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THE EFFECT OF PAINT MARKING ON PREDATION RISK IN WESTERN FENCE LIZARDS: A TEST USING CLAY MODELS

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***Abstract.*—The use of color markings (e.g., non-toxic paint, dye, or pens) is frequently employed by herpetologists to track individuals within a population, but effects of these markings on study animals are generally unknown. Markings could affect survival rates, although this can be difficult to determine through mark-and-recapture methods. With clay models, however, we can quantify interactions with predators and measure predation risk associated with color markings. We used 126 clay models of Western Fence Lizards (*Sceloporus occidentalis*) to examine how marking individuals with colored paint influences predation risk, and to determine whether conspicuous colors enhance risk more than inconspicuous colors. We compared avian attacks on unmarked models to attacks on two treatments: models marked with white nail polish or purple polish. We modeled how these treatments are viewed under an avian visual system and found that white markings exhibited greater achromatic contrast against the clay while purple markings exhibited greater chromatic contrast. Models marked with purple paint received significantly fewer attacks than the control, while the attack rate for the white treatment was similar to that of the control. These results show that purple markings could have positively affected survival rates of marked animals. Conducting experiments on the effects of artificial markings might help researchers minimize negative impacts on their study animals. These studies also suggest that markings may have unintended effects by skewing predation risk, thereby potentially influencing research outcomes.**

Key Words.—avian visual model; experimental design; indirect effects; mortality; *Sceloporus occidentalis*; survival; wildlife ecology

INTRODUCTION

Creating visible and identifiable marks on individual animals is often crucial for collecting behavioral or demographic data, particularly when researchers want to observe and track a subset of the study population. Various marking methods are common in herpetofauna such as passive integrative transponder (PIT) tags (Germano and Williams 1993; Keck 1994; Jemison et al. 1995), branding (Clark 1971; Lewke and Stroud 1974), clipping of the toes, tail, or scales (Brown 1997), and use of colored beads (Fisher and Muth 1989; Galdino et al. 2014), non-toxic paints (Simon and Bissinger 1983; Rodda et al. 1988), or visible implant elastomers (Penney et al. 2001), just to name a few. Markings may negatively affect animals if they cause infection, induce abnormal physiological responses (e.g., elevated glucocorticoids), or impede locomotion (Bloch and Irschick 2004; McCarthy and Parris 2004; Langkilde and Shine 2006). Some research has evaluated the effects of various marking techniques on herpetofauna, and most studies find minimal negative effects (Keck 1994; Arntzen et al. 1999; Ott and Scott 1999; Ginnan et al. 2014). Some techniques, however, such as toe clipping, are controversial as evidence suggests that this method can affect fitness-relevant traits such as body condition, locomotion, and performance (Borges-Landáez and Shine 2003; Bloch and Irschick 2004; Hudson et al. 2017), and toe clipping has been shown to affect survival rates in some lizards (Olivera-Tlahuel et al. 2017). This is problematic not only from an ethical standpoint, but also because these effects could alter research outcomes.

Many marking methods alter the visible appearance of animals, which provides researchers a method of sighting marked individuals without repeated capturing and handling. A common method is the use of paint, tattoos, or dyes, which are applied on the external surface of the animal as numbers, symbols, or codes (Twigg 1975; Brown 1997; Hagler and Jackson 2001). The use of colored tags or bands (e.g., bird leg bands) is also a common marking technique that alters the appearance of animals (Murray and Fuller 2000); however, the colors with which researchers mark their study organisms could have differing impacts based on how they are viewed by conspecifics or predators. Coloration can often serve as a form of inter- and intra-specific communication (Watkins 1997; Siddiqi

2004), and colors and patterns can be used to locate vital resources (Prokopy and Owens 1983; Dominy et al. 2003). Previous research has shown that the colors with which birds are banded can influence mate choice. In a classic study, captive Zebra Finches (*Taeniopygia guttata*) were attracted to red, pink, and black leg bands, but repelled by blue and green (Burley et al. 1982; Burley 1986). Even the shirt colors worn by humans can influence animal behavior (Gould et al. 2004; Putman et al. 2017), potentially producing unintended effects on their fitness. Thus, although colored marks may be considered noninvasive, they may elicit behavioral responses from conspecifics or predators based on how organismal sensory mechanisms are attuned to certain visible properties (Endler 1992).

The application of non-toxic paints and colored beads is commonly used to mark lizards for visual identification (e.g., Rodda et al. 1988; Tokarz et al. 2003; Luiselli et al. 2011). Only a handful of studies have been conducted previously to determine whether the use of paint markings negatively affect lizards, and to our knowledge this work has been solely conducted on phrynosomatid lizards in the genera *Sceloporus* and *Uta*. Survivorship of Eastern Fence Lizards (*Sceloporus undulatus*) was unaffected by paint markings placed dorsally at the base of the tail (Jones and Ferguson 1980). A study evaluating the effects of xylene-based paint pens on Side-blotched Lizards (*Uta stansburiana*), however, found increased mortality and altered sleeping behaviors of marked lizards kept in the laboratory (Boone and Larue 1999). A follow-up study on *Sceloporus* lizards found that a line of xylene-based paint applied on the dorsum did not affect survivorship of Striped Plateau Lizards (*S. virgatus*) housed in an outdoor enclosure (Quinn et al. 2001). Similarly, survivorship of Prairie Lizards (*S. consobrinus*) in captivity was unaffected by abdominal latex-based paint markings (Quinn et al. 2001).

While the presence and chemical base (xylene vs. latex) of paints or pigments may have minimal effects on the survival of lizards, their colors could cause differential effects. In free-ranging animals, conspicuous colors might increase predation risk more than inconspicuous colors because they draw attention to the animal. Previous research on Mountain Spiny Lizards (*Sceloporus jarrovii*), however, found that there was no difference in survivorship between lizards marked dorsally with conspicuous colors (yellow, red, or white) versus inconspicuous colors (dark green, tan, navy blue; Simon and Bissinger 1983). Yet, a study on *Sceloporus undulatus* found that there was an increase in tail loss rates during the year that lizards were marked with orange paint compared to the years they were marked with yellow paint, suggesting that orange paint might have increased predation risk (Jones and Ferguson 1980). Because different colors were used in different years, however, the effect of color could not be decoupled from temporal changes in predation. Despite this previous research, the general effect of the colors used to mark lizards remains unclear because of a lack of robust study designs (e.g., lumping multiple colors into a conspicuous category, and using different colors across years), a lack of knowledge on how colors are perceived by predators, and a lack of investigations into effects beyond survivorship (e.g., non-lethal behavioral and physiological effects).

The use of clay models could be advantageous for studying whether color markings influence predation risk in herpetofauna. Models, made from soft oil-based modeling clay, have been previously used to measure predator interactions in lizards, snakes, and amphibians because predators such as birds and mammals leave distinctive marks in the pliable clay after an attack (e.g., Brodie 1993; Watson et al. 2012; Rehberg-Besler et al. 2015; Bateman et al. 2017). A number of studies have been conducted using clay models to test how predators respond to the natural markings or coloration of animals (e.g., to test predator-deterrent functions of colors and patterns; Husak et al. 2006; Madsen 2016). This method can be easily transferred to study how predators view artificial markings. Using clay models is ideal because this method measures predation risk and not survivorship. Attacks left in clay by predators do not necessarily mean that a live animal would have died from the predator interaction. Determinations of predation risk may provide more information than studies on mortality alone, as predation risk includes both direct (i.e., mortality) and indirect (i.e., non-lethal effects) consequences for prey. Indirect effects can lead to alterations in prey behavior and/or physiology (Preisser et al. 2005; Clinchy et al. 2013).

Because color markings are commonly used to visually identify lizards, we conducted research to determine whether the application of color increases predation risk in lizards, and whether the conspicuousness of the color used positively affects risk. We made clay models of Western Fence Lizards (*Sceloporus occidentalis*) and compared predator attack rates among clay lizard models painted with conspicuous color marks or inconspicuous color marks to unmarked control models. We measured the spectral reflectance of the dorsal scales of live *S. occidentalis*, the model clay, and the color markings and used an avian visual model to determine whether our clay models visually resembled live lizards and how predators might perceive the markings (i.e., how conspicuous the markings are in the eyes of avian predators). We hypothesize that predators respond to conspicuous colors. We predict that the conspicuous paint markings would be associated with increased predator interaction in the form of attack

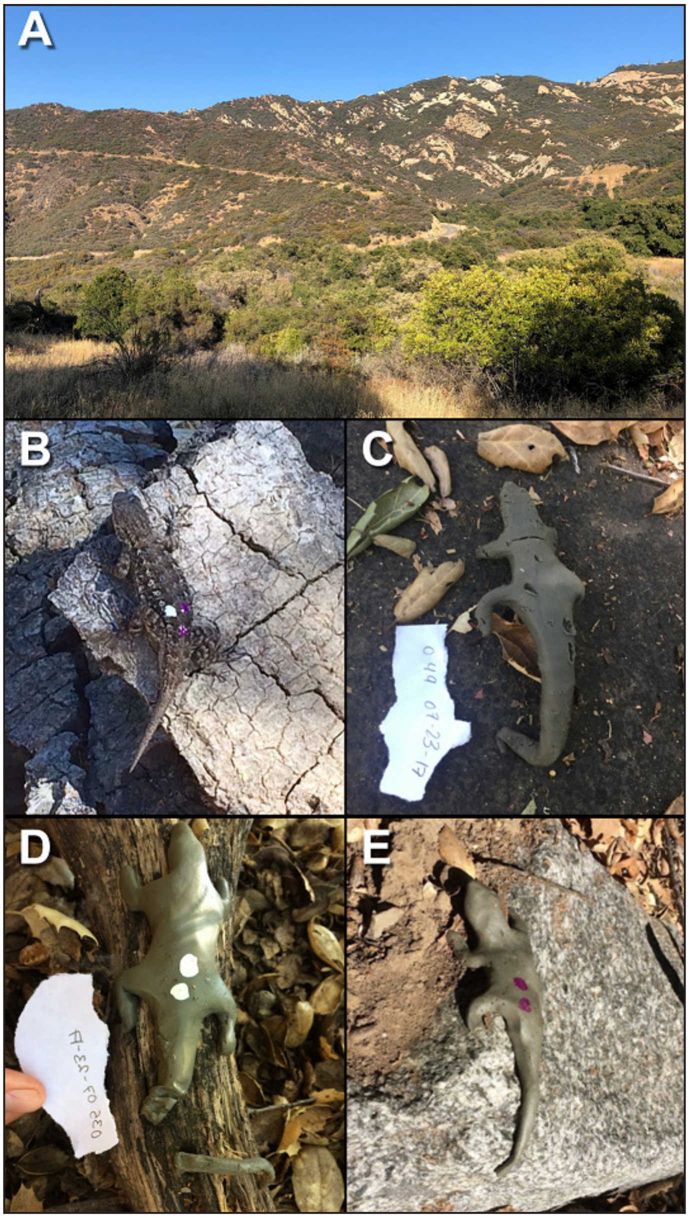

Figure 1. Photographs of the study site and clay lizard models used in this study. (A) The habitat of the study site, which contains a mix of Chaparral, Oak Woodland, and Grassland; (B) A free-ranging Western Fence Lizard (*Sceloporus occidentalis*) marked with a code of two dots of purple paint and one dot of white paint (as part of a separate study at the same site); (C) an unmarked clay lizard model that was found attacked several meters from its original site; (D) a white-marked model that had its tail pulled off by an avian predator (see foot imprint at point of tail break); (E) purple-marked model that was not attacked. Pictures are not to the same scale. (A photographed by Gary Bucciarelli; B-E photographed by Breanna J. Putman).

marks on the clay models and that the inconspicuous paint markings would have similar or slightly increased predator interaction when compared to the control models. Because marking individual animals is a crucial part of many ecological studies of herpetofauna, a better understanding of how various techniques affect survival or behaviors is needed. Such information will be important for allowing researchers to minimize their impact on the biology of their study population.

Materials and Methods

Study site.—We conducted this experiment during the summer of 2017 at the Stunt Ranch Reserve (34.092742°N, 118.657302°W, Fig. 1), a University of California Natural Reserve in California, USA, managed by the University of California, Los Angeles, USA. The reserve is in the Santa Monica Mountains, approximately 35 km west of downtown Los Angeles.

We chose this location because it is not accessible to the public and it contains a sizable population of *Sceloporus occidentalis*. Predators of these lizards include birds of prey, passerine birds, carnivoran mammals, snakes, and possibly sciurid rodents (Barrows and Schwarz 1895; Jaksić et al. 1982; Smith et al. 2016), and those we encountered at our field site were California Scrub-Jays (*Aphelocoma californica*), American Crows (*Corvus brachyrhynchos*), American Kestrels (*Falco sparverius*), Red-shouldered Hawks (*Buteo lineatus*), Red-tailed Hawks (*B. jamaicensis*), Cooper's Hawks (*Accipiter cooperii*), Bobcat (*Lynx rufus*), Coyote (*Canis latrans*), squirrels (e.g., California Ground Squirrels, *Otospermophilus beecheyi*, and Eastern Fox Squirrels, *Sciurus niger*), and snakes, such as young Pacific Rattlesnakes (*Crotalus helleri*), Striped Racers (*Coluber lateralis*), and North American Racers (*C. constrictor*). We suspected that birds and mammals would be the main predators to attack the clay lizard models because snake predators mainly rely on movement and chemical cues to detect prey and past studies using clay lizard models rarely record any snake attacks (Husak et al. 2006; Steffen 2009).

Clay models.—We made 126 clay lizard models using Van Aken Plastalina modeling clay in Sculptor Gray (Van Aken International, North Charleston, South Carolina, USA). This clay is a pigmented, oil-based moldable clay that remains pliable and never hardens. We molded models by hand and used a ruler to verify that they were all the same size with a 7 cm snout-to-vent length and a 9 cm tail length based on the average measurements of *S. occidentalis* taken at the study site (unpubl. data). We divided the 126 clay models into three groups: (1) control, no paint markings; (2) white, painted with two dots of white nail polish at the base of the tail; (3) purple, painted with two dots of purple nail polish at the base of the tail (Fig. 1). We choose these colors because they were being used to mark live free-ranging lizards at the study site (for a separate study), and because to the human eye, white appears as a more conspicuous color, and purple as an inconspicuous color against the dull body coloration of both live lizards and clay lizard models (Fig. 1).

Color analysis.—We used a spectrophotometer to determine whether the gray clay used to make the models resembled live lizards and to determine the conspicuousness of each of our color treatments. We measured absorbance of the unmarked gray clay (i.e., background color), the center of a white paint marking, and the center of a purple paint marking. For each area of interest, we used three different clay lizard models (i.e., three controls, three white, three purple) and took three separate measurements. We measured spectral wavelengths in the lab using an Ocean Optics spectrometer (USB 2000; Largo, Florida, USA) with a fiber optic reflectance probe (Ocean Optics R200-7-UV-VIS) and a pulsed xenon light source (Ocean Optics PX-2) that measured reflectance in a 1.3 mm diameter patch. This method emits light evenly across the visible spectrum, which standardizes how light is transmitted across each measurement (Endler 1990). To reduce glare, the probe was placed at a 45° angle relative to the surface being measured. We measured reflectance relative to a Labsphere certified reflectance standard in software OOIBase32 of Ocean Optics.

We processed reflectance spectra using the R package pavo (Maia et al. 2013). We first summarized reflectance data by calculating brightness, hue, and chroma for each measured surface (gray clay, live lizard dorsum, white paint mark, purple paint mark). These color variables are three standard descriptors of reflectance spectra (Saks et al. 2003; Siefferman and Hill 2005; Montgomerie 2006). Brightness is a measure of the intensity of light reflected from the surface (as a percentage), and we calculated this as the mean reflectance over all wavelengths (300–700 nm). Hue represents the principle color reflected (red, blue, green, etc.) and is determined by the shape of the reflectance spectrum. We calculated hue as the wavelength (in nm) of peak reflectance. Chroma is a measure of spectral purity or saturation of a color. We calculated chroma as the sum of the reflectance values ± 50 nm from the wavelength of peak reflectance (the hue).

We used the receptor noise model of Vorobyev and Osorio (1998) to calculate the Just Noticeable Difference (JND) values for both chromatic and luminance (achromatic) contrasts between the paint markings and the gray background color. We also calculated JNDs between the gray clay and the dorsum of live *S. occidentalis* (taken during another study: Putman et al. 2017) to determine whether our models were visually similar to live lizards. Values of JND < 1 indicated that two regions could not be discriminated, while JND values > 1 indicated that the regions were visually distinct, with higher values indicating greater contrast (Vorobyev and Osorio 1998). We used the vismodel function in pavo to calculate the quantal catch from the visual system of the Peafowl (*Pavo cristatus*), which represents a violet-sensitive (VS) system in birds (Hart 2002), under standard daylight illumination. There is no complete published spectral sensitivity data for all the main predators of lizards at our study site, but visual pigment characteristics are generally conserved across birds (reviewed in Hart 2001). The VS color system is present in raptors and corvids (e.g., crows, ravens, and jays; Ödeen and Håstad 2003), the most commonly seen avian predators at our field site. We used the peafowl visual system because it is the best representative for

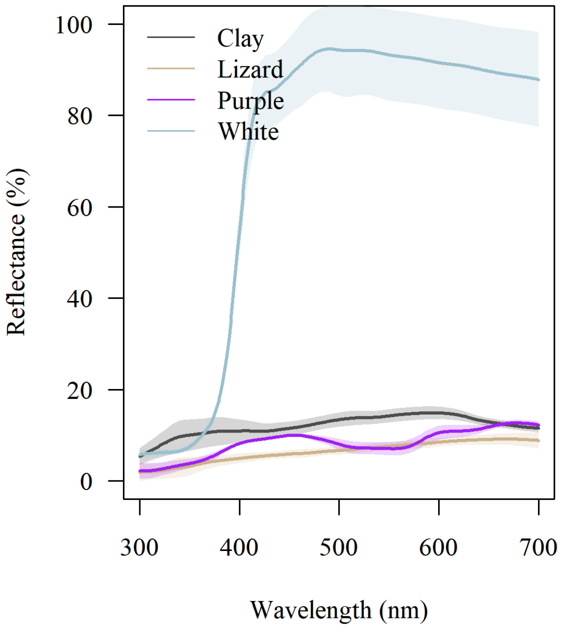

Figure 2. Reflectance spectra (mean ± SD) of the measured surfaces of interest. Ultraviolet range is 300–400 nm and visible spectrum is 400–700 nm.

VS birds for which data are available and past research has used it to represent the vision of raptors and corvids (Håstad et al. 2005). We compared JND values between the following contrast treatments: (1) the unmarked clay and dorsum of *S. occidentalis*, (2) the unmarked clay and white nail polish, and (3) the unmarked clay and purple nail polish.

Model placement and predation detection.—We placed models along a 756 m transect within the reserve alternating among the three treatments and with a distance of approximately 6 m between each placement. We placed models in both open canopy and closed canopy habitats on sites where we observed free-ranging lizards basking on rocks, logs, curb, and a metal pipe that ran along the road), and on the ground. The different treatments were evenly placed between habitats (open vs. closed: $X^2 = 1.249$, df = 2, $P = 0.535$), and among different substrate types ($X^2 = 8.831$, df = 6, $P = 0.183$). All sites were < 1 m off the ground. We laid out models over 2 d and inspected them for predator attacks approximately weekly (7–8 d between each check) starting 23 July and ending 7 August 2017. Although many studies using clay models to assess predation risk check models once after an allotted amount of time (48 h to one week; Shepard 2007; Vervust et al. 2011; Watson et al. 2012), we left our models out for three weeks to allow predators time to locate and attack them. We documented and took pictures of all suspected attacks made on the models and their locations (head, body, limb, tail). If a model appeared to be attacked, we reformed the model and left it in place. Almost all attacks appeared to be from avian predators (Fig. 1).

When models could not be located during inspection (n = 4), they were replaced and marked as attacked. Because the reserve is inaccessible to the public, we are confident that missing models were most likely picked up by a predator, however, it is also possible that missing models were collected by non-predatory rodents (e.g., *Neotoma*) out of curiosity. We initially placed 126 models, but we eliminated 18 from the study that had been placed on the ground because they melted or became covered in leaf litter, and subsequently were not visible to predators. The total number of surviving models that remained accessible to predators was 108.

Statistical analyses.—To determine whether purple and white markings differed in conspicuousness, we compared chromatic and achromatic contrasts between the two using non-parametric Mann-Whitney U tests because transformations failed to normalize distributions and homogenize variances. To determine whether use of color markings increased predation risk in lizards, we used a generalized linear mixed model (GLMM) with a binomial distribution and logit-link function. We set the control treatment as the reference so that each color treatment was compared to the control of no marking, attack (yes/no) as the dependent variable, and model ID as the random effect (because each model was checked multiple times). Finally, we used a Fisher's exact test to determine whether there were differences in the proportions of body regions attacked between white-marked and unmarked models. The purple treatment could not be included in this analysis because the one purple-marked model recorded as attacked was missing (Table 1). We used SYSTAT (v12.0) for Mann-Whitney U tests and made all other tests in R (v3.2.3), and we set alpha to 0.05 for all tests.

Results

Brightness, hue, and chroma were relatively similar among the gray clay, lizard dorsum, and purple paint, compared to the white paint, which reflected a high intensity of light within the visible spectrum (Fig. 2, Table 2). Our clay models were no different in color than live *S. occidentalis* based on the low mean JND value (< 1) of chromatic contrasts; however, the achromatic contrast between the clay model and live lizards had a mean JND value > 1 (Table 3). Purple markings exhibited greater chromatic contrast against the gray clay compared to white ($U = 729.0$, $P < 0.001$; Table 3), suggesting purple is more conspicuous in terms of color than white. White markings, however, exhibited greater luminance contrast compared to purple markings, with a greater absolute difference than the chromatic contrasts ($U = 0.00$, $P < 0.001$; Table 3).

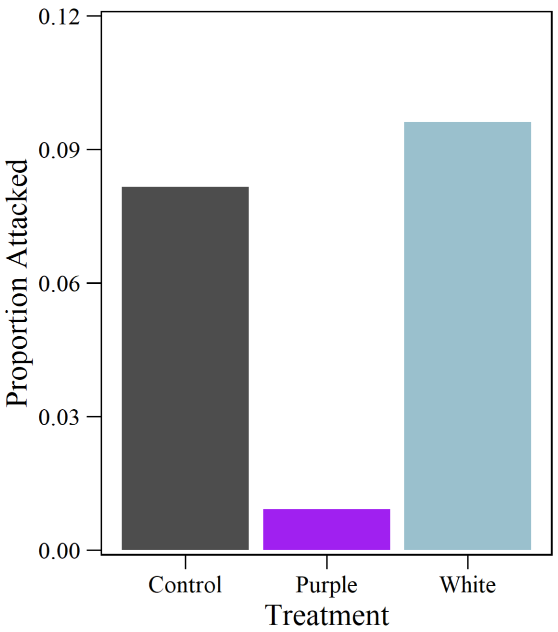

Figure 3. Proportion of clay lizard models attacked under each treatment: control (unmarked), purple-marked, and white-marked.

Table 1. Timing and placement of attacks on clay lizard models. Models were placed in numerical order along a transect spaced at approximately 6 m intervals. The Xs indicate occurrence of an attack on a particular region of the model or whether the entire model could not be found (i.e., missing). Asterisks (*) indicate models that were attacked more than once.

Model ID	Treatment	Date	Head	Body	Limb(s)	Tail	Model Missing
1	Control	23 July			x		
3	Purple	23 July					X
29	White	23 July				x	
35*	White	23 July				x	
49	Control	23 July	x	x	x	x	
68	White	23 July					X
83	White	23 July	x				
106	Control	23 July				x	
44*	White	31 July		x			
55	Control	31 July					X
61	Control	31 July			x		
112	Control	31 July			x	x	
11	White	7 August			x		
35*	White	7 August				x	
44*	White	7 August	x				
47	White	7 August	x			x	
62	White	7 August	x				
79	Control	7 August	x				
103	Control	7 August					X

We monitored 108 models over the course of this study (Control: n = 35; White: n = 36; Purple: n = 37). Predators attacked 19 models along the entire transect length, and only two models were attacked more than once (Table 1), suggesting that a single location/model did not receive a majority of the attacks. Attacked models were often displaced from the location at which they were originally placed (Fig. 1), but they were generally within a few meters from the original location. We were unable to locate models on four occasions.

We made 98 control model observations over the course of the experiment and recorded eight attacks. We made 108 white-marked model observations and recorded 10 attacks, and we made 104 observations of purple-marked models and only recorded a single attack. The attack rate on models with white paint treatment was not significantly different than the control models ($\beta = 0.164 \pm 0.592$, $Z = 0.278$, $P = 0.781$; Fig. 3), but the models in the purple paint treatment experienced a significantly lower attack rate compared to the control models ($\beta = 2.255 \pm 1.11$, $Z = -2.027$, $P = 0.043$; Fig. 3). The odds of an attack on a purple-marked model were 0.105 that of the odds of an attack on an unmarked model, while the odds of an attack on a white-marked model were 1.179 that of the odds of an attack on an unmarked model. Most attacks appeared directed at the head and tail regions of the models, and a few models were attacked across multiple regions (Table 1); however, we failed to detect significant differences in attack location (i.e., body region) between marked and unmarked models (Fisher's exact test: $P = 0.591$).

Table 2. Brightness, hue, and chroma of each measured surface of Western Fence Lizards (*Sceloporus occidentalis*), clay models, and paint marks, calculated from reflectance spectra.

Measured Surface	Brightness	Hue	Chroma
Gray clay	12.11	593	0.30
Lizard dorsum	6.42	666	0.30
Purple mark	8.28	677	0.27
White mark	70.68	490	0.33

DISCUSSION

We sought to identify whether the application of colored paint markings increases predation risk in animals, using *Sceloporus occidentalis* as our study

Table 3. Mean Just Noticeable Difference (JND) values for comparisons between the gray modeling clay and the dorsum of live Western Fence Lizard (*Sceloporus occidentalis*) and the different paint markings. A value < 1 indicates that two regions cannot be distinguished from each other, while a value > 1 indicates that the visual system can perceive a difference between the two with greater values indicating higher contrast.

Comparisons	Chromatic JND	Achromatic JND
Clay background - lizard dorsum	0.88	6.04
Clay background - purple mark	2.31	3.92
Clay background - white mark	1.09	19.13

system. By marking clay models with two dots of purple or white nail polish, we replicated the type of markings that researchers might make on live lizards during fieldwork to facilitate visual identification of study animals (e.g., Tinkle 1967). We found that the use of different colors influenced the rate of attack by predators, which suggests certain markings could affect mortality and/or the behaviors and physiologies of study animals. These results indicate that color matters when marking individuals within population studies, information that may help researchers limit their impact on their study populations.

Unlike past studies, we used a spectrophotometer to measure the spectral reflectances of the color markings and modeled how they might be processed by the visual systems of avian predators. This approach allowed us to determine whether potential avian predators perceive the white paint as a conspicuous color and the purple paint as an inconspicuous color, as they are viewed by the human eye. The most commonly observed avian predators at our study site were scrub jays, crows, and raptors, which have violet-sensitive (VS) visual systems (Ödeen and Håstad 2003; Håstad et al. 2005); thus, our use of a Peafowl visual model to determine conspicuousness was appropriate due to their similar avian VS perception. We found that luminance contrast was higher for the white markings than the purple, which suggests that white is conspicuous against the dull background of the gray clay. White markings did not elicit more attacks than the control without paint marking, however, suggesting that marking lizards with this color would not affect their predation risk.

The lack of an effect of the white paint markings compared to no markings is surprising given the brightness of the white paint and its high level of achromatic contrast against the gray clay. Previous work has shown that bright conspicuous lizards are more prone to predator attacks (Stuart-Fox et al. 2003), and greater contrast against the substrate can lead to more avian attacks in reptiles (e.g., rattlesnakes; Farallo and Forstner 2012). Other research shows that high contrast markings can also reduce predatory attacks because they hinder the predator from detecting or recognizing the prey (Dimitrova et al. 2009). Furthermore, there is evidence that birds use chromatic and achromatic cues in different ways. Achromatic aspects are used for the detection of small objects and for discrimination of visual textures, while chromatic contrasts are used for the discrimination of large objects and color differences (Osorio et al. 1999; Schaefer et al. 2006). Past research has also shown that chromatic contrast is important in resource detection in birds. Birds are more likely to strike fruit with higher chromatic contrast compared to achromatic (luminance) contrast (Cazetta et al. 2009) and prioritize chromatic contrasts when searching for fruits in foliage (Schaefer et al. 2006). Thus, the different attack rates we found between purple-marked and white-marked models could be due to differences in the relative importance of chromatic and achromatic contrasts for resource detection and discrimination in hunting birds.

Contrary to our expectations, clay models of lizards marked with purple nail polish received significantly fewer attacks from predators than the control models without paint markings. Our analyses revealed that purple markings were more conspicuous than white in terms of chromatic contrast for birds with VS perception. Thus, if more conspicuous models are avoided, as in Dimitrova et al. (2009), the higher chromatic contrast of the purple against the gray clay compared to the chromatic contrast of the white against the gray for birds with VS perception could be the mechanism behind the results of our study. Alternatively, conspicuousness might not matter as long as the color can be detected because, as mentioned above, white is more conspicuous in terms of luminance. We found that both colors (white and purple) were detectable against the gray clay, but a different mechanism (e.g., habitat, distance from predator home ranges) besides conspicuousness might be driving the choice to attack by a predator.

We chose to use purple as an inconspicuous color because it appeared as such to the human eye. Purple, however, is also a rare color in nature, and we think that predators may have been dissuaded from attacking purple-marked models because of either predator confusion or predator aversion (Stevens 2007). Confusion could occur if the predators could not recognize the painted model as a potential prey item such as in the case of disruptive coloration (Cuthill et al. 2005). A study on a noxious toad (*Rhinella alata*) found that striped- and diamond-patterned clay models received fewer avian attacks than the unpatterned model (Mcelroy 2016). The patterned models were thought to reflect disruptive coloration, making them less susceptible to bird attacks. Predator aversion could occur if the purple paint mimics warning coloration or patterns that birds naturally avoid (e.g., eyespots; Stevens 2015). We made our markings by placing two circular dots on the dorsal side of the model and these could have potentially been viewed as eyespots. Previous work has shown that more conspicuous spots are more effective at deterring avian attacks (Stevens et al. 2008). The models marked with white may have been equally attacked as the controls because white is a color commonly found on animals, including lizards (Stebbins 2003), so avian predators may perceive the white markings as natural color pattern variation, while purple is unusual. Zebra Finches avoid conspecifics that were banded with unfamiliar compared to familiar colors (Burley et al. 1982; Burley 1986). In addition, there is evidence that birds can show a generalized avoidance response (neophobia) toward resources with novel colors (Marples and Roper 1996; Marples et al. 1998). Our results alone cannot provide support for a mechanism underlying the predatory responses to the clay models, but further research could test the above assumptions.

Overall, we found that markings did not influence the proportion of attacks directed toward different locations on the clay lizard models. Previous studies using clay lizard models show that predators are more likely to attack the head and body regions and avoid the tail (Vervust et al. 2011; Bateman et al. 2014), and that conspicuous tails (e.g., blue colored) could have evolved to misdirect attacks to this non-vital region (Watson et al. 2012; Bateman et al. 2014). Attacks to the head could be fatal, but attacks to the tail are generally thought to be risky, but not necessarily fatal, especially for fence lizards that have tail autotomy (Bateman et al. 2014). Tail loss could ultimately reduce fitness though, through costs to locomotion, physiological stress, and changes in behavior (Bateman and Fleming 2009). In our study, there was no difference in the proportion of tail-directed attacks between painted and control (i.e., unpainted) models suggesting that predators were not attracted to the tail because of our artificial markings. Further research on how predators attack free-ranging fence lizards and if attacks are effective at securing prey would be worthwhile to determine whether tail-directed attacks are fatal for fence lizards at our site.

In summary, researchers should consider how they use colored paints to mark animals because we found that predation risk can be affected by specific colors (in our case, purple reduced risk). Such unintended effects could alter research outcomes; for instance, if researchers are quantifying survival rates or antipredator behaviors. Thus, it could be best to use a single color, use similar colors, or randomize the use of color among treatment groups when marking animals. More studies should also be conducted in the future using different animal species, and different types of markings (e.g., the effect of different symbols or marking locations). Other studies might be conducted on lizards that have different predators and in different habitats. An understanding of the impacts of research methods on animals is not only important from a methodological standpoint (i.e., to reduce bias in data), but also from an ethical one (i.e., reduce harm via increased predation on animals).

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