC chromatograms at various absorbance wavelengths for each species.

Species / absorbance	**214 nm**	**330 nm**	**365 nm**	**400 nm**
Perameles nasuta	43, 85	–	–	37, 39, 47, 78
Isoodon macrourus	51	–	–	37, 39, 48, 78
Dasyurus hallucatus	44, 85	–	–	48, 78
Trichosurus johnstonii	43, 51	2, 12, 19, 23, 25, 27, 28, 37	2, 12, 19, 22, 25, 27, 28, 30, 37	37, 39, 46, 77
Dendrolagus lumholtzi	43, 51	20, 25	20	31, 34, 36
Rattus tunneyi	3, 13, 20, 29, 31, 42	3, 20	3, 20	3, 20
Ornithorhynchus anatinus	46	2	2	2

(a)

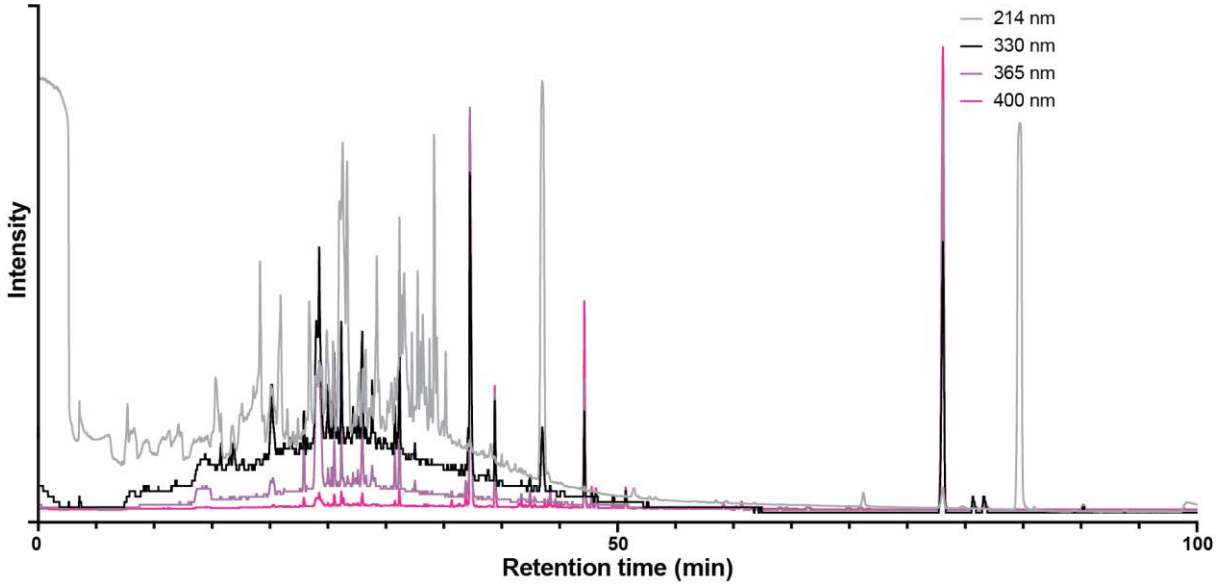


Figure 4.10. RP-HPLC chromatograms of fur extracts monitored at 214, 330, 365 and 400 nm. Intensity levels for each wavelength optimized for clarity. (a) Male long-nosed bandicoot (*Perameles nasuta*).

(b)

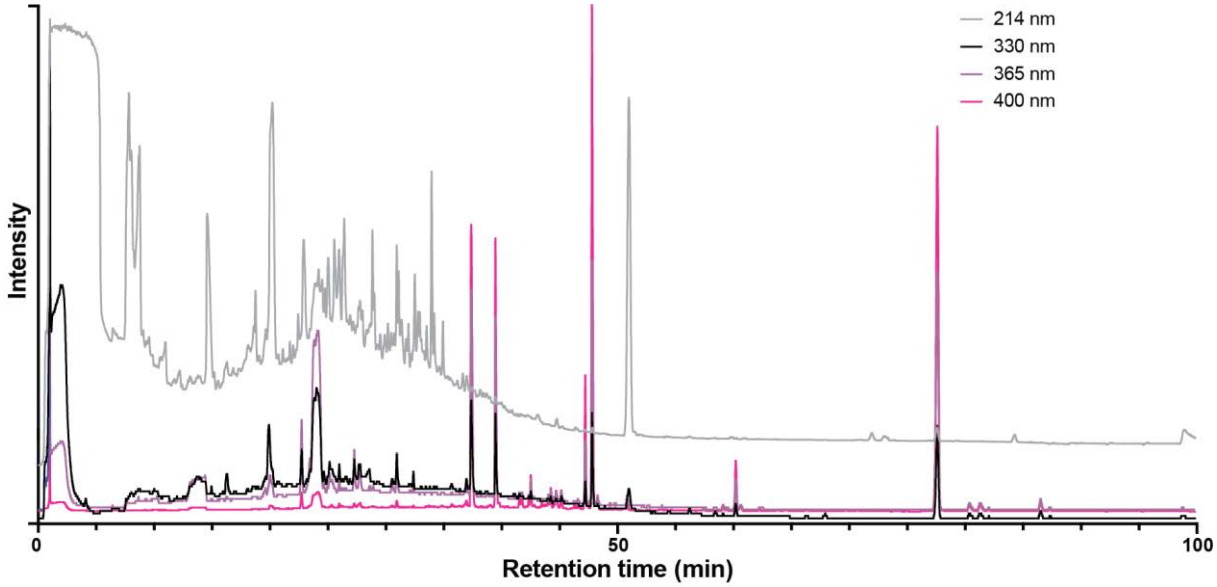


Figure 4.10 (b) Male northern brown bandicoot (*Isoodon macrourus*) RP-HPLC.

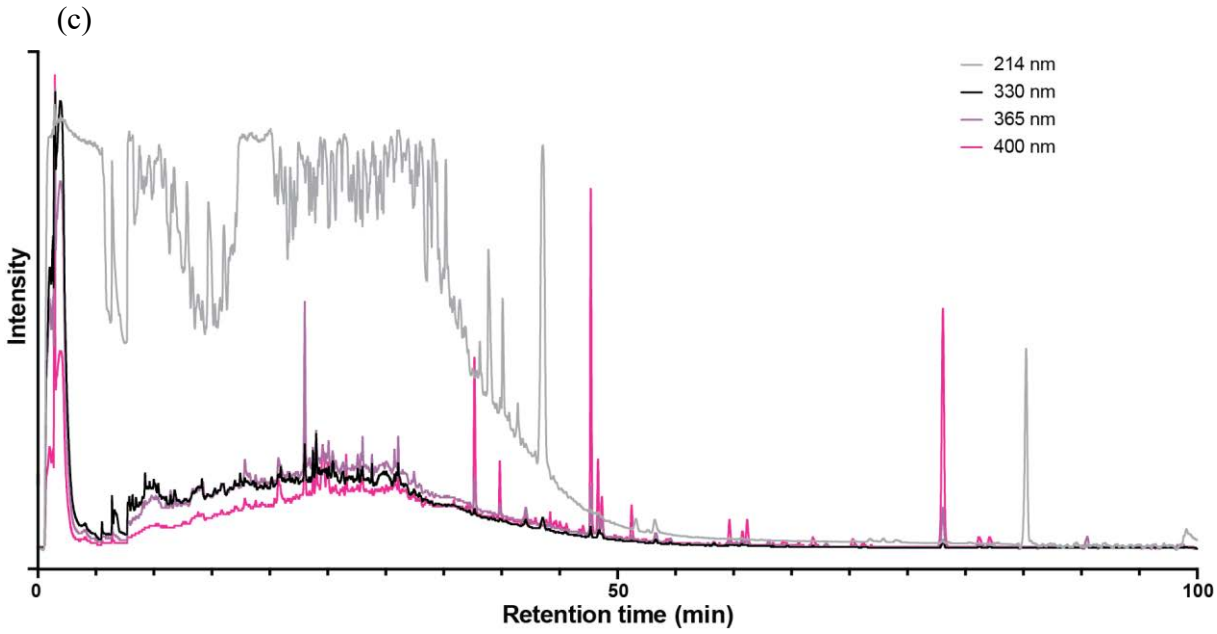


Figure 4.10 (c) Male northern quoll (*Dasyurus hallucatus*) RP-HPLC.

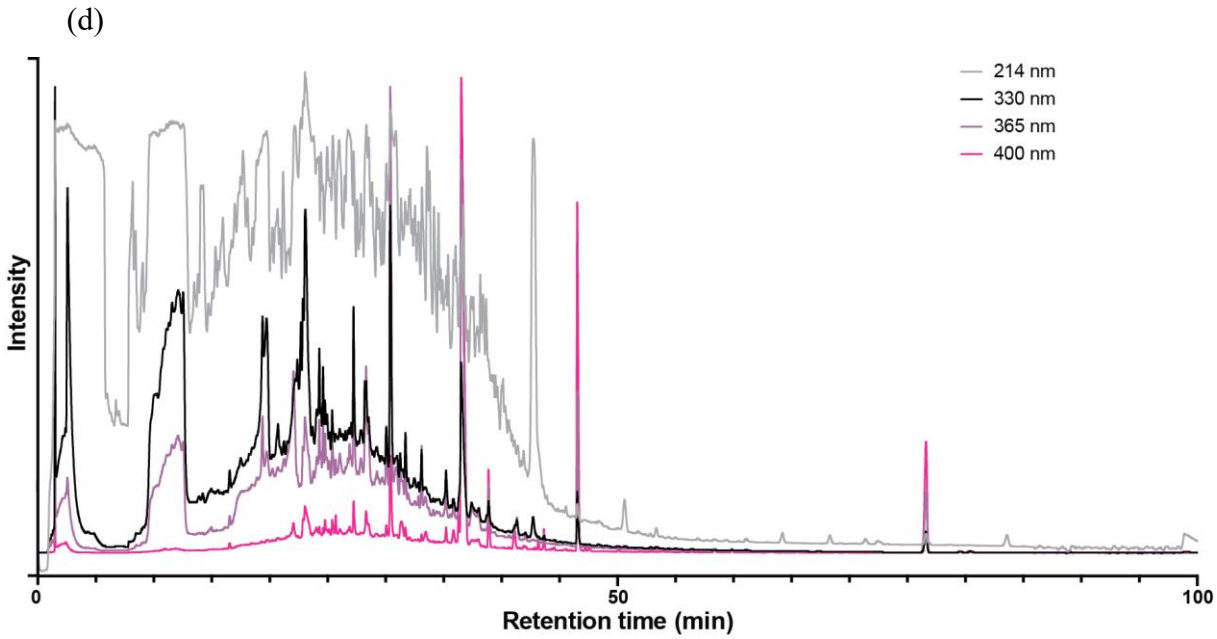


Figure 4.10 (d) Female coppery brushtail possum (*Trichosurus johnstonii*) RP-HPLC.

(e)

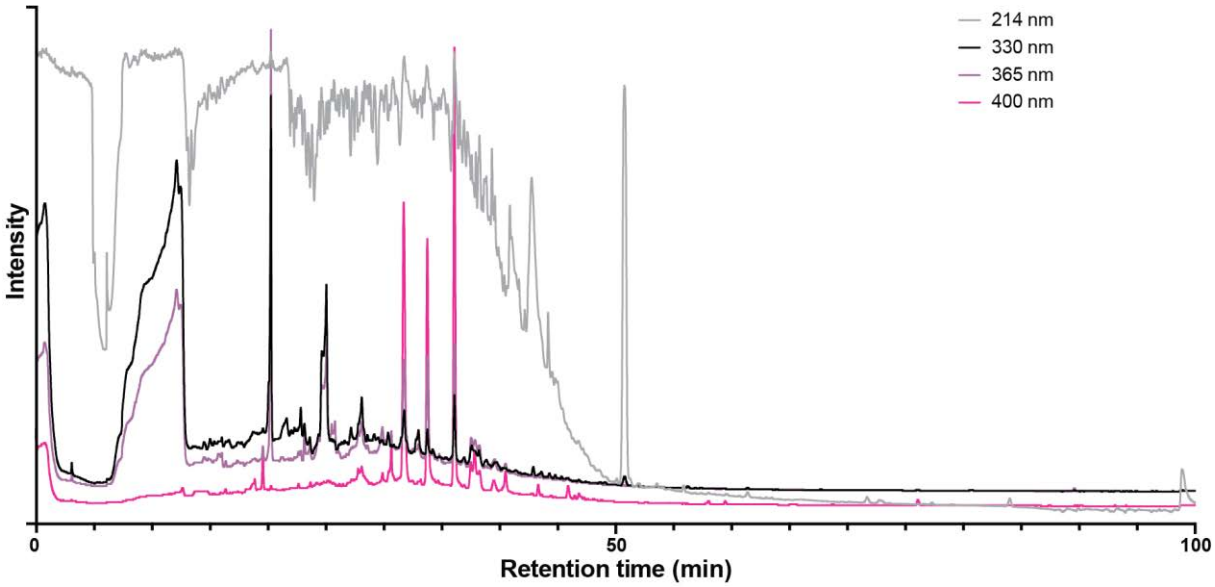


Figure 4.10 (e) Male Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*) RP-HPLC.

(f)

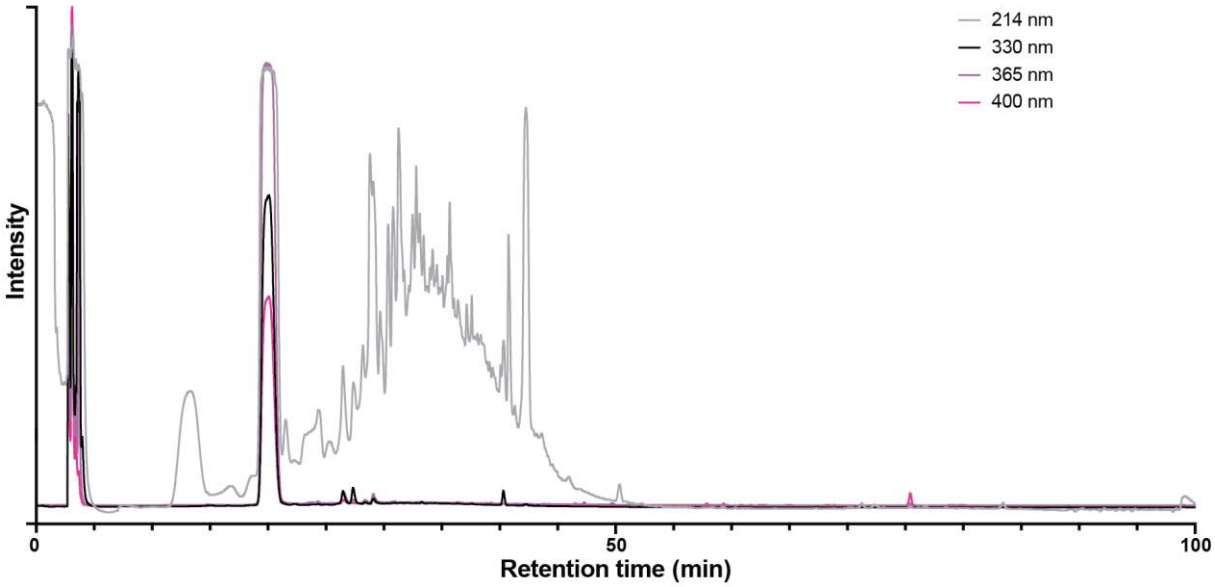


Figure 4.10 (f) Female pale field rat (*Rattus tunneyi*) RP-HPLC.

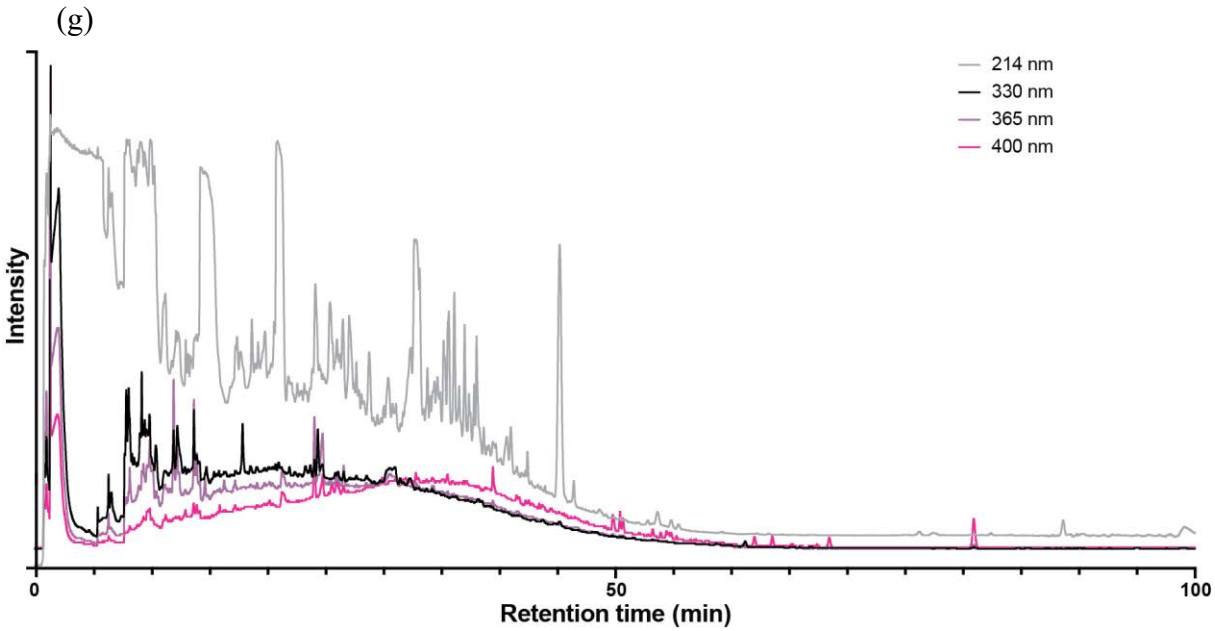


Figure 4.10 (g) Female platypus (*Ornithorhynchus anatinus*) RP-HPLC.

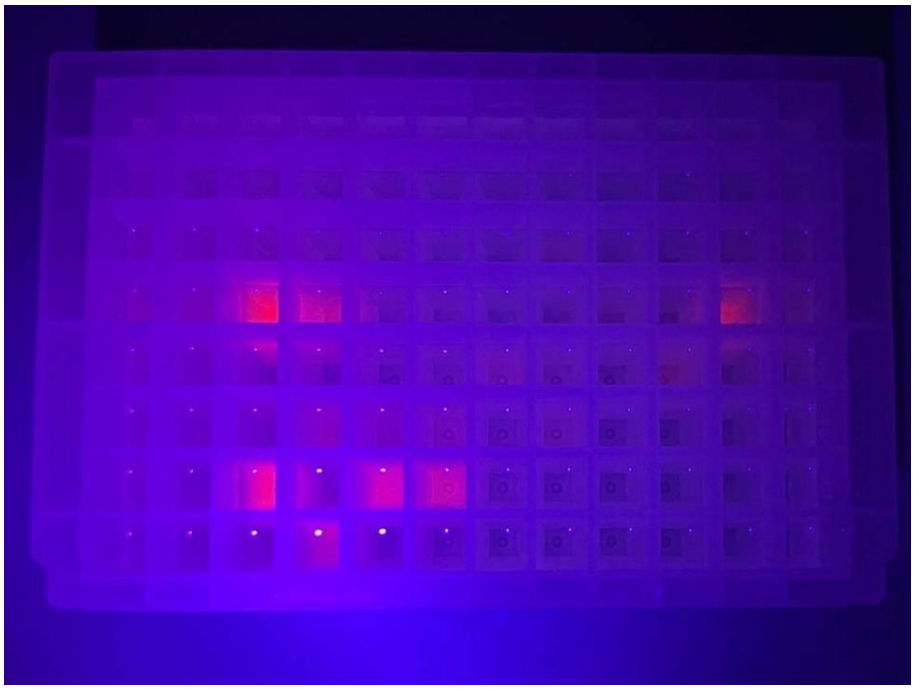


Figure 4.11. RP-HPLC fractions of a sample from a male long-nosed bandicoot (*Perameles nasuta*) observed under a 395–410 nm ultraviolet-violet torch showing the presence of photoluminescent compounds in individual wells (Photo by David Wilson).

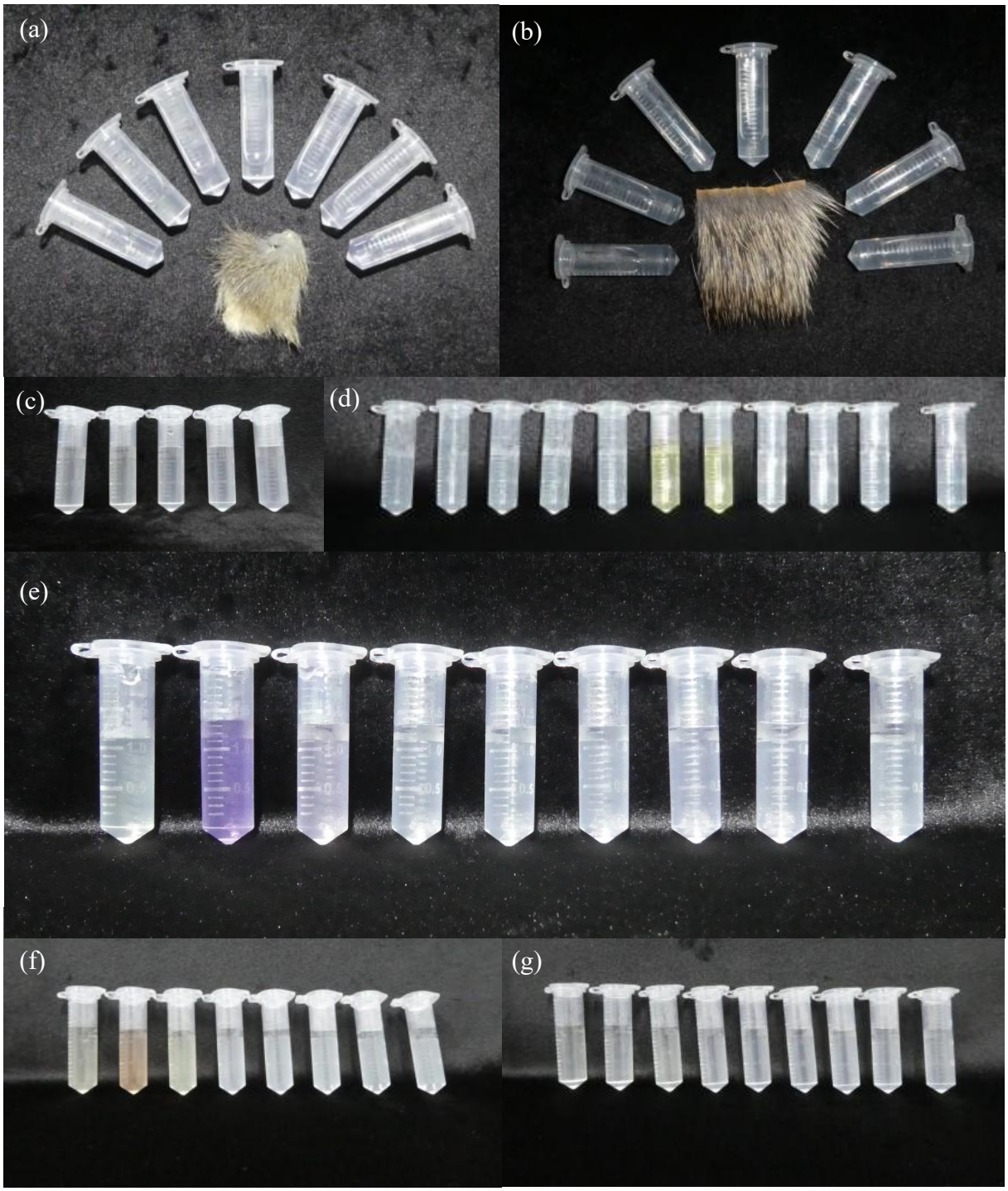


Figure 4.12. Selected RP-HPLC fractions in order of retention time, under white light. (a) Male long-nosed bandicoot (*Perameles nasuta*) (with pelt sample). (b) Male northern brown bandicoot (*Isoodon macrourus*) (with pelt sample). (c) Male northern quoll (*Dasyurus hallucatus*). (d) Male Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*). (e) Female coppery brushtail possum (*Trichosurus johnstonii*). (f) Female pale field rat (*Rattus tunneyi*). (g) Female platypus (*Ornithorhynchus anatinus*).

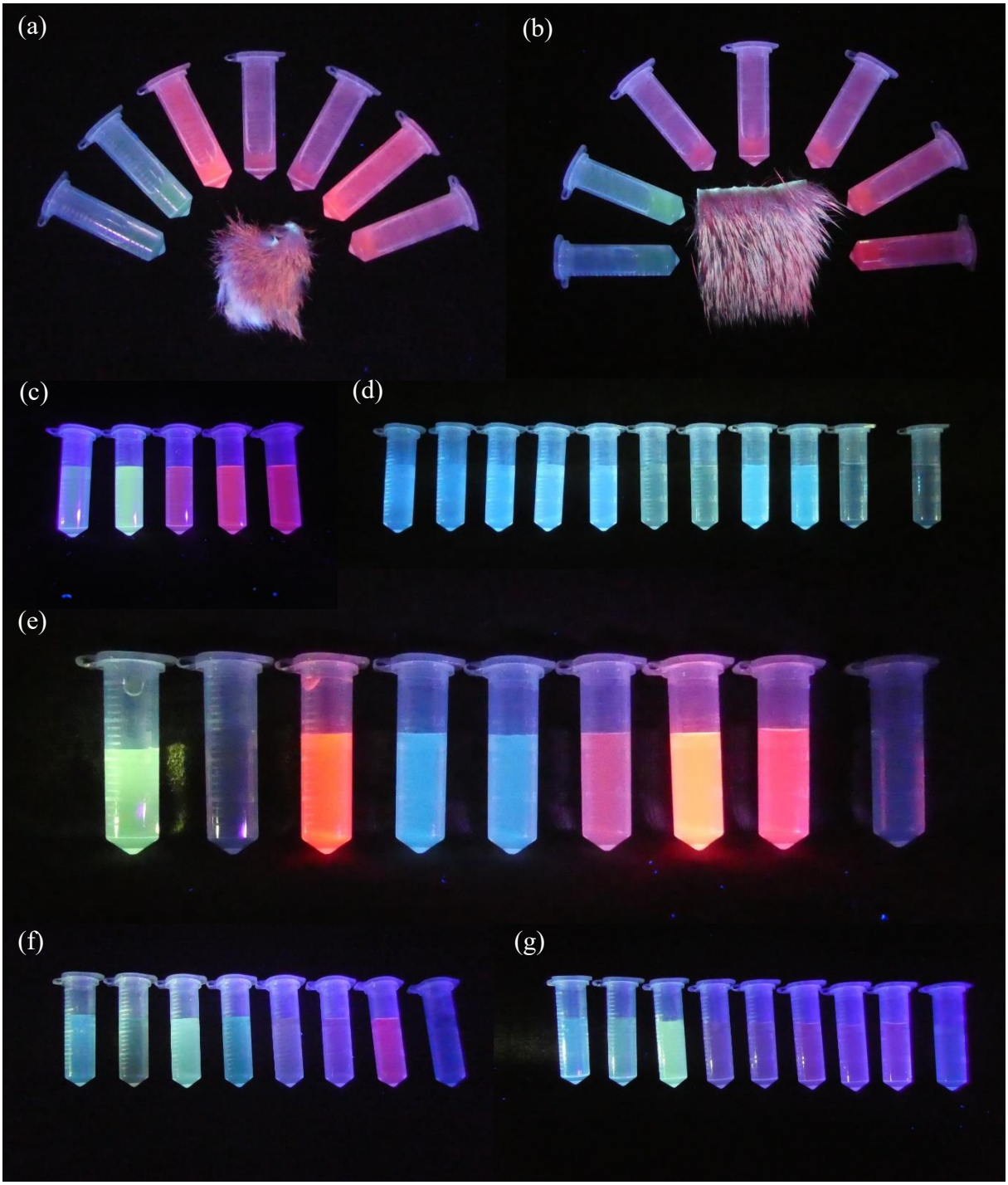


Figure 4.13. Selected RP-HPLC fractions in order of retention time, under 310–410 nm ultraviolet-violet excitation (multiple torches). (a) Long-nosed bandicoot (*Perameles nasuta*) (with pelt sample). (b) Northern brown bandicoot (*Isoodon macrourus*) (with pelt sample). (c) Northern quoll (*Dasyurus hallucatus*). (d) Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*). (e) Coppery brushtail possum (*Trichosurus johnstonii*). (f) Pale field rat (*Rattus tunneyi*). (g) Platypus (*Ornithorhynchus anatinus*). The tube on the far right in (d) to (g) is a non-photoluminescent control RP-HPLC fraction. [Tube photographs may differ from well plate observations made by eye, especially in the blue-shifting of greens.]

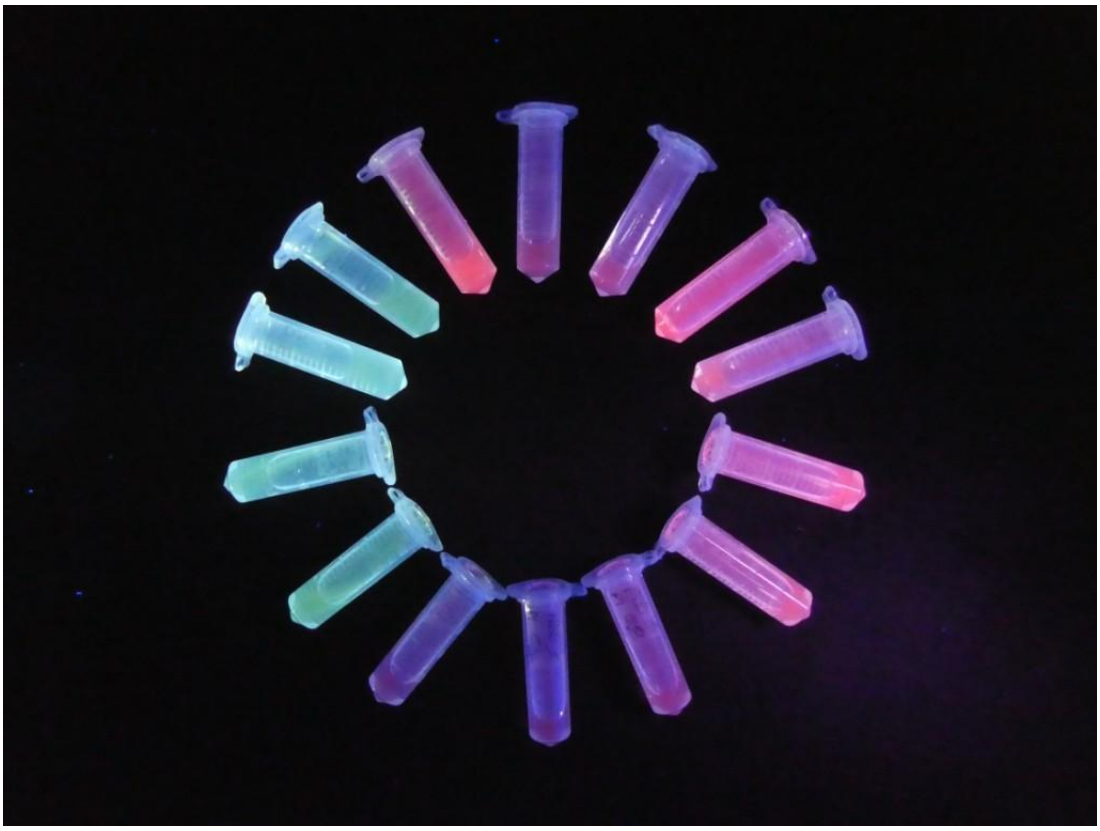


Figure 4.14. Equivalent fractions from long-nosed bandicoot (*Perameles nasuta*) (upper arc) and northern brown bandicoot (*Isoodon macrourus*) (lower arc) fur extracts together.

4.4.6 LC/ESI-MS

The isolated fractions from each species were analysed by LC/ESI-MS to obtain mass and purity data for each fraction. The molecular masses for each fraction are listed along with their photoluminescent characteristics in Appendix D. Based on previous reports of porphyrin molecules present in fur samples, two porphyrin standards (protoporphyrin IX and coproporphyrin I) were also analysed by LC/ESI-MS (Appendix E). Protoporphyrin IX eluted with a retention time of ~66.5 minutes and had a $[M+H]^+$ of *m/z* 563.3271. Coproporphyrin I eluted with a retention time of 30 minutes and had a $[M+H]^+$ of *m/z* 655.3214.

A fraction corresponding to a chromatogram peak with a retention time ~38 min at 400 nm absorption was present for both species of bandicoot and the coppery brushtail possum (long-nosed bandicoot: Plate 1, G3; northern brown bandicoot: Plate 1, G3; coppery brushtail possum: Plate 1, G2). These fractions photoluminesced orange-pink and had monoisotopic masses of 830.2615, 830.3417 and 830.3099 Da respectively, which matched with the monoisotopic mass of uroporphyrin (830.2283 Da).

A fraction corresponding to a chromatogram peak with a retention time of 30 minutes at 370 nm absorption was present for the long-nosed bandicoot and the northern quoll (northern brown bandicoot: Plate 1, H11; northern quoll: Plate 1, H12). These fractions photoluminesced pink and had monoisotopic masses of 654.3169 and 654.3397 Da respectively, which matched with the monoisotopic mass of coproporphyrin (654.2690 Da). A fraction corresponding to a chromatogram peak with a retention time of ~41 minutes at 365 nm absorption was also present for the long-nosed bandicoot (Plate 1, G7). This fraction photoluminesced pink and had a monoisotopic mass of 786.2928 Da, which matched the monoisotopic mass of heptacarboxylporphyrin (786.2385 Da).

All species had a late-eluting peak of ~67 minutes that had a monoisotopic mass matching the monoisotopic mass of protoporphyrin (562.2580 Da). No other molecular weights identified in any other of the samples matched with other previously reported fur luminophores. The LC/ESI-MS chromatograms of the protoporphyrin IX and coproporphyrin I standards, and the fractions for the long-nosed bandicoot (fractions Plate 1 H11 and Plate 2 F1) and coppery brushtail possum (fraction Plate 2 E10), predicted to contain protoporphyrin and coproporphyrin, were compared (Appendix E). The comparison showed the monoisotopic masses of the sample fractions were consistent with the protoporphyrin IX and coproporphyrin I standards. However, the retention time of the protoporphyrin IX standard was ~66.5 minutes, whereas the long-nosed bandicoot (fraction Plate 2 F1) and coppery brushtail possum (fraction Plate 2 E10) fractions eluted at ~67.5 minutes. The retention time of the coproporphyrin I standard was ~30.0 minutes, whereas the long-nosed bandicoot (fraction Plate 1 H11) eluted at ~29.5 minutes. The differences in retention times between the protoporphyrin IX and coproporphyrin I standards and the sample fractions are likely due to the sample fractions containing protoporphyrin and coproporphyrin isomers other than protoporphyrin IX and coproporphyrin I. Following these analyses, the molecules causing photoluminescence in the majority of sample fractions from all species remain unknown or unconfirmed.

4.5 Discussion

I observed photoluminescence in the fur of seven Australian Wet Tropics mammal species, including long-nosed and northern brown bandicoots, northern quoll, coppery brushtail possum, Lumholtz's tree-kangaroo, pale field rat, and platypus. Spectroscopy was useful in determining the exact wavelengths of photoluminescent emission, but could not identify the molecules involved. However, the excitation maxima around 400 nm (Soret band) and emission in the orange-red part of the spectrum is consistent with the spectrographic signature of photoluminescent porphyrins (Shkirman et al. 1999; Ishihara et al. 2014). Whereas the pattern of lesser-absorbing Q-bands can be used to identify different porphyrin molecules using absorption spectroscopy (DiNello and Chang 1978, cited in Toussaint et al. 2023), the emission spectra of coproporphyrin, uroporphyrin and protoporphyrin overlap substantially (Huang et al. 2010; Plavskii et al. 2018). The 633–636 nm emission maxima and 705–706 nm minor peak for both of the bandicoot species are close to the 635 and 705 nm peaks characteristic of protoporphyrin (Rollakanti et al. 2013), a type of porphyrin confirmed in bandicoot fur by LC/ESI-MS. The 624–674 nm lesser peaks of the bandicoot pelts are in the range of coproporphyrin, uroporphyrin and protoporphyrin (Huang et al. 2010; Plavskii et al. 2018). However, the additional ~600 nm peaks expected from these porphyrins were not evident in the spectrographs. Reference spectrographs in water or in acid (Plavskii et al. 2018) may have shifted the emission wavelengths from those displayed in the fur microenvironment. With the different porphyrins so closely overlapping in emission, and no reference emission spectrographs for these molecules *in situ* in fur, the potential for wavelength shifts make it too problematic to identify individual molecules from the emission spectra. The presence of several different porphyrin molecules in the fur may also confound interpretation of the emission spectra.

The RP-HPLC and LC/ESI-MS results indicate the presence of at least four different types of porphyrin molecule in long-nosed bandicoot fur, and at least two different types in northern brown bandicoot fur. Different individual bandicoots were used for the spectroscopy than for the chemistry, and greater sample sizes may result in a greater diversity in both spectrographs and luminophores. However, both species of bandicoot have multiple peaks in the orange-red part of the spectrum, which is consistent with the multiple kinds of porphyrin molecules found by RP-HPLC and LC/ESI-MS. The multiple peaks in the orange-red part of the spectrum of springhare fur also indicates the identification of multiple types of porphyrin molecules (Olson et al. 2021). These results for bandicoots and springhares are at odds with

the results for European hedgehog spines, which showed only a single 654 nm spectrographic emission peak (and a slight hump ~705 nm) at 400 nm excitation, yet the presence of protoporphyrin IX, coproporphyrin III and uroporphyrin III was confirmed by RP-HPLC and ESI+ high-resolution mass spectrometry (HR-MS).

The yellow photoluminescent colour seen on the tips of the brindled bandicoot fur and the yellow photoluminescent RP-HPLC fractions did not manifest in the spectrographs. From detailed examination of dried pelts with different excitation wavelengths (see Appendix C), it is likely that the photoluminescence of the fur tips is from lesser concentrations of the same porphyrin luminophores, with the yellow appearing most conspicuous when excited by 365 nm light instead of the optimal 395–410 nm wavelength, or when the torch is a greater distance from the bandicoot, stimulating a weaker photoluminescence and therefore appearing less red. At higher excitation wavelengths, the same fur can appear pink. The reason for the yellow luminophore(s) isolated from RP-HPLC not showing on the spectrographs could be suggestive of a low concentration compared to the pink luminophores, or of suppression in the fur microenvironment. While some long-nosed bandicoots exhibit a lower-excitation blue and white photoluminescence additional to the pink (see Chapter 3), these colours were not sampled in the fur of those individuals used for either spectroscopy or chemistry.

The bandicoot pelts also gave a ~cyan spectrographic emission, not as pronounced as those emissions presumed to be from porphyrins. This cyan photoluminescence did not appear in the RP-HPLC fractions of bandicoot fur extracts. A broad peak in the cyan region of the spectrum was also recorded for European hedgehog spines (Hamchand et al. 2021). Hamchand et al. (2021) attributed this photoluminescent emission to brown eumelanins. However, in the northern brown bandicoot pelt, the spectrograph of the brown flank fur showed a relatively lower cyan peak than did the pale buff (i.e. less melanin) ventral fur. The melanin-rich rump fur of the long-nosed bandicoot showed the lowest relative cyan peak of all. These results suggest that the presence of melanins may be partially blocking the photoluminescent emission (Jachowicz and McMullen 2011), and that this broad unspecified emission in bandicoots may more likely be due to keratins (Melhuish and Smith 1993; Millington 2020) or intrinsic protein deep-blue photoluminescence (Niyangoda et al. 2017; Tomalia et al. 2019). While melanins do photoluminesce, they do so weakly (Gallas and Eisner 1987; Meredith and Sarna 2006), and generally act as a photoluminescence inhibitor (Rebell 1966; Posudin et al. 2007; Daly et al. 2009). Toussaint et al. (2023) did not associate the general blue emission they recorded from all fur with melanins, but suspected keratins to

be at least partially responsible. The relatively strong cyan peak that Olson et al. (2021) measured from the blue photoluminescent ventral surface, but also from the orange-red photoluminescent dorsal surface, of springhares was not explained.

Fluorescence microscopy observations of Wet Tropics mammals revealed a variation of the location of photoluminescence along the fur shaft; it can be either separate to the pigment granules (zoochromes), or co-occur with pigmentation. When viewed on whole pelts or as RP-HPLC fractions, the photoluminescent colours of fur luminophores spanned from the violet to the red parts of the visible spectrum. However, the ultraviolet (DAPI) filter on the fluorescence microscope only allows emission in a band of deep blue from 420–470 nm. Unfortunately, 450 nm is also the wavelength at which intrinsic deep-blue photoluminescence emits (Niyangoda et al. 2017; Millington 2020). Hence, fluorescence microscopy may not necessarily be capable of distinguishing the luminophores of interest from this ubiquitous background photoluminescence (Tomalia et al. 2019). Additionally, a ubiquitous photoluminescence emanates from the keratin of all fur, which may emit in yellow-green (Pine et al. 1985), cyan (Melhuish and Smith 1993) or blue (Kean and Cutting 2022; Toussaint et al. 2023). Therefore, photoluminescence is observable by microscopy even if the whole animal appears non-photoluminescent by ultraviolet torchlight (Pine et al. 1985). This ubiquitous microscopic photoluminescence could also be elicited because the excitation light emitted by fluorescence microscopes has a higher energy than that of ultraviolet torches. These issues confound the microscopic delineation of actual luminophore photoluminescence in fur. I found the simple close-up examination of whole pelts with appropriate torches (see Chapter 3; Fig. 4.5) a more realistic way of accurately viewing where along the fur shaft the photoluminescence resides. Viewing the fur as it lies naturally on the skin also allows determination of how the differently photoluminescing layers of fur sit in relation to each other, and of the overall effect that the real-life arrangement imparts.

Analysis of fur extracts by RP-HPLC and LC/ESI/MS revealed the presence of isomers of uroporphyrin, heptacarboxylporphyrin, coproporphyrin and protoporphyrin. The protoporphyrin intensity differed and was lower in species that did not have a visible pink photoluminescence throughout their pelts. For the platypus, pink photoluminescence was only found on closer inspection of the remaining clipped fur after the isolation of pink luminophores, but for the Lumholtz's tree-kangaroo and the pale field rat, no pink fur photoluminescence could be detected by the human eye. It is possible that protoporphyrin is a ubiquitous compound in fur that varies in concentration in different species. Small amounts of

protoporphyrin IX, as a precursor to haem, are known to be ubiquitous in living cells (Sachar et al. 2016). Because of its insolubility in water, excess protoporphyrin IX is not excreted through urine, but in the faeces (Ajoka et al. 2006), and also through the liver, which can be problematic (Sachar et al. 2016). To prevent toxic levels building up in the body, an unexplored metabolic pathway of protoporphyrin IX excretion may therefore in part occur in the fur (Toussaint et al. 2023). However, the protoporphyrin in the fur extracts of at least the long-nosed bandicoot and the coppery brushtail possum appeared to be a different isomer of protoporphyrin. No other protoporphyrin isomers are involved in haem biosynthesis, and enzyme specificity was thought to prevent the natural formation of other protoporphyrin isomers from coproporphyrinogen isomers (Burnham 1969, cited in Buldain et al. 1977). However, protoporphyrin XIII can be formed from coproporphyrinogen IV (Al-Hazimi et al. 1976; Mombelli et al. 1976). The identification of other protoporphyrin isomers that can occur in fur remains unknown.

I identified the presence of molecules consistent with uroporphyrin and protoporphyrin in an extract of coppery brushtail possum fur, whereas Nicholls and Rienits (1971) did not identify these or any porphyrins in fur of the closely related common brushtail possum. Common brushtail possum fur had been recorded as having reddish photoluminescence by Bolliger (1944), but not by Nicholls and Rienits (1971). However, porphyrins could be missed because of the use of just water as a solvent, an excitation wavelength too low to excite the luminophores, or because of photobleaching. The highly hydrophobic nature of protoporphyrin means it may have been missed in some chromatography studies due to much longer retention times than other porphyrin molecules (e.g. Olson et al. 2021 cf. Hamchand et al. 2021).

The laboratory methods I used did not identify any non-porphyrin luminophores. Given the range of photoluminescent colours displayed in the RP-HPLC fractions that did not match the profile of porphyrins, there are many more luminophores in fur that remain to be identified. This conclusion is consistent with Nicholls and Rienits' (1971, p. 602) observation that, "A confusing number of fluorescent components is present in some samples", and also the multiple unidentified potential luminophores in squirrel (Sciuridae) fur (Hughes et al. 2022). Tryptophan metabolite luminophores had previously been identified from the fur of several mammal species, including a brushtail possum and a tree-kangaroo (Rebell 1966; Nicholls and Rienits 1971; Pine et al. 1985). With the exception of the porphyrins in hedgehog spines (Derrien and Turchini 1925; Hamchand et al. 2021), tryptophan metabolites

were long thought to be the only known luminophores in fur. It is therefore unexpected that none of the photoluminescent fractions from the Wet Tropics species investigated here had molecules matching the molecular weights of luminophores such as kynurenine or 3-hydroxyanthranilic acid. I took the coppery brushtail possum fur samples from the upper mid flank, a patch chosen because it photoluminesced particularly strong orange/pink, rather than the characteristic purple (Watanabe et al. 1972) of the 3-hydroxyanthranilic acid identified from common brushtail possum fur (Nicholls and Rienits 1971). It is possible that fur from other areas on the coppery brushtail possum contained this luminophore, or that there is a species difference. It is plausible that either the known tryptophan metabolite luminophores were missed, and/or that other luminophores not previously identified in fur were present in the RP-HPLC fractions.

The luminophore colours evident in the fur were not necessarily evident in the RP-HPLC fractions and *vice versa*. Rebell et al. (1956) also noted this, finding a weak blueish photoluminescence in the fur extracts of several domestic species, even if they did not show photoluminescence when viewed as whole animals. The cause of this general blueish photoluminescence in domestic species was not resolved. The fur of all Wet Tropics mammals extracted here yielded yellow photoluminescent fractions, but blue photoluminescent fractions were not common. This observation may simply indicate that the optimal extraction method for pink photoluminescence was not effective at extracting blue photoluminescence from the keratin structure. Whereas both the Lumholtz's tree-kangaroo and the pale field rat showed very similar bright blue photoluminescence when examined whole (Figs. 3.3d and 3.4b), the expected blue photoluminescent fractions were only yielded from the tree-kangaroo fur (Fig. 4.13d cf. 4.13f). When blue photoluminescent fractions from the Lumholtz's tree-kangaroo fur were analysed, their monoisotopic masses did not match any of the known tryptophan metabolites so far identified from fur.

None of the blue or yellow-green photoluminescent RP-HPLC fractions extracted from the fur of Wet Tropics mammals appeared to contain molecules matching keratin proteins. However, keratin was not necessarily extracted from the fur, and keratin proteins would not be expected to be fractioned with a C18 column. The lack of blue RP-HPLC fractions for the brightly blue photoluminescent pale field rat may mean that the blue photoluminescence visible in the fur was caused by keratin proteins, or that any blue-photoluminescing tryptophan metabolites in the fur were not isolated. For the blue photoluminescent fractions extracted from the Lumholtz's tree-kangaroo, more work is required to determine whether the

photoluminescence may be caused by aromatic amino acids, monomeric proteins and non-aromatic amino acids (Niyangoda et al. 2017; Chen et al. 2018), peptides, hetero-atomic sub-luminophores (Tomalia et al. 2019) or an unknown luminophore. At least some of the photoluminescence of keratin is due to the residues of tryptophan, tyrosine and phenylalanine (Longworth 1983, cited in Millington 2020). However, these amino acids maximally emit in the ultraviolet (Yang et al. 2015), and may need to be encased in protein structures to effect bright photoluminescence (Konev 1967; Ormö et al. 1996; Tomalia et al. 2019). No such amino acid residues were identified by my laboratory methods. Nonetheless, keratin causes an intense light-blue photoluminescence of vertebrate claws, scales and fur (Stübel 1911; Jeng 2019).

The principal tryptophan metabolite, kynurenine, becomes more photoluminescent when it is bound into proteins, being barely photoluminescent in its free state (Vazquez et al. 2002). At least for tryptophan residues, the microenvironment within proteins (hydration, softening of the keratin matrix, breakage of disulphide bonds, interactions with some enzymes and side-chain amino acids) can either quench or boost the intensity of photoluminescence (Reshetnyak and Burstein 2001; Jachowicz and McMullen 2011). Therefore, the appearance of luminophores extracted in liquid may not necessarily determine whether a luminophore will be activated in the fur environment. This complication was suspected by Hughes et al. (2022), who extracted luminophores from squirrels that had not exhibited the photoluminescence in their pelts. However, Hughes et al.'s (2022) excitation wavelength for determination of whole-pelt photoluminescence was 395 nm, changing to 350 nm for fur extracts. Higher excitation wavelengths of around 400 nm are optimal for porphyrins (Goldoni 2002), yet lower wavelengths are optimal for the excitation of the tryptophan metabolites in fur (Rebell 1966; Nicholls and Rienits 1971). Melanin can also act to quench the photoluminescence in fur (Rebell 1966; Posudin et al. 2007; Daly et al. 2009). If chemical analysis can isolate luminophores not otherwise visible in the fur itself, this suggests that those luminophores can have no optical function *in situ*.

4.6 Conclusion

In this chapter, I examined the luminophore composition of photoluminescent fur from seven species of mammal from the Wet Tropics of Far North Queensland, Australia. Numerous luminophores were present in each fur sample. Some luminophores were found to be common across different species, with a luminophore likely to be a protoporphyrin isomer other than protoporphyrin IX identified in all species tested. A second common luminophore identified in two species is likely to be a coproporphyrin isomer other than coproporphyrin I. Potential uroporphyrin and heptacarboxylporphyrin isomers were also present in some species. Many of the luminophores isolated in this study remain to be identified. For some species, the observed colour of the fur photoluminescence or fur extraction was not necessarily predictive of the actual luminophores present. This is the first study to show the extent of the luminophore composition across species of marsupial, monotreme and placental mammal fur from one bioregion of Australia.

Chapter 5

Effects of photobleaching and specimen preservation on photoluminescence in fur

5.1 Abstract

The fur of some mammals is photoluminescent due to the presence of different luminophores, principally porphyrin derivates and tryptophan metabolites. I conducted a series of experiments to determine whether exposure to light and wet preservation altered the presentation of photoluminescence in pelts. I primarily used two species, the northern brown bandicoot (*Isoodon macrourus*) as an example of pink photoluminescence, which is commonly caused by porphyrin luminophores, and the laboratory rat (*Rattus norvegicus*) as an example of blue-white photoluminescence, which is commonly caused by tryptophan metabolite luminophores. I used bandicoot pelts to test how long fur could be exposed to sunlight before the pink photoluminescence was compromised. I also used pelts from a bandicoot and rat to assess how resistant photoluminescence was to wet preservation. Pink photoluminescence and blue photoluminescence exhibited different stabilities in various conditions of preservation. The pink photoluminescence observed in bandicoots suffered visibly noticeable photobleaching within two minutes of direct sun exposure. Both types of photoluminescence were mostly lost when fur was stored in ethanol for six months. These findings indicate that studies on photoluminescence in mammal fur based solely on museum specimens should be interpreted with caution, with consideration given to the method of preservation and exposure to light.

5.2 Introduction

Recent studies describing the photoluminescence of mammal fur have largely been based on museum specimens (Anich et al. 2021; Toussaint et al. 2023), although some studies have verified photoluminescence in living or freshly killed individuals (Kohler et al. 2019; Olson et al. 2021; Pynne et al. 2021; Tumlison and Tumlison 2021). A limitation of studies based solely on museum specimens is whether the photoluminescence is inherent to the animal itself, or a function of the preservation method. To date, there have been no dedicated attempts to document the loss (or gain) of photoluminescence as fur pelts are put through taxidermy processes and exposed to light.

The blueish photoluminescence of some mammal species, such as laboratory rats (*R. norvegicus*), is caused by various tryptophan metabolites (Rebell et al. 1957, Rebell 1966). Tryptophan-based photoluminescence in live animals is known to gradually degrade under light exposure over a matter of months (Schäfer et al. 1997; Posudin 2007; Longo et al. 2013). Diurnal mammals such as sheep (*Ovis aries*) (Collins 1992) and humans (*Homo sapiens*), (Daly et al. 2009) continue displaying photoluminescence despite regular sunlight exposure.

Other mammal species, such as bandicoots (family Peramelidae), display bright pink photoluminescence (Reinhold 2020, 2021). Pink-red photoluminescence in the pelage of several unrelated species results from porphyrins, which are also suspected to be responsible for the pink photoluminescence in bandicoot fur (Toussaint et al. 2023; see Chapter 4). Kohler et al. (2019) found pink photoluminescence retained in dry-preserved flying squirrel (*Glaucomys* spp.) pelts dating back to the late 1800s, with similar characteristics observed in wild animals, although not assessed in the same conditions. However, other studies have reported more vivid reddish photoluminescence in live or fresh animals than in preserved museum specimens (Olson et al. 2021; Tumlison and Tumlison 2021). Pine et al. (1985) observed that specimens of American opossums (Didelphidae) collected relatively recently photoluminesced more intensely than older specimens, and that living zoo animals exhibited greater photoluminescence. However, once the initial loss of photoluminescence had occurred after death, intensity did not degrade with length of time since collection (Pine et al. 1985). Pine et al. (1985) had determined that some species of opossums (including the Virginia opossum, *Didelphis virginiana*), that had only been examined as museum specimens, did not photoluminesce. However, after later examining live or fresh examples, Ronald H. Pine found photoluminescence in the fur of these same species, and now believes that strong

photoluminescence may occur in all species of opossums (e-mail from Ronald H. Pine, University of Kansas, Lawrence, Kansas, 17 February 2021). Opossum fur photoluminescence was correlated with flushes of pink and red pigments in white light, which also faded from the pelt soon after death in several species, with some specimens kept in the dark fading after several years (Pine and Handley 1984; Pine et al. 1985).

By the 1980s, Ronald H. Pine had already deduced that sun exposure affected photoluminescence pre- and/or post-mortem: "I discovered that if someone brought me a roadkilled *Didelphis virginiana*, I could tell which side of the animal was down on the pavement and which side was up in the sunlight. The fur that had been on the pavement side fluoresced and the side that had been in the sun did not. Clearly, the fluorescent substance(s) were destroyed by the sunlight." (e-mail from Ronald H. Pine, University of Kansas, Lawrence, Kansas, 18 February 2021). The degradation of photoluminescent molecules by exposure to light has been termed 'photobleaching' (Ericson et al. 2003). In at least two other species of opossums, the cause of this labile pink photoluminescence has since been found to be porphyrins (Toussaint et al. 2023). Photobleaching has since been demonstrated in experimental trials using bustard (Otididae) feathers, which showed that they lost their salmon-pink porphyrin colouration (an indicator of photoluminescence) with 12 to 25 minutes of exposure to sunlight (Galván et al. 2016).

In contrast to fading of photoluminescence from specimens, artificial bright photoluminescent greenish or yellowish stains are sometimes inadvertently added during taxidermy or fumigation of dry-preserved skins (Pohland 2007). Some preservation chemicals that have photoluminescent properties are routinely detected on mammal specimens (Goldberg 1996; Sirois et al. 2010; Kehoe and Becker 2017). Some pest control treatments used in the transportation of specimens are of particular concern. From 1886 to the 1930s, hydrogen cyanide, and the cyan-green photoluminescent (with an additional distinctive emission peak at 258 nm, Gackowska et al. 2003) carbon disulphide, were used as fumigants (Bond and Monro 1984). After the 1930s, the green fluorescent dye, methyl bromide, was commonly used in its gaseous form to treat museum specimens (Rajendran and Parveen 2005), and as an international shipping and quarantine fumigant (Haack et al. 2011; United States Environmental Protection Agency 2022).The pelts of taxidermied mammals on public display have variously been washed, soaked in a tanning solution, and faded skin and fur areas painted or airbrushed with acrylic paints, dyes and other pigments to resemble their

original colours (Queensland Museum 2010–2022; Nunan et al. 2012), rendering them unusable for examination of natural photoluminescence.

Apart from a single comparison by Tumlison and Tumlison (2021) of eastern moles (*Scalopus aquaticus*) retaining their vivid dull-greenish photoluminescence across dry, formalin-isopropanol and frozen specimens, there have been no targeted investigations into the effects of exposure to light or wet versus dry preservation on the fur photoluminescence of mammal specimens. Toussaint et al. (2023) observed that alcohol-preserved opossum specimens had a higher intensity of porphyrin photoluminescence than dry-preserved hedgehog (*Erinaceus* spp.) and flying squirrel specimens, but preserved specimens were not compared with fresh material of the same species, nor were pelts compared before and after preservation.

The research presented here builds on the observations of Pine et al. (1985) and Olson et al. (2021) that live or fresh animals display brighter photoluminescence than that of museum specimens. Specifically, I timed the duration of photobleaching of pink photoluminescence, and tested the effects of wet preservation on the retention of photoluminescence in fur. This study recommends caution in the interpretation of photoluminescence solely from preserved museum specimens.

5.3 Methods

5.3.1 Photobleaching

A pilot study using laboratory rat (n = 16, 10 male and 6 female) pelts demonstrated that blue photoluminescence was not degraded with 18 hours (2 days) of exposure to direct sunlight. I initially tested the effects of sunlight on the pink photoluminescence of northern brown bandicoot (*Isoodon macrourus*; n = 3, 2 male, 1 female) pelts. I sourced fresh roadkill bandicoots from the Pine Creek Yarrabah Road around Kamma, in the south of Cairns, Far North Queensland, Australia. Carcasses were retrieved in the pre-dawn, wrapped in aluminium foil, and transported in a cold box until use that day. Animals were skinned, and the skins stored in a refrigerator (4° C) prior to the experiment. Pelts were each cut into 20 squares measuring 4–5 cm across. Five squares each were cut from the ventral side, dorsal side, and each flank. Two triangles (approximately 4–5 cm base) were cut from the head of each animal.

One square of fur from each location on each animal (n = 12 samples) was wrapped in aluminium foil and labelled as Time (T) = 0. These served as the controls of no light exposure, and kept indoors. All other squares were laid out on brown cardboard on an open area of lawn under direct sunlight. The weather was partly cloudy with the temperature approximately 29 °C (01 October 2021). Randomisation of the length of sunlight exposure in half-hour intervals that each square (from each area of each animal) would receive was determined by the throwing of a die. Sun exposure began at 11:30 am, with one square from each locality on each animal being wrapped in aluminium foil and brought inside at T = 0.5 hour, T = 1 hour and T = 1.5 hours (at 1:30 pm). All squares (controls and light-exposed) were then refrigerated for 1.5 hours to cool them to the same temperature. Squares were then laid back out into their original bandicoot formations in a dark room, unwrapped and examined with a 395–410 nm torch.

Photobleaching had occurred in pelt squares from a minimum sunlight exposure of 30 minutes (see Results). Therefore, an additional three roadkill northern brown bandicoots were sourced from the same locality (one male and two of unknown sex due to damage) to conduct a second experiment to assess the minimum time at which photobleaching occurred. Only the ventral surfaces were used, as the photoluminescence was easier to see and more uniform. Each ventral pelt strip was cut into five or six squares of the same size as the first experiment. The methodology remained the same, apart from adjusting the timing of exposure to five-minute intervals. Sun exposure began at 11:35 am. Weather conditions were similar to the initial experiment, but one degree warmer (30 °C; 14 October 2021).

Photobleaching was still evident in pelt squares from a minimum sunlight exposure of five minutes (see Results). Therefore, a further two roadkill northern brown bandicoots from the same locality (one male and one unknown sex) (14 October 2021) and three long-nosed bandicoots (*Perameles nasuta*) from Yungaburra (virginal female), Malanda (male) and Lake Eacham (virginal female) on the Atherton Tablelands (04 June 2022) were collected to further refine the photobleaching time. Both species photoluminesced a similar intense pink. The photoluminescence in the northern brown bandicoots was not as uniform as in the previous specimens, so the ventral area was only divided into two pieces each. For each ventral pelt, one piece was kept in darkness as the control and the other piece was cut into a further two pieces. This third northern brown bandicoot experiment commenced at 1:20 pm, at a temperature of 30 °C (14 October 2021). One treatment piece was exposed to direct sunlight for one minute, and the other piece was exposed for two minutes. The experiment was

repeated by cutting the remaining half pelt with good dark pink photoluminescence in half again, and putting half in the sun for two minutes. For the long-nosed bandicoots, each pelt was cut into large pieces of dorsal/flanks and ventral (additionally head for the male). Each piece was then cut longitudinally into two pieces, with the pieces on the left reserved as foil-wrapped controls. This time, both control (wrapped) and treatment (unwrapped) pelt pieces were placed alongside each other in full sun at 11:48 am. Photoluminescence was checked each minute for two minutes, then once at five minutes, and finally at 10 minutes total of sun exposure. The weather was sunny with a temperature of 25 °C (04 June 2022).

5.3.2 Wet preservation

One adult female laboratory rat (Bugs Alive, Cairns, Australia) was skinned, fleshed, salted, dried and photographed under ultraviolet to violet torchlight at both 365 and 395–410 nm light. The pelt showed uniform white photoluminescence over the white areas and blue-white photoluminescence on the light brown head cap (Fig. 5.1a). One adult male northern brown bandicoot was collected from a rural road at Aloomba, south of Cairns. The carcass was skinned, fleshed, salted and dried. The dry pelt was stored fur side down inside a cardboard box in a dark laboratory at James Cook University, Nguma-bada campus, at 24 °C until use. Under 395–410 nm torchlight, the pelt showed uniform strong pink photoluminescence throughout the fur, strongest on the ventral fur, and only absent from the front of the face (Fig. 5.1d). The dorsal and face areas were not used.

A map was drawn of each pelt, with the brown and white areas demarcated on the rat, and the flank and ventral areas demarcated on the bandicoot (Fig. 5.2). For the rat, each side of white fur was divided into six 3–4 cm ~squares, totalling 12 squares of white fur. The brown head was divided into eight sections measuring either 2 x 3 cm, or 1.5 x 4 cm, with the four sections on the right allocated to controls and the four sections on the left allocated to treatments (Fig. 5.1b). For the bandicoot, each flank and ventral area on each side was divided into six 4–5 cm squares each, totalling 12 flank squares and 12 ventral pelt squares (Fig. 5.2). For the white fur of the rat, and for the flank and the ventral fur of the bandicoot, a die was thrown to randomly allocate treatment or control groups to each pelt square. Thus, each pelt section of 12 squares had three squares assigned to each of four treatment or control groups.

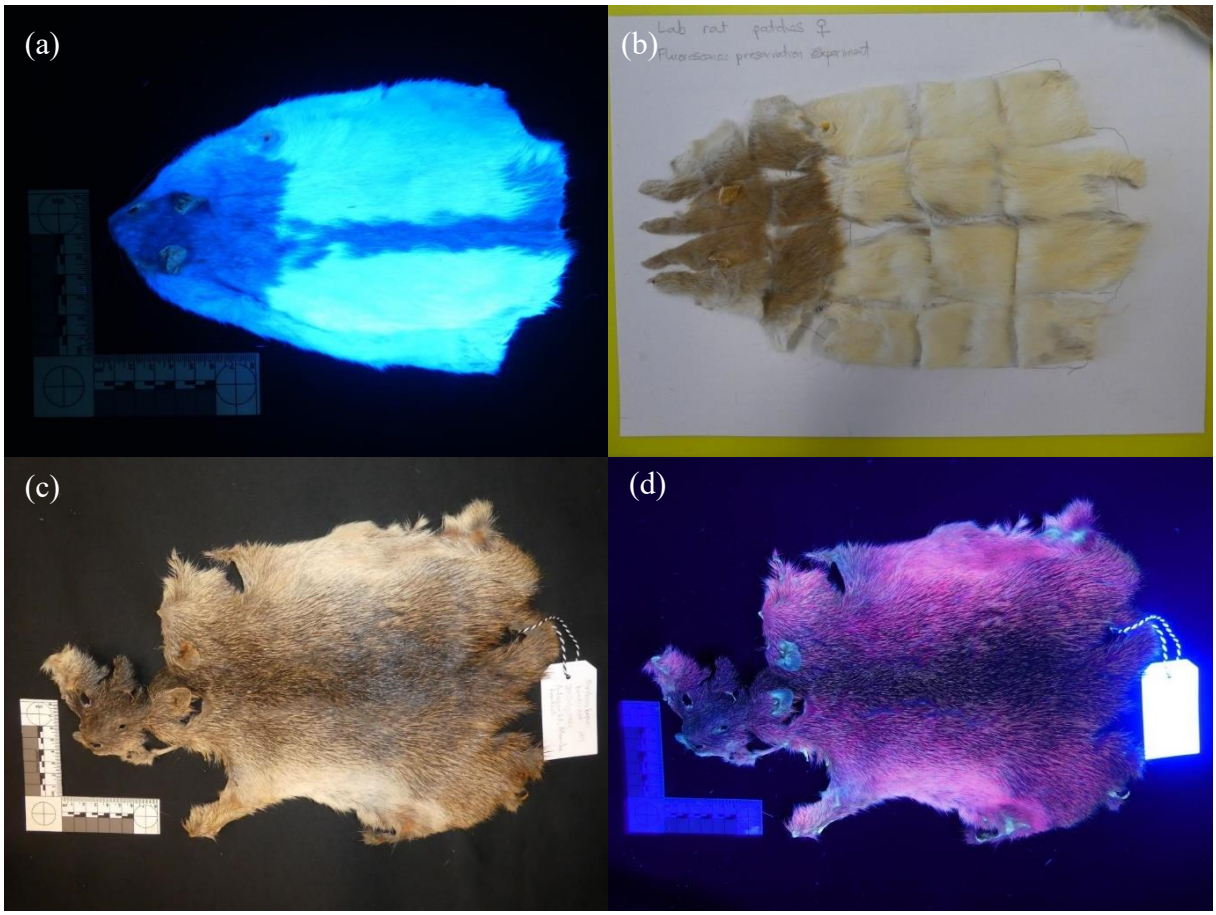


Figure 5.1. Dried pelts in preparation for the wet preservation experiment. (a) Laboratory rat (*Rattus norvegicus*) pelt, white with light brown hood, intact pelt photoluminescing blueish white under 365 nm ultraviolet light (5 s exposure) and (b) mapped out and cut into pieces. (c) Northern brown bandicoot (*Isoodon macrourus*) pelt, brindled brown in white light and (d) photoluminescing pink under 395–410 nm ultraviolet–violet light (6 s exposure).

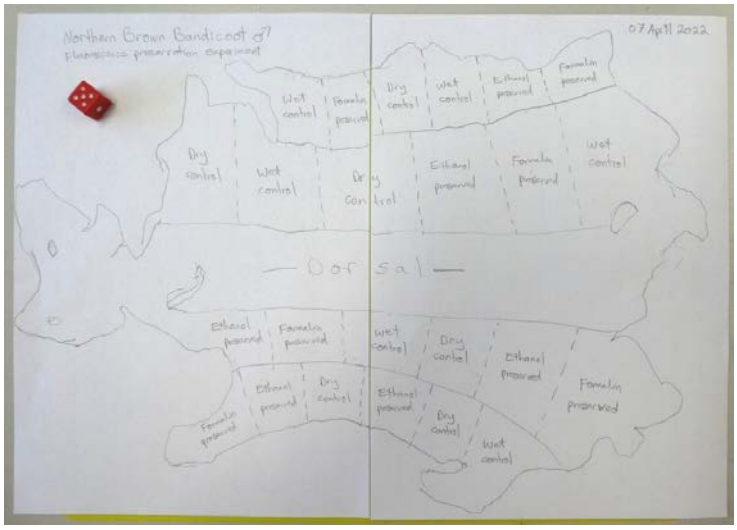


Figure 5.2. Map of bandicoot pelt, with areas demarcated, and squares assigned to treatments.

Pelt squares were assigned to the following treatments and controls: 1) Dry control (rat: n = 5 (3 white + 2 brown); bandicoot: n = 6 (3 ventral + 3 flank)): squares were wrapped in aluminium foil and placed inside a cardboard box; 2) Wet (water) (rat: n = 5; bandicoot: n = 6): squares were placed in a ~90 ml dry glass jar (sealed with a rubber ring and clip-top glass lid) and stored alongside the other treatment groups. After the completion of the wet (ethanol) preservation comparisons, they had Grade 1 water added to compare to the dry controls; 3) Formalin (rat: n = 5; bandicoot: n = 6): squares were placed in a small glass jar filled with formalin (10% Formaldehyde (37%), 90% Grade 1 water) for one week, then rinsed with Grade 1 water and transferred to 70% ethanol (absolute) and 30% Grade 1 water; and 4) Ethanol (rat: n = 5; bandicoot: n = 6): squares were placed in a small glass jar filled with 100% ethanol for one week, then rinsed with Grade 1 water and transferred to 70% ethanol and 30% Grade 1 water. Both formalin and ethanol treatment jars were gently agitated three times in the week before transfer to 70% ethanol. After the fixed, wet-preserved pelt squares were placed into 70% ethanol, jars were placed into a cardboard box (lined and wrapped with aluminium foil) and stored in a metal flammables cabinet at 23 °C for six months (14 April to 15 October 2022). The sequence of fixation followed by longer-term storage was designed to mimic actual conditions used for specimen preservation (Simmons 1999).

After the initial seven days of fixation in either 10% formalin or 100% ethanol, the wet-preserved rat and bandicoot pelt squares were examined *in situ* in comparison to control pelt squares that were dry. After six months, the 70% ethanol-treated pelt squares were removed, rinsed with Grade 1 water and allowed to dry in a dark laboratory for 24 hours, then brushed. The treatment pelt squares were then laid alongside the dry control pelt squares for comparison. The wet (water) pelt squares were then soaked in Grade 1 water for one hour and laid alongside dry control pelt squares for comparison. Rat and bandicoot pelt pieces were examined under 365 nm and 395–410 nm torchlight, respectively. Preservation fluids were examined for photoluminescence straight in control jars, after seven days of pelt square fixation, and after six months of pelt square storage.

5.4 Results

5.4.1 Photobleaching

In the first northern brown bandicoot experiment, all squares, apart from the control squares, were photobleached. The only squares that remained bright pink were those that had not been exposed to the sun (T = 0). Near-complete photobleaching of pink photoluminescence occurred (only a few strands of fur in small pockets retained pink photoluminescence) in all treatment squares of fur from 30 minutes to 1.5 hours of sun exposure. In the second northern brown bandicoot experiment, again only squares not exposed to sunlight retained strong pink photoluminescence. All other squares from 5 to 25 minutes of sun exposure had faded to a much weaker pale pink photoluminescence, or none (Fig. 5.3). The pelt with the strongest, darker pink photoluminescence at the start of the experiment retained some pink photoluminescence compared to the other pelts.

In the third northern brown bandicoot experiment, conducted at one-minute intervals, the ventral fur piece that was in the sun for one minute mostly retained its dark pink photoluminescence. The piece that was in the sun for two minutes had mostly faded like the others that had been exposed for longer time intervals (Fig. 5.3). A repeat of this process produced the same result, with the strong dark pink photoluminescence being mostly bleached out within two minutes, turning it a lesser pale pink.

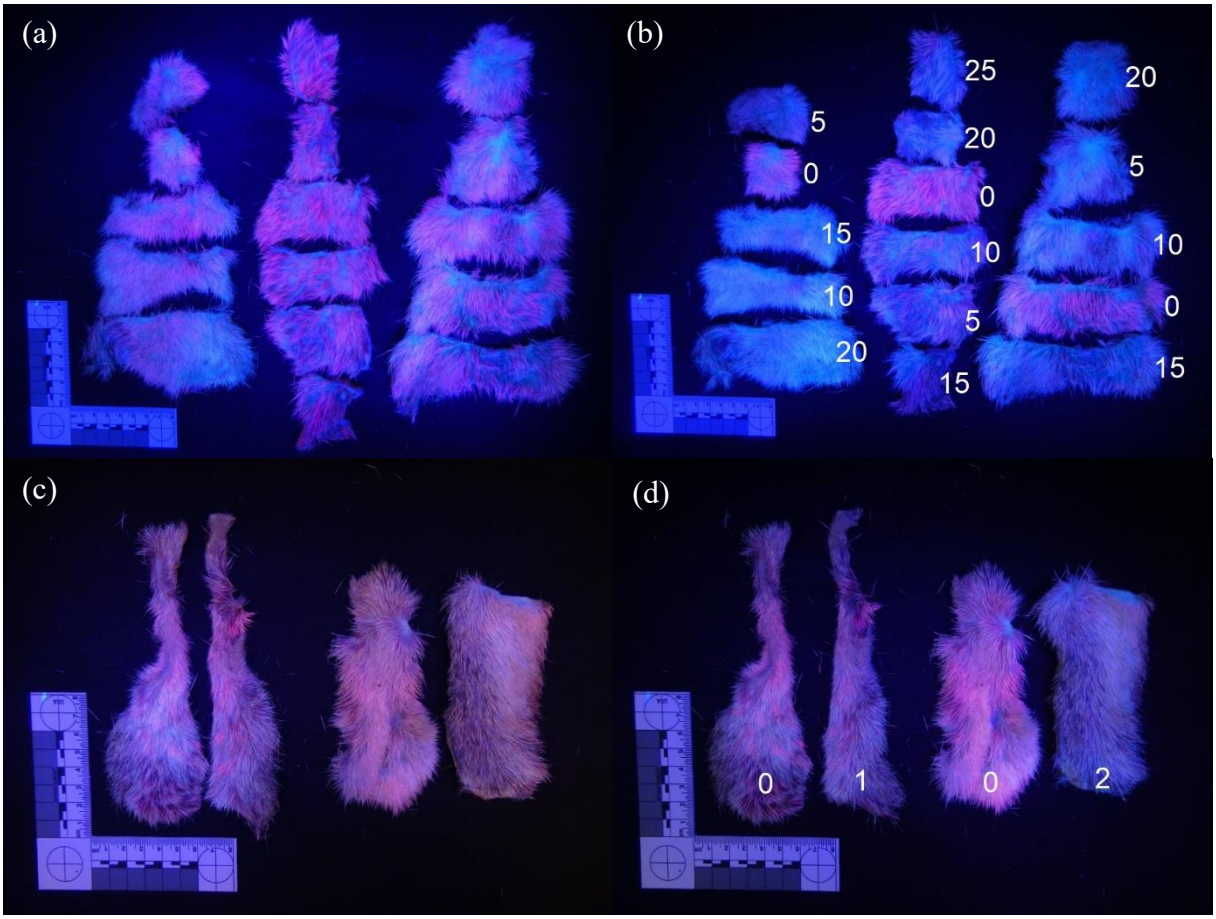


Figure 5.3. Northern brown bandicoot (*Isoodon macrourus*) photobleaching experiments. (a) Three ventral pelts (cut into sections), demonstrating pink photoluminescence with 395 nm torchlight (15 s) before and (b) after photobleaching trials (15 s). (c) Northern brown bandicoot refined experiment with two ventral pelts (cut into halves), photoluminescing under 395 nm torchlight (10 s) before and (d) after photobleaching trials (10 s). Left half of each ventral pair kept in dark, right half of left pair in sun for one minute, right half of right pair in sun for two minutes. Numbers indicate minutes of sun exposure for each treatment; 0 = control kept in the dark.

For long-nosed bandicoot fur, photoluminescence was mostly retained after one minute of sun exposure, but with some loss of depth and strength of colour. After two minutes, the fur showed a marked loss of extent and strength of pink colouration. After five minutes in the sun, photobleaching was obvious in the dorsal/flank fur, but with some retention of pink, while photobleaching was near-complete in the ventral fur and complete on the head. After 10 minutes of sun exposure, while the dorsal/flank fur retained some pink, the ventral fur was completely photobleached apart from a little retained in the Lake Eacham female (Fig. 5.4).

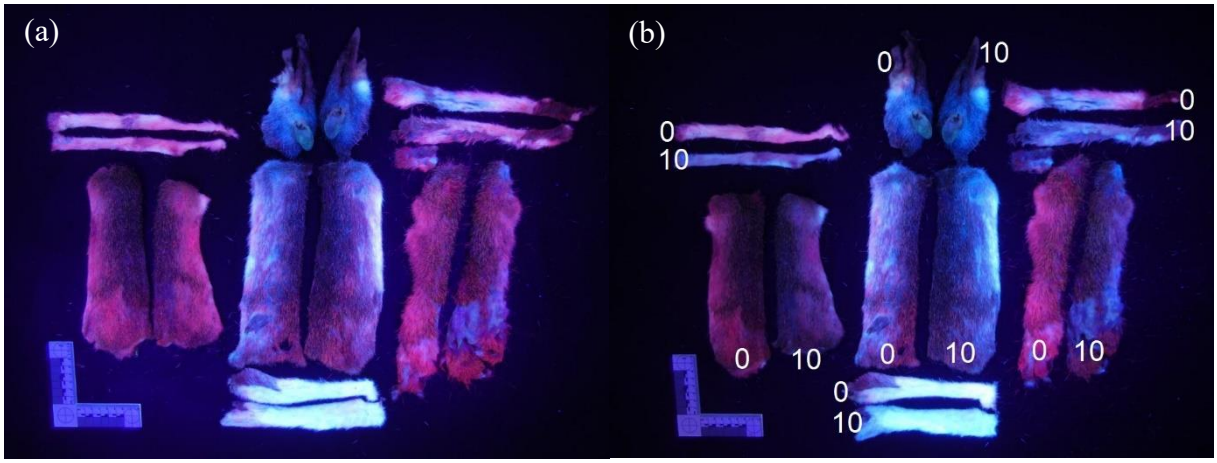


Figure 5.4. Long-nosed bandicoot (*Perameles nasuta*) photobleaching experiment. Three pelts dorsal and ventral (female-male-female, cut in half longitudinally), (a) before and (b) after 10 minutes of sun exposure in photobleaching trials. Under 395–410 nm torchlight (15 s exposure). Numbers indicate minutes of sun exposure for each treatment; 0 = control kept in the dark.

5.4.2 Wet preservation

Photoluminescence was mostly lost from rat fur after seven days of fixation in formalin and ethanol, but was largely retained in bandicoot fur (Fig. 5.5; Table 5.1). Six months after pelt squares were transferred into 70% ethanol, the rat fur photoluminescence was mostly extinguished in the ethanol-fixed treatments, but remained at a lesser intensity in the formalin-fixed treatments (Table 5.1; Fig. 5.6). The pink photoluminescence of the bandicoot fur was faintly retained in pale form, except for the ethanol-fixed flank fur, where it was completely lost (Table 5.1; Fig. 5.6). Wetting (water) of otherwise untreated fur caused some quenching of photoluminescence in rat fur, but not in bandicoot fur (Table 5.1; Fig. 5.7). Photoluminescence of wet rat fur was restored upon drying.

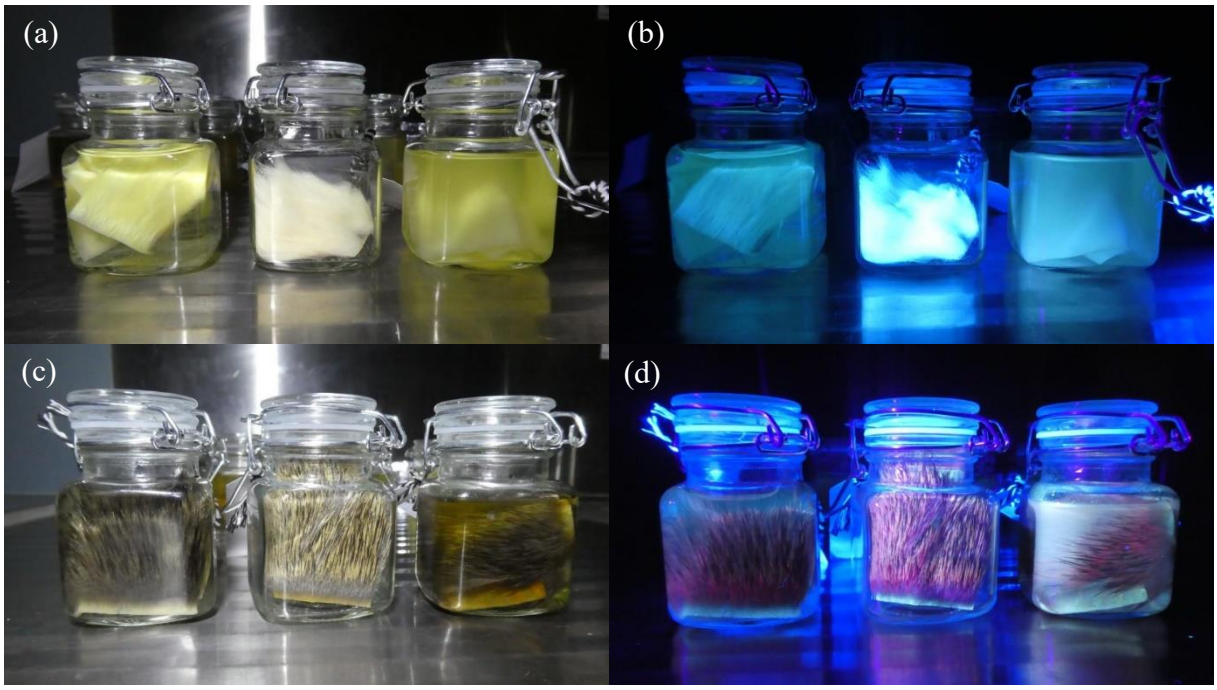


Figure 5.5. Pelt squares *in situ* in jars after seven days of fixation. Left: 10% formalin. Centre: control (dry). Right: 100% ethanol. (a) Laboratory rat (*Rattus norvegicus*) in white light and (b) 395–410 nm torchlight. (c) Northern brown bandicoot (*Isoodon macrourus*) in white light and (d) 395–410 nm torchlight.

Table 5.1. Retention or loss of fur photoluminescence after seven days in wet fixatives, six months in ethanol, or one hour in water, compared to dry control.

Pelt / treatment	Dry control	Wet (water)	10 % formalin seven days	100% ethanol seven days	70% ethanol six months (formalin-fixed)	70% ethanol six months (ethanol-fixed)
Rat brown	Mid blue-white.	Some lessening of blue-white.	~ A third as bright.	~ A third as bright	A little whitish, but brighter pale blue gone.	Completely extinguished. Reflected some purple light.
Rat white	Bright white.	Quench-ing to a quarter or less intensity.	~ A third as bright.	~ A third as bright.	Yellowish, less intense.	Completely extinguished. Reflected some purple light.
Bandicoot flank	Bright strong magenta.	Magenta just as strong.	Retained strong pink.	Retained strong pink.	Magenta retained in pale form.	Magenta completely extinguished.
Bandicoot ventral	Bright strong pink.	Pink just as strong.	Retained strong pink.	Retained strong pink.	Some very pale pink faintly retained.	Some very pale pink faintly retained.

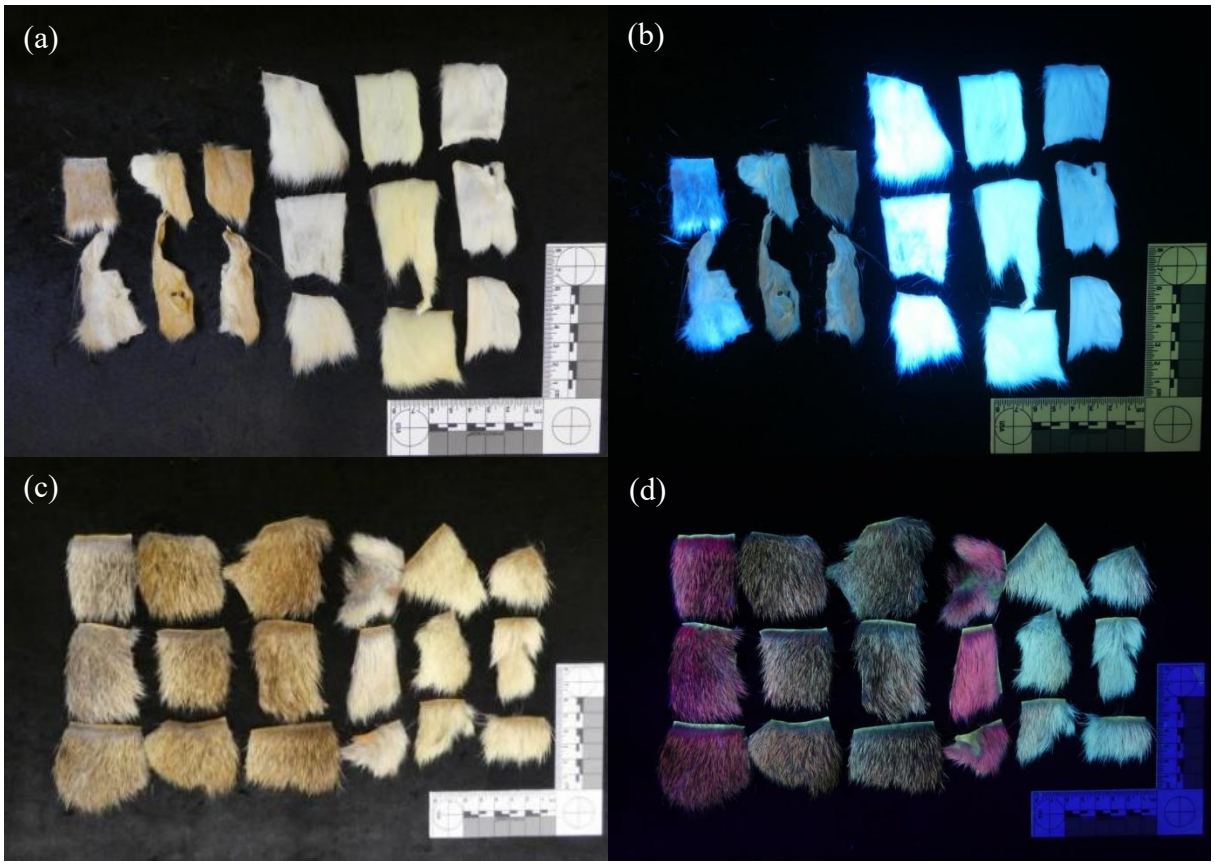


Figure 5.6. Retention or loss of photoluminescence in rat and bandicoot fur after six months in wet (ethanol) preservation. (a) Laboratory rat (*Rattus norvegicus*) pelt pieces after six months of preservation, under white light and (b) 365 nm torchlight (6 s). (c) Northern brown bandicoot (*Isoodon macrourus*) pelt pieces after six months of preservation, under white light and (d) 395–410 nm torchlight (5 s). First column: brown (rat) or flank (bandicoot) control. Second column: 10% formalin-fixed then 70% ethanol. Third column: 100% ethanol-fixed then 70% ethanol. Fourth column: white (rat) or ventral (bandicoot) fur control. Fifth column: white (rat) or ventral (bandicoot) fur 10% formalin-fixed, then 70% ethanol. Sixth column: white (rat) or ventral (bandicoot) fur 100% ethanol-fixed then 70% ethanol.

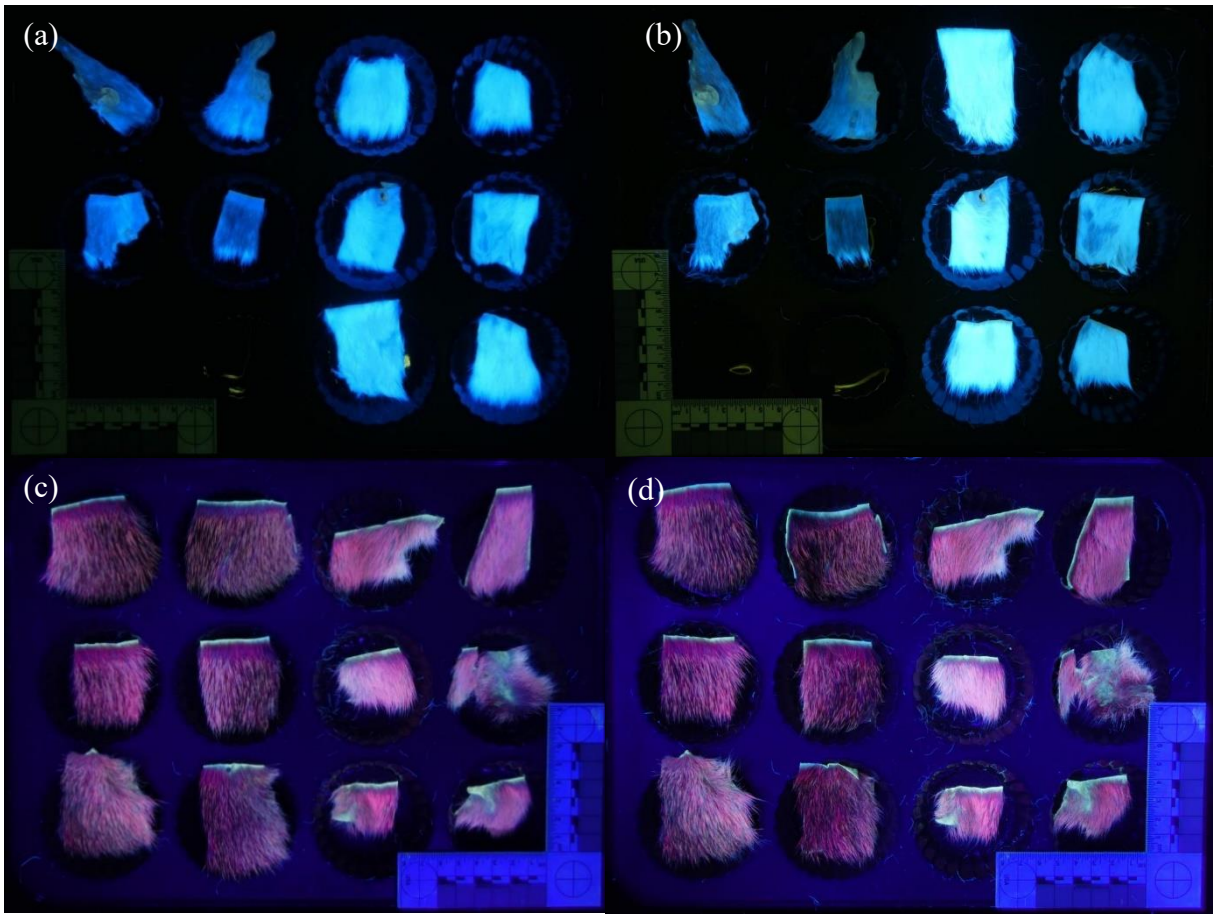


Figure 5.7. Photoluminescence of dry versus wet (water) pieces of rat and bandicoot pelts. (a) Laboratory rat (*Rattus norvegicus*) pelt pieces under 365 nm torchlight (6 s), all dry and (b) after half were soaked in water for one hour. (c) Northern brown bandicoot (*Isoodon macrourus*) pelt pieces under 395–410 nm torchlight (8 s), all dry and (d) after half were soaked in water for one hour. First column: brown (rat) or flank (bandicoot) fur dry control. Second column: brown (rat) or flank (bandicoot) fur wet after soaking in water for one hour. Third column: white (rat) or ventral (bandicoot) fur dry control. Fourth column: white (rat) or ventral (bandicoot) fur wet after soaking in water for one hour.

When control jars of straight preservation fluids were examined for photoluminescence, only the ethanol reacted to 310–410 nm light. Both the ethanol jars photoluminesced pale yellow, with the 100% ethanol jar a little stronger than the 70% ethanol jar. The jar containing 10% formalin remained clear, as did an empty glass jar.

After the 100% ethanol and 10% formalin fixatives had held pelt squares for seven days, their residual photoluminescence changed. When compared with the control jars of straight fixative, the bandicoot pelt had imparted a clear pale green photoluminescence to the 10% formalin, and the rat pelt had imparted a little stronger citrus yellowy green photoluminescence to the 10% formalin. After seven days, the bandicoot pelt turned the photoluminescence of 100% ethanol pale orange, opaque and slightly stronger than the control 100% ethanol. The rat pelt turned the photoluminescence of 100% ethanol more opaque, stronger and more concentrated pale yellowy green than the control 100% ethanol. How much of the fluid photoluminescence was imparted from the fur versus the skin of the pelts cannot be determined. After six weeks of storage in separate jars, the seven-day fixative fluids that were initially photoluminescent green had turned photoluminescent pale yellow.

After six months in 70% ethanol, the fluid from both the ethanol- and formalin-fixed bandicoot pelts was photoluminescent cloudy yellow. The 70% ethanol containing flank fur had turned darker yellow than the 70% ethanol containing ventral fur. The 70% ethanol containing formalin-fixed brown rat fur had turned photoluminescent brownish yellow, and the 70% ethanol containing ethanol-fixed brown rat fur was photoluminescent yellow, similar to the control 70% ethanol. The 70% ethanol containing formalin-fixed white rat fur was photoluminescent yellow, and the 70% ethanol containing ethanol-fixed white rat fur was photoluminescent very pale yellow, also similar to the control 70% ethanol. The photoluminescence of the remaining fluid in the six-month jars appeared a similar yellow colour whether it was excited with 365 nm or with 395–410 nm light. However, when excited with 310 nm light, the rat pelt 70% ethanol photoluminesced purplish clear, and the bandicoot pelt 70% ethanol photoluminesced purplish cloudy.

5.5 Discussion

The results of these tests indicate that studies solely relying on the photoluminescence of museum specimens should be treated with caution. My findings demonstrate that pink photoluminescence, likely caused by porphyrins (Toussaint et al. 2023; see Chapter 4), can be markedly compromised in as little as two minutes in direct sunlight, with bleaching of the head and ventral fur complete at five to ten minutes. The loss of pink photoluminescence from fur occurs in a comparable timeframe to the 12 minutes at which the visible porphyrin colouration is lost from the feathers of the great bustard (*Otis tarda*, Galván et al. 2016). However, if specimens are shielded from light, they can retain their photoluminescence (Kohler et al. 2019).

Photobleaching is likely to confound observations of intra-specific variation in photoluminescence. It also offers a plausible explanation as to why Bolliger (1944) declared long-nosed bandicoots as non-photoluminescent, whereas Reinhold (2021) observed vivid pink photoluminescence in this species. Additionally, the pattern of photoluminescence in two male northern brown bandicoots photographed by Reinhold (2020) is resolved by photobleaching, where the individual retaining its dorsal photoluminescence was lying dorsal side down, while the individual retaining its ventral photoluminescence was lying ventral side down. These observations are consistent with those of Ronald H. Pine for didelphid marsupials (e-mail from Ronald H. Pine, University of Kansas, Lawrence, Kansas, 18 February 2021).

I also found both pink and blue photoluminescence to be affected by wet (ethanol) preservation. At concentrations of 95% and higher, ethanol removes and replaces the water in cells, functioning as a dehydrator, and sometimes a bond disruptor (Stein et al. 2013). Ethanol may also act as a solvent to dissolve some regular pigments (zoochromes) and proteins from specimens (Simmons 1999). My observation that the jars of 10% formalin and 100% ethanol acquired photoluminescence upon initial fixation of the pelt squares suggests that luminophores were leached into the preservation fluids.

A change in the microenvironment of the fur shaft can suppress or boost the effect of luminophores (Jachowicz and McMullen 2011). Sheep's wool has been observed photoluminescing the brightest when completely dry, but not at all when damp (Millson 1943, cited in Collins 1992). In the case of the rat fur in my experiment, the results are consistent with Millson's (1943, cited in Collins 1992) findings, as I found that wet (water) samples

were less photoluminescent than dry samples. Soaking in water seemingly had no effect on the pink photoluminescence of bandicoot fur, either because the fur structure did not easily allow the penetration of water, or because the luminophores were unaffected by hydration (hydrophobic).

Blue photoluminescence, typically a consequence of tryptophan metabolite luminophores (Rebell et al. 1957), is relatively photostable, taking months to degrade in sunlight (Longo et al. 2013). Blue photoluminescence is retained somewhat if fixed in formalin, but is otherwise lost from fur during ethanol preservation. The longer-term preservation of specimens in ethanol demonstrated that both the pink and the blue photoluminescence substantially decreases in preserving solution. Fresh mammal specimens are therefore preferred in studies of fur photoluminescence.

5.6 Conclusion

Mammal fur has different classes of luminophores, each of which give different photoluminescent colours with different degradation profiles. These colours do not necessarily disappear at death, and may be retained indefinitely if appropriately preserved. However, the ease of loss of luminophores from fur highlighted in these experiments reinforces observations in the literature of the extreme lability of some photoluminescent colours. Blue photoluminescence, likely due to tryptophan metabolites, is relatively photostable but ethanol-sensitive. Pink photoluminescence, likely due to porphyrins, is both photosensitive and ethanol-sensitive, whereas it survives initial fixation. Photoluminescence is most accurately studied using living, freshly dead or frozen animals, if their light-exposure history has been recorded. Studying photoluminescence from exposed or preserved pelts will likely yield misleading results, and calls for caution when using museum specimens without comparisons against the fur of living or freshly dead wild animals.

Chapter 6

Does the photoluminescence of rat fur influence animal interactions in the field?

6.1 Abstract

While the photoluminescence of mammal fur is widespread, any potential function based on its optical properties remains speculative. Using paired photoluminescent and non-photoluminescent real-fur rat models in a field experiment, I aimed to test whether nocturnal vertebrates react more frequently to photoluminescent fur than to non-photoluminescent fur. Remote cameras were set out in three different habitats (farmland, rainforest, woodland) in the Wet Tropics of Far North Queensland, Australia, over three full moon and three new moon phases. I recorded the number of interactions with each model, then calculated the pair-wise differences of interactions for photoluminescent and non-photoluminescent models. No animal group (marsupial, placental mammal or avian) showed a preference for either model, on either new moon or full moon, suggesting that they either cannot detect a difference, or that preference is not based on photoluminescent properties. These findings do not support a hypothesis of selective pressure from nocturnal vertebrates acting on the trait of mammal fur photoluminescence.

6.2 Introduction

The appearance of animals can be driven by evolutionary pressures on visual signals (Endler 1992). The colouration of mammal fur can be beneficial in crypsis, aposematism and social signalling (Caro 2013). Mostly limited to the drabness of melanin, some mammals use the simple achromatic contrast of white (absence of melanin) alongside darker fur for signalling in dim light (crepuscular and/or nocturnal landscapes) (Penteriani and Delgado 2017). However, several authors have recently proposed that ventral or all-over fur photoluminescence may have a visual function for nocturnal-crepuscular mammals (Kohler et al. 2019; Anich et al. 2021; Olson et al. 2021; Pynne et al. 2021).

Four hypotheses have been proposed for a visual signalling function of fur photoluminescence (Kohler et al. 2019). Kohler et al. (2019) hypothesised that photoluminescence is adaptive in nocturnal-crepuscular species. This was based on their observations of New World flying squirrels (*Glaucomys* spp.), which are nocturnal-crepuscular, active all year round in low light, have clear ocular lenses, and photoluminescent fur. In contrast, ground-dwelling squirrels (*Tamiasciurus* and *Sciurus* spp.) which are diurnal, hibernate in winter, and have yellow ocular lenses, are non-photoluminescent. However, yellow lens colouration is not common across all diurnal mammals (Hammond 2012; Douglas and Jeffery 2014), and Marshall and Johnsen (2017) explained that yellow long-pass ocular filters could instead facilitate the viewing of photoluminescence.

Kohler et al. (2019) also hypothesised that photoluminescence could be a consequence of remaining active in snowy winter landscapes, where snow cover would reflect ultraviolet light, thereby boosting the photoluminescence of the flying squirrels' ventral surface. However, this hypothesis cannot be expanded beyond the flying squirrels of the New World, as Toussaint et al. (2023) recorded pink photoluminescence in Old World flying squirrels from the warm climate of south-east Asia.

Both Kohler et al. (2019) and Pynne et al. (2021) proposed a visual function for photoluminescence in intraspecific communication. To date, no studies have been conducted on mammals. Although tested in other animals, this hypothesis has only been trialled using artificial ultraviolet lighting to boost photoluminescence, or in natural daylight but with artificial photoluminescent paint (Arnold et al. 2002; Lim et al. 2007; Gerlach 2014; Douglas III et al. 2021).

Kohler et al. (2019), Pynne et al. (2021), Anich et al. (2021) and Olson et al. (2021) hypothesized that photoluminescence is involved in antipredator behaviour. In this context, Kohler et al. (2019) also suggested that the photoluminescence of New World flying squirrels could be Batesian mimicry to resemble photoluminescent pink owls (Strigiformes). Whereas the flying squirrels themselves may be colour-blind (Carvalho et al. 2006), owls may be able to discern colour in low light (Martin 1974; Avilés and Parejo 2012; Potier et al. 2020). However, reddish photoluminescence may only be emitted in response to a strong excitation light; if the excitation light is weak and therefore the emission dim, then the photoluminescence will only appear whitish, rendering the ability to see pink inconsequential (Harvey 1957).

To determine if photoluminescence is ecologically significant, Marshall and Johnsen (2017) proposed that five conditions are required: 1) luminophores should occur in a visible location; 2) the appropriate excitation wavelength must be available, and the emission wavelength must be visually relevant; 3) the emission wavelength should be at maximal sensitivity to the viewer; 4) the natural lighting conditions required for excitation must be available; and 5) visually directed behaviours must change when the photoluminescence is muted. Thus, all hypotheses for a visual function of photoluminescent fur in crepuscular-nocturnal environments principally rest on the premise that natural moonlight or twilight is strong enough to activate the luminophores in fur, and that the mammals themselves, or their predators, must be able to detect the photoluminescence excited by natural light.

The strength of low-wavelength emissions from bright sunlight is enough to excite most natural photoluminescence (Marshall and Johnsen 2017). However, subtle photoluminescence may be masked by the reflectance of bright sunlight (Viitala et al. 1995). At twilight, the overpowering middle wavelengths of the sun taper off, allowing lesser-intensity wavelengths to become more dominant without so much interference from reflection (McFarland and Munz 1975; Endler 1993). It is the lower wavelengths that have the potential to excite photoluminescence that would stand out against an otherwise dark and monochromatic background (Pohland 2007).

The irradiance of the full moon is approximately 1,000 times less than that of twilight, and it lacks the defined peaks of blue and red light, instead mimicking the more gradual spectrum of daylight (McFarland and Munz 1975; Johnsen et al. 2006). Excitation by full moonlight has been tested experimentally and shown to trigger the photoluminescence of

scorpions (*Vaejovis* sp.), with nocturnal flying insects reacting to photoluminescent scorpions on a full moon but not on a new moon (Kloock 2005). However, a subsequent set of experiments on photoluminescent scorpions (*Centruroides granosus*), found that their prey of house crickets (*Acheta domesticus*) did not react to photoluminescence or lack thereof in either laboratory trials with moonlight simulation or in natural outdoor lighting under a half moon (Gálvez et al. 2020).

The plausibility of the excitation of photoluminescence by relatively low-intensity ambient light in a visual function role also relies on the adequate visual sensitivity of the observer to detect the emitted photons. The notion that nocturnal animals may have highly sensitive vision has been previously discarded, with studies predominantly focusing on the importance of olfaction for mammals and hearing for birds of prey (Penteriani and Delgado 2017). However, an emerging body of research is beginning to understand more about the evolution of nocturnal-specific visual systems, and indicates that nocturnal landscapes are visually rich in detail to nocturnal animals (Warrant 2004; O'Carroll and Warrant 2017). Additionally, marsupial and placental mammals differ in the evolutionary retention of visual pigments, with marsupials possessing a third type of cone photoreceptor (Arrese et al. 2002). While some predictions about the range of vision of an animal can be made from eye anatomy, behavioural trials are required to confirm the functional vision of the animal (Jacobs 1993; Arrese et al. 2006; O'Carroll and Warrant 2017).

The idea that mammals have a hidden palette of bright colours is tantalising, but there is as yet no experimental evidence that such photoluminescence could become visible in natural low light. Therefore, I investigated whether wild nocturnal animals preferentially choose to interact with a photoluminescent model compared to a non-photoluminescent model. I deployed pairs of real-fur rat models, one photoluminescent and the other not, and recorded the initial interactions on full moon versus new moon nights. I predicted that, if full moonlight was strong enough to excite the photoluminescence in mammal fur, as it was for Kloock's (2005) scorpions, and if nocturnal vertebrates can see this photoluminescence, wild animals would demonstrate a preference for one model type under a full moon, but not under a new moon. In addition, if small mammals use photoluminescent fur as a means of intraspecific communication that is more visible to themselves than to their predators, I expected that similar-sized mammals would interact more with photoluminescent models, whereas interactions from predators such as dogs (*Canis familiaris*), cats (*Felis catus*) and owls would show no difference. Alternatively, if photoluminescent fur acts as a camouflage mechanism,

then I expected that the photoluminescent models would receive fewer interactions than the non-photoluminescent models on a full moon when their photoluminescence was activated.

6.3 Methods

6.3.1 Ethics statement

This field experiment was conducted under Queensland Department of Environment and Science Research Permit number WA0036056, under the Nature Conservation (Animals) Regulation 2020. All study sites were located on private property, with permission from the landowners. The hairspray used on the models was designed to be safe for use on human hair, so was not expected to have harmful effects on other species. The study was approved by the James Cook University Animal Ethics Committee (approval number: A2768). Wild animals were free to interact with the models or not, and at no time experienced any unexpected adverse events.

6.3.2 Study sites

The study took place between September 2021 and March 2022. Three habitats (described below; Fig. 6.1) on the Atherton Tablelands (Far North Queensland, Australia) were selected to encompass different conditions of sky light, with minimal interference from city skyglow. Faunal composition was factored into site choice to include both ground-dwelling mammals that were of similar size to the models, and nocturnal avian predators. Each site was sampled six times, during three new moon periods and three full moon periods. Due to logistical constraints, the farmland and rainforest sites were sampled concurrently for the first three months, and the woodland site was sampled separately for the second three months.

6.3.2.1. Farmland

The open farmland site (17°14’46” S, 145°31’39” E) encompassed two properties separated by a dirt road, 9 km east of the small town of Atherton (bordering Kairi). The site had some skyglow visible from Atherton, but no local lighting, and provided for full moon illumination under an open sky. The farm on the northern side was a recently harvested sugar cane (*Saccharum* sp.) field bordering a Rhodes grass (*Chloris gayana*) field on the adjoining farm to the east, with fields of legumes at the northern corner. This area was relatively flat. The farm to the south was a young avocado (*Persea americana)* plantation bounded by a dirt road, an older avocado plantation, harvested sugar cane and a fenced, treed creek line and cattle (*Bos taurus*) paddock that the block sloped down towards.

6.3.2.2. Rainforest

The rainforest site (17°17’22” S, 145°38’16” E) encompassed two adjoining hilly private properties in secondary rainforest backing onto a creek, 8 km southeast of the small town of Yungaburra. The canopy was mostly closed but not dense, allowing dappled light through. Each property had household dwellings on rainforest acreage, but there was minimal interference from artificial lighting.

6.3.2.3. Woodland

The woodland site (17°21’26” S, 145°19’48” E) was comprised of ironbark (*Eucalyptus* sp.), red bloodwood (*Corymbia gummifera*) and lemon-scented gum (*C. citriodora*) woodland, with Cypress pine (*Callitris* sp.) thickets, and an understory of native grasses and forbs. The canopy was open, and the site sloped from a granite range down to an annual creek. The woodland habitat provided a mix of filtered light. Being 10 km from the small town of Herberton and in an off-grid part of Watsonville, it experienced no interference from artificial skyglow.

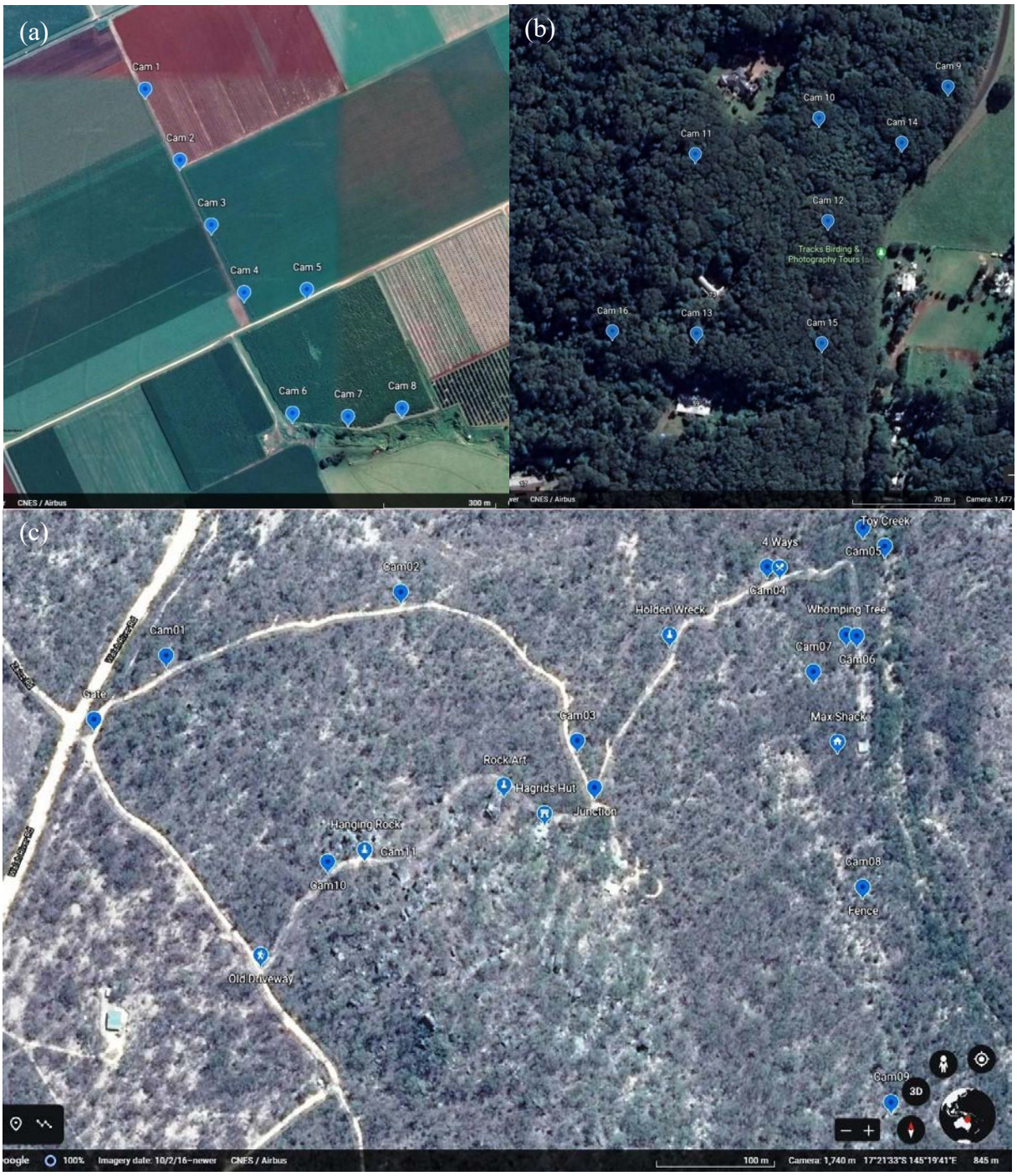


Figure 6.1. Satellite photos of Atherton Tablelands habitats where remote camera stations were set. (From Google Earth, earth.google.com/web/. Imagery date: 07 April 2020).
(a) Farmland habitat, Atherton/Kairi, showing eight camera stations spaced 150–200 m apart.
(b) Rainforest habitat, Lake Eacham, showing eight camera stations spaced ~100 m apart.
(c) Woodland habitat, Watsonville, showing 11 camera stations spaced 100–200 m apart, and navigational waypoints. Scales vary.

6.3.3. Experimental design

The study mostly followed the experimental design of Kloock (2005) but was adapted for interactions with vertebrates rather than flying insects. Thirty-six frozen laboratory rats (*Rattus norvegicus*) of mixed sex and colour, bred on the Atherton Tablelands, were purchased from a commercial supplier (Bugs Alive, Cairns). Rats were used because they are highly photoluminescent (Rebell et al. 1956; Udall et al. 1964; Reinhold 2021). All rats displayed bright light-blue photoluminescence when exposed to 365–410 nm ultraviolet-violet light, with the photoluminescence of white fur most prominent. Rats were skinned, fleshed and salted before the pelts were fitted over a non-photoluminescent grey or black PVC model rat (20 cm straight head-body length). The PVC feet and tail remained exposed. Pelts were stitched into place and craft eyes fitted onto the head. Model rats were allowed to air-dry in a dark, air-conditioned (~24 °C; 50-65% relative humidity) room for two to three weeks. Finished models were paired by sex, colour and size so that both rats in a pair looked similar. Nine pairs were white, two pairs were white with light brown hoods, two pairs were brown, two pairs were black, and one pair was grey. The remaining four rats were used as spares.

To remove luminophores from one rat of each prepared rat model pair (n = 16), pelts were washed in 50 ℃ tap water in a laundry tub for 1.5 hours, with several kettles of boiling water poured over them. This method was a practical way of replicating studies that reduced tryptophan metabolite photoluminescence in fur to approximately one third of its previous intensity (Rebell 1966; Nicholls and Rienits 1971). Photoluminescence was further extinguished using ultraviolet-protectant hairsprays (‘Clarins UVB UVA high protection 30 Sun Care Oil Mist’ hair oil; ‘Batiste dry shampoo & colour protect, with UV filter to protect fade’ for white rat pelts; ‘Batiste dry shampoo beautiful brunette’ and ‘divine dark’, and ‘Tony & Guy brunette’ hairspray for brown pelts). When illuminated with 365–410 nm torches, the suppressant methods used were effective in removing the glow, with the photoluminescent rat appearing much brighter than the non-photoluminescent rat of each pair (Fig. 6.2). Regular ‘Schwarzkopf’ or ‘Woolworths homebrand’ hairspray was then sprayed over all models to mask differing odours. When rain showers were forecast, model rats were also sprayed with ‘Maseur Weather Guard boot and garment spray’. The suppression of photoluminescence in the fur of the non-photoluminescent models was checked before each field session, to ascertain that there was a marked difference in photoluminescence within each pair of rats. Ultraviolet-protectant sprays were reapplied as needed. Damaged rats were repaired or replaced with spare matching rats as necessary.

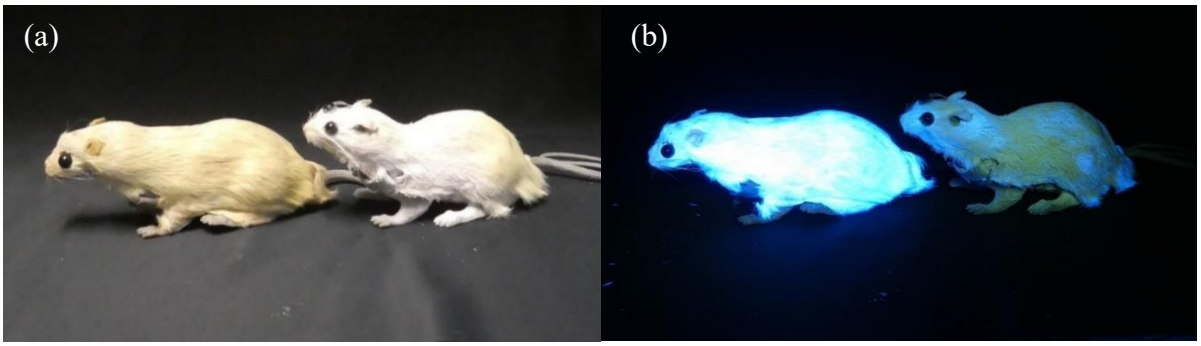


Figure 6.2. Visual difference between photoluminescent (left) and non-photoluminescent (right) white rats of the same pair (a) under white light and (b) under 365 nm ultraviolet light. Photographs taken after six field deployment sessions.

6.3.4. Field experiment

Within each habitat, camera stations were set out for three nights at a time at each full moon and new moon phase, apart from one full and new moon set at the woodland site, which were each left out for four nights because the full moon was fullest in the morning, dividing more evenly over four nights than three. Weather was mostly clear or partly cloudy; however, it cannot be known whether there was a cloud over the moon at the time of each interaction. Decisions were made on weather conditions a couple of days before each field trip. Full moon trips would have been postponed had the forecast been for overcast and rainy conditions, but this was not necessary.

Sixteen remote cameras were used (n = 13 PR700 20MP 1080P 120°Detecting Range Hunting Trail Camera Waterproof Hunting Scouting Camera with Auto IR Filter for Wildlife Monitoring; n = 2 Anaconda 16MP Trail Camera Camo cameras; n = 1 6MP 1080P Hunting Trail Camera Infrared Security Night Vision Waterproof Cam). All cameras had a trigger speed of 0.2–0.6 s, with a 20 m passive infrared (PIR) sensing distance. Different camera models were used because of some camera failures prior to commencement. Videos were chosen over photographs or marks left on models to better capture animal behaviours (Akcali et al. 2019). Cameras were set with high sensitivity and to record in 1080P resolution infrared video with a 2 s delay between videos. Videos were set to record for 20 s.

Camera stations were set as far apart as possible within the confines of each property. For the open farmland, four cameras were set at 200 m intervals on a line of old fence posts dividing the upper adjoining fields, and four cameras were set at 150 m intervals on the fence posts bounding the lower creek line or dirt road. In the rainforest, four cameras were set 100

m apart along narrow tracks on each of the two properties. In the woodland site, initially 13 camera stations were spaced 100–200 m apart along tracks. This number was reduced to nine stations towards the end of the experiment as some rat models were damaged irreparably. Only these nine stations were used in the statistical analyses for the woodland.

Cameras were placed 70 cm above the ground facing a small clearing and tilted downwards to frame the model pairs. Any obscuring vegetation was cleared. For each camera station, the model pair (one photoluminescent and one non-photoluminescent model) was set on natural ground (mostly dirt, grass or leaf litter) 1.5 m directly in front of the camera post/tree. Setting of remote cameras was adapted from Gillespie et al. (2015). Non-photoluminescent synthetic black cord was used to tether the models to the camera post/tree to prevent them from being carried off by predators. Models were placed two body lengths apart (= 40 cm), facing each other and the camera at a 45° angle. Within each habitat, half of the camera stations had the photoluminescent model on the left, and half had the photoluminescent model on the right. The side the photoluminescent model was placed on was alternated once within each habitat to reduce bias. Pair sides were kept consistent within each full/new moon pair.

6.3.5 Behavioural observations

Interactions between wild animals and the model pairs were scored only on first approach; i.e. the rat model that was interacted with first, regardless of subsequent interactions with the other model. This ensured the greatest chance of the interactions being based on sight, and before the infrared light from the camera interfered with natural illumination. Interactions where animals were foraging in the leaf litter and accidentally touched a model in the process of sniffing food from the ground were not counted. Video sequences more than ten minutes apart, or where there was a group size of two or more animals in the same frame both interacting with models, were counted as separate events. Only the first model interaction of an animal was scored if it was observed coming back to the models in numerous videos without a significant time lapse. Wild animals were identified to species where possible. I also categorized animals into broad taxon groups (Bird, Marsupial or Placental mammal). Only interactions between sunset and sunrise were used in the final analyses. While lighting phase (golden hour, civil twilight, nautical twilight, astronomical twilight or dark) was recorded for each interaction, sample sizes were not sufficient to allow for robust statistical analyses.

6.3.6 Statistical analyses

All statistical analyses were conducted using RStudio (RStudio Team 2020, version 1.0.153; R version 4.1.2). Data were tested for normality using the Shapiro-Wilk test and transformed where necessary. I first used a generalised linear mixed effects model (LMER; lmerTest package, Kuznetsova et al. 2020) with negative binomial distribution to assess whether the number of interactions with a model was affected by habitat (Farmland, Rainforest or Woodland), time (Replicate 1, 2 or 3), moon phase (New or Full) or model type (Photoluminescent or Non-photoluminescent). These factors were all included as fixed categorical factors. I also included camera number as a random effect. Thereafter, I ran a linear (LM) model with Gaussian distribution to assess whether the number of interactions was affected by taxon (Bird, Marsupial or Placental). Habitat, taxon, model type and the interaction between taxon and model type were included as fixed factors.

I then calculated the pair difference for each camera station at each habitat as the number of first interactions with the photoluminescent model minus the number of first interactions with the non-photoluminescent model. More first interactions with the photoluminescent model indicated a positive pair difference, while more first interactions with the non-photoluminescent model indicated a negative pair difference. Data for pair differences were first transformed using the orderNorm function (bestNormalize package, Peterson 2022). I ran an LMER with Gaussian distribution to assess whether pair differences were affected by moon phase. Habitat, replicate, moon phase and animal type were included as fixed effects, and camera number was again included as a random effect. Significant differences in the main effects were identified using Tukey's post hoc tests (emmeans package, Lenth et al. 2022).

6.4 Results

6.4.1 General observations

Eleven species of marsupial, at least nine species of placental mammal and four species of bird interacted a combined total of 142 times with the models between sunset and sunrise (Appendix F). Only dogs, cats and long-nosed bandicoots (*Perameles nasuta*) were recorded interacting in all habitats. Rodents interacted with the models in farmland and rainforest, but not in woodland. Northern quolls (*Dasyurus hallucatus*, Fig. 6.3) were only observed in the woodland habitat, where they interacted enthusiastically with the models. Eastern grass owls

(*Tyto longimembris*, Fig. 6.4) were only observed during one moon session, in the open farmland. The only rare species recorded was the Mareeba rock-wallaby (*Petrogale mareeba*), in the woodland habitat.

Figure 6.3. A northern quoll (*Dasyurus hallucatus*) choosing a rat model in woodland.

Figure 6.4. Eastern grass owls (*Tyto longimembris*) interacting with a model pair, and each other, in open farmland.

6.4.2 Number of interactions

I found no significant effect of model type on the number of interactions (GLMER: $\chi^2_1 = 0.00$, $p = 0.969$). However, there was a significant effect of replicate number on the number of interactions with the models, irrespective of model type ($\chi^2_2 = 9.58$, $p = 0.008$). There was a sequential decline, with significantly more interactions in the first testing session (n = 72 interactions) than the last testing session (n = 30; Fig. 6.5). In addition, moon phase had a significant effect on the total number of interactions, irrespective of model type ($\chi^2_1 = 4.87$, $p = 0.027$), with significantly more interactions occurring during the new moon (Fig. 6.6). There was no significant effect of habitat type ($\chi^2_2 = 3.80$, $p = 0.150$) on the number of interactions, but the number of interactions was affected by the random effect of camera number ($\chi^2_2 = 20.74$, $p < 0.001$).

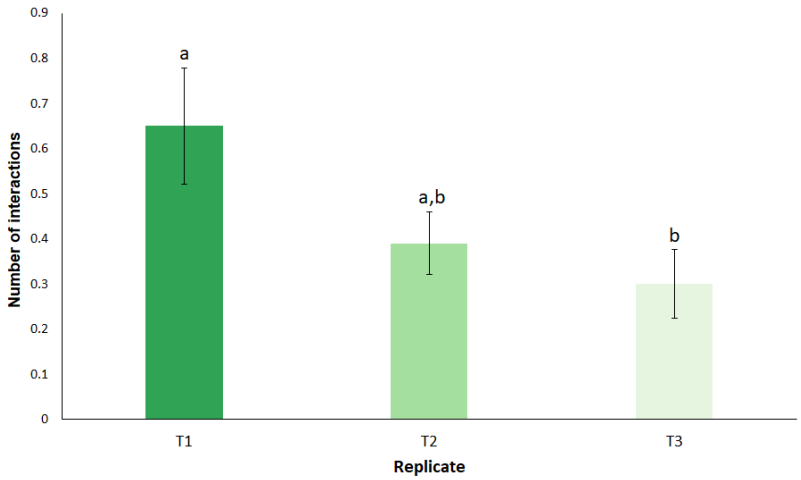


Figure 6.5. Mean ± SE number of interactions with all models (Photoluminescent and Non-photoluminescent) from all habitats (Farmland, Rainforest and Woodland) and both moon phases (New and Full) over time (Replicates T1-3). Different letters indicate a significant difference.

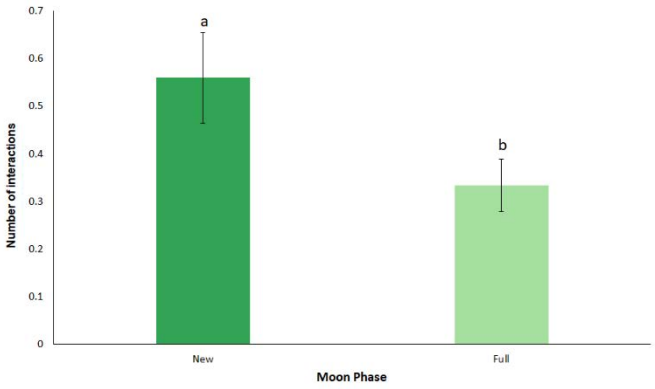


Figure 6.6. Mean ± SE number of interactions with all models (Photoluminescent and Non-photoluminescent) from all habitats (Farmland, Rainforest and Woodland) on New moon versus Full moon phases. Different letters indicate a significant difference.

While there was no significant effect of model type (Photoluminescent or Non-photoluminescent) on the number of interactions (LM: $F_{1,10} = 0.08$, $p = 0.780$), there was a significant effect of animal type on the number of interactions ($F_{2,10} = 13.57$, $p = 0.001$), with marsupials (15.17 ± 1.83) interacting with the models significantly more than placental mammals (7.5 ± 1.18) and birds (1.00 ± 0.52), and placentals interacting with the models significantly more than birds (Fig. 6.7).

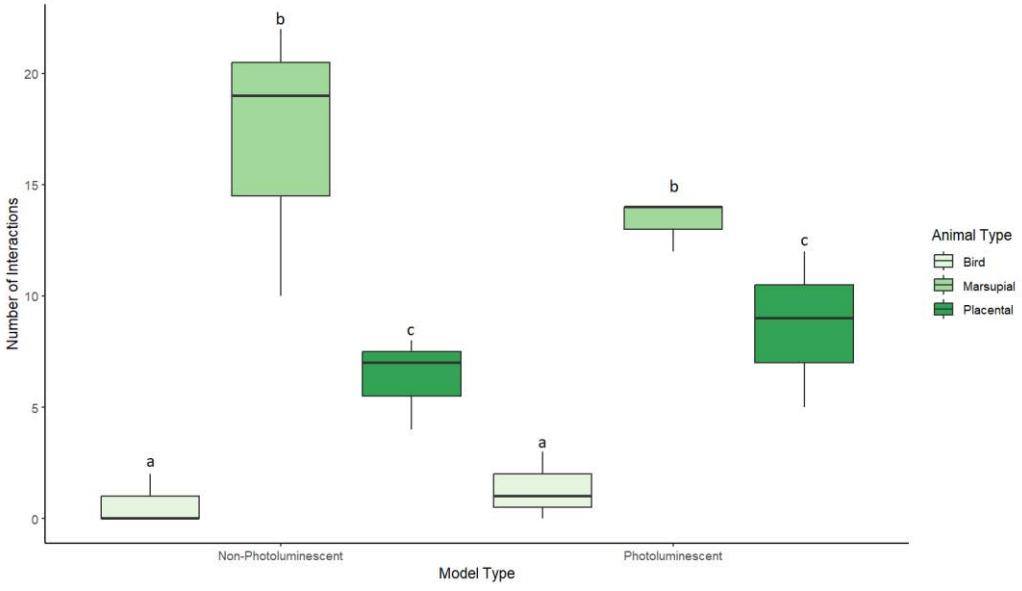


Figure 6.7. Mean ± SE number of interactions with Photoluminescent and Non-photoluminescent models from all habitats (Farmland, Rainforest and Woodland) on both moon phases (New and Full) from three different animal types (Bird, Marsupial and Placental). Different letters indicate a significant difference.

6.4.3 Pair differences

There was an overall pair difference of 0 (i.e. equal numbers of interactions with each model type) when pooled for both moon phases. There were no significant effects of habitat (LMER: $\chi^2_2 = 0.60$, $p = 0.739$), replicate ($\chi^2_2 = 1.35$, $p = 0.508$), moon phase ($\chi^2_1 = 0.48$, $p = 0.488$; Fig. 6.8) or camera number ($\chi^2_1 = 0.03$, $p = 0.872$) on the pair differences. In addition, there was no significant effect of habitat ($F_{2,4} = 0.10$, $p = 0.907$) or animal type ($F_{2,4} = 0.93$, $p = 0.466$) on the pair differences.

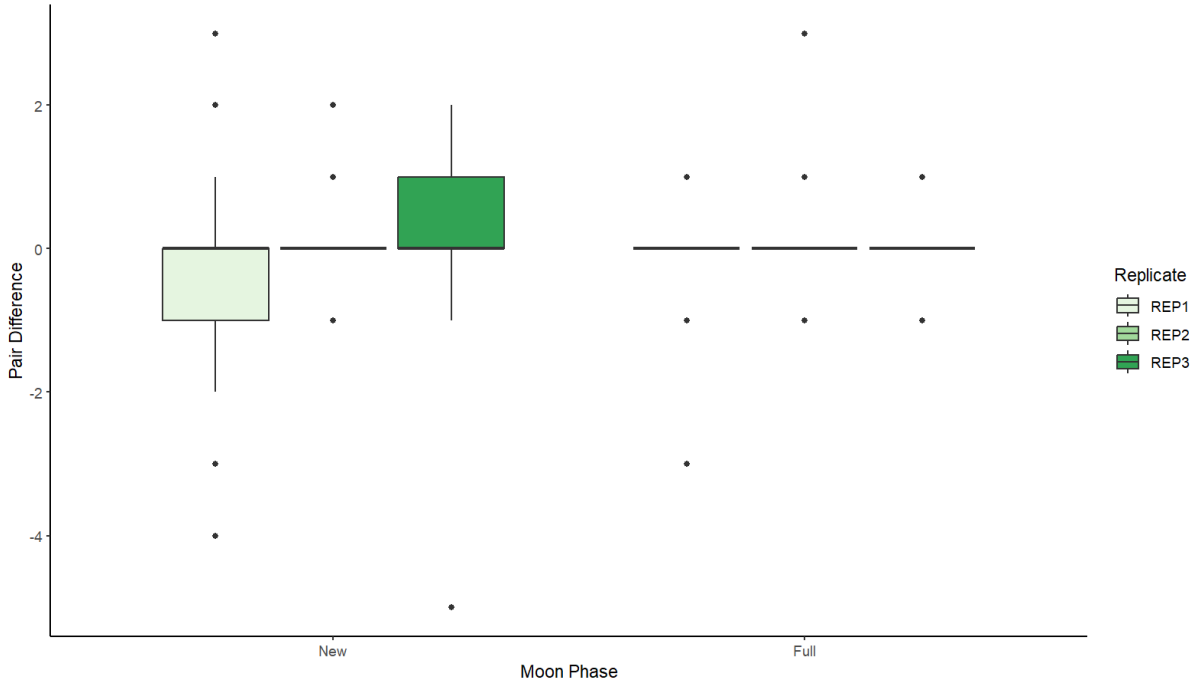


Figure 6.8. Mean ± SE pair difference between Photoluminescent and Non-photoluminescent models from all habitats (Farmland, Rainforest and Woodland) and all animal types (Bird, Marsupial, Placental) on New moon versus Full moon phases.

6.5 Discussion

With speculation increasing about a visual function for fur photoluminescence, this study aimed to test whether wild nocturnal vertebrates would respond to the natural photoluminescence of real fur in natural lighting. The experimental design loosely followed that of Kloock (2005), who concluded that scorpion photoluminescence could be detected by flying insects during a full moon. However, I found no significant difference in preference for photoluminescent mammal models over non-photoluminescent models for any habitat, moon phase or animal type (Birds, Marsupials or Placentals). Marsupial and placental mammals did not differ significantly in their model choices.

There are two possible explanations for these findings: 1) the light of the full moon was not strong enough to excite the photoluminescence in the model fur to a level where it was visible to nocturnal mammals and birds, contrary to Klook's (2005) observations for nocturnal flying insects; or 2) the lack of distinction could mean that, even if nocturnal vertebrates can detect the photoluminescence, it does not affect their behaviour and they have no preference for or against it. Although not testing for intraspecific communication with live rats of the same species, my findings indicate that photoluminescence in nocturnal mammal fur does not provide a visual function for either communication between similar-sized mammals, or predator avoidance as suggested by several recent studies (Kohler et al. 2019; Anich et al. 2021; Olson et al. 2021; Pynne et al. 2021). The lack of behavioural change in response to the trialled moonlight activation of photoluminescence does not meet Marshall and Johnsen's (2017) criteria for ecological significance.

There were significantly more interactions with the models during new moon phases than full moon phases. My findings are consistent with other studies that found that nocturnal mammal activity decreased with increasing moon illumination, suggesting that prey animals may trade-off foraging and predation risk during full, but not new, moon periods (Clarke 1983; Linley et al. 2020). Interestingly, while the sample size was small, limiting further statistical analyses, the few bird interactions I recorded were all on full moon phases (Appendix F), providing further support for enhanced predation risk on full moon nights. The tendency for prey-sized mammals to avoid open spaces on full moon nights would also reduce the opportunity to display their photoluminescence, further suggesting a lack of visual function for photoluminescent fur. A temporally limited signalling colouration that is only

visible on the full moon, from which they shelter, is an unlikely signalling method for smaller mammals.

Habitat appeared to play a role in the moonlight avoidance behaviour of both northern brown (*Isoodon macrourus*) and long-nosed bandicoots, with all 32 farmland bandicoot interactions occurring on new moons, but nearby rainforest bandicoot interactions occurring on either moon phase (Appendix F). This difference suggests that the rainforest habitat blocked a substantial portion of the moonlight from reaching the ground (Endler 1993). If moonlight had triggered the fur photoluminescence of the rat models, then it would be expected to be more effective in unobstructed open farmland than in rainforest, with woodland moonlight intermediate in effectiveness. However, there was no preference for either model in open farmland, negating the probability that intensity of moonlight was sufficient to trigger fur photoluminescence in forested habitats.

Interactions decreased with each session over the three months of trials in each habitat. This decrease in activity with subsequent deployment of the camera stations could be due to habituation to the models (Desforges and Wood-Gush 1975). Animals are known to show an interest to novel stimuli in their environments, which then decreases over time as the animals become familiar with the stimuli (Ewert et al. 2001). Alternatively, weather could explain a decrease in interactions. Some animals are known to become less active with decreasing temperatures (Laurance 1990). However, the mean daily minimum temperature rose by two to three degrees between the first and subsequent replicates (Bureau of Meteorology 2022), suggesting that temperature was unlikely to be a cause of decreased interactions.

The difference in sample sizes between animal types is partially expected to reflect the abundance of these animals at each site. There were more than twice as many marsupials sighted as placentals. Out of all animal sightings recorded on the cameras, 30% of marsupials and 37% of placentals interacted with the models, while only 11% of birds interacted with the models. Many more individuals of marsupials, such as bandicoots and macropods (Macropodidae), were seen on camera than interacted with the models. If seen, cats usually interacted, whereas European rabbits (*Oryctolagus cuniculus*) usually did not. A low percentage of interactions for birds reflects numerous non-interactive sightings of common species such as bush stone-curlews (*Burhinus grallarius*) and Australian brush-turkeys (*Alectura lathami*). Many of the species recorded are not expected to exert any selective

pressure on small to medium-sized mammals, but were merely curious of a new object in their habitat (Masko et al. 2020).

The way an object reflects, absorbs or emits light does not need to have a visual function (Silberglied 1979), given that some rocks and minerals photoluminesce (McDougall 1952; Moses et al. 1997; Othmane et al. 2016), free from any selective pressures to do so. The external absorption of ultraviolet light and its resulting photoluminescence may instead have primarily physiological functions, such as protecting the inner tissues of an animal from damage (Stübel 1911).

6.6 Conclusion

This is the first study on vertebrates testing photoluminescence of real mammal fur in natural lighting conditions in the field. This is also the first study to test whether the photoluminescence of fur is preferentially selected by nocturnal mammals or birds, that is, whether it has the potential for a visual function. I found no evidence for a visual function for photoluminescence in the fur of nocturnal mammals, highlighting that without behavioural tests, a trait function should not automatically be assumed.

This field experiment only tested the response of nocturnal vertebrates to the blueish photoluminescent fur of laboratory rats. The higher excitation wavelengths of porphyrins may mean that another study using a species with pink-red photoluminescent fur could reveal a difference between luminophore types. The relatively stronger and more frequent, albeit brief, excitation wavelengths of twilight could also be a more plausible source of excitation than moonlight. However, it would be difficult to record sufficient sample sizes in these brief twilight periods. Another experiment could test whether photoluminescent fur repels nocturnal flying insects. Diurnal photoluminescence has been largely overlooked because of a recent misconception that mammalian photoluminescence only occurs in nocturnal-crepuscular species. However, because of the ample excitation wavelengths in sunlight, diurnal signalling may be the most plausible optical function, if any, of photoluminescence in fur. For tryptophan-based fur photoluminescence, perhaps a similar experiment to the one presented here, but using direct sunlight and daytime forest shade, would be informative for those species that are diurnal, or that rest amongst foliage during the day.

Chapter 7

General discussion

Spanning the disciplines of physics, chemistry and biology, terrestrial photoluminescence has recently gained increasing attention (Lagorio et al. 2015; Jeng 2019). However, experiments on sunlight excitation of mammal pelage photoluminescence were conducted as far back as the 1700s (Wilson and Beccari 1775), and detailed species-specific documentations have been published since the early 1900s (Stübel 1911; Bolliger 1944). This thesis demonstrates that photoluminescence is not 'all or nothing', but that there is a continuum of photoluminosity (Hirst 1927). It also demonstrates that photoluminescence is affected by light, preservation and the types of luminophores that occur in the fur. Finally, this thesis demonstrates that photoluminescence is unlikely to have a visual function in nocturnal mammals.

7.1 Is fur photoluminescence common?

Recent studies have suggested that mammalian fur photoluminescence is rare. For example, Kohler et al. (2019) claimed to have discovered the first photoluminescently furred animal outside of the opossums (Didelphidae), while Olson et al. (2021) claimed to have documented the first case of photoluminescence in an Old World placental mammal. However, Pine et al.'s (1985) comprehensive work on opossum photoluminescence also cited publications on this characteristic for other mammalian taxa, and Bolliger (1944) had observed that photoluminescent fur was not uncommon. Therefore, I provided a historical context for mammal fur photoluminescence (Chapter 2), bringing together the mostly ignored existing knowledge on the phenomenon, demonstrating that photoluminescence in mammals is reasonably widespread, and so far found in half of all mammal orders. Earlier works all predate the claims of Kohler et al. (2019), Olson et al. (2021) and Pynne et al. (2021).

I then focused the scope of photoluminescent fur in a localised setting to document this phenomenon in mammals from a single region, the Wet Tropics of Australia (Chapter 3).

Numerous species (38 in total) of marsupial, monotreme and placental mammals showed photoluminescence to varying degrees, and brilliant or vivid photoluminescent fur (Rebell et al. 1956; Kohler et al. 2019) facilitated by luminophores (Kricka 2003) was present in 42% of the marsupials and 29% of the placental mammals. Vividly photoluminescent fur was spread across marsupial, placental, carnivorous, omnivorous, granivorous, folivorous, ground-dwelling and arboreal mammals, suggesting that these factors do not affect the presence of photoluminescence in general. Photoluminescent characteristics of colour and pattern seemed to be species-specific. My findings agree with Stübel (1911) and Toussaint et al. (2023) that, unless masked by pigmentation, photoluminescence is an inherently ubiquitous feature of mammal fur, as it is of other biological tissues.

7.2 Two types of luminophore and the problem of preservation

Mammal photoluminescence can broadly be categorised into two main types (although more may be discovered) based on the different excitation wavelengths required by specific luminophores, and the resulting different emission colours from fur: 1) tryptophan metabolites are optimally excited at lower wavelengths of 365 nm and below (but have great variability), often producing blueish photoluminescence, although a range of colours can be emitted (Pine et al. 1985); and 2) porphyrins have a distinct spectrographic signature, being optimally excited at higher wavelengths of 395 nm and above, and producing orange-red-pink photoluminescence (Goldoni 2002; Hamchand et al. 2021; Toussaint et al. 2023).

Surprisingly little chemistry on the photoluminescent properties of mammal fur has been conducted since the first fur luminophores were identified by Rebell et al. (1957). The only study on an Australian species was on common brushtail possums (*Trichosurus vulpecula*), and only a single luminophore was identified (Nicholls and Rienits 1971). Using modern laboratory techniques, I identified a protoporphyrin and a coproporphyrin from the fur of a long-nosed bandicoot (*Peramelas nasuta*), and the same protoporphyrin from the fur of a coppery brushtail possum (*T. johnstonii*) (Chapter 4). Interestingly, I also found protoporphyrin in the fur of all other species examined, regardless of whether the species showed pink photoluminescence in its fur or not. I found molecules matching the monoisotopic mass of a uroporphyrin in the long-nosed bandicoot, northern brown bandicoot (*Isoodon macrourus*), and coppery brushtail possum. A molecule matching the monoisotopic mass of heptacarboxylporphyrin was found only in the long-nosed bandicoot.

Although I tentatively identified some of the luminophores responsible for pink photoluminescence as porphyrins, several more, potentially tryptophan metabolite luminophores, remain unidentified. Tryptophan metabolites may be widely spread across taxa (Rebell et al. 1957; Nicholls and Rienits 1971; Pine et al. 1985). Previous studies identified the follicle as the point of entry of luminophores into the fur shaft for opossums (Pine et al. 1985) and, for common brushtail possums, both the root sheaths of the fur and the sweat glands exude bright blue photoluminescence (Nicholls and Rienits 1971).

My observations of different patterns of photoluminescence (Chapter 3) and the recent trend that several studies have been based on museum specimens (Anich et al. 2021; Tumlison and Tumlison 2021; Toussaint et al. 2023) introduced the question as to whether photoluminescence may be affected by external factors such as light exposure and preservation technique (Chapter 5). I found that two minutes of direct sun exposure can cause photobleaching of pink photoluminescence (Chapter 5), indicating considerable degradation of porphyrins (Toussaint et al. 2023). This loss of photoluminescence by photobleaching could explain Bolliger's (1944) observation of non-photoluminescence in long-nosed bandicoots.

In contrast to the extreme lability of porphyrin pink photoluminescence, the light-blue photoluminescence characteristic of tryptophan metabolites is relatively photostable (Daly et al. 2009). However, both light-blue photoluminescence, and pink photoluminescence, were degraded after six months of wet preservation. These results indicate that the history of light exposure, as well as preservation techniques, must be both stated and considered in the interpretation of mammalian fur photoluminescence. My findings indicate that museum-based studies may be a significant underestimate of natural photoluminescence.

7.3 Does fur photoluminescence have a visual function?

A renewed interest in the subject of photoluminescent mammal fur arose with the publication of colour photographs of New World flying squirrels (*Glaucomys* spp.) glowing pink (Kohler et al. 2019). Prior to these first published colour photographs, the possibility of a visual function for photoluminescence had not been proposed for fur. A visual function requires that natural lighting is strong enough to trigger photoluminescence, and that at least some animals can see it (Marshall and Johnsen 2017). The excitation wavelengths of sunlight are strong enough to excite most natural photoluminescence (Marshall and Johnsen 2017). Sunlight

therefore has the potential for frequent excitation of the pelage of diurnal mammals, but the reflectance may overwhelm the contribution of photoluminescence. Experimental evidence for nocturnal triggering of luminophores by moonlight has only been demonstrated for scorpions (Scorpiones), as reacted to by nocturnal flying insects (Kloock 2005). In contrast, I found no evidence that wild nocturnal mammals and birds can detect the photoluminescence of lab rat (*Rattus norvegicus*) fur or, if they can, they show no preference for it (Chapter 6).

This field experiment challenges the recent assumption that the photoluminescence of fur is a visually adaptive trait in nocturnal-crepuscular mammals. Although the glow of fur is striking in artificial torchlight, it appears to have no optical relevance in wild nocturnal landscapes. However, while I found no effect of animal type (bird, marsupial, placental) on model choice, the sample size of nocturnal birds was relatively low. Kohler et al. (2019) suggested a possible function of Batesian mimicry of photoluminescent pink owls (Strigiformes) for North American flying squirrels. Because the visual systems of mammals and birds are different, further testing of this hypothesis may be warranted in future studies, although owls hunt mammal species both with photoluminescent and non-photoluminescent fur (Rose 1996; Clulow et al. 2011; Kearney 2021). At this stage, however, there is little evidence to support a visual function for fur photoluminescence in nocturnal mammals.

With the recent singular focus on the vivid glow of luminophores when excited by low-wavelength light, it is easy to overlook that these molecules have other physiological roles. Tryptophan is an essential amino acid that cannot be synthesised by mammals, so must be gained from the diet (Richard et al. 2009; Yao et al. 2011). It forms part of several metabolic pathways, being transformed into vital products such as melatonin or serotonin via the serotonin pathway (Richard et al. 2009; Yao et al. 2011). When tryptophan is catabolised, the primary metabolite formed is kynurenine (Richard et al. 2009). Interestingly, kynurenine and N-acetyl-kynurenine, both luminophores identified in the fur of lab rats (Rebell et al. 1957), also act as ultraviolet filters when incorporated into the eye lenses of diurnal animals such as humans (*Homo sapiens*, Vazquez et al. 2002) and thirteen lined ground squirrels (*Spermophilus tridecemlineatus*, Hains et al. 2006).

Studies on corals (Scleractinia) and tardigrades (Tardigrada) have determined that photoluminescence may act as a photoprotective shield (Salih et al. 2000; Suma et al. 2020). Photoluminescence removes ultraviolet light at the excitation bandwidth by transmuting it into longer wavelengths (Pohland 2007). If only a narrow excitation wavelength is

transmuted, the organism is left exposed to harmful lower and higher ultraviolet wavelengths. In mammal fur, however, excitation of tryptophan metabolites covers a broad wavelength range, spanning the entire UV-A and UV-B parts of the spectrum (Fukunaga et al. 1982), hence potentially providing broad-spectrum protection from ultraviolet radiation.

Unlike tryptophan, porphyrins can be internally synthesised by mammals and other organisms (Neves and Galván 2020). Porphyrins are best known as a precursor to haemoglobin (Ajoka et al. 2006) meaning that the molecules are ubiquitous in all mammal species regardless of whether they contribute to external photoluminescence. While defects in any of the enzymes involved in the haem biosynthesis pathway may contribute to porphyria, animals showing an over-accumulation of porphyrins do not show it in their fur (Turner 1937; Rivera and Leung 2008). Collectively, the roles of tryptophan and porphyrins in various metabolic pathways argues more for a physiological function than a visual one.

7.4 Summary

Bright external photoluminescence is a reasonably common phenomenon among Wet Tropics mammals, whereas low to mid-level fur photoluminescence is near-ubiquitous. Whilst not a rare trait, intensely vivid photoluminescent fur occurs in less than half of species examined. This finding invites questioning on whether the species that possess it use it in some optical capacity, or whether species-specific metabolic pathways excrete various molecules into the fur, some of which exist as optically inactive luminophores. The continuum of photoluminescent vividness suggests that the presence of luminophores in fur is not special, and that photo-activation may require the concentration of luminophores to pass some threshold. While some of the luminophores in the fur of mammals were consistent with porphyrins, there were several others still awaiting identification. Species-specific metabolic pathways are suggested as the source of these luminophores.

The pink luminophores of bandicoot fur become degraded in a couple of minutes of sun exposure. This characteristic means that vivid external porphyrin-based photoluminescence may only be retained in nocturnal animals, although this requires additional testing for other species. The blue luminophores of rat fur are relatively photostable, but photoluminescence in general is depleted by wet preservation. These limitations necessitate the use of fresh animals, casting doubt on conclusions drawn from museum-based studies of photoluminescence.

The photoluminescence of rat fur is probably not excited by natural moonlight to an extent where it is differentially chosen by nocturnal vertebrates. This finding does not support recent speculations about a nocturnal visual function for photoluminescence, but suggests that photoluminescence may be an unexpressed optical property of fur chemistry. Rebell's (1966) finding that fur in which the expression of photoluminescence is masked by melanin contains equivalent concentrations of luminophores as does brightly photoluminescent fur, and the observation that some species have greater photoluminescence in the base of the fur than in the tips, that is, the fur has to be parted to reveal the most vivid colouration (Hamchand et al. 2021; Appendix C; Fig. 3.2d), renders the prospect of selection on optical properties unlikely. The continuum in degrees of intensity, near-ubiquitousness and presence in keratin, coupled with the sources being both essential dietary molecules and internally synthesised molecules (that may be excreted when over-abundant, and are photosensitive when expressed), all point to the photoluminescence of fur not being as optically relevant as recently thought.

7.5 Future directions

This thesis has joined some of the pieces of the photoluminescent fur puzzle, but there are still numerous questions that remain to be answered. There are still many gaps in knowledge regarding the prevalence of different types of photoluminescence in mammals, particularly whether external, physiological or species characteristics (e.g. habitat, diet, body condition, nocturnality) affect the excretion of luminophores into the fur, the interpretation of which may be confounded by lability. There are also many luminophores present in fur that remain to be identified, thus studies on fur chemistry in Australian mammals should continue to build on the seminal work of Nicholls and Rienits (1971).

One of the primary limitations with studies on photoluminescence in mammals is a lack of consideration of the light exposure history of specimens, as well as the effects of different preservation techniques on mammal pelts. Future studies need to examine live or freshly dead animals where possible, to gain a better understanding of the intensity and prevalence of photoluminescence in mammals. However, if photoluminescence serves no visual function, then the continued documentation of the optical properties (e.g. relative brightness) of fur under low wavelength light seems merely academic. Finally, the key to understanding a possible adaptive function for photoluminescence may lie in the physiological functions of the molecules involved and their biochemical pathways.

References

Abercrombie M., Hickman M., Johnson M.L., Thain M. 1992. *The new Penguin dictionary of biology*. Penguin Books, Ringwood, Australia.

Ajioka R.S., Phillips J.D., Kushner J.P. 2006. Biosynthesis of heme in mammals. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1763(7):723–736.

Akcali C.K., Pérez-Mendoza H.A., Salazar-Valenzuela D., Kikuchi D.W., Guayasamin J.M., Pfennig D.W. 2019. Evaluating the utility of camera traps in field studies of predation. *PeerJ* 7: e6487.

Al-Hazimi H.M., Jackson A.H., Ryder D.J., Elder G.H., Smith S.G. 1976. Specificity of coproporphyrinogen oxidase: conversion of coproporphyrinogen-IV into protoporphyrin-XIII. *Journal of the Chemical Society, Chemical Communications* 6:188–189.

Allegri G., Costa C., Biasiolo M., Arban R., Bertazzo A., Cardin de Stefani E.L. 1990. Tryptophan, copper and zinc in hair of healthy subjects. Correlation with differences in hair pigmentation. *The Italian Journal of Biochemistry* 39(4):209–215.

Anich P.S., Anthony S., Carlson M., Gunnelson A., Kohler A.M., Martin J.G., Olson E.R. 2021. Biofluorescence in the platypus (*Ornithorhynchus anatinus*). *Mammalia* 85(2):179–181.

Arnold KE, Owens IP, Marshall NJ. 2002. Fluorescent signaling in parrots. *Science* 295(5552):92.

Arrese C.A., Beazley L.D., Neumeyer C. 2006. Behavioural evidence for marsupial trichromacy. *Current Biology* 16(6):R193–R194.

Arrese C.A., Hart N.S., Thomas N., Beazley L.D., Shand J. 2002. Trichromacy in Australian marsupials. *Current Biology* 12(8):657–660.

Avilés J.M., Parejo D. 2012. Covariation between bill colouration and fitness components in a nocturnal bird. *Journal of Avian Biology* 43(6):565–570.

Baird C. 2015. What makes a “fluorescent” highlighter marker so bright? *Science Questions with Surprising Answers*. West Texas A&M University. https://wtamu.edu/~cbaird/sq/2015/05/15/what-makes-a-fluorescent-highlighter-marker-so-bright/. Accessed 19 Apr 2021.

Barnicot N.A. 1956. The pigment, trichosiderin, from human red hair. *Nature* 177(4507):528–529.

Bertazzo A., Biasiolo M., Costa C.V., de Stefani E.C., Allegri G. 2000. Tryptophan in human hair: correlation with pigmentation. *Il Farmaco* 55(8):521–525.

Blythman M.D., Sansom J.L., Lohr M.T. 2016. Use of ultraviolet light to help age nightjars, owlet-nightjars, frogmouths and owls. *Corella* 40(4):98–99.

BOC Sciences. 2022. BODIPY 493/503 *Methyl bromide*. https://probes.bocsci.com/bodipy-493503-methyl-bromide-item-712.html. Accessed 12 July 2022.

Bolliger A. 1944. On the fluorescence of the skin and the hairs of *Trichosurus vulpecula*. *Australian Journal of Science* 7(1):35.

Bond E.J., Monro H.A.U. 1984. *Manual of fumigation for insect control* 54. FAO, Rome, Italy.

Brennan P. 1917. Will o' the Wisp. *Scientific American* 10 March 1917, 116(10):261.

Buldain G., Diaz L., Frydman B. 1977. Synthesis of protoporphyrin XIII and protoporphyrin III. *The Journal of Organic Chemistry* 42(18):2957–2960.

Bureau of Meteorology. 2022. Daily minimum temperature. Walkamin Research Station. *Climate Data Online*. http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_nccObsCode=123&p_display_type=dailyDataFile&p_startYear=2022&p_c=-193535191&p_stn_num=031108. Accessed 12 December 2022.

Camacho C., Negro J.J., Redondo I., Palacios S., Sáez-Gómez P. 2019. Correlates of individual variation in the porphyrin-based fluorescence of red-necked nightjars (*Caprimulgus ruficollis*). *Scientific Reports* 9:19915.

Caro T.I.M. 2013. The colours of extant mammals. *Seminars in Cell & Developmental Biology* 24(6–7):542–552.

Carvalho L.D.S., Cowing J.A., Wilkie S.E., Bowmaker J.K., Hunt D.M. 2006. Shortwave visual sensitivity in tree and flying squirrels reflects changes in lifestyle. *Current Biology* 16:R81–R83.

Chattaway F.W., Barlow A.J.E. 1954. The fluorescent materials produced in vivo by certain dermatophytes. *Microbiology* 11(3):506–511.

Chen X., Luo W., Ma H., Peng Q., Yuan W.Z., Zhang Y. 2018. Prevalent intrinsic emission from nonaromatic amino acids and poly (amino acids). *Science China Chemistry* 61(3):351–359.

Churchill S. 1998. *Australian bats*. New Holland, Sydney, Australia.

Clarke J.A. 1983. Moonlight's influence on predator/prey interactions between short-eared owls (*Asio flammeus*) and deermice (*Peromyscus maniculatus*). *Behavioral Ecology and Sociobiology* 13(3):205–209.

Clulow S., Peters K.L., Blundell A.T., Kavanagh R.P. 2011. Resource predictability and foraging behaviour facilitate shifts between nomadism and residency in the eastern grass owl. *Journal of Zoology* 284(4):294–299.

Collins S. 1992. *Studies on the natural fluorescence of wool and wool grease*. Doctoral dissertation, City University of London, London, United Kingdom.

Cornelius III C.E., Ludwig G.D. 1967. Red fluorescence of comedones: production of porphyrins by *Corynebacterium acnes*. *Journal of Investigative Dermatology* 49(4):368–370.

Covacevich J., Easton A. 1974. *Rats and mice in Queensland*. Queensland Museum Booklet 9. Queensland Museum, Brisbane, Australia.

Croce A.C. 2021. Light and autofluorescence, multitasking features in living organisms. *Photochem* 1(2):67–125.

Czarnecki C., Manderino R., Parry D. 2022. Reduced avian predation on an ultraviolet-fluorescing caterpillar model. *The Canadian Entomologist* 154(1), e10:1–7.

Daly S., Bianchini R., Polefka T., Jumbelic L., Jachowicz J. 2009. Fluorescence and coloration of grey hair. *International Journal of Cosmetic Science* 31(5):347–359.

Dawson T.J, Webster K.N., Maloney S.K. 2014. The fur of mammals in exposed environments; do crypsis and thermal needs necessarily conflict? The polar bear and marsupial koala compared. *Journal of Comparative Physiology* B 184(2):273–284.

De Brauwer M., Hobbs J.P.A., Ambo-Rappe R., Jompa J., Harvey E.S., McIlwain J.L. 2018. Biofluorescence as a survey tool for cryptic marine species. *Conservation Biology* 32(3):706–715.

del Rosal B., Villa I., Jaque D., Sanz-Rodríguez F. 2016. *In vivo* autofluorescence in the biological windows: the role of pigmentation. *Journal of Biophotonics* 9(10):1059–1067.

Department of Physics, Imperial College. 2022. *SCIENCEphotoLIBRARY. Emission spectrum of mercury*. https://www.sciencephoto.com/media/1675/view/emission-spectrum-of-mercury. Accessed 10 July 2022.

Department of Environment and Science, Queensland. 2013. Mammals of Wet Tropics (WET) bioregion, *Wetland*Info *website*.

https://wetlandinfo.des.qld.gov.au/wetlands/facts-maps/wildlife/?AreaID=bioregion-wet-tropics-wet&Kingdom=animals&Class=mammals. Accessed 30 August 2022.

Derrien E., Turchini J. 1925. Nouvelles observations de fluorescences rouges chez les animaux. *Comptes Rendus Séances Société Biologie* 92:1030–1031.

Desforges M.F., Wood-Gush D.G.M. 1975. A behavioural comparison of domestic and mallard ducks. Habituation and flight reactions. *Animal Behaviour* 23(3):692–697.

Douglas III H.D., Ermakov I.V., Gellermann W. 2021. Brighter is better: bill fluorescence increases social attraction in a colonial seabird and reveals a potential link with foraging. *Behavioral Ecology and Sociobiology* 75(10):144.

Douglas R.H., Jeffery G. 2014. The spectral transmission of ocular media suggests ultraviolet sensitivity is widespread among mammals. *Proceedings of the Royal Society B: Biological Sciences* 281(1780):20132995.

Endler J.A. 1992. Signals, signal conditions, and the direction of evolution. *The American Naturalist* 139:S125–S153.

Endler J.A. 1993. The color of light in forests and its implications. *Ecological Monographs* 63(1):1–27.

Ericson M.B., Grapengiesser S., Gudmundson F., Wennberg A.M., Larkö O., Moan J., Rosen A. 2003. A spectroscopic study of the photobleaching of protoporphyrin IX in solution. *Lasers in Medical Science* 18(1):56–62.

Ewert J.P., Buxbaum-Conradi H., Dreisvogt F., Glagow M., Merkel-Harff C., Röttgen A., Schürg-Pfeiffer E., Schwippert W.W. 2001. Neural modulation of visuomotor functions underlying prey-catching behaviour in anurans: perception, attention, motor performance, learning. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 128(3):417–460.

Flesch P., Rothman S. 1945. Isolation of an iron pigment from human red hair. *Journal of Investigative Dermatology* 6(4):257–270.

Flieger M., Bandouchova H., Cerny J., Chudíčková M., Kolarik M., Kovacova V., Martínková N., Novák P., Šebesta O., Stodůlková E., Pikula J. 2016. Vitamin B2 as a virulence factor in *Pseudogymnoascus destructans* skin infection. *Scientific Reports* 6:33200.

Fox D.L. 1944. Biochromes. *Science* 100(2604):470–471.

Fukunaga Y., Katsuragi Y., Izumi T., Sakiyama F. 1982. Fluorescence characteristics of kynurenine and N′-formylkynurenine, their use as reporters of the environment of

tryptophan 62 in hen egg-white lysozyme. *The Journal of Biochemistry* 92(1):129–141.

Gackowska J., Olszewski R., Zubek M. 2003. Fluorescence emission of carbon disulfide in low-energy electron collisions. *Radiation Physics and Chemistry* 68(1-2):133–136.

Gallas J. M., Eisner M. 1987. Fluorescence of melanin-dependence upon excitation wavelength and concentration. *Photochemistry and Photobiology* 45(5):595–600.

Galván I., Camarero P.R., Mateo R., Negro J.J. 2016. Porphyrins produce uniquely ephemeral animal colouration: a possible signal of virginity. *Scientific Reports* 6:39210.

Galván I., Delgado M.D.M., Camarero P.R., Mateo R., Lourenco R., Penteriani V. 2018. Feather content of porphyrins in Eurasian eagle owl (*Bubo bubo*) fledglings depends on body condition and breeding site quality. *Integrative Zoology* 13(5):569–578.

Gálvez D., Nieto C., Samaniego P. 2020. Test of the prey-attraction hypothesis for the scorpion fluorescence. *Neotropical Biodiversity* 6(1):172–177.

Gerlach T., Sprenger D., Michiels N.K. 2014. Fairy wrasses perceive and respond to their deep red fluorescent coloration. *Proceedings of the Royal Society B: Biological Sciences* 281(1787):20140787.

Giaimo C. 2020. Platypuses glow under blacklight. We have no idea why. *The New York Times* 13 November 2020. https://www.nytimes.com/2020/11/13/science/platypus-glow-ultraviolet.html. Accessed 14 June 2021.

Giese A.C., Leighton P.A. 1937. Phosphorescence of cells and cell products. *Science* 85(2209):428–429.

Gillespie G. R., Brennan K., Gentles T., Hill B., Low Choy, J., Mahney T., Stevens A., Stokeld D. 2015. *A guide for the use of remote cameras for wildlife survey in northern Australia*. National Environmental Research Program, Northern Australia Hub. Charles Darwin University, Casuarina, NT.

Goldberg L. 1996. A history of pest control measures in the anthropology collections, National Museum of Natural History, Smithsonian Institution. *Journal of the American Institute for Conservation* 35(1):23–43.

Goldoni A. 2002. Porphyrins: fascinating molecules with biological significance. *ELETTRA Highlights.* 2001–2002:64–65.

Gouterman M., Khalil G.E. 1974. Porphyrin free base phosphorescence. *Journal of Molecular Spectroscopy* 53(1):88–100.

Gray R., Karlsson C. 2022. *101 years of biofluorescent animal studies: trends in literature, novel hypotheses, and best practices moving forward*. https://ecoevorxiv.org/repository/view/3834/. Accessed 16 February 2022.

Haack R.A., Brockerhoff E.G. 2011. ISPM No. 15 and the incidence of wood pests: recent findings, policy changes, and current knowledge gaps. *The 42nd annual meeting of the International Research Group on Wood Protection*, Section 3, Queenstown, NZ; p.11. IRG/WP-11-30568. https://www.nrs.fs.fed.us/pubs/39922. Accessed 12 July 2022.

Hains P.G., Simpanya M.F., Giblin F., Truscott R.J.W. 2006. UV filters in the lens of the thirteen lined ground squirrel (*Spermophilus tridecemlineatus*). *Experimental Eye Research* 82(4):730–737.

Hakanson E.C., Hakanson K.J., Anich P.S., Martin J.G. 2022. Techniques for documenting and quantifying biofluorescence through digital photography and color quantization. *Journal of Photochemistry and Photobiology* 12:100149.

Hamchand R., Lafountain A.M., Büchel R., Maas K.R., Hird S.M., Warren M., Frank H.A., Brückner C. 2021. Red fluorescence of European hedgehog (*Erinaceus europaeus*) spines results from free-base porphyrins of potential microbial origin. *Journal of Chemical Ecology* 47(6):588–596.

Hammond B.R. 2012. The visual effects of intraocular colored filters. *Scientifica* 2012: 424965.

Harvey E.N. 1957. *A history of luminescence from the earliest times until 1900*. Memoirs of the American Philosophical Society 44. Philadelphia, Pennsylvania, United States of America.

Herman B., Frohlich V.E.C., Lakowicz J.R., Murphy D.B., Spring K.R., Davidson M.W. 2015. *Fluorescence microscopy. Basic concepts in fluorescence.* National High Magnetic Field Laboratory, The Florida State University. https://micro.magnet.fsu.edu/primer/techniques/fluorescence/fluorescenceintro.html. Accessed 12 May 2021.

Hill G. 2010. *Bird Colouration.* National Geographic, Washington DC, United States of America.

Hirst H.R., 1927. Ultra-violet radiation as an aid to textile analysis. *Journal of the Textile Institute Transactions* 18(10):T369–T375.

Huang Z., Zeng H., Hamzavi I., Alajlan A., Tan E., McLean D.I., Lui H. 2006. Cutaneous melanin exhibiting fluorescence emission under near-infrared light excitation. *Journal of Biomedical Optics* 11(3):034010.

Huang W., Liu Q., Zhu E.Y., Shindi A.A.F., Li Y.Q. 2010. Rapid simultaneous determination of protoporphyrin IX, uroporphyrin III and coproporphyrin III in human whole blood by non-linear variable-angle synchronous fluorescence technique coupled with partial least squares. *Talanta* 82(4):1516–1520.

Hubbard R., Kropf A. 1965. On the colors of visual pigment chromophores. *The Journal of General Physiology* 49(2):381–385.

Hudon J. 2005. Considerations in the conservation of feathers and hair, particularly their pigments. In: *Proceedings of the CAC/ACCR 31st Annual Conference (Fur trade legacy. The preservation of organic materials)*, Jasper, AB, Canada; p. 127–147.

Hughes B., Bowman J., Stock N.L., Burness G. 2022. Using mass spectrometry to investigate fluorescent compounds in squirrel fur. *PLOS One* 17(2):e0257156.

Hurley C.M., McClusky P., Sugrue R.M., Clover J.A., Kelly J.E. 2019. Efficacy of a bacterial fluorescence imaging device in an outpatient wound care clinic: a pilot study. *Journal of Wound Care* 28(7):438–443.

Ishihara S., Labuta J., Van Rossom W., Ishikawa D., Minami K., Hill J.P., Ariga K. 2014. Porphyrin-based sensor nanoarchitectonics in diverse physical detection modes. *Physical Chemistry Chemical Physics* 16(21):9713–9746.

Jachowicz J., McMullen R.L. 2011. Tryptophan fluorescence in hair—Examination of contributing factors. *Journal of Cosmetic Science* 62(3):291–304.

Jacobs G.H. 1993. The distribution and nature of colour vision among the mammals. *Biological Reviews of the Cambridge Philosophical Society* 68(3):413–471.

Jaworski Z., Stanek M., Sobotka W. 2017. Mineral composition of the hair coat of Polish Konik horses raised in nature reserves and barns. *Journal of Elementology* 22(3):857–867.

Jeng M.L. 2019. Biofluorescence in terrestrial animals, with emphasis on fireflies: a review and field observation. In: Suzuki H., editor. *Biochemistry*. Vol.4, *Bioluminescence–analytical applications and basic biology*. IntechOpen, London, United Kingdom; p. 87–111.

Johnsen S. 2012. *The optics of life: a biologist's guide to light in nature*. Princeton University Press, Princeton, New Jersey, United States of America.

Johnsen S. 2016. How to measure color using spectrometers and calibrated photographs. *Journal of Experimental Biology* 219(6):772–778.

Johnsen S., Kelber A., Warrant E., Sweeney A.M., Widder E.A., Lee Jr R.L., Hernández-Andrés J. 2006. Crepuscular and nocturnal illumination and its effects on color perception by the nocturnal hawkmoth *Deilephila elpenor*. *Journal of Experimental Biology* 209(5):789–800.

Kean Z., Cutting L. 2022. 'Exciting' new thylacine discovery shines light on Tasmania's extinct marsupial. *ABC News*. https://www.abc.net.au/news/2022-08-21/thylacines-glow-under-ultraviolet-light-tasmanian-tiger/101346906. Accessed 21 September 2022.

Kearney S.G. 2021. Diet of an eastern grass owl *'Tyto longimembris*' in Northern New South Wales. *Australian Field Ornithology* 38:44–48.

Kehoe A.B., Becker M.J. 2017. Arsenic and old pelts: deadly pesticides in museum collections. *History of Anthropology Newsletter* 41. http://histanthro.org/notes/arsenic-and-old-pelts/. Accessed 11 July 2022.

Kellie A., Dain S.J., Banks P.B. 2004. Ultraviolet properties of Australian mammal urine. *Journal of Comparative Physiology* A 190(6):429–435.

Kessel D., Rossi E. 1982. Determinants of porphyrin-sensitized photooxidation characterized by fluorescence and absorption spectra. *Photochemistry and Photobiology* 35(1):37–41.

Kloock C.T. 2005. Aerial insects avoid fluorescing scorpions. *Euscorpius – Occasional Publications in Scorpiology* 2005(21):1–7.

Kloock C.T., Kubli A., Reynolds R. 2010. Ultraviolet light detection: a function of scorpion fluorescence. *The Journal of Arachnology* 38(3):441–445.

Kohler A.M., Olson E.R., Martin J.G., Anich P.S. 2019. Ultraviolet fluorescence discovered in New World flying squirrels (*Glaucomys*). *Journal of Mammalogy* 100(1):21–30.

Konev S.V. 1967. *Fluorescence and phosphorescence of proteins and nucleic acids*. (Udenfriend, S., translation editor). Plenum Press, New York, New York, United States of America.

Kozikowski S., Wolfram L., Alfano R. 1984. Fluorescence spectroscopy of eumelanins. *IEEE Journal of Quantum Electronics* 20(12):1379–1382.

Krause G.H., Weis E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Biology* 42(1):313–349.

Kricka L.J. 2003. *Optical methods: a guide to the "-essences"*. American Association for Clinical Chemistry, Washington DC, United States of America.

Kuznetsova A., Brockhoff P.B., Christensen R.H.B., Jensen S.P. 2022. Package '*lmerTest'*. https://cran.r-project.org/web/packages/lmerTest/lmerTest.pdf.

Lagorio M.G., Cordon G.B., Iriel A. 2015. Reviewing the relevance of fluorescence in biological systems. *Photochemical & Photobiological Sciences* 14:1538–1559.

Lamb J.Y., Davis M.P. 2020. Salamanders and other amphibians are aglow with biofluorescence. *Scientific Reports* 10:2821.

Latham R.M. 1953. Simple method for identification of least weasel. *Journal of Mammalogy* 34(3):385.

Laurance W.F. 1990. Effects of weather on marsupial folivore activity in a north Queensland upland tropical rainforest. *Australian Mammalogy* 13(1):41–47.

Leaver I.H. 1978. On the room temperature phosphorescence of wool keratin. *Photochemistry and Photobiology* 27(4):439–443.

Lee T.C., Shih T.H., Huang M.Y., Lin K.H., Huang W.D., Yang C.M. 2018. Eliminating interference by anthocyanins when determining the porphyrin ratio of red plant leaves. *Journal of Photochemistry and Photobiology B: Biology* 187:106–112.

Lennox F.G., Rowlands R.J. 1969. Photochemical degradation of keratins. *Photochemistry and Photobiology* 9(4):359–367.

Lenth R., Buerkner P., Giné-Vázquez, I., Herve M., Jung, M., Love J., Miguez, F., Riebl H., Singmann H. 2022. Package '*emmeans*'. https://cran.r-project.org/web/packages/emmeans/emmeans.pdf.

Lim M.L., Land M.F., Li D. 2007. Sex-specific UV and fluorescence signals in jumping spiders. *Science* 315(5811):481.

Linley G.D., Pauligk Y., Marneweck C., Ritchie E.G. 2020. Moon phase and nocturnal activity of native Australian mammals. *Australian Mammalogy* 43(2):190–195.

Longo V.M., da Silva Pinheiro A., Sambrano J.R., Agnelli J.A.M., Longo E., Varela J.A. 2013. Towards an insight on photodamage in hair fibre by UV-light: an experimental and theoretical study. *International Journal of Cosmetic Science* 35(6):539–545.

Macel M.L., Ristoratore F., Locascio A., Spagnuolo A., Sordino P., D'Aniello S. 2020. Sea as a color palette: the ecology and evolution of fluorescence. *Zoological Letters* 6:1–11.

Main D. 2020. We knew platypuses were incredible. Now we know they glow, too. *National Geographic* 12 November 2020. https://www.nationalgeographic.com/animals/article/glowing-platypus. Accessed 20 March 2021.

Marshall J., Johnsen S. 2017. Fluorescence as a means of colour signal enhancement. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 372(1724):20160335.

Martin G.R., 1974. Color vision in the tawny owl (*Strix aluco*). *Journal of Comparative and Physiological Psychology* 86(1):133–141.

Masko M., Domino M., Lewczuk D., Jasinski T., Gajewski Z. 2020. Horse behavior, physiology and emotions during habituation to a treadmill. *Animals* 10(6):921.

Mazel C.H., Cronin T.W., Caldwell R.L., Marshall N.J. 2004. Fluorescent enhancement of signaling in a mantis shrimp. *Science* 303(5654):51.

Mazel C.H., Fuchs E. 2003. Contribution of fluorescence to the spectral signature and perceived color of corals. *Limnology and Oceanography* 48(1/2):390–401.

McDougall D.J. 1952. Some factors influencing fluorescence in minerals. *American Mineralogist: Journal of Earth and Planetary Materials* 37(5-6):427–437.

McFarland W.N., Munz F.W. 1975. The visible spectrum during twilight and its implications to vision. In: Evans G.C., Bainbridge R., editors. *Light as an ecological factor*: II. Blackwell Science Publications, Oxford, United Kingdom; p.249–270.

Meisner D.H. 1983. Psychedelic opossums: fluorescence of the skin and fur of *Didelphis virginiana* Kerr. *Ohio Journal of Science* 83(2):4.

Menkhorst P., Knight F. 2011. *Field guide to the mammals of Australia*. 3rd ed. Oxford University Press.

Michiels N.K., Anthes N., Hart N.S., Herler J., Meixner A.J., Schleifenbaum F., Schulte G., Siebeck U.E., Sprenger D., Wucherer M.F. 2008. Red fluorescence in reef fish: a novel signalling mechanism? *BMC Ecology* 8:16.

Millington K.R. 2006. Photoyellowing of wool. Part 1: Factors affecting photoyellowing and experimental techniques. *Coloration Technology* 122(4):169–186.

Millington K.R. 2020. Anomalous fluorescence of white hair compared to other unpigmented keratin fibres. *International Journal of Cosmetic Science* 42(3):289–301.

Mills M.G., Patterson L.B. 2009. Not just black and white: pigment pattern development and evolution in vertebrates. *Seminars in Cell & Developmental Biology* 20(1):72–81.

Mombelli L., McDonald E., Battersby A.R. 1976. Enzymic formation of a tricarboxylic porphyrin and protoporphyrin-XIII from coprogen-IV. *Tetrahedron Letters* 17(13):1037–1040.

Mosca L., De Marco C., Fontana M., Rosei M.A. 1999. Fluorescence properties of melanins from opioid peptides. *Archives of Biochemistry and Biophysics* 371(1):63–69.

Moses T.M., Reinitz I.M., Johnson M.L., King J.M., Shigley J.E. 1997. A contribution to understanding the effect of blue fluorescence on the appearance of diamonds. *Gems & Gemology* 33(4):244–259.

Murthy K.V., Virk H.S. 2014. Luminescence phenomena: an introduction. In: Virk H.S., editor. *Defect and diffusion forum*. Vol. 347. Trans Tech Publications Ltd.; p.1–34.

National Center for Biotechnology Information. 2022. *PubChem Compound Summary*. https://pubchem.ncbi.nlm.nih.gov/compound/. Accessed 05 December 2022.

Needham A.E. 2012. *The significance of zoochromes* (Vol. 3). Springer Science & Business Media, Berlin, Heidelberg, Germany.

Negro J.J., Bortolotti G.R., Mateo R., García I.M. 2009. Porphyrins and pheomelanins contribute to the reddish juvenal plumage of black-shouldered kites. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 153(3):296–299.

Neves A.C.O., Galván I. 2020. Models for human porphyrias: have animals in the wild been overlooked? Some birds and mammals accumulate significant amounts of porphyrins in the body without showing the injurious symptoms observed in human porphyrias. *BioEssays* 42(12):2000155.

Newman C., Buesching C.D., Wolff J.O. 2005. The function of facial masks in "midguild" carnivores. *Oikos* 108(3):623–633.

Nicholls E.M., Rienits K.G. 1971. Tryptophan derivatives and pigment in the hair of some Australian marsupials. *International Journal of Biochemistry* 2(11):593–603.

Niyangoda C., Miti T., Breydo L., Uversky V., Muschol M. 2017. Carbonyl-based blue autofluorescence of proteins and amino acids. *PLOS One* 12(5):e0176983.

Nunan E., Levinson J., Elkin L., Rogge C., Sybalsky J., Pollak B. 2012. In their true colors: developing new methods for recoloring faded taxidermy. *Objects Specialty Group Postprints* 19:25–44.

O'Carroll D.C., Warrant E.J. 2017. Vision in dim light: highlights and challenges. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 372(1717):20160062.

Ocean Optics. 2013. *OceanView Spectrometer Operating Software*. Version 2.0.8, 05 March 2021. Ocean Optics, Inc., Dunedin, Florida, United States of America.

Olson E.R., Carlson M.R., Ramanujam V.S., Sears L., Anthony S.E., Anich P.S., Ramon L., Hulstrand A., Jurewicz M., Gunnelson A.S., Kohler A.M. 2021. Vivid biofluorescence discovered in the nocturnal springhare (Pedetidae). *Scientific Reports* 11:4125.

Ormö M., Cubitt A.B., Kallio K., Gross L.A., Tsien R.Y., Remington S.J. 1996. Crystal structure of the *Aequorea victoria* green fluorescent protein. *Science* 273(5280):1392–1395.

Othmane G., Allard T., Vercouter T., Morin G., Fayek M., Calas G. 2016. Luminescence of uranium-bearing opals: origin and use as a pH record. *Chemical Geology* 423:1–6.

Pailthorpe M.T., Nicholls C.H. 1972. ESR studies of the low temperature irradiation of keratin and its component amino acids. *Photochemistry and Photobiology* 15(5):465–77.

Pawelek J.M., Körner A.M. 1982. The biosynthesis of mammalian melanin: the regulation of pigment formation, the key to disorders such as albinism and piebaldism, may also offer some clues for the treatment of melanoma. *American Scientist* 70(2):136–145.

Penteriani V., Delgado M.D.M. 2017. Living in the dark does not mean a blind life: bird and mammal visual communication in dim light. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 372(1717):20160064.

Peterson R.A. 2022. Package '*bestNormalize*'. https://cran.r-project.org/web/packages/bestNormalize/bestNormalize.pdf.

Pine R.H., Abravaya J.P. 1978. Notes on the Brazilian opossum *Monodelphis scalops* (Thomas) (Mammalia: Marsupialia: Didelphidae). *Mammalia* 42(3):379–382.

Pine R.H., Handley C.O.J. 1984. A review of the Amazonian short-tailed opossum *Monodelphis emiliae* (Thomas). *Mammalia* 48(2):239–245.

Pine R.H., Rice J.E., Bucher J.E., Tank D.J., Greenhall A.M. 1985. Labile pigments and fluorescent pelage in didelphid marsupials. *Mammalia* 49(2):249–256.

Plavskii V.Y., Mikulich A.V., Tretyakova A.I., Leusenka I.A., Plavskaya L.G., Kazyuchits O.A., Dobysh I.I., Krasnenkova T.P. 2018. Porphyrins and flavins as endogenous acceptors of optical radiation of blue spectral region determining photoinactivation of microbial cells. *Journal of Photochemistry and Photobiology B: Biology* 183:172–183.

Pohland G. 2007. *Spectral data of avian plumage*. Doctoral dissertation, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany.

Posudin Y. 2007. *Practical spectroscopy in agriculture and food science*. Science Publishers, Enfield, Connecticut, United States of America.

Potier S., Mitkus M., Kelber A. 2020, October. Visual adaptations of diurnal and nocturnal raptors. *Seminars in Cell & Developmental Biology* 106:116–126.

Prötzel D., Heß M., Schwager M., Glaw F., Scherz M.D. 2021. Neon-green fluorescence in the desert gecko *Pachydactylus rangei* caused by iridophores. *Scientific Reports* 11:297.

Pynne J.T., Castleberry S.B., Conner L.M., Piper C.W., Parsons E.I., Gitzen R.A., Duncan S.I., Austin J.D., McCleery R.A. 2021. Ultraviolet biofluorescence in pocket gophers. *The American Midland Naturalist* 186(1):150–155.

Queensland Museum. 2010–2022. *Taxidermy*. https://www.qm.qld.gov.au/Explore/Find+out+about/Behind+the+Scenes/Taxidermy. Accessed 25 August 2022.

Rajendran S., Parveen K.H. 2005. Insect infestation in stored animal products. *Journal of Stored Products Research* 41(1):1–30.

Rakovan J. 2021. Coming to terms with fluorescence: a short glossary of common terms in the context of mineral fluorescence. *Rocks & Minerals* 96(1):20–23.

Rao S., Rajkumar A., Ehtesham M., Prathiba D. 2008. Autofluorescence: a screening test for mycotic infection in tissues. *Indian Journal of Pathology and Microbiology* 51(2):215.

Rebell G. 1966. Kynurenine in rat hair. *Nature* 209:913–914.

Rebell G., Lamb J.H., Mahvi A., Lee H.R. 1957. The identification of L-kynurenine as the cause of the fluorescence of the hair of the laboratory rat. *Journal of Investigative Dermatology* 29(6):471–477.

Rebell G., Mahvi A., Lamb J.H. 1956. Paper chromatographic separation of fluorescent materials in normal rat hair. *Journal of Investigative Dermatology* 27(4):259–262.

Reinhold L. 2020. Fluorescent forests: of mushrooms and marsupials. *The Queensland Mycologist* 15(1/2):5–12.

Reinhold L. 2021. Mammals with fluorescent fur: observations from the Wet Tropics. *North Queensland Naturalist* 51:1–8.

Reinhold L. 2022. Photoluminescent yellow wing markings of eastern tube-nosed fruit bats (*Nyctimene robinsoni*). *North Queensland Naturalist* 52:69–74.

Reis P.J., Schinckel P.G. 1963. Some effects of sulphur-containing amino acids on the growth and composition of wool. *Australian Journal of Biological Sciences* 16(1):218–230.

Reshetnyak Y.K., Burstein E.A. 2001. Decomposition of protein tryptophan fluorescence spectra into log-normal components. II. The statistical proof of discreteness of tryptophan classes in proteins. *Biophysical Journal* 81(3):1710–1734.

Richard D.M., Dawes M.A., Mathias C.W., Acheson A., Hill-Kapturczak N., Dougherty D.M. 2009. L-tryptophan: basic metabolic functions, behavioral research and therapeutic indications. *International Journal of Tryptophan Research* 2.

DOI: https://doi.org/10.4137/IJTR.S2129.

Riedler R., Pesme C., Druzik J., Gleeson M., Pearlstein E. 2014. A review of color-producing mechanisms in feathers and their influence on preventive conservation strategies. *Journal of the American Institute for Conservation* 53(1):44–65.

Rippon J.A. 2013. The structure of wool. In: Lewis D.M., Rippon J.A., editors. *The coloration of wool and other keratin fibres*. John Wiley & Sons in association with the Society of Dyers and Colourists, Bradford, United Kingdom; p.1–42.

Rivera D.F., Leung L.K.P. 2008. A rare autosomal recessive condition, congenital erythropoietic porphyria, found in the canefield rat *Rattus sordidus* Gould 1858. *Integrative Zoology* 3(3):216–218.

Rollakanti K.R., Kanick S.C., Davis S.C., Pogue B.W., Maytin E.V. 2013. Techniques for fluorescence detection of protoporphyrin IX in skin cancers associated with photodynamic therapy. *Photonics & Lasers in Medicine* 2(4):287–303.

Rose A.B. 1996. Notes on the diet of the barn owl *Tyto alba* in New South Wales. *Australian Bird Watcher* 16(8):327–331.

Roulin A., Almasi B., Rossi-Pedruzzi A., Ducrest A.L., Wakamatsu K., Miksik I., Blount J.D., Jenni-Eiermann S., Jenni L. 2008. Corticosterone mediates the condition-dependent component of melanin-based coloration. *Animal Behaviour* 75(4):1351–1358.

Roy J.K., Brown R.R., Price J.M. 1959. 8-Hydroxyquinaldic acid: the red fluorescent substance in the urine of pyridoxine-deficient rats. *Nature* 184(4698):1573–1574.

RStudio Team. 2020. *RStudio*: Integrated Development for RStudio, PBC, Boston, Massachusetts, United States of America. Version 1.0.153; https://www.rproject.org; R version 4.1.2, https://cran.rstudio.com.

Sachar M., Anderson K.E., Ma X. 2016. Protoporphyrin IX: the good, the bad, and the ugly. *Journal of Pharmacology and Experimental Therapeutics* 356(2):267–275.

Salih A., Larkum A., Cox G., Kühl M., Hoegh-Guldberg O. 2000. Fluorescent pigments in corals are photoprotective. *Nature* 408(6814):850–853.

Schäfer K., Goddinger D., Höcker H. 1997. Photodegradation of tryptophan in wool. *Journal of the Society of Dyers and Colourists* 113(12):350–355.

Schnell T., Bentley K., Hayes E., Rick M. 2001. Legibility distances of fluorescent traffic signs and their normal color counterparts. *Transportation Research Record* 1754(1):31–41.

Shimomura O., Johnson F.H., Saiga Y. 1962. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *Journal of Cellular and Comparative Physiology* 59(3):223–239.

Shkirman S.F., Solov'ev K.N., Kachura T.F., Arabei S.A., Skakovskii E.D. 1999. Interpretation of the soret band of porphyrins based on the polarization spectrum of N-methyltetraphenylporphin fluorescence. *Journal of Applied Spectroscopy* 66(1):68–75.

Silberglied R.E. 1979. Communication in the ultraviolet. *Annual Review of Ecology and Systematics* 10:373–398.

Simmons J.E. 1999. Storage concerns for fluid-preserved collections. *Conserve O Gram* 11(3):1–4.

Sirois P.J., Poulin J., Stone T. 2010. Detecting pesticide residues on museum objects in Canadian collections – A summary of surveys spanning a twenty-year period. *Collection Forum* 24(1–2):28–45.

Smith G.J. 1995. New trends in photobiology (invited review) Photodegradation of keratin and other structural proteins. *Journal of Photochemistry and Photobiology B: Biology* 27(3):187–198.

Smith G.J., Markham K.R., Melhuish W.H. 1994. The presence of photoreactive *β*-carboline fluorophores in weathered wool keratin. *Photochemistry and Photobiology* 60(3):196–198.

Smith G.J., Melhuish W.H. 1985. Fluorescence and phosphorescence of wool keratin excited by UV-A radiation. *Textile Research Journal* 55(5):304–307.

Smith G.J., Thorpe M.R., Melhuish W.H., Beddard G.S. 1980. Fluorescence of tryptophan in keratin. *Photochemistry and Photobiology* 32(6):715–718.

Soop K. 2005. A contribution to the study of the cortinarioid mycoflora of New Zealand, III. *New Zealand Journal of Botany* 43(2):551–562.

Sparks J.S, Schelly R.C, Smith W.L, Davis M.P, Tchernov D., Pieribone V.A, Gruber D.F. 2014. The covert world of fish biofluorescence: a phylogenetically widespread and phenotypically variable phenomenon. *PLOS One* 9(1):e83259.

Stein E.D., White B.P., Mazor R.D., Miller P.E., Pilgrim E.M. 2013. Evaluating ethanol-based sample preservation to facilitate use of DNA barcoding in routine freshwater biomonitoring programs using benthic macroinvertebrates. *PLOS One* 8(1):e51273.

Stockdale P.M., Mackenzie D.W.R., Austwick P.K.C. 1965. *Arthroderma simii* sp. nov., the perfect state of *Trichophyton simii* (Pinoy) comb. nov. Sabouraudia: *Journal of Medical and Veterinary Mycology* 4(2):112–123.

Stokes G.G. 1852. XXX. On the change of refrangibility of light. *Philosophical Transactions of the Royal Society of London* 142:463–562.

Stübel H. 1911. Die fluoreszenz tierischer gewebe in ultraviolettem licht. *Pflüger's Archiv für die Gesamte Physiologie des Menschen und der Tiere* 142:1–14.

Suma H.R., Prakash S., Eswarappa S.M. 2020. Naturally occurring fluorescence protects the eutardigrade *Paramacrobiotus* sp. from ultraviolet radiation. *Biology Letters* 16(10):20200391.

Sumita M., Terayama K., Suzuki N., Ishihara S., Tamura R., Chahal M.K., Payne D.T., Yoshizoe K., Tsuda K. 2022. De novo creation of a naked eye–detectable fluorescent molecule based on quantum chemical computation and machine learning. *Science Advances* 8(10):eabj3906.

Taboada C., Brunetti A.E., Pedron F.N., Neto F.C., Estrin D.A., Bari S.E., Chemes L.B., Lopes N.P., Lagorio M.G., Faivovich J. 2017. Naturally occurring fluorescence in frogs. *Proceedings of the National Academy of Sciences* 114(14):3672–3677.

Tomalia D.A., Klajnert-Maculewicz B., Johnson K.A.M., Brinkman H.F., Janaszewska A., Hedstrand D.M. 2019. Non-traditional intrinsic luminescence: inexplicable blue fluorescence observed for dendrimers, macromolecules and small molecular structures lacking traditional/conventional luminophores. *Progress in Polymer Science* 90:35–117.

Toussaint S.L., Ponstein J., Thoury M., Métivier R., Kalthoff D.C., Habermeyer B., Guilard R., Bock S., Mortensen P., Sandberg S., Gueriau P. 2023. Fur glowing under ultraviolet: in situ analysis of porphyrin accumulation in the skin appendages of mammals. *Integrative Zoology* 18(1):15–26.

Troscianko J., Stevens M. 2015. Image calibration and analysis toolbox–a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution* 6(11):1320–1331.

Tumlison C.R., McAllister C., Robison H., Connior M., Redman R., Trauth S., Port P. 2019. Vertebrate natural history notes from Arkansas, 2019. *Journal of the Arkansas Academy of Science* 73(1):39–45.

Tumlison C.R., Tumlison T.L. 2021. Investigation of fluorescence in selected mammals of Arkansas. *Journal of the Arkansas Academy of Science* 75(1):29–35.

Turner G.G., Meteyer C.U., Barton H., Gumbs J.F., Reeder D.M., Overton B., Bandouchova H., Bartonička T., Martínková N., Pikula J., Zukal J. 2014. Nonlethal screening of bat-

wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. *Journal of Wildlife Diseases* 50(3):566–573.

Turner W.J. 1937. Studies on porphyria. I. Observations on the fox squirrel, *Sciurus niger*. *Journal of Biological Chemistry* 118:519–530.

Udall S.L., Briggs F.P., Pautzke C.F., Janzen D.H. 1964. Fluorescence studies. In: *Wildlife Research: problems programs progress 1963. Activities of the Branch of Wildlife Research in the Bureau of Sport Fisheries and Wildlife for the calendar year 1963*. Circular 188. United States Department of the Interior, Fish and Wildlife Service and Bureau of Sport Fisheries and Wildlife, Washington DC, United States of America; p. 64.

United States Environmental Protection Agency. 2022. *Methyl bromide*. https://www.epa.gov/ods-phaseout/methyl-bromide. Accessed 12 July 2022.

Valeur B. 2001. Introduction: On the origin of the terms fluorescence, phosphorescence, and luminescence. In: Valeur B., Brochon J.C., editors. *New Trends in Fluorescence Spectroscopy*. *Springer Series on Fluorescence, vol 1.* Springer, Berlin, Heidelberg, Germany; p.3–6.

Valeur B., Berberan-Santos M.N. 2011. A brief history of fluorescence and phosphorescence before the emergence of quantum theory. *Journal of Chemical Education* 88(6):731–738.

van den Berg C.P., Troscianko J., Endler J.A., Marshall N.J., Cheney K.L. 2020. Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. *Methods in Ecology and Evolution* 11(2):316–332.

Vazquez S., Aquilina J.A., Sheil M.M., Truscott R.J., Jamie, J.F. 2002. Novel protein modification by kynurenine in human lenses. *Journal of Biological Chemistry* 277(7):4867–4873.

Viegas M.S., Martins T.C., Seco F., Do Carmo A. 2007. An improved and cost-effective methodology for the reduction of autofluorescence in direct immunofluorescence studies on formalin-fixed paraffin-embedded tissues. *European Journal of Histochemistry* 51(1):59–66.

Viitala J., Korplmäki E., Palokangas P., Koivula M. 1995. Attraction of kestrels to vole scent marks visible in ultraviolet light. *Nature* 373(6513):425–427.

Visser A.J.W.G, Rolinski O.J. 2014. *Basic photophysics*. American Society for Photobiology. http://photobiology.info/Visser-Rolinski.html. Accessed 08 Apr 2021.

Wang B., Yang W., McKittrick J., Meyers M.A. 2016. Keratin: structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration. *Progress in Materials Science* 76:229–318.

Warrant E. 2004. Vision in the dimmest habitats on earth. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural and Behavioral Physiology* 190:765–789.

Warren M.W. II. 1982. *Quantitative analysis by room-temperature phosphorescence*. Doctoral dissertation, University of Illinois, Urbana-Champaign, Illinois, USA.

Watanabe M., Ohkubo K., Tamura Z. 1972. Studies on carcinogenic tryptophan metabolites —I: enzymatic formation and hydrolysis of glucuronide of 3-hydroxyanthranilic acid. *Biochemical Pharmacology* 21(9):1337–1346.

Weagle G., Paterson P.E., Kennedy J., Pottier R. 1988. The nature of the chromophore responsible for naturally occurring fluorescence in mouse skin. *Journal of Photochemistry and Photobiology B: Biology* 2(3):313–320.

Wiedemann E. 1888. Ueber fluorescenz und phosphorescenz i. abhandlung. *Annalen der Physik* 270(7):446–463.

Wilson B., Beccari, J.B. 1775. *A series of experiments relating to phosphori and the prismatic colours they are found to exhibit in the dark: by B. Wilson, FRS and Member of the Royal Academy at Upsal. Together with a translation of two memoirs, from the Bologna Acts, upon the same subject, by J.B. Beccari, Professor of Philosophy at Bologna*. J. Dodsley in Pall-Mall, T. Becket in the Strand, and G. Kearsly in Fleet-Street; Mess. Dollond in St. Paul's Church-Yard, and Mr. Smith in the Strand, Opticians, London, United Kingdom.

Wojcikowska-Soroczynska M., Radosz A., Fiedorczuk E. 1987. Quantitative appearance of pigments in hairs of sheep and selected animal species. *Annals of Warsaw Agricultural University SGGW-AR. Animal Science* (Poland) 21:17–21.

Wolff C., Corradini P., Cortés G. 2005. Congenital erythropoietic porphyria in an African hedgehog (*Atelerix albiventris*). *Journal of Zoo and Wildlife Medicine* 36(2):323–325.

Wołowiec P., Michalak I., Chojnacka K., Mikulewicz M. 2013. Hair analysis in health assessment. *Clinica Chimica Acta* 419:139–171.

Yang H., Xiao X., Zhao X., Wu Y. 2015. Intrinsic fluorescence spectra of tryptophan, tyrosine and phenyloalanine. In: *Selected Papers of the Chinese Society for Optical Engineering Conferences*; October and November 2016; 10255, p. 1199–1206.

Yao K., Fang J., Yin Y.L., Feng Z.M., Tang Z.R., Wu G. 2011. Tryptophan metabolism in animals: important roles in nutrition and health. *Frontiers in Bioscience* 3(1):286–97.

Appendix A. Torches used to elicit photoluminescence in specimens.

310 nm (Tao Yuan, 10–15 mW, 160 mA, 1 x LED UVB, 1 x 18650 lithium-ion cell)

365 nm (LED shop, 3w, LED, 3 x AAA cells)

365 nm (Olight i5UV Camo, 1,500 lumens, LED, 1 x AA cell)

365 nm (UV Beast V3 UVB-V3-365, 5400 uW/cm^2 UV irradiance, 1350nW UV radiant intensity, 3.7 Volts, 3 x LED, 2 x 18650 lithium-ion cells)

380 nm, range 340–420 nm (Procontechnology ST3386UV, 4 x LED, 3 x AAA cells)

395 nm (Capsulone LD1345, LED, 3 x AAA cells)

395–410 nm (Dulex DX-502B, 3.7 Volts, LED, 1 x 18650 lithium-ion cell)

470 nm (UltraFire H-b3, 283 lumens, 3.7 Volts, 3 x XP-E2 LED, 1 x 18650 lithium-ion cell)

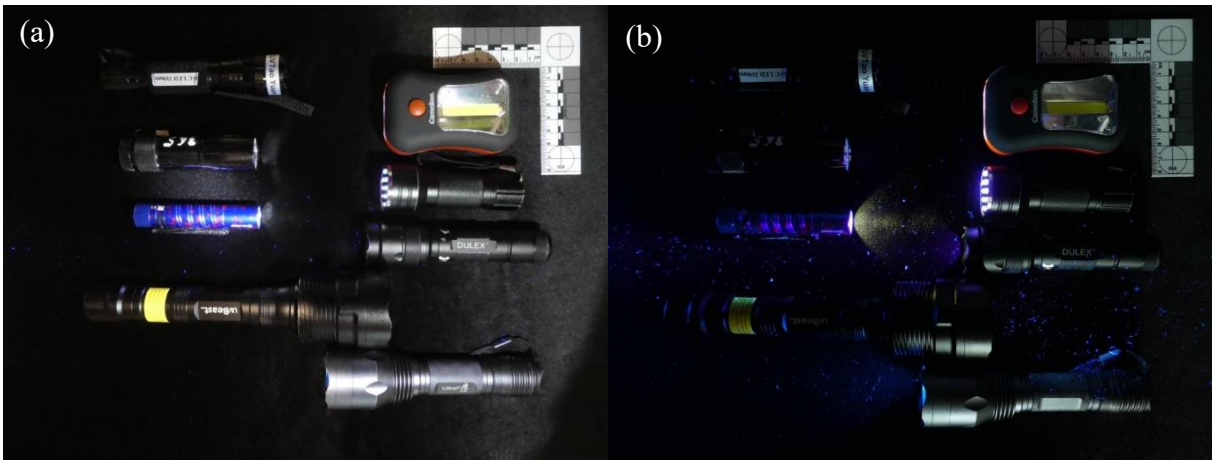


Figure A.1. The ultraviolet-violet-blue torches listed above. In sequential order from top left down then top right down. (a) In white light and (b) in their own light.

Appendix B. Photographic guide to the qualitative classification of photoluminescent intensity.

The brightness of whitish photoluminescence was assessed on the ‘glow’, which is generally not captured by photography. The pink photoluminescence did not glow in the way the whitish photoluminescence did, so was classified on the saturation and intensity of colour change. Both the brightness of glow and the colour qualities of photoluminescence must have the same excitation wavelengths, and in some cases the same torch, to be comparable.

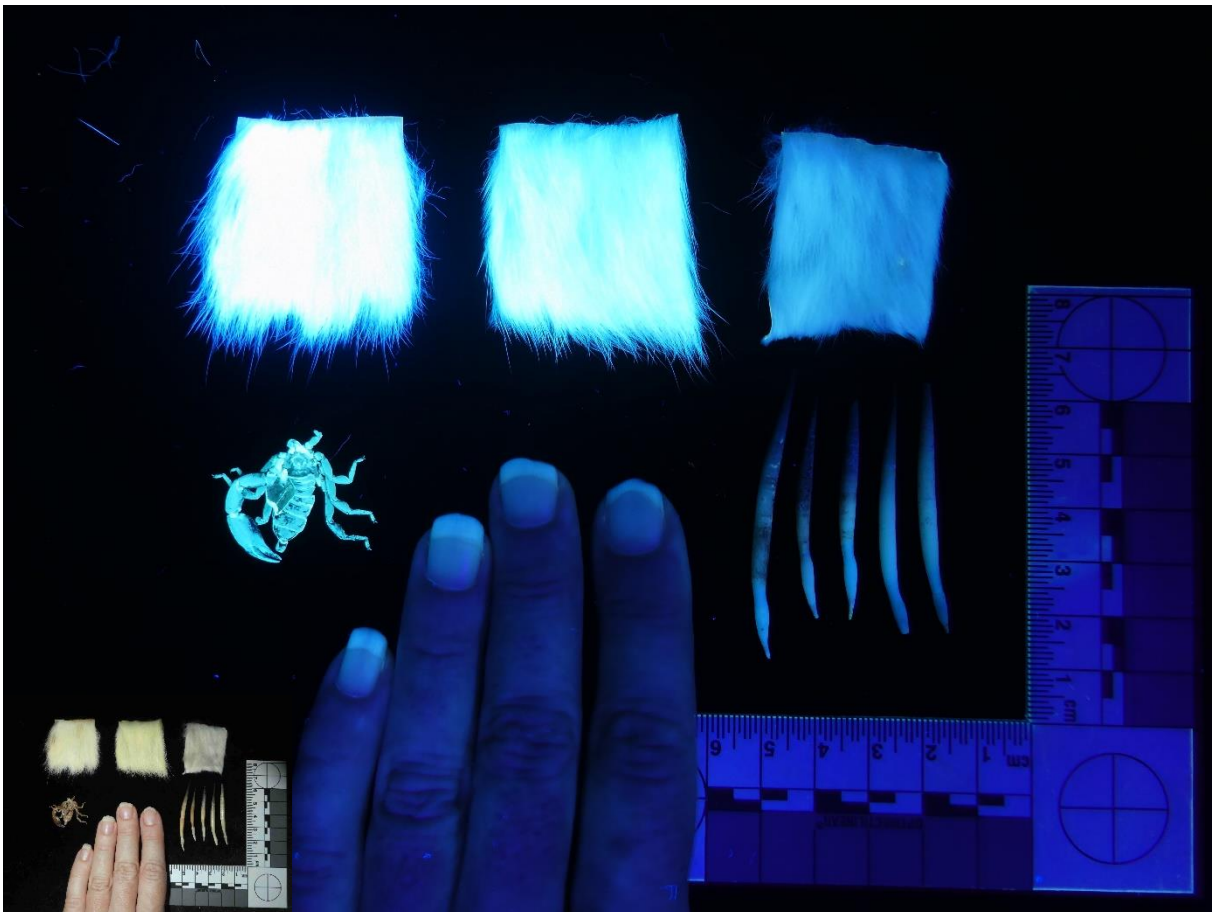


Figure B.1. Photographic comparison of qualitative brightness, blue-white photoluminescence. Top row left to right: ‘bright’, ‘mid’ and ‘none’ laboratory rat (*Rattus norvegicus*) fur. Lower row: photoluminescence of a scorpion (*Hormurus* sp.) exuviae, human (*Homo sapiens*) fingernails and echidna (*Tachyglossus aculeatus*) spines. Keratin alone yields a mid-level glow. Note: the echidna spines are dark at the tips, and the brightest sections are hidden when viewing the whole animal. Main: with 395–410 nm Dulex torchlight, 6 s exposure. Inset: with white flash.

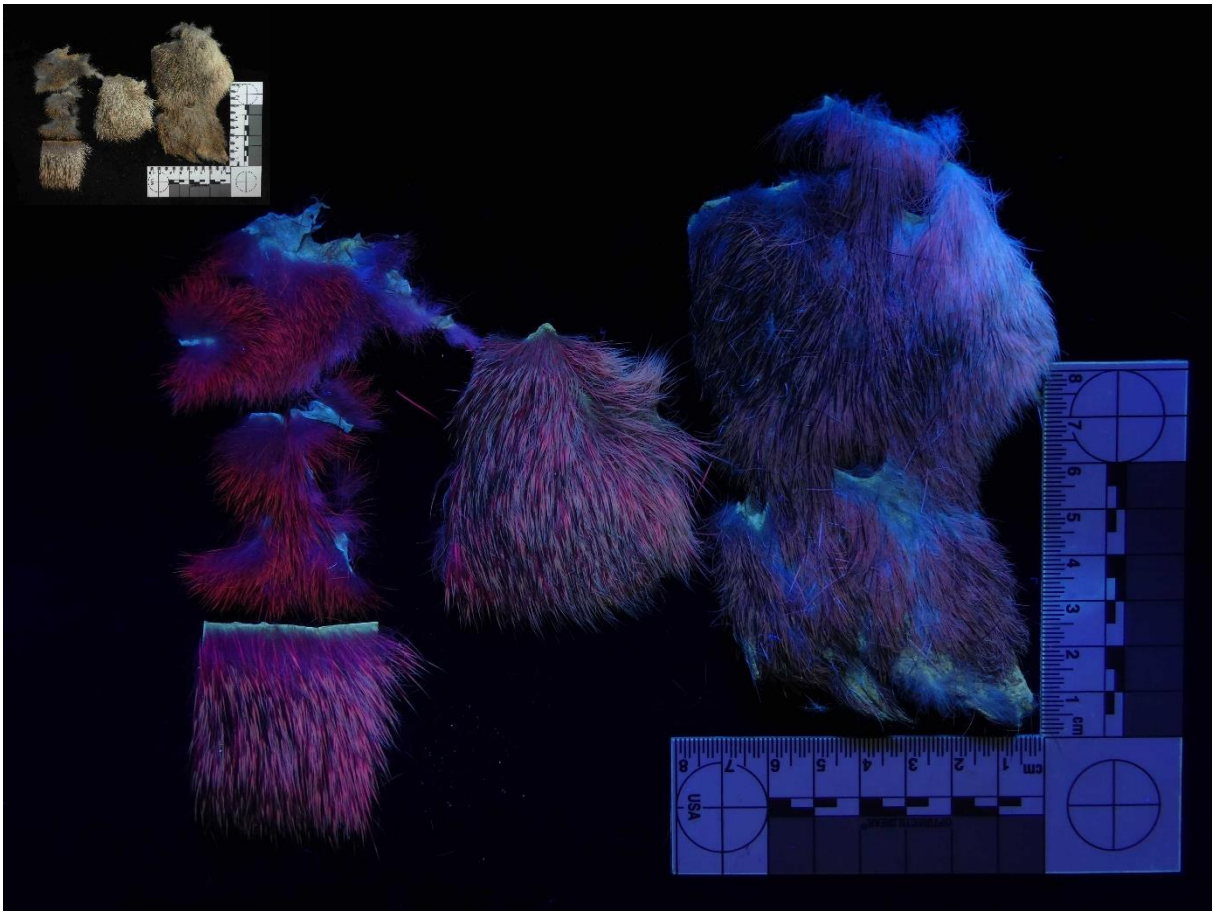


Figure B.2. Photographic comparison of qualitative colour, pink photoluminescence. Left column: bright (top: extremely vivid; bottom: bright). Middle: mid. Right: subtle. Left top two and right: long-nosed bandicoots (*Perameles nasuta*). Left bottom and middle: northern brown bandicoot (*Isoodon macrourus*). Main: with 395–410 nm Dulex torchlight, 10 s. Inset: with white flash.

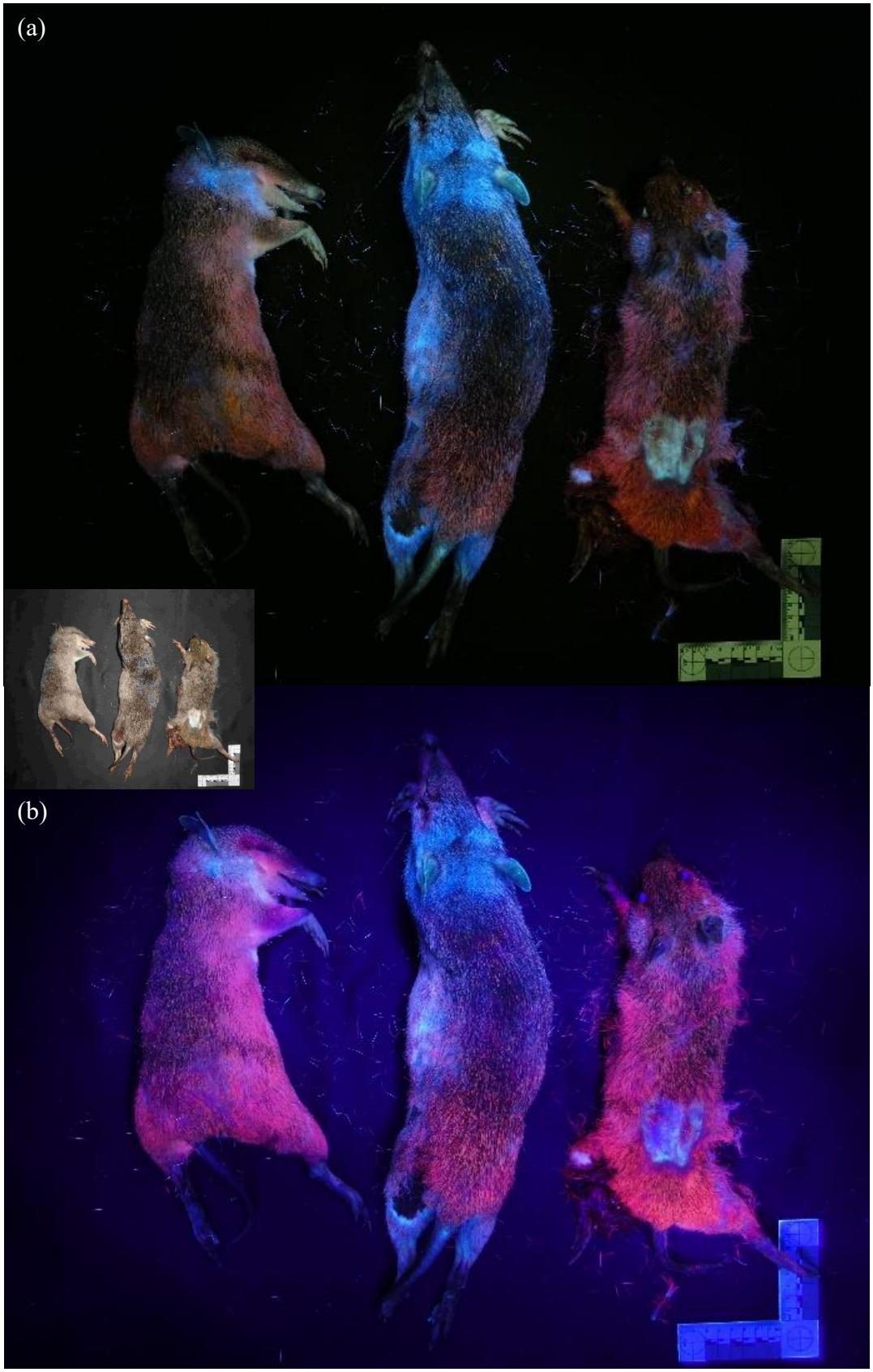


Figure B.3. Comparative effect of excitation wavelength on photoluminescent vividness. Long-nosed bandicoots × 3, dorsal (female-male-female). (a) At 365 nm, OLight 20 s (mid pink, but bright blue) and (b) at 395–410 nm, Dulex 10 s (bright pink, but lesser blue). Inset: with white flash.

Appendix C. Table C.1. Observations of photoluminescence in mammals of the Wet Tropics. Taken directly from carcasses.

Species	Number examined	Wavelength (nm)	Dorsal and flank fur	Ventral fur	Notes
Monotremes					
(Order: Monotremata. Families: Ornithorhynchidae and Tachyglossidae)					
Platypus (*Ornithorhynchus anatinus*)	**2** 1 female 1 ?	310–365	Silvery grey over fur, but tail absorbing.	Silvery grey throughout the fur, especially on sides anterior to hindquarters.	Both frozen (1 thawed). The pale photoluminescent green is in the tips of the fur, not the whole strand. The proximal portion of fur reflects purple light. [Incidental live observation of 2 individuals at dusk with 395 nm torch: fur remained dark and reflected some purple light; photoluminescence barely discernible.]
		380–410	Faint moss green flecked with hairs of pale green. Pale green at sides. Not so much on tail.	Yellowish green or pale green-grey; green over lower sides and back legs. Ventral flank: some dusky pink in otherwise light brown fur tips. Some orangey pink whole strands. Some pale pink in distal half of soft underfur; in the light brownish rather than the grey underfur, so it contributes to the brown colouration. Pale pink in distal half of other thicker fur interspersed with the grey non-photoluminescent underfur. Pink not evident at the whole pelt level, only when clipped fur examined separately.	
Short-beaked echidna (*Tachyglossus aculeatus*)	**2** 2 ?	310–365	None.	None in fur, but some blue skin near cloaca.	Both fresh roadkills.
		380–410	Dorsal and neck fur same kind of dull moss green as platypus. Moreso on tops of front paws. Spines pale greenish yellow.	Greenish-yellow. Very dull moss green. Blue skin towards cloaca.	

Marsupials					
Quolls, antechinuses and dunnarts (Order: Dasyuromorphia. Family: Dasyuridae)					
Rusty(?) antechinus (*Antechinus adustus(?)*)	**2** 2 male	310–365	Blue-white throughout underfur; tips remain brown. Brightest photoluminescence at 365 nm.	Some blue-white through fur but mostly absorbing; remaining rusty.	Frozen (1 thawed).
		380–410	Blue-grey-white through fur but mostly absorbing. Slight pink wash around rump.	A little yellowish white but mainly absorbing.	
Northern quoll (*Dasyurus hallucatus*)	**9** 8 males 1 female	310–365	White spots glow slightly white. Some orange on tops of paws.	White spots slightly white. Genital fur of males white, but rest of ventral fur absorbs, remaining cream.	8 frozen (2 thawed), 1 fresh roadkill. One female and a couple of males have strong dark pink photoluminescence only on one side; suspect that the pink luminophores are readily lost when exposed to the sun.
		380–410	Three males have yellowish green photoluminescence on side of neck/shoulder and one has greenish white chest patch. The chest patch of the fresh roadkill is bright lemon yellow. Orange-pink over feet. Four individuals (both sexes) have bright pink photoluminescence variously occurring over face, head, neck, flanks, hindquarters, legs and tops of paws. Bright pink goes through both brown flank fur and white spots. On white spots, pink can be evident all the way along the hair shaft, but there is some white at the tips. In others, bright strong pink occurs in the basal 2/3 of fur in white spots.	Most have rose pink to apricot wash ventrally, particularly at edges and between legs. The most prominent has bright fairy floss pink on ventral fur, testes and under tail.	

Dunnart (*Sminthopsis* sp.)	**1** 1 male	310–365	Tips of fur pale pink, but not as bright as at 395 nm.	Whitish, but not as bright as at 395 nm.	Frozen.
		380–410	Tips of fur pale pink.	Whitish.	
Bandicoots (Order: Peramelemorphia. Family: Peramelidae)					
Northern brown bandicoot (*Isoodon macrourus*)	**20** 10 male 6 female 4 ? (+ 4 pouch young)	310–365	Bright pink, more on flank and head than dorsal. Fur pink at the base, dark in the middle and yellow at the tips. Pink/orange yellow not as bright as at 395 nm. Can also have blue-white photoluminescence on flanks.	Not really photoluminescent at 310 nm. Pinky orange or pale pink. Can also have bright white photoluminescence on different strands of fur.	All fresh roadkill. Individuals vary in how much strong pink photoluminescence they have in their fur, even if they were not exposed to sunlight after death. Pouch young at least up to 34 mm head length did not photoluminesce, only reflecting purple. [Incidental live observation: bright orange photoluminescence.]
		380–410	Very bright magenta pink with yellow tips. Yellow tips not as bright as pink. Over head, neck, tops of paws and flanks. Darker brindling where there is darker fur over the dorsum. In the brightest individual, the hot pink peppering through the dorsal fur is from the thickest, wholly white, strands of fur. White photoluminescence less so than pink which becomes stronger and darker at 395 nm.	Strong to very strong neon pink or orange-pink. Particularly bright under chin. The brightest pink is in the thicker fur, lacking at base where it is grey, but wholly pink for distal 4/5. Often brighter than dorsal and flank fur. Fainter in some individuals. Some pink strands have white at the very tips. Fine short fur under base of tail photoluminesces white. White ventral photoluminescence less so, and more absorbing, at 395 nm.	
Long-nosed bandicoot (*Perameles nasuta*)	**14** 8 male 5 female 1 ?	310–365	Fresher individuals have deep orange-pink over head, particularly on cheeks with a red ring around the eye. Dorsal mostly dark brindled with pink	Not really photoluminescent at 310 nm. At 365 nm, white or bright blueish white with hint of pink. White	13 fresh roadkill, 1 frozen (thawed). Most individuals all brindled brown in

			strands. Flanks brindled pink-orange with yellow, some with black or white bands. Deep pink photoluminescence patchy on some individuals. Two virginal females with blueish white on neck. Larger males with pale flanks have blueish white on top of head, neck, face and flanks; dorsal mostly yellow with some pink and blue-white.	photoluminescence brighter than pink.	white light. Some individuals grey fawn with more white extending from ventrum and wide black bands over hindquarters. The 5 freshest individuals, not yet in rigor mortis, have the brightest photoluminescence.
		380–410	Brighter hot pink. Dorsal and flank fur is magenta pink in the proximal section, then reflects for a section before photoluminescing yellow or orange at the tips. The yellow is not as bright as the orange-pink. Yellow brighter at 365 nm. Pink sometimes strongest on the rump. Strong bright pink mixed with yellow gives appearance of orange at some angles. Larger pale-flanked males have both white and bright pink photoluminescence on flanks.	Pink photoluminescence brighter than white. In the larger pale-flanked males, white stronger than pink. In the freshest individuals, wildly vivid orange-pink. In some, white photoluminescence appears washed with pink tips whereas other hairs are all pink. Layer of denser, short, soft fur photoluminesces white; interspersed with longer, thicker hairs that photoluminesce wholly pink, giving the effect of a pink wash over white.	
Wallabies and possums (Order: Diprotodontia)					
Feathertail glider (Family: Acrobatidae)					
Feathertail glider (*Acrobates pygmaeus*)	**1** 1 female	310–365	None.	None.	Frozen.
		380–410	Slight apricot pink to face.	Slight apricot pink.	

Wallabies, tree-kangaroos and pademelons (Family: Macropodidae)					
Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*)	**2** 2 male	310–365	Pale blue in the underfur all over. Photoluminescence along shaft of fur until tips where it remains brown. Tail not photoluminescent apart from base.	Pale blue in the underfur all over.	Both frozen (thawed).
		380–410	Photoluminescence not nearly as evident as at 365 nm.	Photoluminescence not nearly as evident as at 365 nm.	
Red-legged pademelon (*Thylogale stigmatica*)	**3** 3 male 1 adult 2 young	310–365	Some white photoluminescence of pale grey fur but mostly reflecting. Brown fur absorbs.	Quite bright blue-white glow on pale buff fur. Absorbing on darker fur.	All fresh roadkills. Minimal exposure to sunlight.
		380–410	Much less white, but some photoluminescence of yellow tips. Grey fur turns strong light purple which is brighter than the usual reflectance. Brown fur absorbs. Slight pink tinge to some tips or bases of fur. Subtle dusky pink photoluminescence more common in tips of flank fur.	White glow only very mild. Very slight pale pink in some pale brown fur or where pale grey is at base of some fur.	
Black wallaby (*Wallabia bicolor*)	**1** 1 ?	310–365	A little light grey, maybe from white strands of fur. Mostly stays dark.	Faint white glow over lighter fur.	1 decaying roadkill.
		380–410	Light greyish and yellowish, but only very slight.	Paler fur turns green-yellow, but not much glow.	
Gliders and striped possums (Family: Petauridae)					
Striped possum (*Dactylopsila trivirgata*)	**9** 7 male 2 ?	310–365	Brilliant white neon, almost greenish blue-white, glow on white stripes. Black stripes remain black. Tail tip lacks the vivid blue-white glow.	Brilliant white glow. Fur intermediate to stripes and ventral fur photoluminesces pale grey.	8 frozen (thawed), 1 decaying roadkill.

		380–410	White glow, but not as distinct as at 365 nm. Almost greenish tint to the glow.	White glow, but not as distinct as at 365 nm.	
Krefft's glider (*Petaurus notatus*)	**4** 2 male 2 female	310–365	At 310 nm, pale blueish white all over. At 365 nm, milder blueish white all over.	At 310 nm, almost-blue white glow. At 365 nm, white glow almost gone, except maybe just on the tail tip.	All frozen (3 thawed).
		380–410	Not really photoluminescent.	At 380 nm, edges stand out more than other areas. At 395–410 nm, mild dusky pink colour over entire ventral fur of 3 individuals.	
Brushtail possums (Family: Phalangeridae)					
Coppery brushtail possum (*Trichosurus johnstonii*)	**3** 1 male 2 female	310–365	The one fresh night time female roadkill, not yet in rigor mortis, showed pale purply blue-grey photoluminescence on the face, skin and basal half of underfur. Colour more prominent in 310 nm than in 365 nm light. The others not really photoluminescent, but a little white-grey in parts.	At 310 nm, fresh female has light greyish purplish blue, especially on face and arms. Fresh male mostly glows white, with small areas of dusky pink. Chest gland of fresh roadkill male: a quarter of fur photoluminesces white in whole strands; rest of fur absorbs.	1 fresh female roadkill: collected at night. 1 fresh male roadkill: dorsal surface exposed to sun for several hours. 1 frozen female (thawed). [Incidental live observation of 1 male with 365 nm torch: glowed blue-white with a reddish chest patch.]
		380–410	At 395–410 nm, in the fresh female, basal half of flank fur (that at 310nm was pale purply blue) now quite orangey-pink; distal half remains pale ginger. Rump fur pink throughout, with one patch particularly strong orange-pink. In the fresh male, ginger parts of fur	At 380 nm, the blue-grey is gone. Fresh male mostly glows whitish yellow, with patches of pink. Chest gland: a quarter of fur photoluminesces pale yellow. Some strong pink through the ginger fur in patches of whole strands.	

			turn pale yellow and brighter light ginger. Yellow tips peppered through darker absorbing fur. In the frozen female, subtle pinkish orange over all fur, enhancing existing russet colour. Not on tail.		
Common brushtail possum (*Trichosurus vulpecula*)	**3** 1 male 1 female 1 ?	310–365	Bright sky-blue photoluminescence on paler parts, particularly bright on tops of back feet. Tinge of dusky pink on hindquarters, back legs and base of tail.	Strong sky-blue photoluminescence on a third of the fur, rest absorbing.	1 frozen, 1 old roadkill, 1 fresh roadkill. [Incidental live observation: photoluminescing a little, yellow on the underside and faint green on the edges of the legs. General white glow of ventral fur and inside ears.]
		380–410	Silvery grey or sky-blue photoluminescence still there, but much milder. Pink much more prominent. Most fur is dark pink underneath with silvery grey tips. Pink extends over all fur on dorsal surface and through tail. Back of neck is pink at base of fur, getting less towards distal. On flank and hindquarters, pink is absent from base of fur, more along shaft and brightest at tip. At base of tail, pink only occurs in the distal half of fur.	White not as bright. Most fur is quite strong orangey pink. Though only a wash of pale pink in small patches on the female whose ventral fur was exposed to an overcast sun for several hours.	
Bettongs (Family: Potoroidae)					
Rufous bettong (*Aepyprymnus rufescens*)	**2** 1 male 1 ?	310–365	Blueish white at 365 nm, brightest on hindquarters. Underfur glows light purplish white; brown absorbs. Some pink on flank.	White and faint light pink.	1 fresh roadkill: dorsal surface had been exposed for a couple of hours, but shady and overcast. 1 decaying roadkill.
		380–410	Hardly discernible faint blueish-grey photoluminescence. Dorsal surface	Most of fur photoluminesces pink, whole strands in uniform fairy floss	

			has dark pink strands, but white tips often remain white. The flanks have patches of stronger and brighter pink – whole strands but darker towards the tips.	pink.	
Northern bettong (*Bettongia tropica*)	**9** 6 male 1 female 2 ?	310–365	Photoluminescence hardly perceptible, maybe slightly grey-white or a little yellow of the buff fur tips.	Not really photoluminescing, just absorbing white.	All frozen. Suspect individual retention of pink photoluminescence is related to sun exposure. The fur on the inside of an ear of one female photoluminesces mild green-blue-white at 365–410 nm.
		380–410	Individuals with the most pink photoluminescence retain it throughout the fur. Pinkest on face, head, neck, flank, legs, rump, base of tail, with some down the dorsal side of the tail. Pink can be in distal or proximal half of the fur shaft, or along whole strands. Some darker pink in proximal half of fur and in whole strands – tips tend to be paler pink. The pale buff-white tips mostly remain white, particularly in dorsal fur. Pink is particularly mottled on flanks.	Photoluminescence not so much in ventral fur. Some have pink along edges, between thighs, throughout ventral fur or in very small patches of salmon pink. Faint pink photoluminescence occurs in distal half of fine fur, but may be along whole strand. Ventral side of tail fur photoluminesces mild greenish yellow.	
Ringtail possums and greater gliders (Family: Pseudocheiridae)					
Greater glider (*Petauroides volans*)	**2** 2 ?	310–365	Pale grey.	White. Plus some white on white patch under tail.	Both frozen.
		380–410	Same grey on dorsal fur but mostly reflecting. No pink photoluminescence detected.	White chest absorbing. White under tail photoluminesces a little. No pink photoluminescence detected.	
Green ringtail possum	**10** 1 male	310–365	Pale yellow scattered throughout. Yellow tips turn blueish white. Some	Mild white photoluminescence. Darker parts absorbing.	8 frozen, 1 old roadkill, 1 fresh

(*Pseudochirops archeri*)	1 female 8 ?		pale grey fur photoluminesces pale pink. On the old roadkill, the tail has grey-white photoluminescence throughout with a little dusky pink and fur tips pale green-yellow. Photoluminescence subtle.		roadkill collected at night. Photoluminescence only really evident on roadkills and QPWS freezer specimen, not on 7 JCU freezer specimens.
		380–410	Fur mostly reflects. Yellow tips appear bright pale greeny yellow, more distinctive in UV than in white light, more on flanks than on dorsal. Flecks of white fur photoluminesce white or pale green. Some tufts of dusky pink fur in pale grey-brown underfur. On old roadkill, the pale brown tail fur is dusky pink throughout, with tips of fur pale yellow. The rest of the fur absorbs. On fresh roadkill, white patches behind ears photoluminesce white. Stripes stand out more in the UV than in white light; yellow.	Mid-ventral white, edges of ventral pink wash. Inside legs and under tail dull pinkish orange. Fur mostly absorbing, except pale buff parts that were absorbing at lower wavelengths are now pale pink.	
Herbert river ringtail possum (*Pseudochirulus herbertensis*)	**3** 1 male 2 female	310–365	At 310 nm, faint grey over dorsal fur. At 365 nm, grey only very faint. Tail tip not as bright as ventral fur.	At 310 nm, white glow over ventral and shoulder fur. At 365 nm, stronger pure white glow over ventral fur. Much less so in females than in male.	All frozen. The white skin of the tail tip of the male photoluminesces, not the relatively sparse fur which appears dull and yellowish in both 365 and 395–410 nm light.
		380–410	One female has pale greenish photoluminescence to tips of fur all over excepting tail; no pink. The other female has a pink wash in the basal	Definite white glow of ventral fur in male. Not so much in females.	

			half of fur on flanks and tops of legs. The male just reflects light from his dorsal fur.		
Common ringtail possum (*Pseudocheirus peregrinus*)	**1** 1 male	310–365	Cheek greenish white. Greenish white also flecked through fur.	Tail and ventral fur white.	Frozen. [Incidental live obs., distant: absorbed white, maybe glowing.]
		380–410	Cheek greenish white. Greenish white also flecked through fur.	Tail and ventral fur white.	
Placentals					
Rabbits (Order: Lagomorpha. Family: Leporidae)					
European rabbit (*Oryctolagus cuniculus*) [naturalised]	**3** 1 male 1 female 1 ?	310–365	Not really photoluminescent.	Mild whitish. Underside of tail and rear end bright white.	All fresh roadkill. Skin inside of ears photoluminesces white.
		380–410	Mostly absorbing, but pink in some light tan sections of fur.	Mostly absorbing, but pale pink in pale buff tips and strands of fur. Paler sections of tail white. Some small patches of pale buff fur on tail have whole strands photoluminescing pale pink.	
Rats and mice (Order: Rodentia. Family: Muridae)					
Black-footed tree-rat (*Mesembriomys gouldii*)	**1** 1 ?	310–365	Fur in general not really photoluminescent.	Fur in general not really photoluminescent.	Frozen.
		380–410	Pinkish wash over head and shoulders and orange over nape of neck.	Not really photoluminescent.	
Prehensile-tailed rat (*Pogonomys mollipelesus*)	**1** 1 female	310–365	Not really photoluminescent.	Not really photoluminescent.	Frozen.
		380–410	Pink over dorsal fur. Not bright, but changes fur from russet brown to dusky pink.	White on ventral fur.	

Giant white-tailed rat (*Uromys caudimaculatus*)	**1** 1 female	310–365	Blue-grey in underfur.	Light orange.	Frozen (thawed). [Incidental live observation with 365 nm torch: ventral fur photoluminesced white.]
		380–410	Pink on face. Orange flecked through dorsal and neck fur. Orange photoluminescence is in the distal tips of the fur, not the underfur. The fur with mild orange photoluminescence is also pale orange in white light; may be stained from lying in blood.	More orange.	
Pale field rat (*Rattus tunneyi*)	**1** 1 female	310–365	Pale blue photoluminescent underfur with brindled brown tips.	Tips photoluminesce pale/white. Underfur absorbs.	Fresh roadkill.
		380–410	Similarly photoluminescent pale blue brindled with brown.	Similarly photoluminescent blue-white.	
Bush rat (*Rattus fuscipes*)	**1** 1 female	310–365	Distinct blue-white photoluminescence through fur, more in flanks than in dorsal.	Distinct blue-white photoluminescence purer where less melanin.	Frozen (thawed).
		380–410	Photoluminescence more grey-white than blue-white. Photoluminescence brightest in distal tips of fur.	Photoluminescence more grey-white than blue-white.	
Black rat (*Rattus rattus*) [naturalised]	**2** 1 male 1 ?	310–365	Bright blueish white. Photoluminescence brighter on tips of fur. Darker blue-grey underneath, and paler tending to white at tips.	Bright blueish white.	1 rotting then frozen (thawed) + 1 fresh suburban carcass. [Incidental live obs. with 365 nm torch: brilliant blueish white of all fur, not skin.]
		380–410	Bright light blue to pale greenish blue.	Bright light blue to pale greenish blue.	
Delicate mouse (*Pseudomys delicatulus*)	**1** 1 female	310–365	Bright blueish white over all fur, but brindled where more melanin.	Bright blueish white.	Frozen (thawed).
		380–410	Photoluminescence less than at 365 nm, more white than blue.	Does not glow as much as at 365 nm, but more white photoluminescence in ventral fur than over whole mouse.	

Bats (Order: Chiroptera)					
Flying foxes and fruit bats (Family: Pteropodidae)					
Eastern tube-nosed fruit bat (*Nyctimene robinsoni*)	**5** 3 male 2 female	310–365	Light blue photoluminescence mostly restricted to the head, rest mild grey.	Fur photoluminesces light blue with a distinct glow, similar in brightness to that of a brushtail possum. One specimen photoluminesced only mildly grey, but brighter around the eye. Eyes pale blue.	All frozen (3 thawed). Yellow skin spots photoluminesce yellow at all wavelengths, brightest neon yellow at 365 nm. Some brown skin inside wings and along bones on ventral side, around eye and muzzle, also photoluminesces yellow. Ventral wing photoluminescence more pronounced in males. Penises photoluminesce bright yellow.
		380–410	Light blue photoluminescence mostly restricted to the head, rest mild grey. Less intense than at 365 nm.	Fur photoluminesces mid light blue, not as bright at these higher wavelengths.	
Spectacled flying-fox (*Pteropus conspicillatus*)	**2** 2 female	310–365	Blue-grey photoluminescence through ginger collar. Some blue-grey flecked through main fur.	Blue-grey photoluminescence through ginger collar. Some blue-grey flecked through main fur. Eye rings a little. Also eyes and teeth.	Frozen (thawed).
		380–410	Photoluminescence more greenish grey. Ginger fur gives yellowish photoluminescence. Photoluminescence quite subtle.	Grey-blue photoluminescent strands throughout fur. Photoluminescence less so on one than the other. Photoluminescence quite subtle.	
Little red flying-fox	**1** 1 female	310–365	Grey photoluminescence at head.	Grey over head, but absorbing at neck.	Frozen (thawed).

(*Pteropus scapulatus*)				Pale blue eyes.	
		380–410	Brighter greenish grey photoluminescence over head. Photoluminescence quite subtle.	Slight yellowy greeny grey on head and strip where wings meet body. Photoluminescence quite subtle.	
Microbats (Family: Vespertilionidae)					
Gould's wattled bat (*Chalinolobus gouldii*)	**1** 1 female	310–365	Fur does not photoluminesce.	Fur does not photoluminesce.	Frozen (thawed). Some white glow of teeth, and skin of feet and genital area.
		380–410	Fur does not photoluminesce.	Fur does not photoluminesce.	
Eastern bent-wing bat (*Miniopterus schreibersii*)	**2** 1 male 1 female	310–365	No photoluminescence of fur.	Male: no photoluminescence of fur. Female: Pale grey on lower abdomen.	Both frozen. Ventral wing bones photoluminescing bright pale blue, visible through membranes. Female: claws light blue.
		380–410	Male: very mild greenish yellow photoluminescence over fur tips. Female: no photoluminescence.	Male: very mild greenish yellow photoluminescence over fur tips. Female: mild pale grey, prominent on lower abdomen.	
Eastern free-tailed bat (*Mormopterus ridei*)	**1** 1 female	310–365	No photoluminescence.	Some very mild light blue of the throat fur. Hardly discernible.	Frozen.
		380–410	No photoluminescence.	No photoluminescence.	
Eastern horseshoe bat (*Rhinolophus megaphyllus*)	**1** 1 female	310–365	No photoluminescence of fur.	Faint blueish-grey photoluminescence on tips of fur.	Frozen. Ventral wing bones photoluminescing light blue, visible through membranes.
		380–410	Mild greenish yellow photoluminescent band around back of neck.	Stronger photoluminescence, greenish yellow, but still mild.	
Forest bat (*Vespadelus* sp.)	**1** 1 female	310–365	A little grey-white tips to the fur.	Bluey grey-white photoluminescence on fur tips, particularly under chin.	Frozen (thawed). White glow of teeth.
		380–410	Slight yellow-grey on fur tips. Photoluminescence quite subtle.	Yellow-grey on fur tips and under chin, but milder than at 365 nm. Photoluminescence quite subtle.	

Dingo (Order: Carnivora. Family: Canidae)					
Dingo (*Canis lupus dingo*) [naturalised]	**1** 1 male pup	310–365	Not really photoluminescent; just paw pads and claws.	Not really photoluminescent.	Frozen (thawed).
		380–410	Just paw pads and claws.	Not really photoluminescent.	

Appendix D. Table D.1. Molecular masses and colours of selected RP-HPLC fractions.

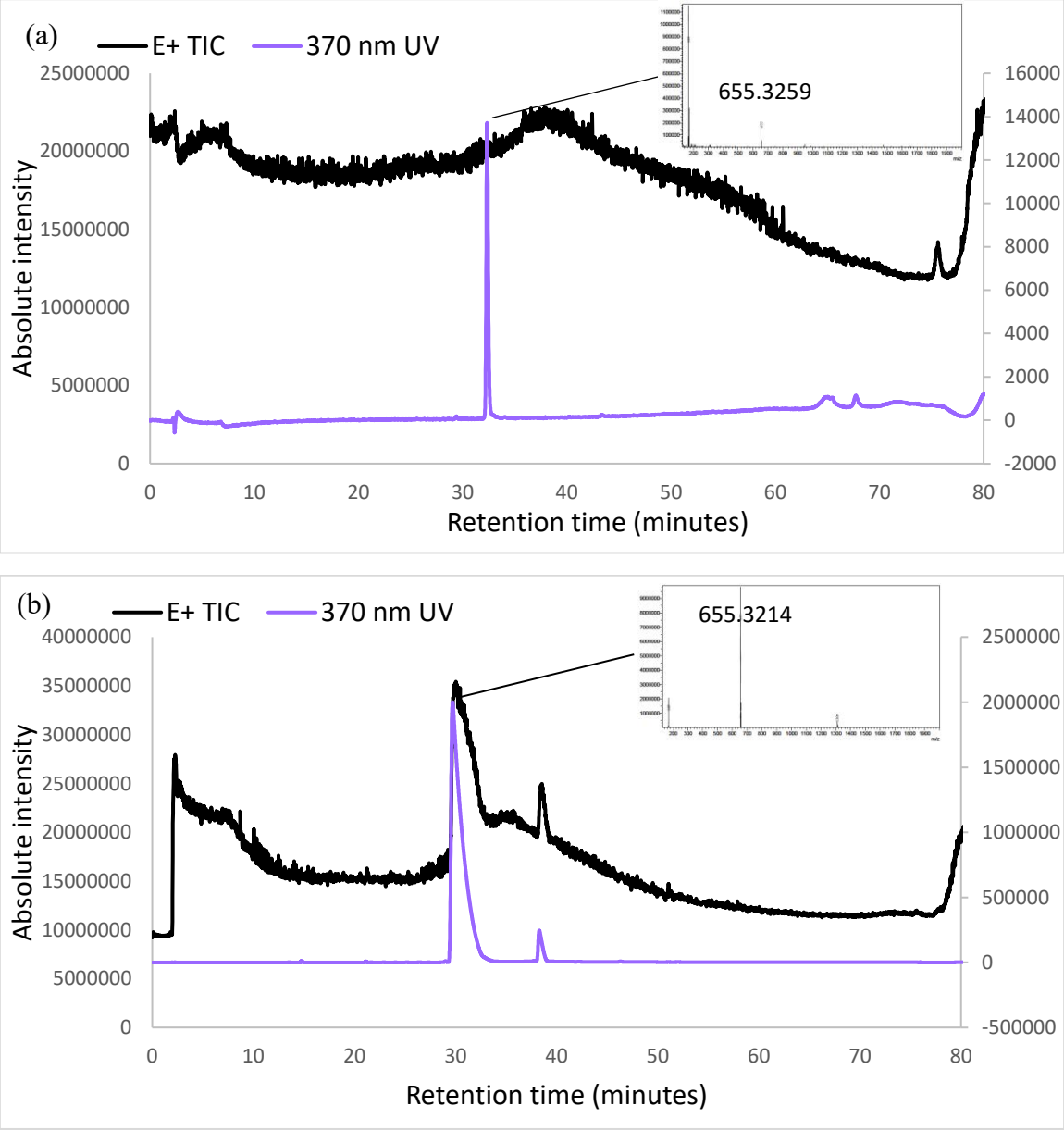
Species	**RP-HPLC fraction**	**Colour** (colourless in white light unless otherwise stated)	**Masses observed (m/z)**	**Potential compounds**
Perameles nasuta	P1 D5	Photoluminescent **mild yellow**	501.2937 295.1647 381.1709 408.1499 533.2503 516.2367 432.2104 603.2643 630.2874	? ? ? ? ? ? ? ? ?
	P1 D10	Photoluminescent **mild yellow**	246.1843 377.1408 352.1740 523.2879 761.4003	? ? ? ? ?
	P1 E5	Non-photoluminescent pink visible in white light	606.3652 386.1760 1016.4341 473.1981 530.2732 572.2774 629.3230 743.3622 686.3218	? ? ? ? ? ? ? ? ?
	P1 G3	Photoluminescent **hot pink** (pink visible in white light)	347.2253 831.2615	? Uroporphyrin
	P1 G7	Photoluminescent **bright pink**	787.2928 274.2935	Heptacarboxyl-porphyrin
	P1 H11	Photoluminescent **bright pink**	453.3703 274.3042 655.3169	? ? Coproporphyrin
	P2 F1	Photoluminescent **hot pink** (pink visible in white light)	274.2903 563.3058	? Protoporphyrin
Isoodon macrourus	P1 D5	Photoluminescent **pastel yellow**	246.0678 508.3100	? ?
	P1 D10	Photoluminescent **pastel yellow**	246.1228	?
	P1 G3	Photoluminescent **hot orangey pink**	327.1747 347.1721 831.3417 715.5227	? ? Uroporphyrin ?
	P1 G8	Photoluminescent **hot orangey pink**	327.1825 367.1854 390.2439 787.3524	? ? ? ?

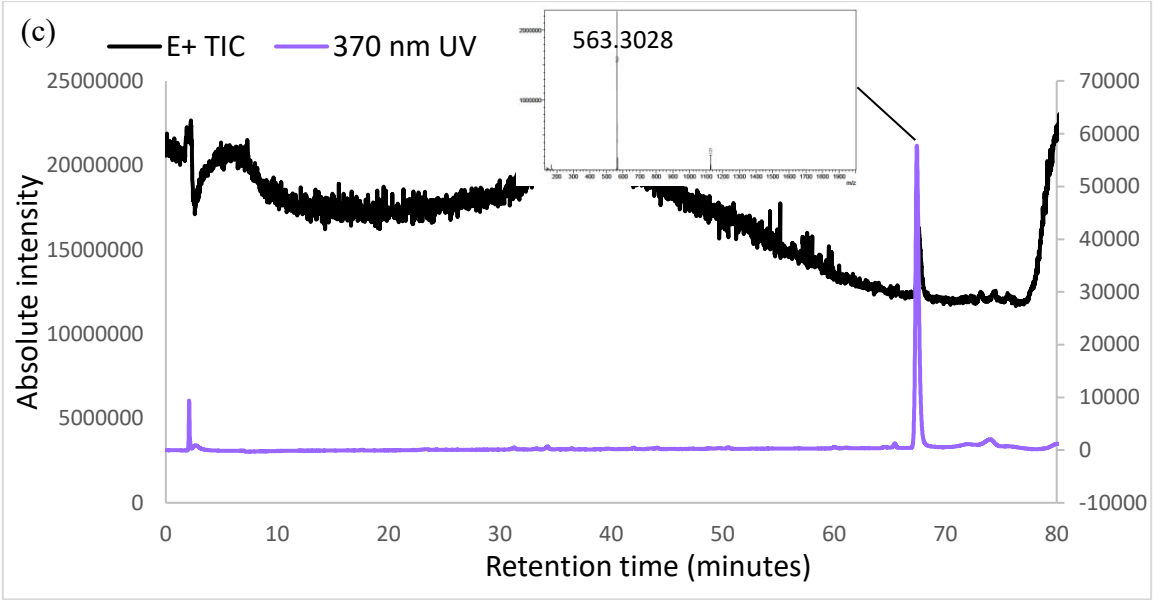
	P1 H12	Photoluminescent **hot orangey pink**	261.1779 323.1380 365.1554 623.4126 2243.1553	? ? ? ? ?
	P2 C1	Photoluminescent **pink**	332.3017 326.3395	? ?
	P2 E12	Photoluminescent **hot orangey pink**	563.3135	Protoporphyrin
Dasyurus hallucatus	P1 A4	Photoluminescent **yellow** (dark yellow visible in white light)	614.7210	?
	P1 D6	Photoluminescent **yellow**	–	?
	P1 D11	Photoluminescent **very bright yellow** (dark yellow visible in white light)	595.3334	?
	P1 G4	Photoluminescent **pink**	–	?
	P1 H12	Photoluminescent **bright pink**	655.3397	Coproporphyrin
	P2 F1	Photoluminescent **bright pink**	563.3103 5082.6775 5067.2158	Protoporphyrin Peptide Peptide
Trichosurus johnstonii	P1 D9	Photoluminescent **bright yellow** [Appeared more **green** when transferred to tube.] (mild dark yellow visible in white light)	329.1003 909.5227 370.1376 215.0978	? ? ? ?
	P1 D10	Non-photoluminescent violet visible in white light	647.3729 263.0495 215.0978	? ? ?
	P1 D11	Non-photoluminescent **strong violet** visible in white light	409.1833	?
	P1 G2	Photoluminescent **bright hot orange-pink** (**dusky pink** visible in white light)	2481.1596 1286.6669 831.3099	? ? Uroporphyrin
	P1 G5	Photoluminescent **pale green** [The green fractions G4 and G5 appeared **blue** when transferred to the tubes.]	1343.7892 1001.4962 408.2166	? ? ?
	P1 H10	Photoluminescent **bright hot orange-pink**	–	?
	P2 E10	Photoluminescent **pink**	563.3113	Protoporphyrin
Dendrolagus lumholtzi	P1 A2	Photoluminescent **very slightly brownish**	316.9976	?
	P1 D10	Photoluminescent **slightly brownish**	909.5470	?
	P1 E2	Photoluminescent **pale blue** in 395–410 nm light; **pale lavender blue** in 365 nm light	629.3307	?
	P1 E3	Photoluminescent **pale orange** in 395–410 nm light; **pale lavender blue** in 365 nm light	443.1900	?

	P1 F4	Photoluminescent **dark yellow**	544.2568	?
	P1 F8	Photoluminescent **dark yellow** (**yellow** visible in white light)	791.4106 800.4366	? ?
	P1 G1	Photoluminescent **dark yellow**	743.3970 301.0101	? ?
	P1 G4	Photoluminescent **light yellow** in 395–410 nm light; **very light blue** in 365 nm light	1377.7473	?
	P2 E9	Photoluminescent **slight dusky pink**	5067.1062 5083.0275 379.3242 563.6330	Peptide Peptide ? Protoporphyrin
Rattus tunneyi	P1 A6	Photoluminescent **slight greenish pastel yellow** [Appeared pale **greenish blueish white** in tube.] (dark yellow visible in white light)	314.1432	?
	P1 A7	Photoluminescent **dark brown with yellow** (rusty orange/ reddish brown visible in white light)	250.0655	?
	P1 A8	Photoluminescent **strong canary yellow** (dark yellow visible in white light)	231.0492	?
	P1 D5	Photoluminescent **lemon yellow** [Appeared **pale greenish** in tube.]	251.0517 292.0798	? ?
	P1 E11	Photoluminescent **faint orange-pink**	–	?
	P1 H11	Photoluminescent **faint pink**	326.3345	?
	P2 A4	Photoluminescent **faint pink**	–	?
	P2 E8	Photoluminescent **bright orange** [Appeared **bright pink** in tube.]	563.3157	Protoporphyrin
Ornithorhynchus anatinus	P1 A4	Photoluminescent **very faint yellow** [Appeared **cyan** in tube.] (mild dark yellow visible in white light)	316.9849	?
	P1 D7	Photoluminescent **yellow** [Appeared **cyan** in tube.] (**very faint dark yellow** visible in white light)	246.0642 221.1065 254.0855	? ? ?
	P1 E1	Photoluminescent **yellow** (**very faint dark yellow** visible in white light)	329.1053 370.1384	? ?
	P1 G8	Photoluminescent **faint pink** (**very faint dark yellow** visible in white light)	277.0861	?
	P1 H4	Photoluminescent **faint pink**	–	?
	P2 A6	Photoluminescent **faint pink**	233.1318 247.1357	? ?
	P2 B2	Photoluminescent **faint pink**	285.2151	?
	P2 F7	Photoluminescent **pink**	563.3314	Protoporphyrin

Appendix E. Figure E1. LC/ESI-MS chromatograms and mass spectra (m/z) of long-nosed bandicoot and coppery brushtail possum RP-HPLC fractions and porphyrin standards.

(a) Long-nosed bandicoot Plate 1 H11. (b) Coproporphyrin I standard. (c) Long-nosed bandicoot Plate 2 F1. (d) Coppery brushtail possum Plate 2 E10. (e) Protoporphyrin IX standard.

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370 nm UV
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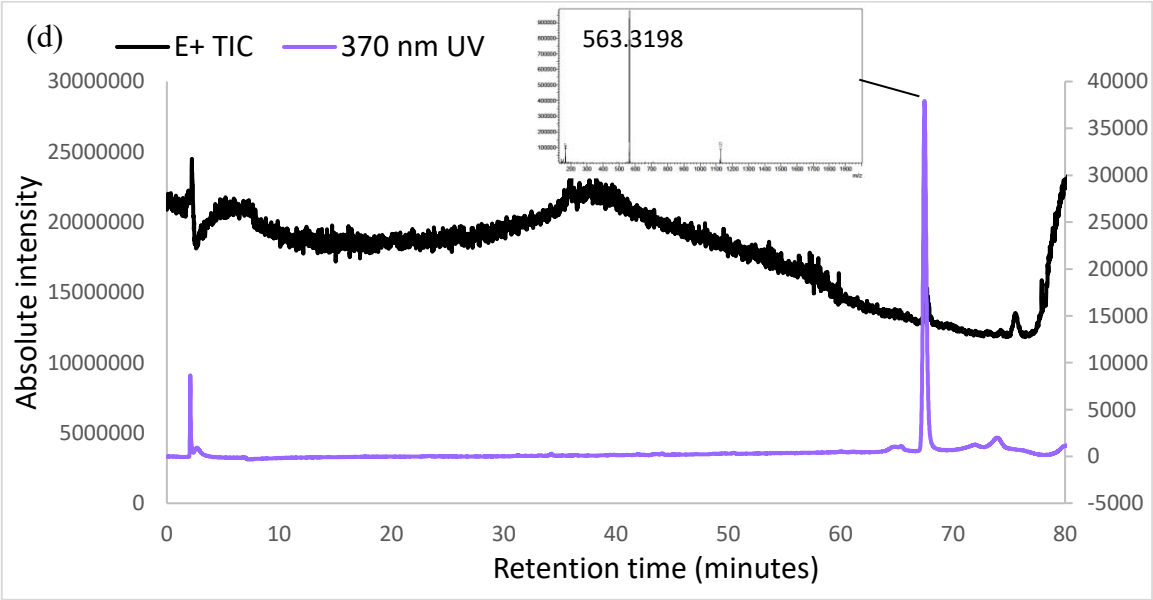
Absolute intensity
Retention time (minutes)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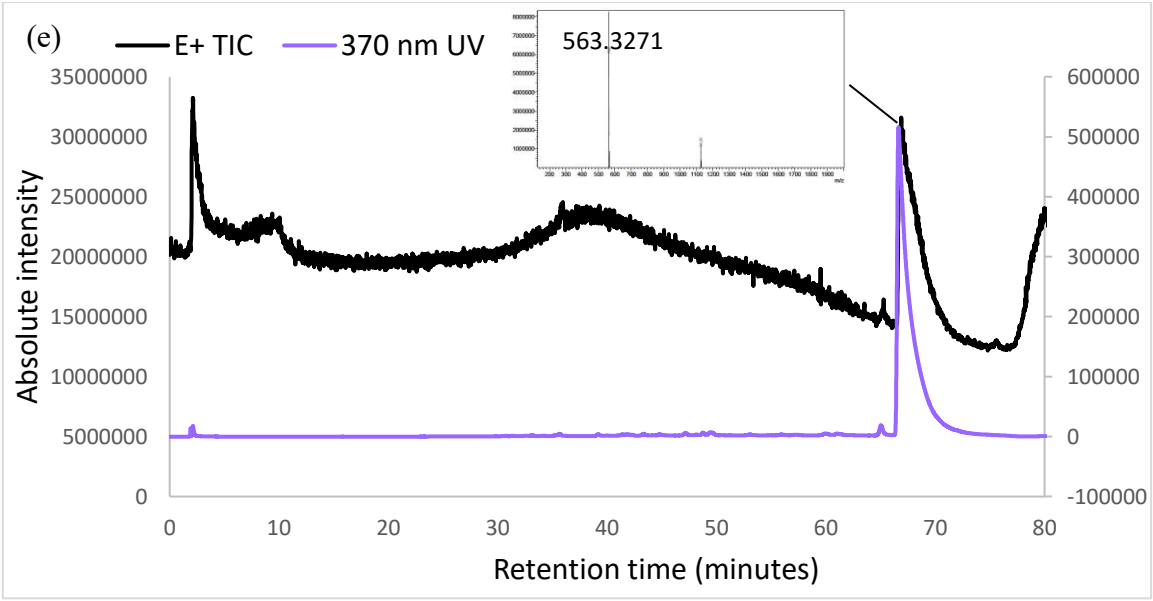
(e)
E+ TIC
370 nm UV
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Absolute intensity
Retention time (minutes)

Appendix F. Table F.1. Taxonomic distribution of wild mammals and birds interacting with rat models in each habitat.

Including all camera stations, but excluding 18 interactions (two marsupials, five placentals and 11 birds) that occurred during golden hours (i.e. outside of sunset–sunrise).

Photo = Photoluminescent; Non = Non-photoluminescent.

Species	**Total**	**Full Moon**		**New Moon**	
		Photo	Non	Photo	Non
Open farmland					
Marsupial mammals					
Isoodon macrourus	20	0	0	8	12
Perameles nasuta	12	0	0	6	6
Sminthopsis virginiae	1	0	0	0	1
Placental mammals					
Canis lupus	5	2	1	2	0
Felis catus	4	2	1	0	1
Oryctolagus cuniculus	1	0	0	0	1
Melomys spp.	6	0	2	4	0
Rattus rattus	1	0	0	0	1
Rodentia	3	1	0	1	1
Birds					
Accipiter fasciatus	1	1	0	0	0
Tyto longimembris	3	2	1	0	0
Burhinus grallarius	1	0	1	0	0
Totals	**58**	**8**	**6**	**21**	**23**
Rainforest					
Marsupial mammals					
Perameles nasuta	12	3	2	6	1
Isoodon macrourus	5	1	0	3	1
Thylogale stigmatica	3	0	1	1	1
Trichosurus vulpecula	3	0	3	0	0
Antechinus sp.	1	0	0	0	1
Placental mammals					
Canis lupus	3	0	3	0	0
Felis catus	1	1	0	0	0
Uromys caudimaculatus	4	0	0	1	3
Hydromys chrysogaster	1	0	0	1	0
Melomys sp.	1	1	0	0	0
Rattus fuscipes	1	0	0	0	1
Rodentia	1	1	0	0	0
Totals	**36**	**7**	**9**	**12**	**8**

Woodland					
Marsupial mammals					
Trichosurus vulpecula	15	2	8	2	3
Aepyprymnus rufescens	8	1	1	2	4
Dasyurus hallucatus	5	1	2	2	0
Petrogale mareeba	3	1	2	0	0
Macropus agilis	1	0	1	0	0
Macropus giganteus	1	0	0	0	1
Perameles nasuta	1	0	0	1	0
Placental mammals					
Canis lupus	9	5	1	2	1
Felis catus	3	0	0	2	1
Oryctolagus cuniculus	1	0	0	0	1
Birds					
Alectura lathami	1	1	0	0	0
Totals	**48**	**11**	**15**	**11**	**11**
Grand totals	**142**	**26**	**30**	**44**	**42**