Rats don't glow in the moonlight:

behavioural study doesn't support visual function of fluorescence in fur

Moonlight isn't strong enough to make rat fur glow, dashing hypotheses of a visual function for photoluminescence (fluorescence and/or phosphorescence) in nocturnal mammals.

Although scientists working on fur fluorescence since 1911¹ never assumed the phenomenon to have any optical relevance in nature, speculations that nocturnal mammals use fluorescence as a *Secret Squirrel* means of communication have accompanied colour photos on the internet since 2019².

Researchers from James Cook University, led by zoologist Linda Reinhold, aimed to find out if wild nocturnal mammals and birds reacted to fluorescent fur any more or less often than non-fluorescent fur. This question is fundamental for any studies going on to test what a visual function might be.

Ms Reinhold and her team modified Carl Kloock's landmark 2005³ study demonstrating that nocturnal flying insects could see the fluorescence of scorpions on a full moon, but not on a new moon. Instead of using sticky traps for invertebrates, they used remote cameras for vertebrates.

At each of three habitat sites (open farmland, woodland and rainforest), Ms Reinhold set eight pairs of model rats, each covered in a real-fur pelt from lab rats locally bred for pet food. Within each of the pairs, the fluorescent fur of one rat was left in its natural brilliance and coated with regular hairspray, and the fur fluorescence of the other rat was extinguished with UV-protectant hairspray. The 'rats' were set out in front of remote cameras at dusk, and brought in at dawn. To test whether moonlight triggered the fluorescence, each camera station was set out over both full moon and new moon phases.

"If moonlight set off the fluorescence, and if wild vertebrates reacted to it, we would expect to see a preference for either the fluorescent or the non-fluorescent rats," said Ms Reinhold. "As it turned out, there was zero preference towards either fluorescent or non-fluorescent coats." The results cast doubt on speculations of fur fluorescence being somehow optically relevant specific to nocturnal animals.

In some ways, this study ends the recent speculation of a *Secret Squirrel* visual function of nocturnal mammal fluorescence. But this study is a world first, and as such, many more studies, using different species, native rats, possums, perhaps narrowing down predator-vs-prey and conspecifics, are bound to follow. This first study only used the bright blueish white fluorescence of rat fur, not the pink-red fluorescence of some other species, triggered by longer wavelengths. A future study may support the case for twilight, but with only a 20-minute window at either end of the night, it would take some effort to get enough of a sample size in this lighting condition. But for the studies that are to follow, they must also be of real fur in natural lighting, because disco lighting doesn't represent nature.

"We wanted to contribute to answering the existing hypotheses of visual function in *nocturnal* mammals, but visual function of fluorescence in *diurnal* mammals may in fact be more likely because of the abundance of excitation wavelengths in sunlight," said Ms Reinhold. Moonlight only yields around 1000th the strength of twilight, and 1/400,000th the strength of sunlight, and is not dominant in UV wavelengths. We know that sunlight is strong enough to activate the fluorophores, but the fluorescence is drowned out by reflectance. There is some evidence⁴ that diurnal birds can detect fluorescence that humans can't, indicating a specialised filtering system in the lenses of their eyes.

"So, rats don't glow in the moonlight (at least not to vertebrate eyes), but there are mammals, birds, insects, all fluorescing brightly around us in the sunlight, though we can't see it because we don't have the filtering in our eyes to cut out the glare," said Ms Reinhold.

"I would like to see the next set of experiments done in daylight conditions, in direct sunlight or dappled woodland shade, to test if various types of fluorescence may act as attractant or deterrent," said Ms Reinhold. Perhaps the most likely candidate would be a nocturnal animal that uses its colouration as camouflage where it rests amongst foliage under dappled sunlight during the day, i.e. the tube-nosed fruit bat⁵.

The fur of lab rats is among the most brilliantly fluorescent of mammals. Their fluorescence was discovered while scientists in the USA were looking for ringworm with UV light in the 1950s⁶. Those scientists went on to isolate the fluorophores (fluorescent molecules in the fur) as tryptophan metabolites, the same class of fluorophore as that which makes scorpions fluoresce. The wild mammals that interacted with Reinhold's 'rats' also generally have fluorescent fur⁷.

Unlike *bio*luminescence, which makes light in the darkness through a chemical reaction in living cells, *photo*luminescence means 'glow-in-the-light' – it can only shine back light that is shone onto it initially. When the light shone onto an object is ultraviolet, appearing 'invisible' to us, and the wavelengths it converts it to are in the visible range of the spectrum so that we can see them (often blue, green and red), the light becomes visible to us, and the object appears to glow. It's often called 'poor man's bioluminescence'. Contrary to popular misconception, UV vision is not at all involved in seeing this kind of fluorescence, as it *turns* the invisible light *into* visible light. We don't need to see the light coming out of the UV torch, only the light coming off the fur.

Linda Reinhold: Zoologist, James Cook University

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Link to photos and videos (credits: Linda Reinhold) for free use by the media: https://1drv.ms/f/s!AsOsQ2EsnmASgfhm3e0BBRUoMRa8_Q?e=efvX40

The article published this week in Australian Journal of Zoology:

Reinhold L.M., Wilson D.T. and Rymer T.L. 2024. Does the photoluminescence of rat fur influence interactions in the field? *Australian Journal of Zoology* <u>https://www.publish.csiro.au/zo/ZO23021</u>

Further reading:

¹ Reinhold L.M., Rymer T.L., Helgen, K.M. and Wilson D.T. 2023. Photoluminescence in mammal fur: 111 years of research. *Journal of Mammalogy* **104(4)**: 892-906. <u>https://doi.org/10.1093/jmammal/gyad027</u>

- ² Kohler AM, Olson ER, Martin JG, Anich PS (2019) Ultraviolet fluorescence discovered in New World flying squirrels (*Glaucomys*). *Journal of Mammalogy* **100**: 21-30. <u>https://doi.org/10.1093/jmammal/gyy177</u>
- ³ Kloock CT (2005) Aerial insects avoid fluorescing scorpions. *Euscorpius Occasional Publications in Scorpiology* **2005**: 1-7. <u>https://doi.org/10.18590/euscorpius.2005.vol2005.iss21.1</u>
- ⁴ Czarnecki C, Manderino R, Parry D (2022) Reduced avian predation on an ultraviolet-fluorescing caterpillar model. *The Canadian Entomologist* **154**: e10. <u>https://doi.org/10.4039/tce.2021.57</u>
- ⁵ Reinhold L (2022) Photoluminescent yellow wing markings of eastern tube-nosed fruit bats (*Nyctimene robinsoni*). North Queensland Naturalist **52**: 69-74. <u>https://www.researchgate.net/profile/Linda-Reinhold-</u> <u>2/publication/365359760_Photoluminescent_yellow_wing_markings_of_Eastern_Tubenosed_Fruit_Bats_Nyctimene_robinsoni.pdf</u>
- ⁶ Rebell G, Lamb JH, Mahvi A, Lee HR (1957) The identification of L-kynurenine as the cause of the fluorescence of the hair of the laboratory rat. *Journal of Investigative Dermatology* 29: 471-477. <u>https://doi.org/10.1038/jid.1957.123</u>
- ⁷ Reinhold L.M. 2023. Widespread fluorescence in the fur of mammals of the Australian Wet Tropics. Proceedings of the Royal Society of Queensland **132**: cover & 3-40. <u>https://doi.org/10.53060/PRSQ23.5</u>