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DIPLOMA THESIS

Aposematic Coloration in Alpine newt

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Declaration:

I hereby declare that I am the sole author of the thesis entitled: " Aposematic coloration in Alpine newt." I duly marked out all quotations. The used literature and sources are stated in the attached list of references.

In Prague on 18.04.2019

Petr Cakl

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Abstract

Adaptation can be explained as a response of animal species to parameters of the environment in which they live. Salamander (*Caudata*, Scopoli, 1777) species can respond with high phenotypic plasticity to changes in the environment and exhibit a wide variety of adaptations which help them increase the chance of survival. One of the most exciting traits which are displayed by several Salamander species is either cryptic or aposematic body coloration. We established an experiment with artificial clay models resembling Alpine newts, which we divided into three categories according to different color type: Black, black/orange, and orange. In July 2016 and 2017 we randomly spread out 60 individuals from each color type (total 180) to test whether aposematic coloration has a direct effect on predation and predator composition. The testing locality was established at the north of the Czech Republic, within Mělník county, in the mixed coniferous forest with several water bodies. Results showed that aposematic individuals were attacked less, and by a different predator than individuals with cryptic coloration.

Keywords: Phenotypic plasticity, Adaptation, *Ichthyosaura alpestris*,

Abstrakt

Adaptaci můžeme vysvětlit jako reakci živého organizmu na parametry prostředí, ve kterém žijí. Zástupci řádu ocasatých obojživelníků (*Caudata*, Scopoli, 1777) jsou schopni reagovat s vysokou, fenotypickou plasticitou na změny v jejich habitatu. Vykazují širokou škálu adaptací, které jim pomáhají zvyšovat šanci na přežití. Jedním z nejzajímavějších rysů, které vykazují některé druhy ocasatých obojživelníků je kryptické nebo aposematické zbarvení těla. Vytvořili jsme experiment s umělými hliněnými modely připomínající Čolka horského, které byly rozděleny do tří kategorií: Černá, černá/ oranžová a oranžová. V červenci, v letech 2016 a 2017 jsme náhodně rozmístili 60 modelů od každé barevné varianty (celkem 180), abychom otestovali, zda mělo zbarvení vliv na dravce, respektive druh predátora. Testovací lokalita se nacházela na severu České republiky v okrese Mělník, ve smíšeném lese s několika umělými vodními tělesy. Výsledky ukázaly, že aposematicky zbarvení jedinci byli atakováni méně, a jiným typem predátora, než jedinci se zbarvením kryptickým.

Klíčová slova: Fenotypická plasticita, Adaptace, *Ichthyosaura alpestris*,

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1. Introduction

During its lifespan, the organism is continuously forced to respond to many variable ecological parameters within its environment. One factor that is difficult to adapt due to its biological nature is predation (Ruxton et al. 2004). Successful predation leads to the killing of the prey, transferred to its reproductive failure (Bengtson 2002). From the evolutionary point of view, predation affects more prey than the predator (Dawkins et Krebs 1979). Unsuccessful predation is essential for anti-predatory adaptations (Vermeij 2002), which can be defined as the long-term response of the organism to the influence of ambient selection pressures. Determining specific environmental pressures is possible through studies that focus their interest on the impact of specific components of the habitat where the individual exists (Luiselli et al., 2005). Some authors describe the predatory pressure as the primary selective force influencing the morphological and behavioral traits of animals (Lima et Dill 1990). Generally, the prey can respond evolutionally to predatory pressure by predator avoidance mechanism, or by reducing the possibility of successful predation, anti-predatory mechanism. Both of these mechanisms fall under different selective regimes, and the evolution of one type of survival mechanism means decreasing selection for the other (Brodie et al., 1991).

Most amphibians do not live long enough to become sexually mature, and if they live at this stage, many of them do not live long enough to produce offspring (Alford et Richards 1999). The size, soft tissue, slow motion, and the absence of significant defensive structures are amphibian specific parameters that reduce overall ability to resist predatory pressure. These specifics may be the reason why amphibians effectively use a whole range of behavioral and physiological defense mechanisms that increase their ability to withstand predatory pressure, Fig. 1. Amphibians are more likely to use a type of strategy that reduces the likelihood of direct interaction with the predator.

Before attack itself predator asses the prey with a purpose to choose the most efficient strategy. Functional assessment of the prey is crucial for successful predation, killing and eating eatable prey leads predator to attack similar kind of prey because of its morphology and coloring (Barrett 2005). In a case where predator encounters a specific type of prey for the first time, aposematic signalization can help him with identification, therefore efficient attack strategy.

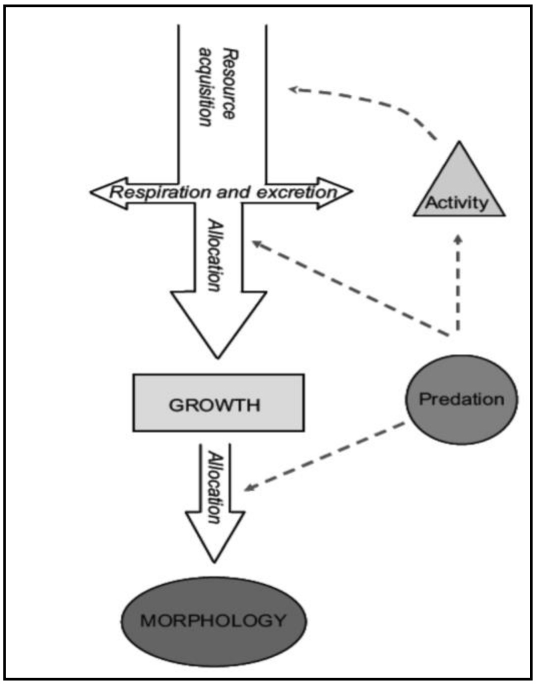

Fig. 1. Diagram showing energy distribution and direct/indirect (dashed line) impact of the predatory risk on prey morphology. Adapted from (Bourdeau et Johansson 2012).

Aposematism can be explained as a contrast coloring on an epidermal layer of an organism which is informing its surroundings about their potential unpalatable or poison state (Santos et al. 2003). Aposematic signalization often acts synergistically as an anti-predator mechanism, and during the mating process.

The aposematic color signals are mostly combinations of red, orange or yellow (rarely white) with black less often with dark brown or blue. The combination of all three or four colors is not excluded (Komárek, 2004). These combinations increase the external contrast of the individual, which improves signal efficiency while helping to accelerate the predator's education process (Aronsson et Stille-Bamberale, 2009). Simple, more memorable patterns such as strips and spots are commonly known as signals that prevent individuals from re-attacking by the predator (Stevens et Ruxton 2012). Nevertheless, the meaning of colors and patterns must be understood in the broader sense. When assessing the effective aposematic coloration, we must take into account the ambient light conditions (aquatic, a shade of the rainforest, desert). The visual perception of colors by the particular predator. The significance of the distance from which the individual is detected. The color contrast can be perceived within an individual or against a particular type of background. Hegna et al. 2011 conducted a field experiment with 840 clay models of poison dart frogs to find out whether contrasting, aposematic color patterns can affect predation rates. They find out that is not the contrasting pattern that affects predation rates, butinsteadr on which background aposematic individual appears. Thus, the perception of cryptic or aposematic patterns can be depending on distance and background.

We can establish two fundamental theories for retaining aposematic coloration by species in case of predatory pressure. Reluctance to attack more conspicuous prey than predator encountered before and faster future recognition of the prey (Järvi et al. 1981, Leimar et al. 1986).

The type of aposematic defense depends on the preferences of individual species and a type of predator (Komárek 2004). Aposematic species are often distributed across smaller isolated habitats, where they tend to form smaller sub-populations (Lee et Speed 2010). It is likely that aposematic coloring and chemical defense requires specific food sources (Lee et al. 2011). The carotenoid pigments are responsible for many examples of aposematic colors such as: red, orange and yellow (Macedonia et al., 2000). Many food sources providing bright aposematic coloring are poisonous. Therefore, they are usable by amphibian species only because of specific antioxidant and immune processes (McGraw et Ardia 2003, King et al., 2012). If the individual is in a resource-restricted environment, and predatory pressure from mammals (color blindness, night activity) prevails, aposematic signaling may be a disadvantageous strategy.

Many amphibians are enhancing the efficacy of aposematic signalization via warning positions, accompanied by decreased activity and increased skin secretion (Brodie et Gibson 1969, Bajger 1980). Some species combine the cryptic dorsal coloration of the body with an aposematic ventral coloring, which, when confronted with a predator, exhibits (e.g., Unken reflex) (Brodie 1977). Aposematic signaling is often accompanied by secondary defense mechanisms (granular gland secretion). In general, we can divide the secrets as unpalatable or toxic. Unpalatable secrets repulse the predator, and effects of toxic secrets can kill the predator (Brodie 1983).

Selection pressures unrelated to predation (temperature, UV-B radiation, genetic differences) may cause changes in aposematic signaling, especially in smaller subpopulations (Gomez et Théry 2004, Mochida 2010). Amphibians can cope with these various pressures by optimizing trade-offs between coloring and activity. It is possible that a higher rate of activity associated with broader food selection could be one of the determining factors of the evolution of aposematic signaling? Contradictory selection pressures can select opposing defense strategies

(García et al. 2003). The food composition of aposematic amphibians is considerably more diverse than in cryptic species (Santos et al., 2003; Anthony et al., 2008). Higher activity of the aposematic amphibians and the associated migration tendencies reduce the likelihood of predator generalization and thus maintain the advantage of the aposematic signaling (Lee et Speed 2010). The form and extent of generalization are essential for both, the development of aposematic signaling and the development of mimics (Ruxton et al., 2008). The mimetic individual mimics the aposematic species and increases its chance of survival when confronted with a visually oriented predator (Brodie et Brodie 1980).

The advantage of this strategy is conditioned by the earlier experience of the predator with a truly aposematic individual rather than a mimetic individual. E.g., individuals of *Pseudotriton ruber* (Latreille, 1801) mimics larvae of different salamander species *Notophthalmus viridescens* (Rafinesque, 1820), which investigated the positive effect on the reduction of the predatory risk in *Pseudotriton ruber* individuals (Howard et Brodie 1973). Similar results were obtained with *N. viridescens* larvae and the species of *Plethodon cinereus* (Green,1818).

In specific amphibian populations, the aposematic coloration can be selected by sex selection (Sheldon et al. 2003). Amphibians are therefore able to take specific forms of assessment of selective pressures. They can then use that type of strategy, which is most advantageous at the time. Choosing aposematic strategy also has negative impacts. A colorblind or inexperienced predator can easily attack a conspicuous individual in the environment. However, it suggests that the benefits resulting from the use of conspicuous colors are not only associated with predation.

Understanding the evolution of the aposematic signals requires a focus on their diversity, Fig. 2: Examples of aposematic and cryptic coloration within *Caudata* species. The variety of the aposematic signals leads to the question of what makes the aposematic signal effective in the given environment. Some individuals from species *Cynops pyrrhogaster* (Boie, 1826) which inhabit isolated islands, displays more aposematic patterns than those, individuals from the same species, on the mainland Mochida (2009). The visually-oriented predators such as birds are more frequent on the islands, while mostly colorblind mammals predominate in the inland. The similarities in the aposematic pattern can be explained as a result of the combination of the predatory pressure from the particular predator type in a specific environment with several variable parameters (Mochida 2011).

Cryptic coloring, morphology or its combination is a very effective way to prevent detection by predators who are orientated mostly by vision (Vitt et Caldwell 2009, Mochida 2010). A cryptic individual tries to blend into the background on which it is predominantly occupying. The nature of the cryptic pattern may differ geographically, even within different populations of the same species (Wang et Shaffer 2008, Mochida 2009, Mochida 2011). The type of coloration of the same species may vary even within different altitudes (García et al. 2003). When we assume that the individual is placed in a relatively homogeneous environment, it must be able to react to background changes depending on light conditions during the day or season. The optimal strategy, in this case, is a certain degree of cryptic coloration that fits a set of various backgrounds (Ruxton et al. 2004).

The coloring may not be impeccable, but it must be effective at the distance to which the appropriate predator usually detects the individual. The rate of uniformity of cryptic patterns may be related to the rate of predation pressure in a particular environment (Endler 1980). Therefore, the variety of cryptic pattern should decrease on individuals occurring on a more uniform background.

An essential part of the cryptic strategy used by amphibians is disruptive coloration (Ruxton et al. 2004). The significance of this type of coloration remains the same, to resemble the colors of the background to minimize the risk of detection by the potential predator, e.g., *Afrixalus fornasini*, (Bianconi, 1849). The main difference over cryptic coloring is in a variety of patterns of stains, streaks, bands or dots that blend with a more varied background and make it even more difficult for the predator to identify the prey. A more significant number of patterns all over the body distorts the outline of an individual. Therefore, the predator is not able to accurately focus his attack (Vitt et Caldwell 2009)

Many amphibian species add various morphological features to the cryptic coloration. E.g., head grooves, jagged bands, ridges along the outer edges of the limbs and a large number of different types of warts help to make it difficult for effective predator attack (Vitt et Caldwell 2009). Other forms of cryptic signaling include those that contribute to escape predator detection by affecting other perceptual organs than vision, e.g., sound and olfactory signals (Ruxton 2009). What kind of amphibian species generally use cryptic strategy more often than aposematic? We can imagine an individual inhabiting a habitat limited by a particular predator structure, e.g., other than visually oriented, which leads to lower activity and narrower food niche. Animals using cryptic strategies are often limited in some ways, e.g., limited activity since motion acts antagonistically with the cryptic coloration (Stevens et al. 2012).

Direct amphibian anti-predatory mechanisms involve many of stereotypical behavioral mechanisms. Such as immobile positions, various twisting and shaking movements including aposematic patterns on specific parts of the body (Brodie et Gibson 1969).

Fig. 2. Examples of aposematic and cryptic coloration within *Caudata* species. a)The aposematic coloration of *Pseudotriton ruber*, Van Laar, L. (2014); b) cryptic coloration of the ventral side of the body with contrasting aposematic coloration of the abdominal side of the body in *Taricha rivularis*, Ghegenbart (2006); c) uniform cryptic coloration of *Salamandra atra*, Huntke T. (2008); d) yellow aposematic spots on the black background of the body of *Salamandra salamandra*, Descouens D. (2012).

1.1. *Caudata*

Caudata accountss for about 9% of all species of amphibians on the planet. The clade of *Caudata* contains as of April 2019, 728 species (Amphibia Web 2019), which are spread mainly in the temperate and subtropical zone of the northern hemisphere. Part of the species is also inhabited by tropical regions of Asia, Central, and South America.

The most abundant species representation is found in North America, which is inhabited by representatives of eight families. The most numerous of all families are the *Plethodontidae*; by April 2019, this family has over 473 species (AmphibiaWeb 2019).

They occur in aquatic, semi-aquatic and terrestrial environments (Vitt et Caldwell 2009). Some species of *Plethodontidae*, *Bolitoglossidae*, and *Aneides* are well adapted to life on the trees. *Plethodon* and *Hydromantes* show adaptations suitable for climbing on the stone surface (Jaekel et Wake 2007). Characteristic features of *Caudata* consist of a long tail, thin body with four short legs. Morphological specifics of the clade vary across developmental stages and families. From smaller individuals of *Plethodontidae* 3 cm, *Rhyacotritonidae* 8 - 10 cm, medium-sized *Salamandridae* 7 - 30 cm up to the family where the length can reach or exceed values 1 m, such as *Amphiumidae* and *Cryptobranchidae*. The shape of the body differs from family to family. The salamanders with a robust body, limbs and the short, round head can be found in the family *Ambystomatidae* (Vitt et Caldwell 2009). Individuals of elongated, snake-like shape, with significantly reduced limbs of the family *Amphiumidae* (Dodd 2010). The *Cryptobranchidae* family includes the world's most giant living amphibians (up to 1.8 m) with large folds of skin along the sides of the body. These folds increase the surface of the skin for better oxygen absorption, due to the absence of gills and small almost nonfunctional lungs, and the skin is the exclusive respiratory organ (Ultsch 2012).

The way that of receiving oxygen differs depending on the presence/absence of lungs or gills. They can be internal, external (e.g., *Necturus* and *Siren* sp.) or in the form of gill slits (e.g., *Necturus* and *Siren* sp.). Individuals from *Plethodontidae* family lack a set of lungs and breathing is allowed by the skin and the tissue lining in the oral cavity (Cogger et al. 1998).
The activity of tailed amphibians is predominantly nocturnal and is associated with a search for a food source, and in the reproduction period with mating (Vitt et Caldwell 2009, Denöel et Doellen 2010).

During their terrestrial phase, adult individuals usually use the cover of stones, wood, vegetation, and underground burrows. While in their aquatic they are found in the smaller, streams with low torrents and natural or artificial water reservoirs, where they use stones and aquatic vegetation (Arnold et Ovenden 2002).

We have previously stated that over the lifespan, *Caudata* species alternate between two life stages: water (breeding) and terrestrial. For longer migrations, *Caudata* species use the geomagnetic compass (Diego-Rasilla et al., 2008). At shorter distances, they use to orientate the stimulus in the form of the olfactory signals surrounding the reproduction site (Malmgren 2002, Sinsch et al., 2006, Mathis et Unger 2012).

Species which alternate between water and terrestrial environment display gender and interspecies differences in he ttiming of migration to reproduction sites (Wells 2010). These differences are tailored to maximize reproductive success (Semlitsch 2008). Males often come to reproduction sites earlier than females (Semlitsch et al., 1993). The early arrival of males increases their mating opportunity. The late arrival of females increases the choice of a suitable partner (Douglas 1979). Another possible reason for the late arrival of the female individuals is a broader level of food sources for juvenile individuals (Harris 1980). In temperate climate adults usually correspond to their seasonal activities associated with reproduction, i.e., migrating to aquatic environments where courtship takes place, then mating and laying eggs.

The reproductive behavior of most male amphibians is complicated and includes a variety of elements, e.g., engagement dance, amplexus (Verrell et Mabry 2003, Wilczynski et Lynch 2011). For successful mating under adverse light conditions, when visual communication is prevented, male individuals instead prefer to find a female with the help of olfactory signals (Denöel et Doellen 2010).

The eggs are placed in large clumps into the water, vegetation partially immersed in water or directly on the wet soil, which is flooded after during spring (Vitt et Caldwell 2009, LeGros 2012). Terrestrial representatives of *Caudata*, e.g., *plethodontidae* reproduce in terrestrial part of the habitat and eggs are placed directly on the ground or in cavities of trees and rock formations with sufficient humidity (Chippindale et al. 2004, Ringia et Lips 2007).

Some female individuals of species, e.g., *Taricha granulosa* (Skilton, 1849), *Ambystoma maculatum* (Shaw, 1802) choose the laying site depending on the presence of a potential predator that could endanger juveniles (Yurewicz 2004, Gall et al., 2012). Female individuals of *Ichthyosaura alpestris* choose upon temperature conditions of a particular microhabitat (Dvorak et Gvodik 2010). Numerous *Caudata* representatives are displaying nursing care (Croshaw et Scott 2005). Female species of the family *Ambystomatidae* and *Plethodontidae* form a terrestrial nest, where they protect the eggs with their bodies until hatching (Jenkins et al., 2006; Trauth et al. 2006). This method of protecting eggs can serve as a defensive mechanism against predation or microbes (Trauth et al. 2006) and increases the chance of hatching eggs (Croshaw et al. 2005).

Male individuals of the family *Cryptobranchidae* protect the nest in the water nest until hatching (O'Shea et Halliday 2001). Adult individuals of several species show loyalty to specific reproduction sites (a few square meters) (Bonato et Fracasso 2003, Semlitsch 2008). The evolution of this behavior can be attributed to the use of a favorable and relatively stable water reservoir in a known location rather than a search for a new one (Semlitsch 2008).

The development of an individual begins with the aquatic larval stage, which changes after metamorphosis into an adult capable of terrestrial life. Direct development without the larval stage is widespread among species that give birth to a smaller number of fully metamorphosed juveniles (e.g., *Plethodontidae*, *Salamandridae*, *Ambystomatidae*) (García-París et al. 2003, Velo-Anto'n et al. 2007, Pough 2007).

Juvenile individuals are morphologically similar to adults. The main differences are in the body size and excess or absence of specific physiological structures. In some species, juveniles are characterized by the presence of external gills and tail fin, the absence of eyelids, specific jaw structures and possibly less developed limbs (Davit-Béal et al., 2007, Crump 2009). The food composition of juveniles predominantly consists of arthropods, ringworms, diatoms, insect larvae, e.g., *Chironomidae* and *Odonata*, algae, and debris (Pough 2007, Schriever et Williams 2013). Species that alternate between aquatic and terrestrial environment on the regular bases depend not only on the characteristics of the breeding pond (Jakob et al., 2003) but also on a suitable terrestrial habitat (Todd et al., 2009). The movement of amphibians on land is predominantly related to the search for terrestrial shelters (temporary migration), food search or dispersion (Bar-David et al., 2007). Juveniles choose migratory corridors unintentionally, the selection is likely to be related to species preferences for the environment and age of individuals (Dodd et Cade 1998, Malmgren 2002, Walston et Mullin 2008).

2. Objectives

Several species of newts are distributed across Europe. Some have dull-colored abdomen *Triturus vulgaris*, others display aposematic coloration with a different kind of spotted pattern *Triturus cristatus*, and some species display aposematic coloration without spots or stains *Triturus alpestris*.

We focused on the *Triturus alpestris* (Laurenti, 1768), which has an abdominal side colored with shades of yellow to fiery red.

Coloring of the abdominal side of the body may have two functions:
1. Sexual selection, males have a brighter coloring at abdominal side than females.
2. Defensive, it should protect individuals from predators.

In this study, we will focus on the pros and cons of the aposematic signalization in terms of predation, and or defensive behavior. We set up a field experiment with artificial clay models resembling Alpine newts to record which type of predator is most common in the given environment. Furthermore, we will test which type of color is more advantageous against mammal respectively bird predator.

3. Materials and methods

3. 1. Locality

We established the testing locality in Northern Bohemia, 40 km north of Prague, municipality Jeviněves (Latitude: 50.353457, Longitude: 14.330578), Fig. 4. The average annual temperature 9 – 10 °C and the average annual rainfall 450 – 500 mm (Czech Hydrometeorological Institute 2017). Locality consists mainly of the mixed coniferous forest with two artificial water bodies. As a testing area, we set up a square 265 x 265 meter wide Fig. 3. Which gave us in total 70 225 m^2 of the testing area, and we spread out randomly 180 individuals from each color variation. Each artificial model covered approximately 390 m^2. We labeled testing locality with white flags which we put upon the trees.

Fig. 3. Areal photogrph of the testing site with experimental grid (50°21'10.7"N 14°19'44.6"E). Retrieved and modified from https://www.google.cz/maps/.

There are no records of Alpine newt presence in this are. Nevertheless, there are records of Alpine newts in Mělník county. It is likely that predators found at the test site have never encountered with the color combination displayed by the Alpine newt. Furthermore, they have no experience with the given prey.

Significant Salamander species: *Salamandra salamandra* (Linnaeus, 1758) (own observation).

Significant bird predators: *Buteo buteo* (Linnaeus, 1758), *Dendrocopos major* (Linnaeus, 1758) (own observation), *Chroicocephalus ridibundus* (Linnaeus, 1766), *Garrulus glandarius* (Linnaeus, 1758) (own observation), *Phasianus colchicus* (Linnaeus, 1758) (own observation, Pica pica (Linnaeus, 1758), *Picus viridis* (Linnaeus, 1758) (own observation), *Pernis apivorus* (Linnaeus, 1758), *Falco subbuteo* (Linnaeus, 1758), *Falco tinnunculus* (Linnaeus, 1758) (own observation). The species which were not observed personally are listed at the Atlas of breeding distribution of birds in the Czech Republic website (http://birds.cz/avif/atlas_karta.php?ctverec=5652ca).

Significant mammal predators: *Martes martes* (Linnaeus, 1758) (own observation), *Mustela nivalis* (Linnaeus, 1766) (own observation), *Vulpes vulpes* (Linnaeus, 1758) (own observation),

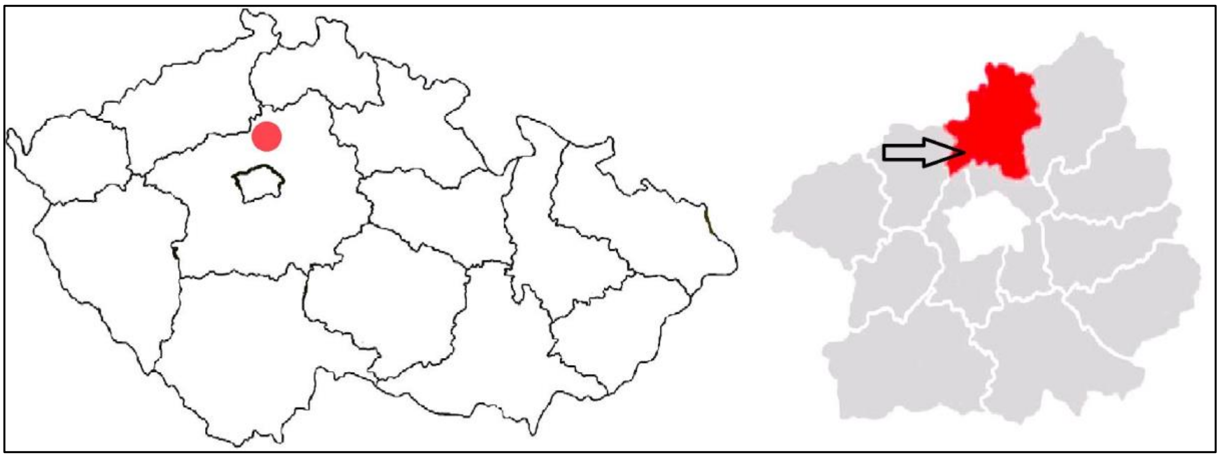

Fig. 4. Position of testing locality Jeviněves in Mělník county (50.3451° N, 14.3381° E). Created in Adobe Photoshop by Petr Cakl (2019).

3. 2. Target species

Alpine newt, *Mesotriton alpestris* (Laurenti, 1768)

Fig. 5. Male Alpine newt, *Ichthyosaura alpestris*, Bartz R. (2008)

3.2.1. Diagnosis

The Alpine Crested Newt Fig. 5., is an amniotic poikilothermic four-legged vertebrate, an amphibian (*Caudata*). During life, it alternates between two phases: water - for reproduction, and land. While in water phase males differ from other *Caudata* species, in the Czech Republic, by distinguishable bluish to blue coloring. Also, during the water phase males have on their back yellow colored crest with black spots. Females are colored at this time with prevailing green color combined with dark gray and brown shades. They both sexes also have uniformly orange-colored whole abdominal part of the body, which is mostly without spots or stains. The head is smooth, free from grooves, the body has an oval shape on an imaginary cross-section. The length of the body belongs to the middle-aged members of the order. Females usually do not exceed the length of 12 cm, male 9 cm.

The larvae are without significant pigmentation after hatching from the egg. With gradual development is a noticeable increase in the dark pigment. Coloring of larvae is usually in shades of ocher to dark brown. The fin hem is marbled.

3.2.2. Distribution within the Czech Republic

The occurrence of the Alpine newt is mainly linked to higher altitudesand largee forest complexes (Roček 1992). It mostly occurs at heights above 400 m a.s.l. Its occurrence below this limit is unique and is bound to the forests, mainly in the Hradec Králové, Mělník and Děčín areas (Mikátová et Vlašín, 1998). Below the altitude of 400 m a.s.l. and outside of the extensive forest complexes is the Alpine newt found mainly in headwaters areas or inverted valleys (Roček 1992).

3.2.3. Defensive behavior

At the time of the terrestrial phase, the most common defense mechanism is immobile position, which protects the newts from predators reacting mainly to movement. This behavior contrasts significantly with wildflicking iff we capture a newt at the time of the water phase (Brodie 1977, Griffiths 1996). The passive element of defense in females is the cryptic coloring of the ventral side of the body at the water phase. In the terrestrial phase both sexes display cryptic signals from ventral sides. Also, aposematic coloring abdominal sides of the body with shades of yellow to fiery red, which contrast sharply with thedark-coloredd ventral side. To emphasize it, when threatened, Alpine newts rarely show off Unken reflex. It is a position in which the body twists to the side, slightly raising its head and curled its tail (Griffiths 1996, own observation). An active element of the defense of the newt is trying to wriggle out of grip, rarely trying to bite an attacker and probably even vocal signaling, reminiscent of the faintly sounding liberating voice of frogs (own observation). Anessentialt role in protecting newts against predators plays secretion of aromatic scents, chemically based on alkaloids. They are excreted from granular skin glands evenly distributed over the body surface (Rehák 1992, Griffiths 1996). The contraction of glandular muscle cells secretes the toxins. To a greater extent, this happens when an individual is at risk. These toxins cause, when prolonged contact with human skin, redness of the affected area, slight swelling and slight burning. The experimental exposure of mountain newt larvae predation to dragonfly larvae has led to more pronounced dark pigmentation, relative head enlargement, and relative tail enlargement, in comparison with newt larvae that were not exposed to predation (Van Buskirk et Schmidt 2000).

3. 3. Models

Models were made from black and orange non-toxic polymer FIMO clay fabricated by Staedtler company (STAEDTLER Mars GmbH & Co. KG). FIMO conforms to the European Standard EN 71 part 5 and bears the ACMI-Seal AP nontoxic. Models were made by hand individually, with the help of basic sculptural tools. Nevertheless, we tried to make all individuals identica. Alsoo we tried to create the models look similar to our target genus *Ichthyosaura*. SVL (Snout-Vent Length) was 120 mm; the average width was 13 mm. Models were made in three color variations: black, orange, black/orange (dorsal = black; ventral = orange). The color hue of orange-colored models was matching as much as possible the color of the animals in the wild. We chose the uniform black color as a compromise between the shades of dark colors often finds on the dorsal part of the newt body. The total number of models were 180. 60 Individuals from each color variation Fig. 6.

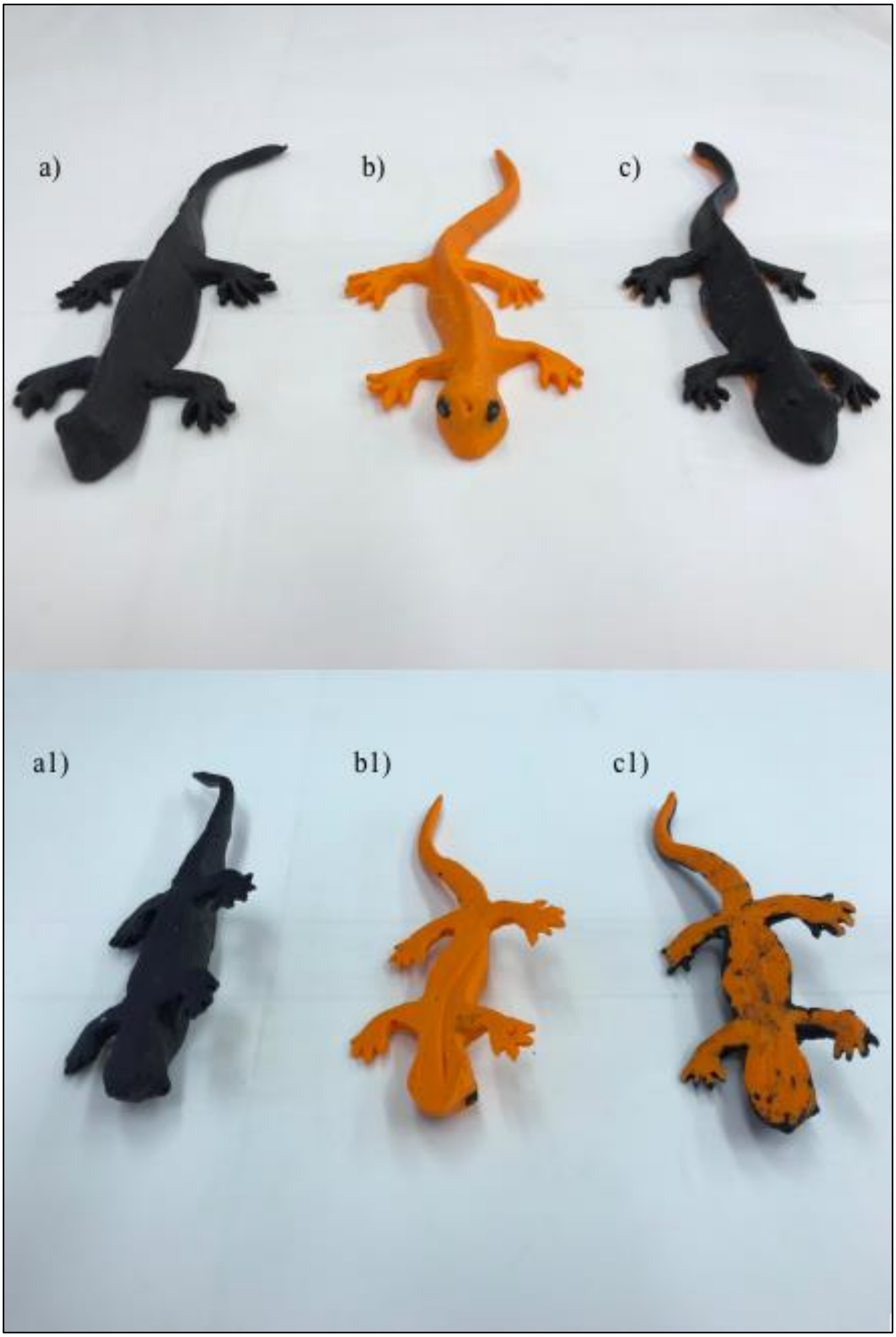

Fig. 6. Models used in our study from both sides; a) black model type, b) orange model type, c) black/orange model type.

After finishing the modeling process, we placed models into an electric oven (MORA VT 536 BX). According to the manual from manufacturer FIMO needs about 30 minutes at 130 °C to harden it. From our previous experience in that point, the surface was for predators hard to penetrate. Therefore, it was difficult to distinguish any marks after a predatory interaction. We had to change the time and the temperature to 20 minutes at 110 °C. This ensured that models were more penetrable. Thus, we were able to classify the type of the injury according to a particular type of a predator (mammal/bird).

Before placing the models, we scouted the area for human paths and or any influence that could be disruptive for its natural state. Each model was placed randomly into a particular square. Within given square we found an ideal place for placing the model, then we blindly grab a model from a bag with all models combined and strap it to its fixed location. While strapping models to a specific location we used plastic self-binding straps to prevent moving of the particular model. FIMO clay allowed some extent of movement of the model but prevented complete removal by a predator. Some models were strapped to vegetation or dead-wood to pose more naturally. Each attachment point of the self-binding strap was carefully masked to prevent color disruption of the whole model. The placement of each particular model was labeled with the yellow flag for re-capturing. In 2016 we placed the modelsforg one day on July 7th. In 2017 we placed models on July 10th. We removed models after seven days of screening in both years. All plastic straps and all the models were removed, to reduce any ecological trace we could leave at the testing locality, excluding missing individuals. Missing individuals were not included in statistical analysis.

4. Results

Each model was assessed individually for any marks after predation. After evaluating predatory marks, we decided to divide our analysis into two categories. One with bird-like predatory marks (U, V shapes) and one with mammal-like predatory marks (teeth imprints, claw imprints) Fig. 7 - 9.

Fig. 7. Example of mammal predator interaction.

Fig. 8. Example of bird predator interaction.

Fig. 9. Example of mammal and bird predator interaction.

4. 1. Statistical analysis

Overall, 360 models were placed in July 2016 and 2017 (60 from each of the three color variations). Years did not differ among the number of models with "hit" ($\chi^2 = 2.07$, $P = 0.15$). Altogether 269 models (74.72%) were re-captured with traces of manipulation in both years, which is more than the average number of models ($\chi^2 = 88.01$, $P < 0.001$). This means that newt models were found in the field by predators and that they showed interest in them.

If we look at the distribution of the types of predator attacks, then most were purely mammalian interactions 133, followed by solely bird interactions 72. The remaining number of models were without injury or were not recaptured. Some attacks could not be unambiguously assigned to a bird / mammalian predator. It could also be a combined attack of both predators on one model. Therefore, B (both possibilities) attacks can be assigned to both bird and mammalian predators. That is why we have calculated the birds + both possibilities, and it has been shown that the results copy the solely bird interaction results Tab. 1., when again the least popular were the orange models.

However, in the case of merging the results of mammals with B (both possibilities) values, the results are not significant Tab. 2. In particular, there is a different number of attacks between years on solely orange models Fig. 10 - 11.

We used ANOVA with the interaction of two factors (color * year) with binomial distribution, i.e., GLM model.

For our statistical analysis we used STATISTICA™ software (StatSoft 2012), version 12.

The charts were modified in MS Excel® and Adobe Photoshop®.

effect	degree of freedom	Chi-x2	p
year	1	0,01345	0,907
color	2	63,64981	0,000
year*color	2	1,38771	0,499

Tab. 1. Avian interactions + Both possibilities

effect	degree of freedom	Chi-x2	p
year	1	0,098	0,753
color	2	0,523	0,769
year*color	2	1,459	0,482

Tab. 2. Mammal interactions + Both possibilities

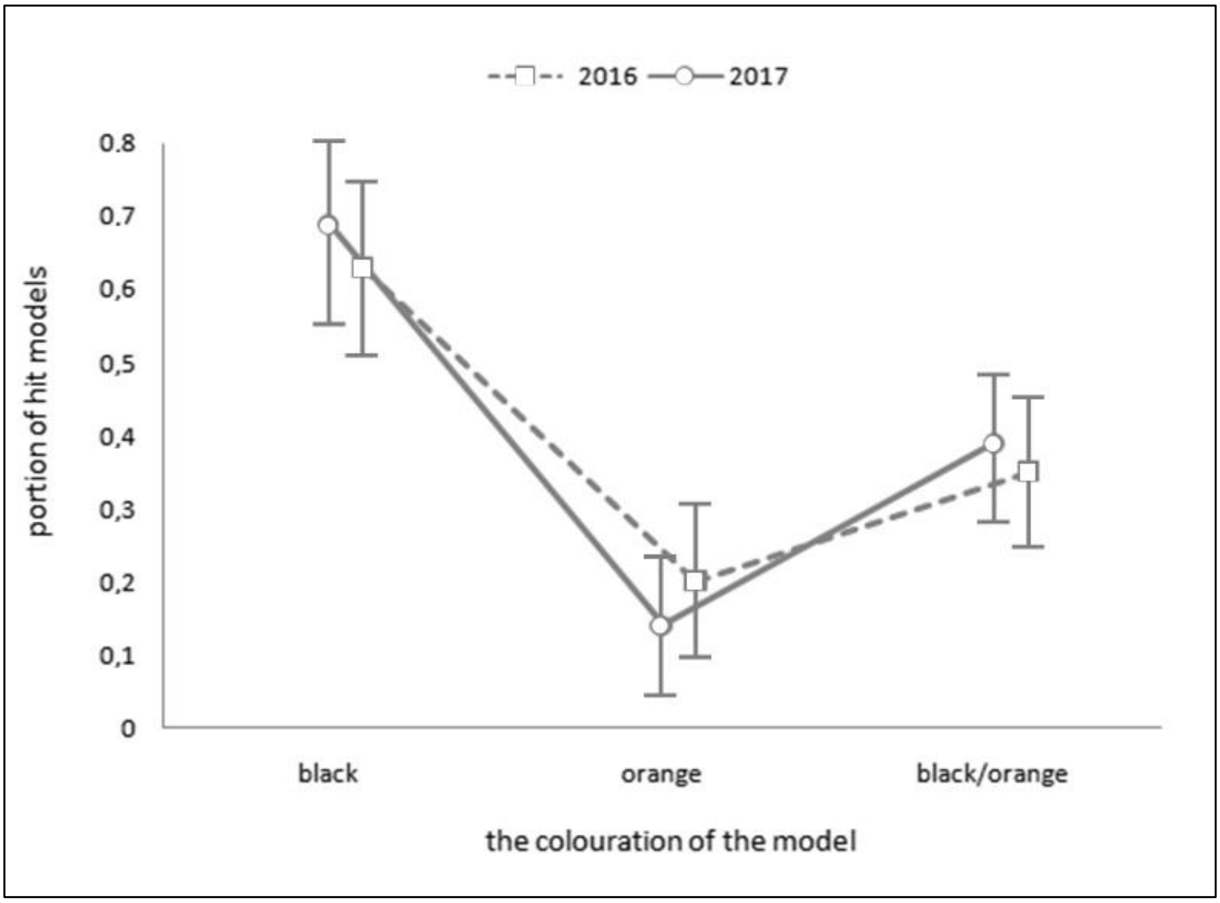

Fig. 10. Portion of bird interactions + both possibilities in years 2016 (dashed line) and 2017 with confidence intervals.

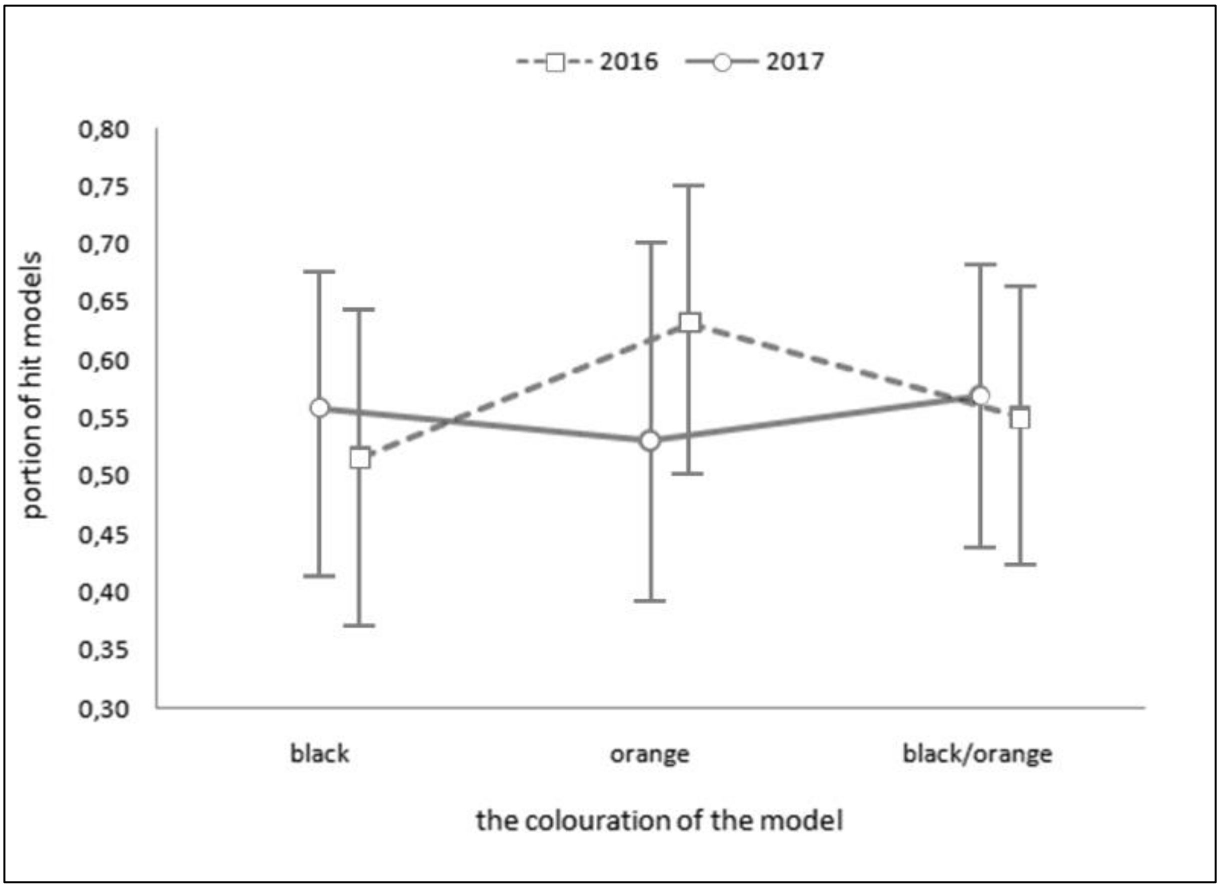

Fig. 11. Portion of mammal interactions + both possibilities in years 2016 (dashed line) and 2017 with confidence intervals.

5. Discussion

It is possible, that the original defensive function of warning coloration holds another function in some species. The transition from aposematic to cryptic coloring and vice versa is not an unusual phenomenon and it requires a substantial change in the way of using habitat e.g., (Ruxton et Sherratt 2006, Przeczek et al. 2008). What leads newts and salamanders to such behavioral change? We created simplified model, focusing on specific adaptation component. The output of this study could partially determinate function of aposematic display in newt species.

We tested whether there is a connection between coloration of an Alpine newt individual and predatory composition, respectively which color attracts which type of predator. We established a field experiment consisting of 180 artificial individuals resembling newt species. Models were made in three color options orange, black and black/orange, 60 individuals from each color category. We set up a 265 x 265 m square and randomly placed all 180 individuals. After seven days long testing period we collected and assessed models for predation marks. We conducted two screenings in July 2016 and July 2017.

According to predation marks caused by mammal predators, we find out there is no significant connection between specific color type and the number of attacks from mammal predators. All three color types were attacked with a similar pattern in both screenings.

We can assume that orange (aposematic) color has a direct effect on visually oriented predators, such as bird species. It seems that mostly colorblind mammals had no preferable color type within our color variations.

Alpine newt combines cryptic and aposematic coloration. Cryptic from the ventral side and aposematic from the abdominal side. Aposematic coloration is advantageous when considering bird predators, but it can be highly disadvantageous in terms of mammal predation. It is probable that the development of aposematic coloration of Alpine newt goes hand in hand with the development of behavioral reaction which increases its effect.

Only a few authors conducted similar field experiment as we did. One of the main differences is a number of used model organisms and environment which they used as a testing site.

For example, Hegna et al. 2011 used 840 clay models of poison dart frogs in Costa Rica. That is almost five times more than we used in our study. Also, the environment of the rain forest of Costa Rica plays a crucial role in terms of enhanced environmental pressures, in this case, predation, which leads us to question. Which variables of the environment would significantly alter our results? Is it just a number of used model organisms and environment, which would make a difference? Alternatively, should we pay more attention to the color composition of models, aposematic patterns (spots, stains) and background on which we would place them? Future field experiment should be conducted in an area with predators which already came across this type of aposematic prey to obtain more significant results. This study should serve as a guideline for using this type of model organism in geographical conditions of the Czech Republic.

6. Conclusion

We found out that color plays a significant role in predatory interactions. Many more screenings have to be made to find out a more definite pattern of aposematic signalization within our geographical conditions. Furthermore, we need to raise the total number of artificial models, work with new design possibilities (3-D print), apply other measurable environmental factors, such as wavelength measurements of particular color combination, background color, temperature, humidity, locality of interaction into our future experiments.

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8. Appendix

1st mammal and avian screening July 2016.

Mammals and avians first screening
0 = no interaction
1 = interaction
A = avian
M = Mammal
B = Both possibilites

barva	hit*	hit	barva	hit*	hit
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
1	0	A	1	1	A
2	0	A	2	1	A

2	0	A	2	1	A
2	0	A	2	1	A
2	0	A	2	1	A
2	0	A	2	1	A
3	0	A	3	1	A
3	0	A	3	1	A
3	0	A	3	1	A
3	0	A	3	1	A
3	0	A	3	1	A
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barva	hit*	hit	barva	hit*	hit
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Fig. 12. Model of newt species made by 3-D printer.