

Is the Northern Spectacled Salamander *Salamandrina perspicillata* aposematic? A preliminary test with clay models

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Abstract. Aposematism is a visual communication system in which bright and contrasted coloured prey warn predators about their unprofitability. The Northern Spectacled Salamander *Salamandrina perspicillata*, a small terrestrial salamander endemic to Italy, displays a uniform dark dorsal colouration and a contrasted ventral side in which a bright red colour is displayed by coiling the tail over the body. In amphibians, this behaviour, known as “*Unkenreflex*”, is usually considered to be aposematic. In this study, we used realistic plasticine replicas to test this aposematic hypothesis in the Northern Spectacled Salamander. Of the 199 clay models placed in a natural habitat, 165 (83%) were recovered and 39 (24%) showed some sign of predation. The head of the models was more attacked than expected by chance ($P = 0.042$), suggesting that potential predators were perceiving models as real prey. However, there were no differences in the proportion of dorsal ($18/83 = 22\%$), and ventral ($21/82 = 26\%$) models attacked by predators. Therefore, contrary to expectations our experiment did not support the aposematic hypothesis. However, predation experiments with clay models have limitations and our results should be considered as preliminary, deserving further research to better understand the Northern Spectacled salamander prey-predator system.

Keywords. Animal replicas, plasticine, predation, salamander, *Unkenreflex*.

Many animal taxa evolved conspicuous colourations associated with different mechanisms of antipredator defence such as claws, beaks, teeth, spines, stings and a variety of toxic compounds that are actively inoculated or passively delivered to predators (Stevens, 2013; Caro and Allen, 2017). This visual communication system is known as aposematism (Steven, 2013), a widespread phenomenon that independently evolved many times in different amphibians lineages (Wells, 2007). Aposematic colourations are usually associated with a variety of toxic compounds that are produced or sequestered and stored in specialised glandular skin glands (e.g., Wells, 2007; Rojas, 2017; Demori et al., 2019). Several species of aquatic and terrestrial salamanders exhibit a variety of combinations of red, orange yellow or white marks usually displayed on brown or black backgrounds. In

salamanders, conspicuous colourations are typically assumed to act as aposematic warning anti-predatory signals (e.g., Wells, 2007; Lüddecke et al., 2018). In fact, several alkaloids (e.g., tetrodotoxins) and other toxic compounds are isolated from the skin of newts and salamanders, reinforcing the assumption that these contrasted colourations are associated with unpalatability (e.g., Yotsu-Yamashita et al., 2007, 2017; Preissler et al., 2019).

Usually, bright colour patterns are usually displayed on the dorsal surface of amphibians. However, some salamanders are dorsally cryptic while possessing aposematic colourations on the underside. These species display their ventral bright colours by exposing the venter by coiling laterally (e.g., the Asian newt *Paramesotriton deloustali*) or coiling the tail above the body (e.g., the North American newt *Taricha rivularis* and the Alpine

newt *Ichthyosaura alpestris*) (see Fig. 14.30 in Wells, 2007). This latter anti-predatory behaviour is known as “*Unkenreflex*”, first described in the fire-bellied toad *Bombina bombina* (Hinsche, 1926). This peculiar behaviour, i.e. exposing bright ventral colouration by arching the body, is displayed also by the two species of Spectacled salamander belonging to the genus *Salamandrina* Fitzinger, 1826: *S. perspicillata* and *S. terdigitata* (Angelini et al., 2007). However, Lanza (1967) casted doubts on the aposematic function of *Unkenreflex* in *Salamandrina*, because this genus does not possess the parotoid glands typical of many toxic salamanders, while Utzeri et al. (2005) presented some anecdotal evidence for unpalatability. Therefore, the function of the black, white and red coloured ventral side and of the red tail of *Salamandrina* remains uncertain, although no alternative explanation has been proposed.

To better understand if the ventral colouration of *S. perspicillata* could represent an aposematic signal, we made a predation experiment by using of clay replicas representing the dorsal and ventral patterns of the focal species. Experiments using this technique are non-invasive and easy to perform in natural habitats, but problems and limitations should be also taken into consideration (Bateman et al., 2017; Rössler et al., 2018). In the present study, clay models were exposed in the species’ natural habitat and predation rates on different model types compared. If the aposematic hypothesis holds true, we expected that models with a conspicuous red colouration typical of the body underside and tail would be attacked less frequently than those bearing a dark dorsal appearance.

Salamandrina perspicillata (Savi, 1821), the Northern Spectacled Salamander, is endemic to central and northern Italy (Angelini et al., 2007). This species is found from the sea level up to about 1900 m, in Mediterranean vegetation areas and in humid broadleaf woodlands (Angelini et al., 2007; Romano et al., 2009). Adults usually range from 70 to 100 mm in total length and are fully terrestrial, with the exception of gravid females that enter water bodies for spawning (Angelini et al., 2007). The dorsal colouration is characterised by a very dark or black dorsal pattern with, during the terrestrial phase, a matt appearance. On the salamander’ head the presence of a characteristic whitish or yellowish mark between the eyes suggested the common species name “spectacled” (Fig. 1). The ventral surface displays a conspicuous combination of black and white in its central and anterior part, while the posterior part of the abdomen, cloaca and tail, and the inferior part of all four limbs is brightly coloured in red (Angelini et al., 2007; Fig. 1). When disturbed, *S. perspicillata* sometimes displays its conspicuous ventral colouration by coiling its

tail above the body in an *Unkenreflex* posture (Lanza, 1967; Angelini et al., 2007).

The Northern Spectacled Salamander’ replicas were made from plasticine, a soft material prepared from clay, wax and oil. Clay models are malleable, retain predatory marks (Kuchta, 2005; Salvidio et al., 2017) and have been used to analyse predation in relation to colour polymorphism and aposematism in amphibians (e.g., Kraemer et al., 2016; Paluh et al., 2014).

Clay models were obtained from a 3D printer template. We imported a photographic image of *S. perspicillata* in the software “Rhinoceros”. The template was printed in PLA (a plastic material) with the “Wanhao Duplicator 6” printer (Supplementary Material Fig. S1). Models, possessing a total length of 79 mm, were hand painted with acrylic water-soluble red, black and white colours (Maimeri Polycolor # 220, 530 and 021, respectively). The model head, torso and tail surfaces were measure by ImageJ software (Schneider et al., 2012) giving these relative proportions: head (17.35%), torso (27.33%) and tail (55.32%).

The experiment took place, from October 26th to November 1st 2020, in North-western Italy, Province of Genova, in a mixed humid deciduous forest at about 900 m a.s.l. (44°34’00”N; 9°08’10”E). In this area the Northern Spectacled salamander is widespread along forest streams and in the leaf litter. During autumn, Northern Spectacled Salamanders can be found active on the forest floor also during daytime (Salvidio et al., 2012). Models showing the ventral or dorsal coloration were alternatively placed every 2 m on stones, fallen branches and moss. After 6 days, models were examined in the field with the aid of a ×20 geologist lens and removed. Clay models were scored as attacked, if they showed evident predatory marks on any body part, excluding the limbs. In many cases, predators could be identified as mammals, if tooth marks were evident, or as birds, if V-shaped peck marks were observed (Fig. 1). In some cases, however, the predator could not be identified. Predations on dorsal and ventral-coloured models were compared by means of Fisher exact test or χ^2 , while absolute frequencies of attacks on the different body parts were compared to the expected frequencies, proportional to the relative surfaces of the model parts, by means of a χ^2 goodness-of fit test (Zar, 2005). We set the significance level at $\alpha = 0.05$ and all tests were performed with PAST 4.03 software (Hammer et al., 2001).

Of the 199 models placed on the forest floor (99 dorsal and 100 ventral), 165 (83%) were recovered (82 dorsal and 83 red). The total number of models attacked was 39 (24%), 18 dorsal and 21 ventral models (Table 1). Head, torso and tail of the dorsal and ventral-coloured models

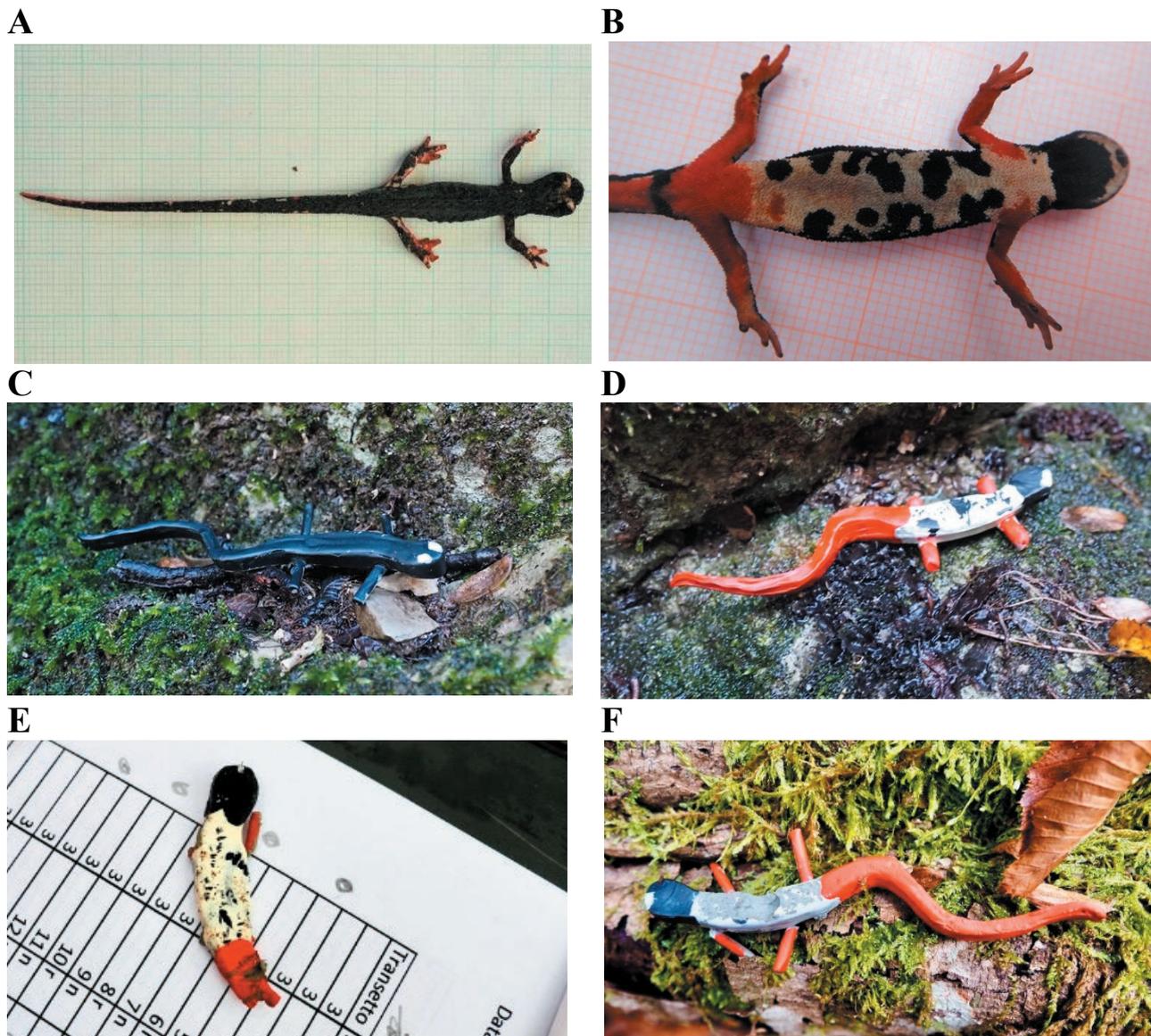


Fig. 1. Dorsal (A) and ventral (B) view of live Northern Spectacled Salamander *Salamandrina perspicillata*; dorsal (C) and ventral (D) view of clay models displayed in the field; E) clay model showing a bird V-shaped beak mark on the tail; F) clay model showing mammal teeth marks on the torso.

received a similar number of attacks ($\chi^2 = 3.25$, $df = 2$, $P = 0.172$). Overall, there was a tendency that more attacks were aiming to the head than expected by chance (16 observed versus 9.19 expected attacks; Fig. 2), this difference being statistically significant (head vs torso + tail: goodness-of-fit $\chi^2 = 4.147$, $df = 1$, $P = 0.042$). Dorsal and ventral-coloured models were attacked with similar frequencies: 18 dorsal and 21 ventral models (Fisher exact test, $P = 0.715$; Table 1).

In our experiment the models' head was attacked more than expected by chance. Therefore, potential

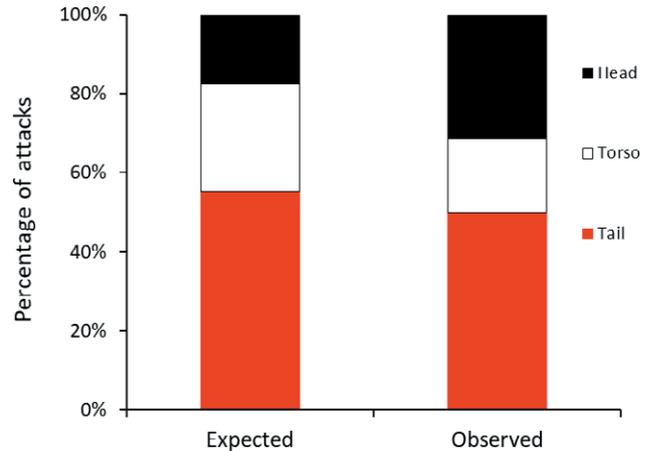
predators were perceiving the salamander replicas as real prey, suggesting that our experimental setting was successful. Contrary to expectations in case of aposematic colouration, clay models painted with conspicuous colours and representing the contrasted ventral side of the Northern Spectacled Salamander were attacked with similar frequencies as clay models displaying the dorsal colouration. This finding may be surprising, given that to date no alternative hypothesis to aposematism has been discussed in detail for this species. However, Utzeri et al. (2005) described the occurrence

Table 1. Distribution of attacks and supposed predators on the different body parts of *Salamandrina perspicillata* clay models.

Body part attacked/predator	Model		
	Black	Red	All
Head/bird	2	0	2
Head/mammal	4	3	7
Head/unidentified	0	0	0
Torso/bird	0	0	0
Torso/mammal	2	3	5
Torso/unidentified	1	0	1
Tail/bird	2	2	4
Tail/mammal	4	8	12
Tail/unidentified	1	0	1
Entire model/bird	0	0	0
Entire model /mammal	2	5	7
Entire model/unidentified	0	0	0
Intact models	64	62	126
Missing models	17	17	34
Total	99	100	199

of salamanders showing their ventral coloration in the field, during male-male contests and possibly during sexual encounters. Moreover, a possible deimatic function for *Salamandrina unkenreflex* was suggested during the review process by both reviewers. Deimatism is a behaviour by a sender that produces a sudden change in shape, colour or emits noises. This unexpected behaviour of the prey may cause hesitation or recoil in the predator (Umbers et al. 2017). Unlike aposematism, deimatism does not require predator learned aversion and may be unrelated to unpalatability or armed defence. These alternative hypotheses should deserve attention in future studies on the colourations of the Spectacled salamanders.

In any case, predations trials using motionless animal replicas have several shortcomings and should be always interpreted with caution (Bateman et al., 2017; Rössler et al., 2018; Costa et al., 2020). Actually, studies using clay modes in well-established prey-predator systems, as in the Amazonian poisonous frog *Adelphobates galactonotus* (Rojas et al., 2015) and the Brazilian venomous coral snake *Micrurus corallinus* (Banci et al., 2020), were not able to validate the aposematic function of bright and conspicuous colourations. In fact, many predators use prey movements to search, spot and select their preferred prey items (Paluh et al., 2014). Therefore, it is possible that movement is playing a relevant role in the prey-predator system involving the Northern Spectacled Salamander, and that motionless models were not recognised as aposematic by

**Fig. 2.** Percentages of expected and observed attacks on different body parts of clay models of the Northern Spectacled Salamander *Salamandrina perspicillata*. Expected percentages are proportional to the relative surfaces of the model parts (see text).

predators. For example, red models of the Dendrobatid Poison Frog *Oophaga pumilio* equipped with a moving mechanism were attacked less frequently than moving brown models, suggesting that in this species aposematic warning signals need to be broadcasted through an association of colour and behaviour to become effective (Paluh et al., 2014). Indeed, combined factors may increase the specific recognition of visual signals as aposematic and reinforce learning abilities of predators (Stevens, 2013). Unfortunately, we do not have enough information about the prey-predator system of the Northern Spectacled Salamander in its natural habitat and, in particular, if the main predators are birds or mammals (Angelini et al., 2007). However, starting from our results, it could be possible to perform new and more accurate experiments to tests for aposematism (i.e., with moving models and displaying the replicas in different positions), or for alternative explanations such as deimatism or intra-specific communication, that could explain the significance of the bright ventral colouration and of the peculiar behaviour of *S. perspicillata* known as *Unkenreflex*.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <<http://www.unipv.it/webshi/appendix>> manuscript number 10229

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