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Short Note

Full pelage Ultra-Violet fluorescence occurs in both lesser horseshoe bat, *Rhinolophus hipposideros* (André, 1797) and Blasius's horseshoe bat *R. blasii* Peters, 1867

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Abstract

Ultra-Violet (UV) fluorescence has been observed and reported in a wide range of living organisms from lower plants to mammals. In animals, its function has been attributed to a range of behaviours including signalling in mate selection, camouflage, and mimicry, but in many cases its function is unclear, and it may be non-adaptive. Here we report on full pelage UV fluorescence in both *Rhinolophus hipposideros*, the lesser horseshoe bat and *R. blasii*, Blasius's horseshoe bat but it is restricted to just these two species within the European Rhinolophidae. The fluorescence in *R. hipposideros* was only observed in adult bats of both sexes and not in juveniles (at least until they were six months old). It is unlikely this phenomenon has any function in the ecology or behaviour of either the species, as rhinolophids lack the short wavelength opsins in their cones to detect light emitted at these wavelengths. It may be maladaptive, as some nocturnal predators may have the ability to detect the fluorescence. Potentially, the differing responses of adults and juveniles have uses in the monitoring of species, especially concerning confirming maternity colonies and estimates of the productivity of colonies. In the case of *R. blasii*, it may also aid in the identification of this cryptic species that often roosts with other medium-sized horseshoe bats.

Introduction

Fluorescence is the process where radiation at higher energy levels (shorter wavelengths) are absorbed by particular molecules, causing those molecules to emit light at a lower energy level (longer wavelengths). This process frequently involves ultra-violet (UV) light, often resulting in the generation of emissions in the visible spectrum; in natural systems this process is dependent on the UV component of sunlight. The spectral quality of UV light reaching the Earth's surface varies diurnally and seasonally, and with altitude and latitude. UV radiation is strongest in the tropics and at high altitudes, due to the thinness of the ozone layer and the reduced air mass between the Earth's surface and the edge of the atmosphere respectively. Although light is at its lowest intensity at night, moonlight still contains a proportion of UV radiation, and is relatively more abundant at twilight compared with daylight (Spitschan et al., 2016). All of these factors will impact on the degree of fluorescence over diurnal and seasonal cycles (Zhang et al., 2020).

UV fluorescence has been reported in biological substances as far back as the 19th century, with the initial observations being made in plants; in recent years it has been reported increasingly widely in a range of different taxa (Lagorio et al., 2015). UV fluorescence has been recorded in a range of mammals, it occurs in some marsupials (Pine et al., 1985; Travouillon et al., 2022), rodents (Nummert et al., 2023; Olson et al., 2021; Sobral and Souza-Gudinho, 2022), insectivores (Hamchand et al., 2021) and bats (Reinhold, 2022; Travouillon et al., 2022). The function of UV fluorescence in animals has been attributed to signalling around mate choice (Garcia and de Perera, 2022), a type of Batesian mimicry where prey species emit a similar fluorescence to their predators (Kohler et al., 2019), camouflage against vegetation or habitats that themselves fluoresce (Sparks et al., 2014) or it may simply be the by-product of biochemical processes and have no adaptive purpose (Marshall and Johnsen, 2017).

In July 2024, we opportunistically discovered whole pelage fluorescence in a colony of some 25 *Rhinolophus hipposideros*, lesser horseshoe bat, during fieldwork on Lokrum Island in southern Croatia. The fluorescence occurred under illumination with both 365 nm or 395 nm ultra-violet hand torches and was visible to the naked eye as a light blue glow coming from the fur of the bats, but not from their wing membranes (Fig. 1).

This fluorescence was emitted by adult animals but not from the pups the females were carrying or from newly volant juveniles.

In this study we investigate whether this phenomenon was restricted geographically to *R. hipposideros* in the area around our study site in southern Croatia and whether full pelage fluorescence occurred more widely in the other European Rhinolophidae. We also discuss the po-

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Figure 1 – UV Fluorescing *R. hipposideros* at roost..

Figure 2 – Fluorescent pelage and hairs on the plagiopatagium of the wing of adult *R. hipposideros*..

tential function or non-function of this phenomenon and whether it has potential uses in monitoring or surveying for species.

Materials and methods

To determine whether UV fluorescence was more widespread than just at our Croatian study site, seven colonies of *R. hipposideros* in Britain, nine in Croatia and two in Serbia were tested. To ascertain whether this phenomenon occurred in other European rhinolophids, we tested animals from three maternity colonies of *R. ferrumequinum* in Britain, six in Croatia and two in Serbia. Three colonies of *R. euryale* in Croatia and two in Serbia were visited. *R. blasii* was tested at one site in both Croatia and Serbia, and *R. mehelyi* at one site in Serbia.

During surveys roosts were briefly entered during the day and the bats illuminated using either a 365 nm Luxnovaq or 395 nm Lightfe ultra-violet hand LED torch. A selection of fluorescing animals were photographically documented in situ while roosting using a Nikon Z8 camera fitted with a 70–200 mm Nikkor telephoto lens (UV filter removed). The UV torch was held alongside the camera to illuminate the bats, and the camera was set to an aperture of f2.8 and a shutter speed of 1/30 s, the ISO was varied between 1600 to 16000 depending on the distance to the subject.

A small number of bats in Croatia and Britain were caught and exposed to the UV torchlight to document the response of the pelage more closely. These handheld bats were placed on a black non-UV reflective background. They were illuminated from 1.5 m with the 365 nm Luxnovaq UV hand torch and photographed using a 105mm Nikkor Macro lens with the Nikon Z8 camera set to an aperture of f8 and shutter speed of 1/40s and ISO of 12800. These activities were carried out under the appropriate licences

Results

In addition to the UV fluorescence observed in *R. hipposideros* on Lokrum Island in Croatia, the phenomenon was observed at all colonies of the *R. hipposideros* sites surveyed in Britain, Croatia and Serbia, confirming that this phenomenon is geographically widespread in this species. The pelage of adult *R. hipposideros* of both sexes elicited a strong light blue glowing response, as did tests of the pelage of mummified carcasses of adults found in the roosts.

The pigmented membranes of these bats (wing membranes, the noseleaf, and the tips of the pinnae) elicited no response, although there was a slight response from the unpigmented skin inside the pinnae. While the wing membranes themselves did not fluoresce under UV light, the short hairs on the wings, particularly on the plagiopatagium, did fluoresce (Fig. 2). No fluorescence was observed from the pelage or membranes of juvenile *R. hipposideros*. This included both non-volant animals and volant animals up to the age of 6 months (Fig. 3).

UV fluorescence was also recorded in colonies of *R. blasii* in both Croatia and Serbia (Fig. 4), once again the response was restricted to the fur and not the membranes. All of the *R. ferrumequinum, R. euryale* and *R. mehelyi* we tested elicited no response (Tab. 1).

Discussion

There have been several published studies into the occurrence of UV fluorescence in bats, all nine bat species examined by Travouillon et al. (2022) showed some fluorescence, but the tissues emitting light in their study were mainly membranes and wing bones, with just two of the species tested having fully reactive fur and a further two exhibited fur fluorescence restricted to the neck or parts of the pelage. Tumlison and Tumlison (2021) reported no fluorescence in the eight bat species they surveyed in Arkansas. Toussaint et al. (2022) reported no fluorescence in the one bat species (*Plecotus auritus*) they tested amongst 23 other mammal species, and Gual-Suárez et al. (2024) found bristles on the feet of *Tadarida brasiliensis* fluorescing. Reinhold (2022) describes striking fluorescent wing markings in *Nyctimene robinsoni* along with a full pelage response as a blue glow but in a further seven species she tested, six gave very mild responses on the tips of their fur or on claws and wing bones and one species did not react at all. In our study two of the five European rhinolophids exhibited full pelage fluorescence. On the evidence collected to date, it appears that full pelage fluorescence is relatively unusual in bats.

As to its function, Marshall and Johnsen (2017) suggest a checklist for ecologically significant fluorescence and key amongst these is the spectral sensitivity range of potential viewers. If the UV fluorescence in *R. hipposideros* and *R. blasii* is adaptive and being used to signal to conspecifics, we would expect the species to have the ability to perceive the wavelengths of light being emitted. Some bat species do have vision in short wavelength colours and into UV spectrum (Gorresen et al., 2015), and this is dependent on them having cone photoreceptors in their retina with the short wavelength sensitive opsins (S opsin) required to detect these spectra (Müller et al., 2009). However, Zhao et al. (2009) have shown a divergence in different bat evolutionary lineages regarding the S opsin gene, and it has been lost from the Rhinolophidae, probably due to an evolutionary trade-off in sensory systems, with this and related families evolving more highly sophisticated Constant Frequency echolocation systems and relying less on vision compared with other bats (Jones et al., 2013; Xuan et al., 2012).

If the fluorescence is not for signalling conspecifics, it may be used as a type of Batesian mimicry defending species used against potential predators. In which case we would expect to be able to identify a suitable defended or unpalatable species that is being mimicked. As other bat species are neither unpalatable or defended against larger nocturnal avian or mammalian predators, there are not any other volant mammal subjects to mimic. The closest we can come to a non-volant potential

Table 1 – Results of UV testing at Rhinolophid colonies.

Site and Region	Country	Species	Colony size	Result
Lokrum Island, South Dalmatia	Croatia	*R. hipposideros*	25	Luminescence
Powys, Wales	UK	*R. hipposideros*	298	Luminescence
Shropshire, England	UK	*R. hipposideros*	215	Luminescence
Gower, Wales	UK	*R. hipposideros*	382	Luminescence
		R. ferrumequinum	50	No response
Wiltshire A, England	UK	*R. ferrumequinum*	96	No response
Wiltshire B, England	UK	*R. hipposideros*	10	Luminescence
Somerset, England	UK	*R. ferrumequinum*	75	No response
		R. hipposideros	88	Luminescence
Somerset, England	UK	*R. hipposideros*	45	Luminescence
Monmouthshire, Wales	UK	*R. hipposideros*	213	Luminescence
Kopaonik Mt	Serbia	*R. ferrumequinum*	4	No response
		R. hipposideros	2	Luminescence
Canetova pećina, Eastern Serbia	Serbia	*R. mehelyi*	1	No response
Pećina u dolini Crne reke, Eastern Serbia	Serbia	*R. hipposideros*	4	Luminescence
Gornjak, Eastern Serbia	Serbia	*R. ferrumequinum*	88	No response
		R. euryale	13	No response
Lazareva pećina, Eastern Serbia	Serbia	Mixed colony of *R. euryale* and *R. blasii*	600	Mixed response (180 luminescence, 420 no response)
Ercegovci A, Sibenik-Knin County	Croatia	*R. ferrumequinum*	5	No response
		R. hipposideros	2	Luminescence
Ercegovci B, Sibenik-Knin County	Croatia	*R. hipposideros*	5	Luminescence
Golubic, Zadar County	Croatia	*R. blasii*	50	Luminescence
		R. hipposideros	1	Luminescence
Nova Krslja, Karlovac County	Croatia	*R. ferrumequinum*	24	No response
		R. hipposideros	14	Luminescence
Kordunski Ljeskovac A, Karlovac County	Croatia	*R. ferrumequinum*	2	No response
		R. hipposideros	5	Luminescence
Kordunski Ljeskovac B, Karlovac County	Croatia	*R. hipposideros*	8	Luminescence
Lipovac, Karlovac County	Croatia	*R. ferrumequinum*	9	No response
		R. hipposideros	29	Luminescence
Stara Krslja A, Karlovac County	Croatia	*R. euryale*	3	No response
		R. ferrumequinum	2	No response
		R. hipposideros	9	Luminescence
Stara Krslja B, Karlovac County	Croatia	*R. euryale*	4	No response
		R. ferrumequinum	18	No response
		R. hipposideros	29	Luminescence

unpalatable species of a similar size are shrews, but there is no evidence that they are UV fluorescent (Toussaint et al., 2022), and so it does not appear that this is a case of Batesian mimicry.

In which case, it would appear that UV fluorescence in these rhinolphid species is non-adaptive and probably a by-product of the species' physiological processes (Toussaint et al., 2022). Fur naturally has a degree of photoluminescence because it contains the protein keratin, but this does not explain the luminous response which we have from UV light. The two chemical groups that could potentially cause the fluorescence in the fur of these species are porphyrins or a build-up of tryptophan metabolites (Reinhold et al., 2023). Porphyrins generally emit fluorescence that is pink, orange, and red (Olson et al., 2021), and therefore it is more likely the cause of UV fluorescence in *R. hipposideros* and *R. blasii* are tryptophan metabolites, which fluoresce across a range of the visible spectrum including the shorter blue wavelengths (Pine et al., 1985). A build-up of metabolites over time may also explain why the phenomenon is not observed in juvenile *R. hipposideros* that would not yet have accumulated the tryptophan metabolites needed to elicit the response from UV light, we believe this is the same for juvenile *R. blasii*. At one of our cave study sites in Croatia visited in late August, a group of 20 *R. blasii* elicited no response from UV lights, on a return visit six months later when the over-wintering colony numbered 50 animals the majority of the bats were fluorescing. In common with other rhinolophids, it appears the adults moved out of the maternity colony at the end of the summer leaving the juveniles alone at this time of the year.

Having pelage that glows under UV light appears maladaptive for species that are potentially prone to crepuscular and nocturnal predation. Principal amongst the nocturnal predators are owls; although owls themselves lack S opsins, adaptations to their rod vision enable them to detect bright signals from UV reflecting surfaces, such as some feathers (Höglund et al., 2019) and presumably UV emitting fur. Other potential predators, such as domestic cats, can see in UV (Douglas and Jeffery, 2014) and their hunting could also benefit from this fluorescence. The behavioural adaptation observed in *R. hipposideros* and *R. blasii* foraging at night, flying within, under or close to vegetative clutter (Bücs and Csorba, 2023; Schofield et al., 2022), as well as protecting these species from predation by diurnal predators hunting at dusk and dawn, may also reduce nocturnal predation by predators able to detect the shorter wavelength light the bats are emitting when UV light is present.

The UV fluorescence demonstrated by the adults of these bats may provide those monitoring their population status with an additional tool. Counts of glowing bats versus those not eliciting a fluorescent response inside roosts or as they emerge could be a new tool for identifying maternity roosts and estimating the productivity of single species colonies. In addition, *R. blasii* is a cryptic species that roosts with *R. euryale* in some areas of Europe and the two species are difficult to separate without catching and handling the bats. The use of UV-torches to determine whether *R. blasii* is present and if so, what proportion of the mixed colony they comprise would be a non-invasive means of monitoring this species. This was the case in this study at Lazareva pećina in

Figure 3 – Juvenile *R. hipposideros* showing no UV fluorescence from the pelage.

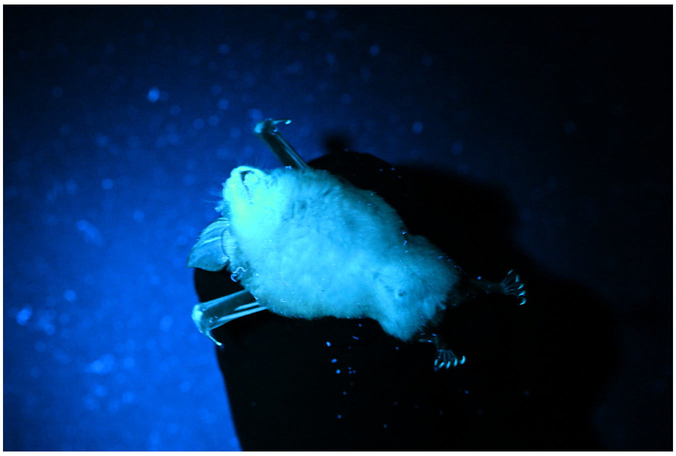

Figure 4 – UV-Fluorescence in adult *R. blasii*.

Serbia, a site known to have a mixed colony consisting of around 70 % *R. euryale* and 30 % *R. blasii.* Surveying the cave with UV-torches resulted in 420 bats eliciting no response and 180 that glowed, in line with the results of mist-netting surveys carried out at the site (own data).

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