

Defensive behaviour in the Italian Stream Frog *Rana italica* (Anura: Ranidae)

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Anuran amphibians exhibit numerous defensive behaviours, including *unkenreflex* and eye-protection (see Toledo et al., 2011 for a comprehensive list of such behaviours). The *unkenreflex* term, first used by Hinsche (1926) and borrowed from German *Unkenreflex* (Unke: fire-bellied toad, and reflex: reflex), is a typical posture displayed by several anuran and urodele species when they are threatened. In anurans, this posture usually consists in arching the body, raising the limbs from the ground such that the forelimbs partially cover the head while the hind limbs partially bend over the frog's back, thus showing the bright colours of the ventral body surface (e.g., *Bombina* ssp., see Guarino et al., 2007; some species of the genera *Melanophryniscus* Gallardo, 1961, *Pseudophryne* Fitzinger, 1843, and *Smilisca* Cope, 1865, see Toledo et al., 2011). Traditionally, the *unkenreflex* position is interpreted as a defence behaviour adopted by individuals from brightly coloured species, more specifically as an aposematic signal advertising their toxicity to predators (e.g., Wells, 2007; Rojas, 2017). However, recent evidence showed that the static *unkenreflex* position and the ventral red colouration of the south American toad *Melanophryniscus cambaraensis* Braun & Braun, 1979, do not prevent predator attacks but reduce their efficiency (Bordignon et al., 2018). The static *unkenreflex* seems to confuse the predator in identifying the toad's head, which may reduce the success rate of predatory attempts. It is possible that the *unkenreflex* movement, and not only the static position, together with the subsequent exhibition of the bright ventral colouration and the secretion of toxins may

constitute an effective aposematic signal in this species. Recently, the aposematic function of the *unkenreflex* has also been questioned in the Italian Savi's Salamander *Salamandrina perspicillata* (Savi, 1821) (Barbieri et al., 2021) highlighting that further research is needed to confirm the significance of this type of defensive behaviour in different amphibian species.

The eye-protection behaviour has been recorded for a wide variety of anuran species (see Toledo et al., 2011; Borteiro et al., 2018) and consists in slightly arching the body upwards and protecting the eyes with the forelimbs (individuals can close the eyes while in the arched posture). A possible function of eye-protection behaviour is to avoid injuries during subjugation, ingestion, and regurgitation by predators (Toledo et al., 2011).

Rana italica Dubois, 1987 (Anura: Ranidae) is a monotypic species endemic to the Italian peninsula where it is distributed from Central Liguria to southern Calabria, mainly along the Apennine chain (Picariello et al., 2007; Guarino et al., 2012; Guarino and Picariello, 2014). During an amphibian field survey on 27 February 2020, we collected an adult female of *Rana italica*, about 50 mm in snout-vent length (SVL), along the Torbido stream that flows about 2,5 km from Pietraroia (Province of Benevento, southern Italy) at 1113 m elevation (41.35953°N, 14.57755°W). When gently manipulated, this individual repeatedly showed the typical eye-protection defensive behaviour of anurans (Fig. 1). This unusual posture was held as long as the animal was in the hands of the researcher (about one minute) and for about ten seconds after being released, before jumping into the nearby stream. We did not capture any additional individual of this species during our survey and could not verify if other individuals also displayed this defensive behaviour.

To our knowledge, our finding is the first report of a defensive posture in *Rana italica*. So far, among European brown frogs, a defensive behaviour described as *unkenreflex* has been recorded in *R. temporaria* Linnaeus, 1758 (García-París and Esteban, 1989; Haberl

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Figure 1. Adult female *Rana italica* displaying defensive behaviour. Photo by Salvatore Viglietti.

and Wilkinson, 1997; Schlüpman, 2000), *R. dalmatina* Bonaparte, 1838 (Stojanov, 2005), *R. macrocnemis* Boulenger, 1885 (Carretero et al., 2011) and *R. graeca* Boulenger, 1891 (Jablonski et al., 2019). Nevertheless, according to Toledo et al (2011), the term *unkenreflex* seems inappropriate for the European brown frogs as it is usually attributed to warning of skin toxins, and the terminology “eye-protection” should be used instead. Concerning the defensive posture of *R. graeca*, it has also been hypothesised to be a variant of thanatosis, simulating death in an attempt to be inconspicuous in order to increase the survival changes (Jablonski et al., 2019). *Rana italica*, like the other European brown frogs, does not have any bright colouration or anti-predatory toxicity, suggesting that aposematism is unlikely. Rather, it is plausible that the defensive behaviour we observed is meant to avoid or reduce injuries during subjugation and ingestion. Moreover, it is also possible that the eye-protection posture confuses predators by reducing their ability to identify the frog’s head and determine the direction of the attack, as shown in the Brazilian toad *Melanophryniscus cambaraensis* (Bordignon et al., 2018). However, several questions remain open. The fact that the here observed defensive posture was never previously reported for *R. italica* strongly suggests that such behaviour occurs in this species only occasionally; if so, what are the display-inducing factors? Furthermore, if defensive strategies are generally displayed when the animal is threatened and cannot escape, why have similar eye-protection behaviours never been reported in studies involving

the capture and handling of individuals of *R. italica*? We cannot exclude that this occasional behaviour was never recorded in *R. italica* simply because of a lack of attention from researchers. Therefore, more targeted studies are needed to establish how often and under what specific conditions the eye-protection defensive posture occurs in different population of *R. italica*. Furthermore, it is intriguing that a defensive behaviour categorised as *unkenreflex* has been recorded only in four species of the European brown frogs so far.

To conclude, we report for the first time a defensive behaviour in *R. italica* and hypothesise that it functions as an eye-protection mechanism and/or to confuse predators. Furthermore, we suggest that similar behaviours, previously categorised as *unkenreflex* in other European brown frogs (with exception of *R. graeca*), are probably unrelated to aposematism and the release of toxic substances, as typically documented in the fire-bellied toads of the genus *Bombina*. The evolutionary history and adaptive value of these behaviours should be better assessed in future studies, and the different postures, generally described as anuran *unkenreflex*, probably require a more detailed redefinition in different taxa.

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