Ultraviolet reflectance, absorption and fluorescence in frogs: Observations from the Wet Tropics

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Abstract

Eight species of frog in the Wet Tropics of Far North Queensland were viewed in the field under ultraviolet torchlight. The skin of the frogs variously reflected, absorbed and fluoresced the light. These preliminary observations into the external fluorescence of Australian frogs are of a phenomenon that has been observed in amphibians across Europe and the Americas.

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Introduction

When low wavelength (ultraviolet, violet or blue) light is shone onto an object, its surface can do one or a combination of three things. The object can reflect the light (the surface bounces back the same colour photons as were shone onto it), absorb the light (the colour remains much the same as it appears under regular torchlight) or fluoresce (change colour and/or appear to glow brighter). For part of a molecule (a fluorophore) to fluoresce, it absorbs photons of a particular wavelength, re-emitting some of them at another wavelength (Johnsen 2012). Visible fluorescence is usually any colour of longer wavelength (higher up the rainbow) than the ultraviolet/violet light emitted from the torch, regardless of the original colour of the object's surface. Objects can also fluoresce at the same wavelength as their original colour. These properties are inherent to different types of molecules or pigments found in nature (Johnsen 2012; Marshall & Johnsen 2017; Jeng 2019). Fluorescence is widely known from the oceans, but the study of fluorozoology is still an emerging field in terrestrial environments (Lagorio *et al*. 2015; Jeng 2019; Macel *et al*. 2020).

Fluorescence is a type of photoluminescence, whereby an object can only glow when excited by an external light source. Photoluminescence differs from chemiluminescence in which an organism uses a chemical reaction to produce its own cold light in darkness (Johnsen 2012). Historical accounts of chemiluminescence in frogs come from the jelly-like substance of their eggs, found glowing in swamps across Europe (Holder 1887). In Suriname however, the Crackling and Luminescent Frog of Rolander (*Rana typhonia*) probably acquired its intermittent bioluminescence from eating fireflies (Lavilla *et al*. 2010).

The other type of photoluminescence is phosphorescence. Phosphorescence is similar to fluorescence, but exists in an intermediate state before emission and usually continues as an afterglow when the light source is switched off (Valeur & Berberan-Santos 2011). *Phosphor*escence lasting two to four seconds has been observed in the ventral skin, but not in the dorsal skin, cornea or lens of frogs in North America (Giese & Leighton 1937). Phosphorescence of organic substances in general was found to last longer when excited by wavelengths lower than 300 nm than when excited by violet and blue light (Giese & Leighton 1937).

*Fluor*escence was first described in the skin of the Edible Frog (*Rana esculenta*) in Europe (Stübel 1911; Hadjioloff 1929). Using light of 300 to 400 nm, Stübel (1911) described a light blue fluorescence in the non-pigmented areas of the frog's skin and a weak yellow fluorescence in partially pigmented skin. The eyes of the Edible Frog fluoresced light blue, with internal organs and bones fluorescing shades of blue and yellow (Stübel 1911). The young tadpoles of some frogs have a green fluorescence in their skin, which turns to blue fluorescence as they grow (Gourévitch 1939 cited in Reeder 1940). Hama (1953) called the fluorophores in the dorsal skin, ventral skin and eyes of the Black-spotted Pond Frog (*Pelophylax* (formerly *Rana*) *nigromaculatus*) of East Asia "Rana-chromes". These substances, which fluoresce sky blue and yellow, were thought to have physiological importance relating to folic acid and ocular function (Hama 1953). Fluorophores giving blue fluorescence in the brown skin of the European Common Toad (*Bufo vulgaris*) were likewise called "Bufo-chroms" (Goto 1963). Studies on several frog species have confirmed some such skin fluorophores to be ranopterins, a type of pteridine (Hadjioloff & Zvetkova 1970; Zvetkova 1999).

Fluorescence has since been found to be reasonably widespread in amphibians in the Americas (Taboada *et al.* 2017; Deschepper *et al*. 2018; Lamb & Davis 2020). South American Tree Frogs (*Hypsiboas punctatus*) produce fluorescence with chemical compounds occurring in lymph, with skin glands also containing variants of the fluorophores (Taboada *et al.* 2017). The translucent skin of the frogs is yellowish-green under white light, but excitation wavelengths of 390 to 430 nm (ultra-violet-violet-blue) induce cyan-blue fluorescence over the whole frog, excepting for the eyes (Taboada *et al.* 2017). A closely-related species, the Canal Zone Tree Frog (*Boana rufitelus*), found in Costa Rica, changes from yellow to fluorescent blue when exposed to 365 nm ultraviolet light (Deschepper *et al.* 2018). Diurnal Pumpkin Toadlets (*Brachycephalus ephippium*, *B. pitanga* and *B. rotenbergae*) in Brazil are coloured yellow-orange-red in white light, but fluoresce cyan-blue or pale yellow under 365 to 395 nm ultraviolet light. However, it is their bony plates that fluoresce, only visible externally because their skin is so thin (Goutte *et al.* 2019; Nunes *et al.* 2021). Five families of American frogs (including those with non-translucent skin) fluoresce under blue wavelengths of 440 to 460 nm, with one species fluorescing less under ultraviolet wavelengths of 360 to 380 nm (Lamb & Davis 2020). However, twenty-one other frog species, from Costa Rica and Colombia, did not fluoresce under 385 nm ultraviolet light (Thompson *et al.* 2019).

Several substances isolated from the skin of frogs have the potential to fluoresce. The skin secretions of Carvalho's Surinam Toad (*Pipa carvalhoi*), from Brazil, are from the fluorescent tryptophan derivative kynurenic acid (Mariano *et al.* 2015). In the Australian frog genus *Litoria*, various peptide skin secretions have multiple bioactive functions including antimicrobial, antifungal, analgesic, smooth muscle contraction and courtship pheromones (Pukala *et al.* 2006; Jackway *et al.* 2009). Dahl's Aquatic Frog (*Litoria dahlia*) excretes peptides that contain residues of the fluorescent amino acid tyrosine. Additional to acting as an amphibian defence peptide, these dahleins function in inhibiting the formation of a free radical, nitric oxide (Wegener *et al.* 2001). An amino acid forming part of a peptide secreted from the translucent skin of the Red Tree Frog (*Litoria rubella*) matches the fluorophore kynurenine, a minor addition to tryptophyllin skin secretions which act to deter predators (Ellis-Steinborner *et al.* 2011). Tryptophyllins contain residues of the fluorescent amino acid tryptophan (Steinborner *et al*. 1994), though the fluorescence is not always in the visible spectrum. Tryptophyllins and rubellidins are peptides, which are intrinsically fluorescent.

Observations

While out in forests around Cairns (Bayview Heights, Kuranda, Watsonville and Ravenshoe), Far North Queensland, over the course of 2020 and 2021, I opportunistically observed eight species of frog (mostly males) with an ultraviolet torch (unbranded 395 nm, 20 – 22 lumen (~2 watts), 3xAA cell, 51 LED). On the final night I used several torches of different wavelengths to test for fluorescence. Ultraviolet photographs of frogs were taken with an unfiltered Panasonic Lumix TZ80 camera, on a tripod with a 10 second exposure. The fluorescence characteristics of each species is listed in Table 1. Additional observations of reflectance, absorption and their overall appearance are given below.

Table 1. Observations of fluorescence in some frogs of North Queensland's Wet Tropics.

Species	Date	Location	Colour of fluorescence; excitation wavelength	Part of frog fluorescing	Figure no.
Eastern Sedge-frog (*Litoria fallax*)	19/08/2021	Glendinning Road, Ravenshoe	Light blue, slight; 365 nm	Eyes	-
Northern Stony-creek Frog (*Litoria jungguy*)	08/11/2020	Barron River causeway, Little Road, Kuranda	Light yellow, but not much more than in white light; 395 nm	Ventro-lateral surface	1 right
	25/11/2020	Owens Creek, Myola Road, Kuranda	Yellow, but not much more than in white light; 395 nm	Thigh markings	-
	19/08/2021	Glendinning Road, Ravenshoe	Distinctly yellow; cyan-blue; 365 nm	Ventral surface and thigh markings; eyes	-
Kuranda Treefrog (*Litoria myola*)	08/11/2020	Barron River causeway, Little Road, Kuranda	Light blue; 395 nm	Large irregular patch over dorsal surface and hind leg	2 right
Common Mistfrog (*Litoria rheocola*)	06/05/2020	Clarkes Creek, Ivan Evans Walk, Bayview Heights	Cyan-blue; 395 nm	Eyes	-
Green-eyed Treefrog (*Litoria serrata*)	21/09/2020, 05/10/2020	Jum Rum Creek, Jum Rum Creek Conservation Park, Kuranda	Blue, very subtle	Various skin, mottled	-
Wood Frog (*Papurana daemeli*)	21/09/2020, 05/10/2020	Jum Rum Creek, Jum Rum Creek Conservation Park, Kuranda	Cyan-blue; 395 nm	Small spots over dorsal surface, toes, eyes	3 right
Ornate Burrowing Frog (*Platyplectrum ornatum*)	27/11/2020	Janetta Creek, Jeffrey Road, Kowrowra	Did not fluoresce; 395 nm	-	-
Montane Toadlet / Tableland Gungan (*Uperoleia altissima*)	05/03/2021	Walsh River Road, Watsonville	Pale red; 395 nm	Areas on the toes	-

*Eastern Sedge-frog (*Litoria fallax*)*

Several wavelengths were tested on one individual: 310 nm, 365 nm, 395 nm, 395-410 nm and 470 nm. The frog reflected the light of all of them uniformly from its dorsal and lateral surfaces. However, the eyes fluoresced light blue at 365 nm, but only slight compared to the other species.

*Northern Stony-creek Frog (*Litoria jungguy*)*

In white torchlight, these frogs were mostly brown over their dorsal surfaces with yellowish undersides (Fig. 1 left). The amount of yellow varied between individuals. When the 395 nm ultraviolet torch was shone from a distance, the dorsal surfaces of the frogs mostly absorbed the

Figure 1. Northern Stony-creek Frog photographed with regular camera flash (left) and in 395 nm ultraviolet torchlight (right). At right it is absorbing ultraviolet light on its dorsal surface, with the yellow ventral surface slightly enhanced by fluorescence.

Figure 2. Kuranda Treefrog (left) showing (at right) ultraviolet reflection, absorption and blue fluorescence on the dorsal surface and hind leg.

Figure 3. Wood Frog (left) mostly reflecting the ultraviolet light (right) but with fluorescent pale blue-cyan eyes and mildly fluorescent toe.

light, remaining brown (Fig. 1 right). This absorption made them stand out from the background, making them relatively more visible than with a regular torch. The ventral surfaces were a brighter yellow, indicating absorption with the possibility of fluorescence, but this varied between individuals. If an individual was more yellow in visible light, it did not mean it was brighter in ultraviolet light. Under 395 nm ultra-violet torchlight the thigh markings were bright yellow, but not much more so than their appearance under white light.

Three individuals (including a pair in amplexus) were later tested with a range of wavelengths of 310 nm, 365 nm, 395 nm, 395-410 nm and 470 nm. All three frogs strongly absorbed the various wavelengths on their dorsal surfaces. Their eyes fluoresced cyan-blue at 395 nm, but much more distinctly at 365 nm. Illumination at 365 nm also elicited yellow fluorescence of the ventral surface and thigh markings, which were otherwise a relatively pale buff colour in white light.

*Kuranda Treefrog (*Litoria myola*)*

In regular white light, a large irregular but distinct patch over the dorsal surface was a pattern of greenish-brown blotches (Fig. 2 left). In ultraviolet light, both this dorsal area and a patch on the hind leg fluoresced light blue (Fig. 2 right). The rest of the frog variously reflected the ultraviolet light (appearing purple) or absorbed it (appearing pale grey). The frog had an all-over patchy appearance in ultraviolet light. The pattern of ultraviolet reflection, absorption and fluorescence on the frog's dorsal surface camouflaged it as well under ultraviolet light as its pattern of greens and greys camouflaged it under visible light. From some angles its eyes also appeared to fluoresce, similarly to that seen in the Northern Stony-creek Frog, Common Mistfrog and Wood Frog.

*Common Mistfrog (*Litoria rheocola*)*

This frog reflected the purple light of the ultraviolet torch over its body, but the eyes mildly fluoresced cyan-blue.

*Green-eyed Treefrog (*Litoria serrata*)*

These frogs mostly reflected the ultraviolet light in a mottled pattern. Subtle blue fluorescence occurred in small patches, but because of the mottling and the varying angles and brightness of the torch on the skin it was difficult to isolate. The species remained just as camouflaged in purple/ blue/red under ultraviolet light as it was in green/ brown/cream in regular torchlight.

*Wood Frog (*Papurana daemeli*)*

Similarly to the Northern Stony-creek Frog and Common Mistfrog, the eyes of the Wood Frogs (Fig. 3 left) fluoresced pale cyan-blue (Fig. 3 right). Small spots over the dorsal surface of some individuals also fluoresced cyan-blue as did the toes. The rest of the frog appeared dark purple, mostly reflecting the ultraviolet light.

*Ornate Burrowing Frog (*Platyplectrum ornatum*)*

This frog did not fluoresce under 395 nm light.

*Montane Toadlet / Tableland Gungan (*Uperoleia altissima*)*

The Montane Toadlet mostly absorbed the ultra-violet light, but areas on the toes fluoresced pale red.

Discussion

The few frog species so far observed in the Wet Tropics of Far North Queensland have fluoresced only very mildly compared to the stunning all-over fluorescence reported for some frogs from the Americas. The skin has a subtle change of colour more than a glow. However, testing a greater range of ultraviolet and blue wavelengths may excite vivid fluorescence not seen at 395 nm. In the two Wet Tropics species that were tested at a range of wavelengths, the lower ultraviolet wavelength of 365 nm was better at exciting the fluorescence of the eyes, and for the Northern Stony-creek Frogs, their ventral surfaces and thigh markings as well. Frogs have not been specifically tested for their emission of fluorescence versus phosphorescence at varying wavelengths.

The colour of a frog's skin acts in camouflage and/or signalling, and the peptides exuded from skin have a host of bioactive functions. The fluorescence of some of these molecules may be a purely incidental feature of their chemical structure. Although the skin secretions of Australian frogs have been analysed in depth for their chemistry, such studies were done in the context of bioactive properties and not on the whole-animal visual effect of fluorescence. However, a visual function was recently proposed for the fluorescence of frogs. Although not yet tested experimentally, it was suggested that South

American Tree Frogs may have their fluorescence excited by twilight, and be able to see it and use it in intraspecific signalling (Taboada *et al.* 2017).

Frogs are active mostly in the evenings, when the ambient ultraviolet light that may excite fluorescence is relatively dominant. However, the shift from day to night is complex and occurs over hours, with illumination going from being spectrally neutral, to long-wavelength (red/orange) dominated, then short-wavelength (blue/violet) dominated, then spectrally neutral or long-wavelength dominated again depending on moonlight (Johnsen *et al.* 2006). Perhaps the mottled pigments of some treefrogs in the Wet Tropics mean the animals can sit out the changing lighting conditions, remaining camouflaged the whole time, changing similarly with the spectral qualities of the variously fluorescing lichen-covered branches where they are situated. Daytime camouflage colours are necessary to keep hidden from diurnal visual predators, but ultraviolet fluorescence may play a role in nocturnal camouflage when the animals are more active and exposed. Alternatively, it may benefit some species of frog to hide in the daytime yet be conspicuous at night. Perhaps fluorescence can act as a visual warning of toxicity to nocturnal predators. Further research could investigate if there is a correlation between the visible fluorescence and predator defence properties of skin secretions. Such a correlation would only be useful however if the frogs and/or their predators can detect fluorescence in natural light.

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