NATURAL HISTORY NOTE

Ocular Biofluorescence Due to Ultra-violet Excitation in California Red-legged and Foothill Yellow-legged Frogs, in central California, USA

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Biofluorescence is a widespread natural occurrence that has been reported in a variety of organisms (Lagorio et al. 2015). It generally occurs when tissue absorbs electromagnetic radiation (i.e., light) at one wavelength and reflects that light back at lower wavelengths, typically resulting in the emission of florescent light (Marshall and Johnson 2017, Lamb and Davis 2020). Selected invertebrate and vertebrate fauna demonstrate biofluorescence (Lawrence 1954, Babu et al. 2002, Honkavaara et al. 2002, Maxwell and Johnson 2002, McGraw and Nogare 2004) but the degree to which this phenomenon occurs is only recently being investigated. The ecological function of biofluorescence is not well understood. Some studies, however, provide evidence of a role in visual communication (e.g., defense, mate choice; Lagorio et al. 2015, Kohler et al. 2019). Amphibians have only recently been examined for biofluorescence with a wide range of results, from tiny spot patterns to complete skin florescence (Muñoz 2018, Gray 2019, Lamb and Davis 2020, Whitcher 2020). Here we report a case of ocular biofluorescence in two ranid frogs (*Rana draytonii* and *R. boylii*) in California.

While conducting a workshop to detect and identify declining amphibians, we surveyed Copeland Creek, Sonoma County, California (38.335657 N, 122.578036 W, elev. 700 m). Our surveys were conducted in August 2022, began at 2000 hrs and ended at 2330 hrs, and included the use of hand-held flashlights which were used to detect eye shine of amphibians (Corben and Fellers 2001). Amphibian surveys were conducted while two or more biologists walked either side of the creek searching the creek margin, as well as up to 10 m into the upland. We measured and weighed captured amphibians and inspected them for PIT tags and ectoparasites. All animals were released at capture locations after processing.

Over three days, we conducted nighttime surveys along an ephemeral creek, with weather conditions being clear, approximately 15-20° C. We captured 20 adult and 91 subadult California Red-legged Frogs (*R. draytonii*), 10 adult Foothill Yellow-legged Frogs (*R. boylii*), three adult Pacific Treefrogs (*Hyliola regilla*), and 41 adult Rough-skinned Newts (*Taricha granulosa*). We used a 480-lumen white (COAST® PX1 LED flashlight) light to inspect captured amphibians for ectoparasites, and then placed similar sized frogs in a 19 L bucket to await further processing. We exposed captive frogs and newts to a 365 nm ultraviolet (UV) light (Alonefire® SV003 flashlight) for 5 to 10 seconds. We observed no fluorescence anywhere on the body of Rough-skinned Newts or Pacific Treefrog. A single California Red-legged Frog adult showed an extremely small portion of the eye lid (3 mm long, 1 mm wide) that fluoresced, as well as having intense ocular fluorescence (Figure 1). We noted that all California Red-legged Frog individuals, including adult and young-of-the-year frogs, showed a similar biofluorescence from the eyes (Figures 2a and 2b, and 3). Ten adult Foothill Yellow-legged Frogs that we captured were also found to have the same biofluorescence in the eyes of the adults we examined (Figure 4a and 4b), but showed no reflectance from the skin.

We reviewed recent publications that included photographic illustrations of frog species that were exposed to UV light of a similar wavelength and noted that only two species included ocular biofluorescence. These included the Canal Zone Treefrog (*Boana rufitelus* [= *B. rufitela*]; Deschepper et al. 2018) from Costa Rica, and the Harlequin Treefrog (*Rhacophorus pardalis*) from Indonesia (*https://www.jungledragon.com/image/49946/glow_in_the_eyes.htmlphoto*). Biofluorescence in the eye is reported to be reflected from guanine crystals in the eyes of some vertebrates (Somiya 1980). We point

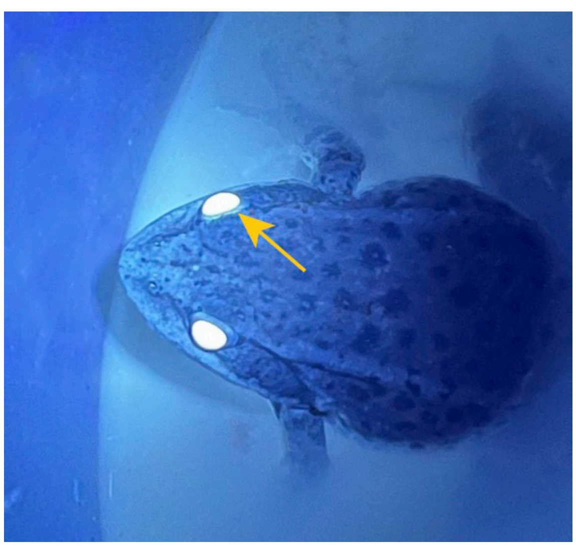

Figure 1. California Red-legged Frog (*Rana draytonii*) adult showing ocular fluorescence and a small patch of the eye lid (yellow arrow) under UV-light (365 nm), in Sonoma County, California.

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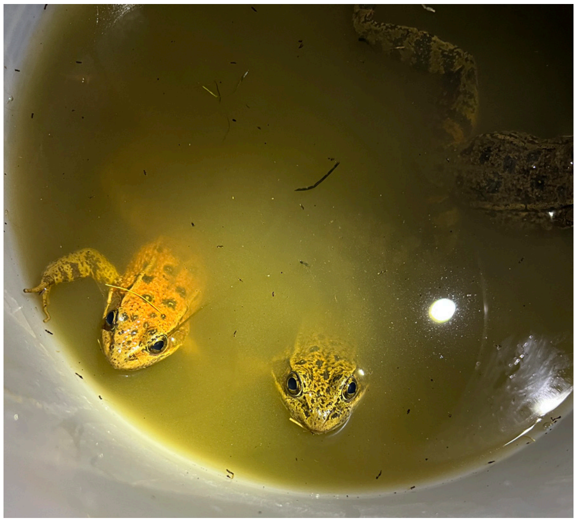

Figure 2a. California Red-legged Frog (*Rana draytonii*) under white light (400 lumens), in Sonoma County, California.

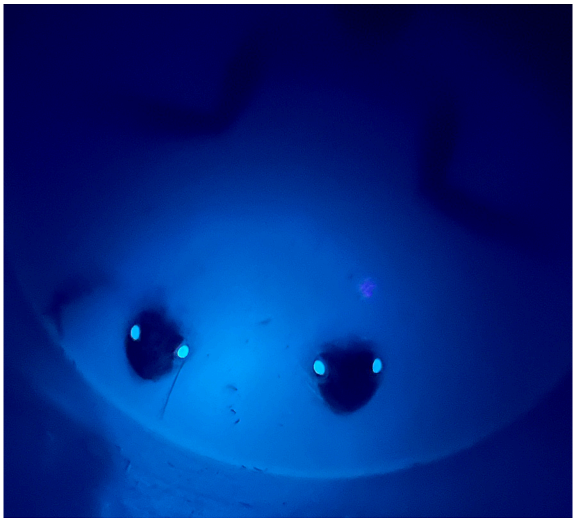

Figure 2b. California Red-legged Frog (*Rana draytonii*) under UV-light (365 nm), in Sonoma County, California.

out that despite previous authors looking at several hundred species of amphibians, only two other species, in addition to those reported here, appear to reflect UV light from the eyes that creates a glow for the observer to detect.

During our survey efforts we made attempts to determine if the use of a UV light could aid in the detection of the species during survey efforts. We determined that the range of the UV light was relatively minimal, even in complete darkness—approximately 5 m. This detectability was exceeded by the use of 480-lumen white light and putative reflectivity of the tapetum lucidum, which was clearly visible up to 50 m, particularly with the use of binoculars (see Corben and Fellers 2001).

Figure 3. Twenty California Red-legged Frog (*Rana draytonii*) post-metamorphic frogs showing ocular fluorescence under UV-light (365 nm), in Sonoma County, California.

Several authors have reported biofluorescence from the skin of amphibians (Taboada et al. 2017b, Gray 2019, Lamb and Davis 2020) but few report any level of biofluorescence from the eyes. The role of biofluorescence has been a subject of consideration by researchers (Honkavaara et al. 2002, Lagorio et al. 2015, Taboada et al. 2017a, Lamb and Davis 2020). Several authors have reported that a range of species may be using biofluorescence as a means of interspecific communication, and even interaction among conspecifics (Lim 2007, Sparks et al. 2014, Prötzel et al. 2018, Lamb and Davis 2020). It is possible that both *R. draytonii* and *R. boylii* use ocular biofluorescence for interspecific detection. Since both species of ranid have this feature, it is also possible that they use it for predatory avoidance (Lagorio et al. 2015, Kohler et al. 2019). Although it is rare to find both California Red-legged Frog and Foothill Yellow-legged Frog at the same location (pers. obs.), UV reflectance may play a role in one species avoiding predation by the other, or possibly in reducing competition for similar food resources (i.e., spatial or temporal avoidance). Future work should include looking at similar adjacent species [i.e., Northern Red-legged Frog (*R. aurora*), Cascades Frog (*R. cascadae*), Mountain Yellow-legged Frog (*R. muscosa*), and Sierra Nevada Yellow-legged Frog (*R. sierrae*)]. It may also be important to determine if other wavelengths of light (e.g., blue light: 440-460 nm) or the use of ocular filters (yellow/orange) may offer increased detection in the field (Lamb and Davis 2020), which may facilitate survey efforts for these declining species.

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Acknowledgements—We thank the Sonoma Mountain Ranch Preservation Foundation for access to the site. Two anonymous reviewers commented on this manuscript and have improved its content, for which we are grateful. We are also grateful to Alex Berryman, Griffin Capehart, Andrea Claassen, Andrew Danos, Greg Davis, Shane Emerson, Tawni Gotbaum, Elizabeth Gutberlet, Isao Kaji, Noon Mokhayesh,

Figure 4a. Foothill Yellow-legged Frog (*Rana boylii*) under white light (400 lumens), in Sonoma County, California.

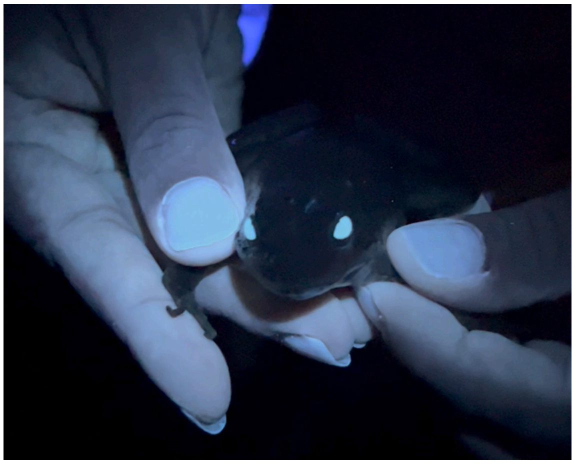

Figure 4b. Foothill Yellow-legged Frog (*Rana boylii*) under UV-light (365 nm), in Sonoma County, California.

Daniellle Montijo, Crecencia Sanchez, Matt Swagler, and Christina Van Oosten for valuable assistance in the field. Sampling was conducted under federal and State permits held by Jeffery T. Wilcox (TE-068745 and SCP-005654) and Jeff A. Alvarez (TE-27427 and SCP-000040).

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