## Original Article

# Defensive posture in a terrestrial salamander deflects predatory strikes irrespective of body size

Alexander L. Myette,[a,]Thomas J. Hossie,[b,] and Dennis L. Murray[b]

aDepartment of Environmental and Life Sciences, Trent University, 1600 W Bank Dr, Peterborough, ON K9J 0G2, Canada and bDepartment of Biology, Trent University, 2140 East Bank Drive, Peterborough, Ontario K9J 7B8, Canada

A wide variety of prey use defensive postures as a means of protection from predators. Many salamanders engage in broadly similar defensive postures, which may function as a warning signal and reduce the probability of attack, or may deflect predator attacks away from vital body parts. The extent to which these strategies (i.e., aposematism and deflection) act exclusively or synergistically, however, remains unknown. We deployed clay salamanders in the field, manipulating size (small, large) and posture (resting, defensive), and documented attack rates across three predator types. Competing risks analysis revealed that attack rates were affected by model size, deployment period, and leaf litter depth at the site of deployment, whereas model posture had no significant effect. Model size and posture did not interact, indicating that defensive posture was ineffective in deterring attack irrespective of prey size. Model prey in the defensive posture received significantly more attacks on the tail irrespective of size, and the defensive posture was more effective at deflecting avian attacks compared to mammal predation. We conclude that defensive posture increases tail conspicuousness without increasing predation risk, and primarily functions to deflect attacks away from vital body parts. The efficacy of deflection may be further increased by tail undulation, however our use of static models means that we cannot exclude aposematic or deimatic functions for such movements. Our results provide important support for the deflection hypothesis in explaining antipredator behavior, and thereby set the stage for additional research targeting the functionality of attack deflection in natural predator–prey encounters.

*Key words:* **antipredator behavior, predator avoidance, taste-rejection, prey selection, warning signal.**

## INTRODUCTION

Predation is an important selective force leading to the evolution of various behavioral and morphological adaptations that mitigate both encounter and attack by predators (e.g., Garcia and Sih 2003; Ruxton et al. 2004; Barnett et al. 2016). Many prey rely on traits that minimize their encounter rate with predators or reduce their detectability to predators (e.g., Garcia and Sih 2003; McElroy 2015). In contrast, animals with secondary chemical defenses (herein "defended prey") often possess conspicuous aposematic patterns, colors, or displays that warn predators of their toxicity and dissuade them from attack (e.g., Mappes et al. 2005). Prey may also possess defenses that increase their chance of surviving a predatory attack upon detection. For example, some animals direct predatory strikes away from vulnerable body parts towards less vital parts (i.e., deflection), which either deters subsequent attack or facilitates their escape (e.g., Cooper and Vitt 1985; Van Buskirk et al. 2004;

Barber et al. 2015). Having detected a predator, prey can behaviorally reduce their chance of being attacked or increase likelihood of surviving an attack by adopting defensive postures. Indeed, prey defensive postures serve a variety of functions including crypsis (e.g., Zhang et al. 2015), masquerade (e.g., Preston-Mafham and Preston-Mafham 1993), mimicry (e.g., Hossie and Sherratt 2014), thanatosis (e.g., Gregory et al. 2007), intimidation (e.g., Peckarsky 1980), aposematism (e.g., Johnson and Brodie 1975), and deflection (e.g., Telemeco et al. 2011). Yet, in many cases, the function of such defensive postures remains poorly understood, as does the process though with they have been shaped through natural selection.

Recently, theoretical and empirical attention has been directed towards understanding the extent to which crypsis and aposematism can act synergistically to affect predation risk in defended prey (e.g., Tullberg et al. 2005; Barnett and Cuthill 2014; Barnett et al. 2016). Indeed, while conspicuous defensive display and crypsis seem to be in conflict, they may operate at different stages of the predator–prey interaction with the former functioning to protect prey following detection by a predator (e.g., Umbers and Mappes 2015).

Address correspondence to A.L. Myette. E-mail: alexandermyette@trentu.ca.

Moreover, prey which only reveal their warning signal once detected or attacked by a predator can mitigate the high detectability of their conspicuous warning signals while still deterring attack upon detection (e.g., Johnson and Brodie 1975; Barnett et al. 2016). Facultative (i.e., postattack) displays may even facilitate avoidance of defended prey in their cryptic form through predator learning (e.g., Kang et al. 2016). Importantly, we might expect the benefit of facultative aposematic displays to vary with prey body size if, for example, body size correlated with prey detectability or toxin load.

Experienced predators often trade-off nutrient and toxin intake when attacking defended prey depending on a variety of factors (e.g., Smith et al. 2016), including body condition (e.g., Barnett et al. 2012), current toxin load (e.g., Skelhorn and Rowe 2007), and availability of alternative prey (e.g., Carle and Rowe 2014). As a result, the body size of defended prey can act as a cue for predators when making decisions on whether an attack would be profitable or not. For example, prey defensive chemical quantity and richness may be higher in larger individuals (e.g., Jeckel et al. 2015; Regueira et al. 2017), and individuals with larger warning signals may be more salient to predators and accelerate avoidance learning (e.g., Remmel and Tammarub 2011). In contrast, large size may augment predation risk through increased prey conspicuousness (e.g., Whitman and Vincent 2008; Remmel et al. 2011; Smith et al. 2014) or because it indicates a larger meal to predators, and thus body size may influence predation risk through conflicting processes. The direction of body size effects vary among prey (e.g., Whitman and Vincent 2008; Remmel and Tammarub 2011; Flores et al. 2015), probably due to the multiplicity of factors involved, including predator type, environment, and prey life history (e.g., Endler and Mappes 2004).

Adult and juvenile salamanders employ a range of antipredator defenses, including defensive posture (e.g., Brodie et al. 1974; Brodie 1977; Brodie et al. 1979). Within Ambystomatidae, these postures typically include tail raising or coiling, body erection, and limb stiffening (e.g., Brodie 1977). This posture is qualitatively similar to the "Unken reflex" (e.g., Hinsche 1926), except that it also can involve tail undulation and does not reveal bright ventral coloration (e.g., Brodie 1983). Many salamander species that engage in defense posturing, including those from Ambystomatidae, also possess defense chemicals which have been shown to be noxious or toxic to predators (e.g., Brodie et al. 1979; Hopkins and Migabo 2010). These chemicals are secreted from the tail either during the defensive display or when the salamander is grasped. Preliminary investigation suggests at least two functions for this behavior: aposematism (e.g., Johnson and Brodie 1975) and deflection (e.g., Brodie and Gibson 1969; Brodie 1977). For example, Pacific newts (*Taricha granulosa*) are considered aposematic (e.g., Johnson and Brodie 1975), however, their defense posture exposes bright ventral coloration making it unclear if the posture itself serves as an aposematic signal or if color is a necessary component. In some lizards, behavioral tail displays are hypothesized to deflect predatory attacks away from the head and body (e.g., Hawlena et al. 2006), although conspicuous tail coloration is probably important to the effectiveness of this behavior (e.g., Watson et al. 2012; Fresnillo et al. 2015). Interestingly, salamanders from the genus *Ambystoma* generally do not possess conspicuously colored tails yet they still perform defensive postures, and the extent to which these postures serve as a warning signal or deflective display has not been resolved.

Here, we use artificial salamander models to test two nonmutually exclusive hypotheses to explain the adaptive value of defensive posturing behavior in terrestrial salamanders: aposematism and deflection. If defensive posture acts as an aposematic warning signal that deters attack by predators, then defensively postured models should be attacked less than those in a resting posture. In contrast, if defensive posture functions to deflect attacks away from the head and torso, then defensive models should receive a greater proportion of attacks on the tail compared to the head and body relative to models in a resting posture. We also sought to test whether the efficacy of defensive posture in reducing predation risk varies with body size. We predicted that defensive posture would be more effective at deflecting attacks for large prey since a larger tail may better attract the attention of a would-be predator. To test these predictions, we conducted a field experiment where we deployed clay salamander models that varied in body posture (resting vs. defensive) and size (small vs. large), and then recorded attack rates by free-roaming predators as well as the location of these attacks on the salamander models. In addition, we quantified the defensive behavior of wild-caught salamanders of varying body size ($n = 75$) to determine whether deployment of the defense is size-dependent.

## METHODS

### Study area

Our experiment was conducted on Pelee Island, Ontario, Canada (Lat. 41.77, Long. −82.65), which is the largest island (42 km²) in the Lake Erie archipelago spanning from Ontario, Canada, to Ohio, USA. Pelee Island is located within Carolinian forest and contains predominantly deciduous forest. Three Ambystomatid salamanders are present on the island; small-mouthed salamanders (*Ambystoma texanum*), blue-spotted salamanders (*A. laterale*), and unisexual polyploids (*Ambystoma laterale - texanum*). The three groups are morphologically similar and possess similar defensive tail postures, which served as inspiration for our generic clay models. Pelee Island has a diverse community of potential salamander predators, including wild turkey (*Meleagris gallopavo*), coyote (*Canis latrans*), raccoon (*Procyon lotor*), fox (*Urocyon cinereoargenteus; Vulpes vulpes*), and other passerine birds (e.g., common grackle *Quiscalus quiscula*, bluejay *Cyanocitta cristata*).

### Salamander predation experiment

Clay model prey are increasingly used to test otherwise intractable questions related to predation risk, prey selection, and antipredator strategies (e.g., Brodie 1993; Kuchta 2005; Bateman et al. 2016). Our clay salamander models were produced following Yeager et al. (2011) and resembled *Ambystoma* spp. salamanders on Pelee Island. Models were constructed by pouring melted plasticine modeling clay into custom silicon molds and further shaping models by hand once removed from molds. Salamander shape and size conformed to our four treatments (large-resting, large-defensive, small-resting, small-defensive; see Figure 1). Large (12 cm total length) and small (6 cm TL) models were consistent in size with adult and juvenile *Ambystoma* salamanders observed on Pelee Island (A. Myette, unpublished data).

Models were deployed at four sites on Pelee Island (separated by 0.82–8.4 km, average = 5.5 km) to test how size and posture affected predation risk. Salamander presence was confirmed at each deployment site, and trail camera footage from the previous year established presence of potential predators (A. Myette, unpublished data). At each site, two 50 m transects were established, with 3 m long perpendicular lines placed at 5 m intervals. Along each perpendicular line, one salamander from each treatment

**Figure 1**
Salamander defensive posture (tail secretion indicated with an arrow) (a), and clay salamander treatments (b), with treatment type shown in the top left of each image: SR, small-resting; LR, large-resting; SD, small-defensive; and LD, large-defensive.

group was placed on top of leaf litter at 1 m intervals, providing 40 salamander models per transect. Our decision to deploy models on top of leaf litter was based on a pilot study from the previous year, which demonstrated comparable predation rates between models deployed under or on top of light structural cover (see Supplementary Material). Models were checked for injury then removed after 27–31 days of deployment. Fresh models were then deployed along new transects, no closer than 50 m from the previous transect. Concurrent with both model deployment and recovery, two individual observers quantified leaf litter depth (i.e., number of leaves before contacting the substrate) and visually estimated canopy cover (see Thornton et al. 2012) at each 5 m intersect. At one end of each transect we deployed a single trail camera (Bushnell® Trophy Cam™, Bushnell, Overland Park, KS, U.S.A or Reconyx® RapidFire™ RC55, Reconyx, Holmen, WI) by fastening the camera to a tree at a height of approximately 1 m. Cameras were armed during the entire deployment period and monitored the presence of various predators at our deployment sites. This process was replicated each month from March to July, for a total of 1600 individual clay models deployed.

When we recovered models, we found that a small percentage were missing (3%) or were chewed to the point that the treatment could not be identified (2%). In such cases, we used deployment order and position of the unidentified model to infer treatment group. Clay models were inspected for attack impressions and all attack marks were photographed. Based on impressions left on attacked models, we recorded suspected predator type as either wild turkey, mammal, or passerine bird. Turkey predation was classified as a separate fate from other birds since: 1) turkey are large-bodied opportunistic predators which spend most of their time on the ground and often scratch at cover and 2) turkey were introduced to Pelee Island in 2002 and they are distinctly important from the

perspective of predation on endangered salamanders on the island (e.g., Hossie 2018). Turkey and passerine attacks were differentiated by size and shape of beak impressions (e.g., Supplementary Figure S1). To test for attack deflection, attack location (head, body, tail) on the model was recorded. Overall, we recorded 712 attack occurrences. The attack impression could not be immediately identified in some cases ($n = 155$, 22%), so the predator identity was temporarily classified as "unknown." Several of these ($n = 60$, 8%) were censored after photo interpretation indicated that these impressions were left by gastropods or arthropods (e.g., Low et al. 2014). Further inspection of attack photos enabled us to bin 26 additional unknown attacks into one of the three fate classes (i.e., "killed" by wild turkey, mammal, or passerine), ultimately leaving only 69 models (10%) with attacks that could not be reliably attributed to a particular known fate category (see Supplementary Material).

## Model detection

We considered that size and posture of our models may affect their detectability to predators, potentially altering attack rates differently depending on treatment. To quantify model salamander detectability, we conducted a detectability experiment where human participants ($n = 22$) searched a 20 × 20m grid (10 min) for our models (see Supplementary Material). We acknowledge the difference in visual capacity between human observers and wild predators, but contend that this work provides at least a coarse assessment of detectability differences among model treatments (e.g., see also Rojas et al. 2014; Rößler et al. 2019). Moreover, all model types were identical in color, so differences color–vision among predator types should minimally affect treatment-related differences in detectability for a given predator. Two-way analysis of variance revealed that defensive posture marginally increased detectability of models to human participants (4% increase, $P = 0.059$), but that there was no effect of body size ($P > 0.20$) or the size–posture interaction ($P > 0.21$) (Supplementary Table S1, Supplementary Figure S2).

## Salamander behavior

We sought to relate our work on models to wild salamanders by subjecting wild-caught salamanders ($n = 75$) to different levels of tactile stimulation. We used stimulus to elicit defense posture and recorded responses as: 1) defensive response (i.e., stiff posture, raised tail), and 2) response intensity (i.e., the level to which the tail was raised; described below). Body size (i.e., mass) was measured for each animal. Simulated threat was applied as: 1) initial flipping of cover object; 2) prodding once at the base of the tail; and 3) prodding multiple times at the base of the tail. Tactile disturbance was based on previous methods (e.g., Brodie 1977). Salamander response intensity was recorded as: 1) none; 2) tail raised moderately; 3) tail at 45°; and 4) tail held vertically. Response intensity was quantified once salamanders had responded, and higher stimuli were not applied after animals had responded.

## Statistical analyses

We used competing risks survival analysis (e.g., Heisey and Patterson 2006) to examine how body size, defensive posture, and the size × posture interaction affected salamander predation risk. The competing risks approach assumes that subjects can only succumb to a single fate; so, we excluded models that had been attacked by >1 predator type ($n = 24$), restricting our analysis to 1576 clay models. Hazard rates were analyzed using a discrete-time framework in R

(R Core Team 2015) using the package *discSurv* (Welchowski and Schmid 2016). We ran exploratory analyses in an attempt to properly classify remaining unknown attacks ($n = 69$). Unknown attacks could not be reliably attributed to a particular known fate category (see Supplementary Material), therefore the competing risks analysis was conducted using three fate classes (turkey, mammal, and passerine), with unknown attacks being censored. A series of candidate models ($n = 26$; Supplementary Table S2) were compared using delta Akaike Information Criterion scores corrected for small sample size ($\Delta AIC_c$) to determine the relative importance of predictor variables for salamander predation risk. Month of deployment, leaf litter depth, and canopy cover were also included in candidate models to account for spatiotemporal and environmental effects, and the top model ($\omega = 0.904$; Table 1) was used to extract hazard coefficient estimates.

Multinomial logistic regression served to determine how body size, posture, and predator type affected model body section attacked. Location of injury was recorded for each attacked salamander model. When clay models were attacked in multiple body locations (48%, $n = 277$), it was impossible to infer which order the attacks occurred, or if multiple attacks were by a single versus multiple predator individuals (see Saporito et al. 2007). Moreover, multiple attacks may not be independent of each other, even if assumed to be by a single predator. Therefore, we contend that the primary position of attack is most appropriate for understanding the role of deflection; so, we restricted our analysis to 52% ($n = 306$) of all clay models that were attacked in a single body section (see also Watson et al. 2012; Fresnillo et al. 2015). Exploratory analyses indicated that model size and posture did not predict the occurrence of multiple attacks, indicating that multiple attack prevalence was equal among treatment groups (see Supplementary Material). Supplementary analyses using full and reduced (i.e., single attack location only) data sets produced comparable results for body section attacked (Supplementary Figure S3, Supplementary Tables S4 and S5).

We analyzed wild salamander behavioral responses using regular and ordinal logistic regression to understand what factors predicted defensive response and response intensity, with stimulus level included as a covariate to account for potential variation in salamander risk perceptions. Body size was included in analysis of defensive behavior; ambient temperature was also included as a predictor because of known effects on defensive behavior in ectotherms (e.g., Mori and Burghardt 2001; Herrel et al. 2007; Careau et al. 2014).

### Animal ethics

All experiments were conducted under local ethical approval from Trent University (Protocol No. 23906) and complied with both Canadian regulations and the Association for the Study of Animal Behaviour's Guidelines for the ethical treatment of animals. Model

detectability experiments with human participants were approved by the Trent University Human Ethics committee (Protocol No. 24471), and were conducted in accordance with the spirit of the Helsinki Declaration of 1975, as revised in 2000.

## RESULTS

We deployed 1600 salamander models through March–July, and overall, 18% ($n = 279$) were attacked by wild turkey, 10% ($n = 160$) by mammals, and 9% ($n = 144$) by passerines (see Supplementary Table S3 for full summary). Only 3% ($n = 42$) of models could not be recovered, 4% ($n = 69$) had unknown impressions, and 1% ($n = 24$) were attacked by multiple predator types. Despite the length of our deployments, only 17% of recovered models were visually obscured by fallen debris (i.e., >50% of the model not visible when viewed from above) at the time of recovery. Critically, the likelihood of being obscured was equivalent between resting and defensive model postures ($X^2 = 1.73$, df = 1, $P = 0.20$), and therefore fallen debris did not influence our ability to evaluate aposematism or deflection mechanisms. The probability of a model being obscured was however influenced by month of deployment ($X^2 = 133.45$, df = 4, $P < 0.001$), site of deployment ($X^2 = 78.67$, df = 3, $P < 0.001$), and model size ($X^2 = 23.38$, df = 1, $P < 0.001$). Notably, our evaluation of the cause of attack quantitatively considers each of these factors (see below).

### Cause of attack

Of the models that were attacked, 28% were large-resting, 27% were large-defensive, 23% were small-defensive, and 22% were small-resting. Competing risks analysis revealed that the best-fit candidate model included body size, month, and leaf litter depth predictors (Table 1; second best model $\Delta AIC_c = 5.1$, $\omega = 0.07$). Posture and the size × posture interaction did not influence cause-specific hazard rates, as indicated by the relatively low importance of these predictors (Table 1). Thus, defensive posture did not increase or decrease the attack rate on salamander models.

Large body size increased risk of attack by mammals (hazard ratio [HR] = 2.12, SE = 0.17, $P < 0.001$), and while size also appeared to increase risk of attack by turkeys, the increase was not significant (HR = 1.27, SE = 0.14, $P = 0.08$; Table 2, Figure 2a). Body size did not influence risk of attack by passerines (HR = 0.96, SE = 0.18, $P = 0.81$). Deeper leaf litter reduced the estimated hazard ratio for attacks by turkey (HR = 0.86, SE = 0.068, $P = 0.023$) and mammals (HR = 0.63, SE = 0.082, $P < 0.001$), but not passerines (HR = 0.97, SE = 0.082, $P = 0.71$).

The month of deployment influenced hazard rates for all causes of mortality, though this effect varied depending on predator type

**Table 1**

AIC$_c$, $\Delta$AIC$_c$, and model weights for a set of candidate models with Q = 3 fates. The top model was used for hazard coefficient estimation. For simplicity, only the top five models are shown (see Supplementary Material for full candidate set)

| Model | AIC$_c$ | $\Delta$AIC$_c$ | $\omega$ |
|---|---|---|---|
| timeInt + Size + Month + AvLL | 3799.1 | 0 | 0.904 |
| timeInt + Size + AvCC + AvLL + Month | 3804.2 | 5.11 | 0.0702 |
| timeInt + Size × Posture + AvLL + Month | 3806.7 | 7.61 | 0.0201 |
| timeInt + Size + Posture + AvCC + AvLL + Month | 3810.0 | 10.96 | 0.00378 |
| timeInt + Size × Posture + AvCC + AvLL + Month | 3811.8 | 12.73 | 0.00156 |

AvCC = canopy cover (%), AvLL = leaf litter depth (number of leaves).

**Table 2**

**Hazard ratios with 95% confidence intervals for the three fate classes analyzed using the best-fit model from a discrete-time competing risks analysis**

| Risk factor | Turkey HR (95% CI) | P | Mammal HR (95% CI) | P | Passerine HR (95% CI) | P |
|---|---|---|---|---|---|---|
| Size | 1.27 (0.97, 1.65)* | 0.0796 | **2.12 (1.51, 2.98)** | **<0.001** | 0.96 (0.67, 1.36) | 0.806 |
| Leaf Litter Depth | **0.86 (0.75, 0.98)** | **0.0226** | **0.63 (0.53, 0.74)** | **<0.001** | 0.97 (0.83, 1.14) | 0.710 |
| Month (April–May) | **44.7 (15.3, 131)** | **<0.001** | **0.33 (0.19, 0.59)** | **<0.001** | **6.02 (2.91, 12.5)** | **<0.001** |
| Month (May–June) | **56 387 (8280, 382 965)** | **<0.001** | **3.49 (1.72, 7.09)** | **<0.001** | **397 (97.3, 1623)** | **<0.001** |
| Month (June–July) | **4.65 (2.11, 10.2)** | **<0.001** | **0.22 (0.13, 0.36)** | **<0.001** | **0.29 (0.12, 0.70)** | **0.00637** |
| Month (July–August) | **1429 (304, 6718)** | **<0.001** | 1.20 (0.64, 2.25) | 0.568 | **28.2 (8.42, 94.2)** | **<0.001** |

Significant ($P < 0.05$) effects are shown in bold; effects that approached significance ($0.05 < P < 0.10$) denoted with an asterisk. Reference level for month is March–April.
CI = confidence interval.
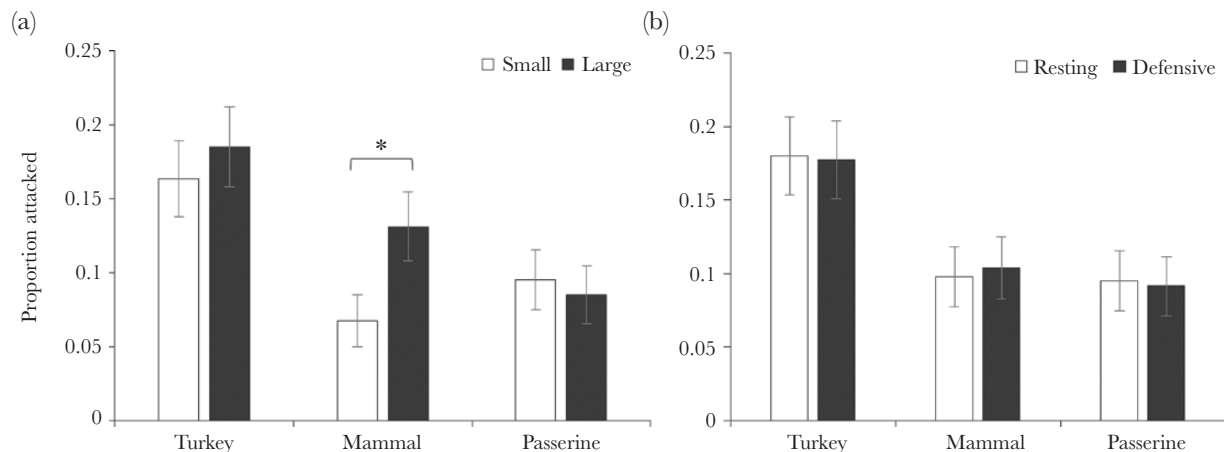


**Figure 2**
Effect of size (a) and posture (b) on cause-specific predation rates, expressed as proportion of clay models attacked and shown with 95% confidence intervals (significant pairwise comparisons denoted with an asterisk; *$P < 0.05$).

(Table 2). Model salamander predation risk was highest for each of the three predator types during the May–June deployment; risk of attack was lowest during the March–April deployment for turkeys, and during the June–July deployment for mammals and passerines (Table 2).

## Attack location

Of the 306 clay models attacked in a single body section, 45% were turkey attacks; 20% were mammal attacks; and 35% were passerine attacks. Models were attacked on the tail (54%) more often than on the head (23%) or body (23%). We found that model posture, but not size or the size × posture interaction, affected attack location, with additional differences being evident across predator types (Supplementary Table S6, Figures 3 and 4). For example, all predators preferred the tail, but turkeys and mammals attacked the head more often than the body, whereas passerines attacked the body more than the head. Multinomial regression indicated that mammals more frequently attacked the head compared to passerines (odds ratio [OR] = 3.13, SE = 0.48, $P = 0.017$), whereas turkeys and mammals tended to attack the tail rather than the body (Turkeys: OR = 3.19, SE = 0.32, $P < 0.001$; mammals: OR = 3.46, SE = 0.44, $P = 0.005$). Defensive posture significantly increased the frequency of tail attacks (OR = 1.95, SE = 0.30, $P = 0.023$); head and body attack frequency was reduced by defensive posture, but not significantly (Figure 3b, Supplementary Table S4). Attacks by

avian predators were more frequently deflected to the tail of defensive posture models, whereas mammalian attacks were not deflected as readily (Figure 4). Leaf litter depth and the leaf litter × posture interaction were not influential on attack location (Supplementary Table S6), suggesting that attack location was influenced primarily by model posture rather than cover.

## Salamander behavior

Our behavioral test elicited antipredator postures in 77% (58/75) of wild salamanders tested (e.g., Figure 1a). While engaging in the defensive posture, less than half (47%) of salamanders performed tail undulations, and very few released secretions (4%). Body size did not influence defensive behavior, although temperature provided a degree of explanatory power in salamander responses to simulated threat (Supplementary Tables S7 and S8). At higher temperatures, both the occurrence of the defensive posturing response in salamanders (OR = 0.90, SE = 0.046, $P = 0.023$) and response intensity (OR = 0.93, SE = 0.030, $P = 0.023$) decreased.

## DISCUSSION

We tested two hypotheses to explain the function of defensive posturing behavior in salamanders: aposematism and deflection. Our analysis of predation rates on model salamander prey found no difference in attack rate on models in the resting versus defensive

(a)

(b)
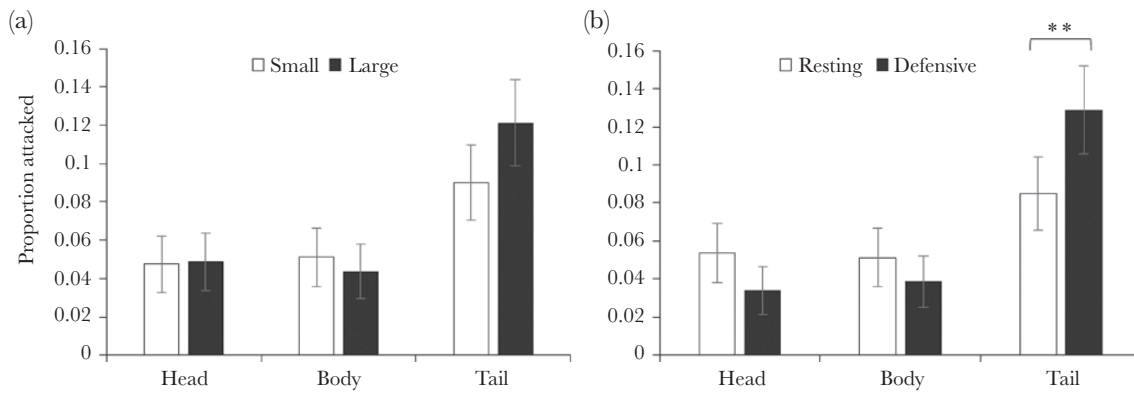


**Figure 3**

Effect of size (a) and posture (b) on the body section attacked of clay salamanders. Data is shown for models attacked in one body section. Values expressed as proportion of attacks that were directed to each body section, and shown with 95% confidence intervals (significant pairwise comparisons denoted with an asterisk; **$P < 0.01$).
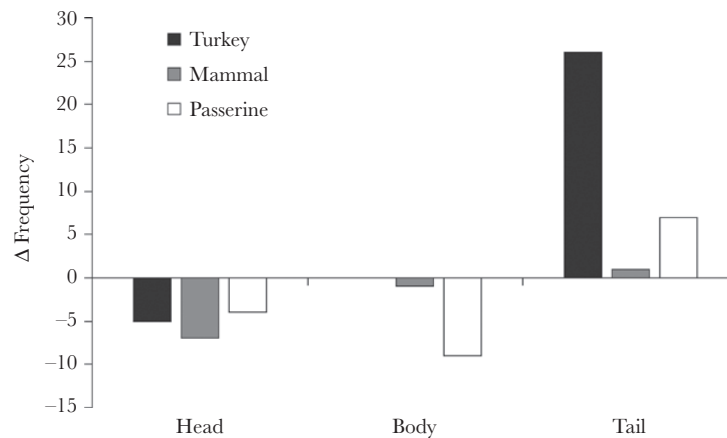


**Figure 4**

The difference in frequency of attacks in each body section between resting and defensive posture models for each predator type ($\Delta$ Frequency = number of defensive models attacked - number of resting models attacked). Data shown are restricted to models attacked only in a single body section.

posture, which does not support the aposematism hypothesis. Body size affected survival, with mammals preferentially attacking large prey, but did not have an interactive effect with posture. Importantly, models in the defensive posture received more attacks on the tail and fewer on the head and body compared to models in the resting posture, supporting a deflection function. Body size did not influence the body section that was attacked, and the extent to which defensive posture effectively directed strikes to the tail and away from the head or body differed among predator types. Overall, our results suggest that salamander defensive posture within Ambystomatidae acts to direct predator attacks to body regions that are nonvital and/or noxious (Duellman and Trueb 1986), but does not deter attack through aposematism. Our research extends previous tests of the aposematism and deflection hypotheses (e.g., Johnson and Brodie 1975; Watson et al. 2012; Fresnillo et al. 2015; Barnett et al. 2016) by identifying the latter as being strongly supported in this system under realistic field conditions. Specifically, we suggest that defensive posture in at least some salamanders facilitates taste-rejection by directing strikes to a noxious organ (tail) which is resistant to attack because it can regenerate quickly and is nonvital.

Our predation experiment did not find evidence of an aposematic function for salamander defensive posture. Defensive posture

did not reduce predation rates, and body size influenced predation risk independently from posture, suggesting that defensive posture was ineffective in deterring attacks, irrespective of prey size. That body size was not used as an aposematic cue is consistent with previous work indicating that predators associate color signals with prey toxicity more rapidly than signals related to prey body size (e.g., Halpin et al. 2013). Increased attack rates on large prey may suggest that predators selected larger prey items, since they are more profitable (e.g., Stephens and Krebs 1986; Manicom and Schwarzkopf 2010). Indeed, even when prey are chemically defended, predators may still preferentially attack and consume prey items that are large (e.g., Halpin et al. 2013) or nutritionally rich (e.g., Cruz-Rivera and Hay 2003; Halpin et al. 2014). While greater attack rates on large prey could also have resulted from easier detection of large models (e.g., Karpestam et al. 2014), our companion trial using human subjects failed to detect size-specific differences in detectability (see Supplementary Table S1). Similar defensive postures likely serve an aposematic or deimatic function in other salamanders (e.g., Hinsche 1926; Johnson and Brodie 1975; Umbers et al. 2015), however, these examples tend to be associated with bright coloration exposed upon posturing, which may intensify the signal and/or independently act as a deterrent. In contrast, most

*Ambystoma* spp. salamanders (as well as our models) lack conspicuous ventral markings and tend to be only mildly noxious to predators (e.g., Brodie 1977). Failure to detect a difference in the attack rate on resting versus defensive models therefore suggests that defensive posture serves an alternative function in this group. That said, we cannot fully exclude the possibility that the movement involved in adopting the defensive posture or tail undulation serve an aposematic function.

Our analysis of body section injury revealed an increase in tail attacks among defensively postured models concurrently with a qualitative reduction in head and body attacks, thereby supporting the deflection hypothesis. Specifically, defensive posture appears to effectively redirect predator attacks toward the tail, which should increase survival during predation encounters. Attack deflection must increase the likelihood of surviving a predation attempt to be an effective defense strategy favored by natural selection (e.g., Humphreys and Ruxton 2018). Some lizards and salamanders use behaviors that attract predator strikes to the tail (e.g., Brodie 1977; Hawlena et al. 2006), and are then able to autotomize their tail, thereby allowing for rapid escape (e.g., Vitt and Cooper 1986). We propose that the defensive posture provides a survival advantage, even in salamanders that are not highly toxic and lack the capacity for tail autotomy, via two complimentary mechanisms. First, deflecting attacks towards the tail, which is less vital and can regenerate following injury (e.g., Young et al. 1983; Voss et al. 2013), reduces potentially lethal attacks to the head or body. Second, directing strikes toward the tail, where noxious defensive secretions are concentrated (e.g., Brodie and Gibson 1969), reduces predator motivation to continue the attack. Thus, in our system, defensive posture probably serves as a generalist defense that facilitates taste-rejection by drawing strikes to the tail, which is resistant to attacks (Brodie and Gibson 1969; Brodie 1983; Duellman and Trueb 1986). Such a strategy should be effective against a wide range of predator taxa, and unlike aposematism, does not require predator learning to confer a survival benefit. Moreover, adopting a deflection strategy that facilitates taste-rejection would be an effective strategy when prey (e.g., *Ambystoma* salamanders) cannot outrun their predators (e.g., turkeys, raccoons). Indeed, approximately 15% of salamanders captured at our study site from 2016 to 2018 showed signs of prior tail damage, potentially indicative of successful attack deflection (T. Hossie, unpublished data). Interestingly, the eyespots of some caterpillars (genus *Papilio*) may similarly direct predator strikes towards a noxious organ (i.e., the osmeterium), possibly to deter predators postattack through taste-rejection (e.g., Blest 1957; Hossie and Sherratt 2013). We note that the efficacy of deflection and/or taste-rejection in reducing subsequent predation is likely to vary among predator types (e.g., Ratcliffe et al. 2003), and with an individual predator's current energetic needs (e.g., Barnett et al. 2007; Barnett et al. 2012) or toxin burden (e.g., Skelhorn and Rowe 2007).

Responses to salamander model size and posture varied between predator types, with predators also differing in their relative frequency of attacks on each body section. Defensive posture appeared to be most effective against wild turkeys, but was less effective against mammals. Pelee Island has been separated from the mainland for approximately 4000 years (e.g., Calkin and Feenstra 1985), and mammals and passerines are native to the island, whereas wild turkeys, while native to North America and sympatric with *Ambystoma* salamanders, were first introduced to the island < 15 years prior to our study (e.g., Hossie 2018). Despite this gap in co-occurrence, *Ambystoma* salamanders on Pelee Island

retain a generalized behavioral defense that appears to be effective against turkeys, but only moderately effective against other predator types. Interestingly, this is consistent with the "multipredator hypothesis" which predicts that antipredator behavior for missing predators can be maintained by the presence of any predators (e.g., Blumstein 2006), however, this defensive behavior could also have been maintained by a modest selective advantage or the absence of selection against the behavior. Humphreys and Ruxton (2018) suggest that predators may vary in their response to deflection defenses according to experience level (over ecological or evolutionary timescales), and that attack deflection may be more effective against naive individuals or novel predators due to the lack of behavioral counter-adaptations. Evidence from other systems also suggests that some predators may avoid the tail when this body part is particularly noxious, or in prey that employ tail autotomy, since this renders tail-directed attacks less profitable (e.g., Dodd and Brodie 1976; Vervust et al. 2011). While it is tempting to ascribe differences in the efficacy of deflection against mammals versus turkeys to predator experience, key differences in predator ecology (e.g., nocturnal vs. diurnal foraging) could also account for these differences. For example, a raised tail may be more conspicuous during the daylight hours, rendering the defensive posture more effective in deflecting strikes of diurnal predators. Birds are known to be capable of taste-rejecting prey using strategic decisions based on their own energetic state and the individual prey's level of chemical investment (e.g., Skelhorn and Rowe 2006a, 2006b). Thus, an intriguing possibility is that attacks to the salamander's tail enable turkeys and passerines to evaluate profitability of the prey item prior to consumption.

These findings contribute to our understanding of defensive postures by demonstrating that prey may use specialized defensive behaviors to draw predatory strikes away from the head and body and towards a nonvital and noxious body part. Although defensive posture has been proposed as an aposematic signal (e.g., Johnson and Brodie 1975), we found no evidence supporting this hypothesis in our system. Following Skelhorn and Rowe (2006a), we suggest instead that the salamanders in our system remain cryptic to avoid predation encounters, but combine noxiousness with specialized postures to facilitate taste-rejection following predator detection. While our models did not mimic the tail undulation behavior of real salamanders, less than half of the salamanders in our behavioral tests performed tail undulation. Further, tail motion is thought to further increase the likelihood of predator attack diversion towards the tail (e.g., Brodie 1977). We therefore contend that our study conservatively tested the deflective function of the defensive posture and suggest that although undulation likely enhances deflection it is not necessary for such postures to confer protection. Our use of static models precludes us from empirically examining possible roles of tail undulation in aposematic or deimatic signaling however, and the degree to which defensive posturing increases the efficacy of color-based aposematic signals remains an interesting avenue for research. Given that some salamanders clearly use defensive postures as part of an aposematic signaling complex (e.g., "Unken reflex" in *Taricha granulosa*, Johnson and Brodie 1975), our research illustrates that natural selection via predation can produce similar defensive behaviors that serve distinct antipredator functions in both toxic prey with conspicuous visual signals and prey that are merely distasteful and cryptic. The lack of tail autotomy in many salamanders that use defensive behaviors to deflect predator strikes (e.g., Brodie 1977) further suggests that the adaptive value of deflection can be realized in a variety of ways that remain to be fully understood.

## SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

## FUNDING

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