Comparing Day and Night Predation Rates on Lizard-like Clay Models

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Abstract.—Clay models of live animals are often used by behavioral ecologists and ethologists to study prey-predator interactions under field conditions. This technique has limitations, however, and often models are displayed in the field for long periods of time without distinguishing between daytime and nighttime attacks. We compared day and night predation rates on lizard-shaped clay models in a temperate ecosystem in northwest Italy. We placed 206 lizard models in two adjacent habitats (i.e., a mixed broadleaf woodland and along an adjacent ecotone) and checked them every morning and evening for three consecutive days (i.e., for 72 h). The model head was attacked more than expected by chance, indicating that predators were perceiving the models as true prey items. The overall observed predation rate was 18% (39/206) and was similar between the two habitats; however, models were attacked more than twice as often during daytime versus nighttime with 28 attacks (71%) during the day and 11 attacks (29%) during the night. Mammal predators attacked the models with similar frequencies during both daytime and nighttime. These findings indicate that, at least in our study area, the predation rate on terrestrial lizards differs between day and night and that mammals are diurnal potential predators of lizards.

Key Words.—animal replicas; field experiment; lizard; plasticine; prey-predator system; study design; techniques

Introduction

Realistic clay models, which are artificial replicas that resemble specific organisms, have frequently been used to study prey-predator interactions (Bateman et al. 2017; Rössler et al. 2018). This technique offers several advantages in comparison with observational or laboratory experiments using live animals. In particular, there are few legal or ethical requirements, if any, and the modeling material is inexpensive and non-toxic (e.g., Bateman et al. 2017; Salvidio et al. 2019; but see Barr et al. 2018). Moreover, clay is easy to shape and color, permitting flexibility in the study design and favoring replicability with large sample sizes. In addition, clay models are malleable and retain marks on their surfaces, often allowing the identification of damaged body parts and of the predators responsible for the attacks (Kuchta 2005; Sato et al. 2014; Salvidio et al. 2017; Orton et al. 2018). This technique, therefore, is appealing to behavioral ecologists and ethologists interested in prey-predator interactions and is increasingly used in studies on cryptic and small-sized animals such as amphibians (Rössler et al. 2018; Salvidio et al. 2019) and reptiles (Bateman et al. 2017).

In reptiles, clay models have been applied to test hypotheses concerning aposematism, crypsis, survival, polymorphism, predation risk, and anti-predatory behavior in many species of terrestrial snakes and lizards (e.g., Madsen 1987; Brodie et al. 1993; Martínez-Freiría et al. 2017; Calderon-Chalco and Putman 2019).

In lizards, clay models have been used to evaluate predation risk and the escape behavior of populations living in different habitats, leading to evidence that different antipredator behaviors may be influenced by differing rates of local predation (Diego-Rasilla 2003; Keehn and Feldman 2018; Hansen et al. 2019). Also, predation rates may be more dependent on prey availability than on predator densities (Nordberg and Schwarzkopf 2019).

Models are usually displayed in the field continuously over several days or weeks, remaining exposed to both night and day predators in weather conditions that may vary. In these cases, unnatural ecological situations may occur. For instance, diurnal predators may have the chance to attack models of nocturnal animals that are secretive and inaccessible during daytime, or vice versa (Salvidio et al. 2019). This problem has been sometimes addressed by means of camera traps that allow the identification of predators and that record the exact time of each attack (e.g., Hansen et al. 2017; Oversby et al. 2018) by exposing models only during the daylight in studies focusing on diurnal animals (e.g., Pérez-Mellado et al. 2014) or by checking the models in the early morning and late afternoon to record nocturnal and diurnal attacks separately (Niskanen and Mappes 2005; Martínez-Freiría et al. 2017). Attacks on models continuously exposed in the field, however, are usually measured without experimental modifications to address this problem, increasing the probability of obtaining spurious results and raising questions about ecological inferences.
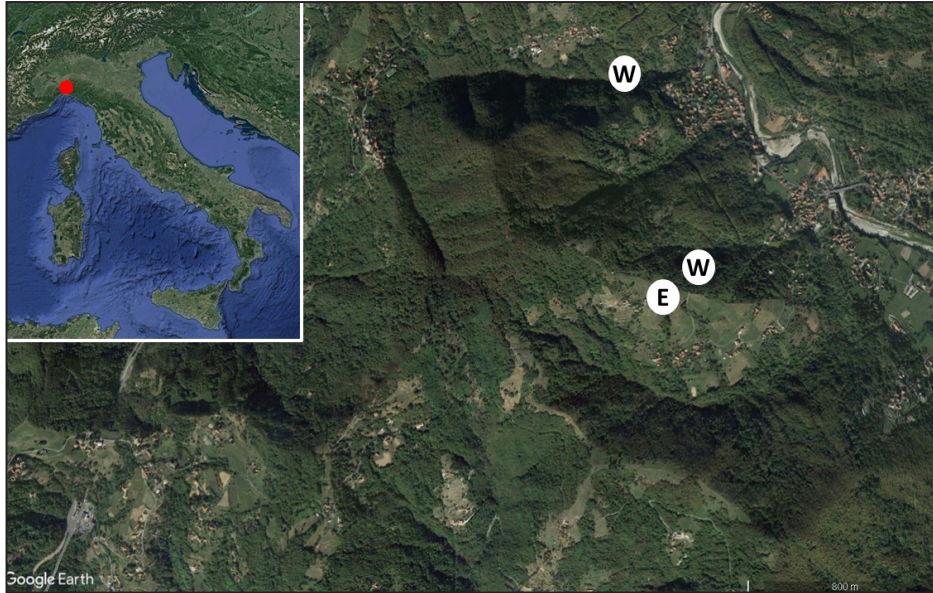
Figure 1. Location of the study site in Italy (red point in the upper left corner inset) and location of the experimental sites within the municipality of Savignone, Province of Genova. Habitat abbreviations are W = woodland and E = ecotone. The scale is indicated in the lower right corner of the image. (Image from GoogleEarth 2019).

To our knowledge, only three studies have addressed day and night predation rates separately using animal clay models that were continuously exposed to field conditions (Seifert et al. 2016; Niskanen and Mappes 2005; Martínez-Freiría et al. 2017). The first study was performed in the Amazon rainforest and used clay models replicating small caterpillars (Seifert et al. 2016). These authors found higher attacks rates during the daytime compared with the nighttime. These findings could be related to fact that these caterpillars are mainly active at the night, possibly to reduce predation risks (Seifert et al. 2016). In the second case, clay models of the venomous snake Lataste's Viper (*Vipera latastei*) were set out in the evening, checked every morning and evening for up to 36 h, and then only attacks that could be attributed to birds were used in the analysis (Niskanen and Mappes 2005). The third study treated attacks occurring during the day (all identified as avian) separately from those occurring during nighttime (all rodent attacks, identified by the marks retained by the model) on clay models of the Iberian adder (*Vipera seoanei*; Martínez-Freiría et al. 2017). In this case, the authors analyzed only attacks attributed to birds because rodents are not acknowledged as predators of this snake (Martínez-Freiría et al. 2017).

We tested predation rates on clay models designed to mimic a diurnal reptile, the Common Wall Lizard (*Podarcis muralis*). The primary aim of our study, given that predation rates may be higher during the day than at night (Seifert et al. 2016), was to compare potential predation rates during the day and night and to determine whether proportions of attacks by different types of predators (i.e., mammals and birds) varied by time of day. We also compared predation rates in two

adjacent habitats in northwestern Italy, a closed mixed broad-leaf woodland and the adjacent ecotone separating the woodland from a mowed field. This ecotone is less shaded than the woodland and, therefore, models were potentially more exposed to avian predation during the day. Therefore, models in the open habitat may experience a higher incidence of avian attacks compared with those in the closed habitat.

Materials And Methods

Study species.—We designed the models to resemble the local Common Wall Lizard, a medium-sized reptile with a snout-vent length ranging from 50 to 75 mm (Biaggini et al. 2011) that is found in all terrestrial habitats in the region (Corti 2004; Biaggini et al. 2011). This species is active exclusively during the day over most of the year during mild and sunny weather (Avery 1978). Many animals listed as potential predators by Biaggini et al. (2011), are present in the study area: Red Fox (*Vulpes vulpes*) and martens (*Martes* sp.) among mammals, Common Buzzards (*Buteo buteo*), Red-backed Shrikes (*Lanius collurio*), House Sparrows (*Passer domesticus*), Little Owls (*Athene noctua*), Barn Owls (*Tyto alba*), and Tawny Owls (*Strix aluco*) among birds, and Western Whip Snakes (*Hierophis viridiflavus*) and Common Vipers (*Vipera aspis*) among reptiles.

Study sites.—We selected the experimental sites to represent relatively undisturbed countryside environments, with little human disturbance, but chose sites that were easily accessible to facilitate the frequent monitoring (Fig. 1). The two woodland

sites were part of the same forested area and were separated by about 1 km. Weather conditions, obtained from the nearest official regional weather station (town of Busalla, Province of Genova. Agenzia regionale per la protezione dell'ambiente ligure. Available from http://93.62.155.214/~omirl/WEB/mappa_sensori.html [Assessed 2 June 2019]), were very similar during both trials; the mean daily temperature ranged from 12.8° to 13.8° C and from 12.4° to 13.9° C in the April and May, respectively. There was no precipitation during April and only 0.6 mm of rain was registered during the May trial.

Field methods.—For the field experiment, we placed 206 clay models near the Village of Savignone (Liguria, northwest Italy) and monitored them from 17–20 April 2019 and from 9–12 May 2019. In April, we placed 64 models in a mixed broadleaf woodland habitat. In May, we placed 142 models in a different woodland (42 models) and along the ecotone (100 models) separating that woodland from a mowed field. We shaped the clay models by hand and painted them with an acrylic brown color (Polycolor #493, Industria Maimeri S.p.A, Mediglia, Milan, Italy; Fig. 2) to resemble the natural color of these lizards. Within each field site (two woodlands and one ecotone), we began from a random location and placed models 3–5 m apart by nonrandomly choosing locations on the soil, in the vegetation, on stones, or on wood debris to attempt to mimic natural lizard behavior. In the field, we placed models on the soil in the vegetation, on stones, or on wood debris to mimic lizard behavior. We displayed all models in the evening of the first day and checked them twice per day, at 0700 and at 1800 for three consecutive days (i.e., six checks in 72 h).

We removed models that showed signs of predation when checked. We scored missing models as predated (Diego-Rasilla 2003). We assigned large tooth marks to mammals and bill marks to birds (Niskanen and Mappes 2005; Salvidio et al. 2017). We scored all other damage as undetermined. We measured the surfaces of the heads, torsos, and tails of 16 randomly selected models in pixels on digital macro-photographs using the software ImageJ 1.52a (Schneider et al. 2012) to calculate the proportion of attacks expected by chance based on their relative proportions. Therefore, we used the proportions 0.224, 0.552, and 0.224 for the head, torso, and tail, respectively. We used two camera traps (Scout Guard, model SG-560; ScoutGuard, China) to collect data on potential predators, one placed in the farthest woodland and the other along the ecotone.

Statistical analyses.—We used Chi-square tests to compare the observed number of attacks on the head, body, and tail with the expected frequencies based on our calculated proportions and to compare the distribution of predation events between habitats. We used a Binomial

Figure 2. Lizard-like clay model used in the predation experiments. The ruler is in centimeters and millimeters.

test to analyze predation frequencies assuming that the probability of being predated during the day was 0.46 because the models were exposed for 11 h (46% of the time) during daytime and for 13 h (54% of the time) during nighttime. We used Fisher's exact test when analyzing 2 × 2 tables and we set the significance level at $\alpha = 0.05$ for all tests.

RESULTS

Overall, there were no differences between the woodland and the ecotone habitat in any of the measured factors. In particular, the temporal attack rate was comparable. In the first day, there were six attacks in the woodland versus 10 in the ecotone, compared with nine versus three on the second day and six versus five on the third day ($\chi^2 = 3.883$, df = 2, $P = 0.143$). In addition, the overall observed predation pattern was extremely similar (Table 1), and there were no significant differences in the proportions of attacked models between habitats: 20/106 (19%) and 19/100 (19%) in the woodland and the ecotone, respectively ($\chi^2 = 0.0006$, df = 1, $P = 0.981$). Finally, the proportions of day and night attacks were almost identical between habitats: 14 (70%) during the day and six (30%) during the night in the woodland, in comparison with 14 (74%) during the day and five (26%) during the night in the ecotone ($\chi^2 = 0.065$, df = 1, $P = 0.798$).

On the basis of these considerations, we pooled the two habitat types in all subsequent analyses. Therefore, the overall predation rate was 18% (39/206) over the 3-d experimental period. In addition, head, body, and tail

Table 1. Lizard-like clay models exposed and attacked in the field experiment.

Site	Models displayed	Attacked by day	Attacked by night	Not attacked
Woodland 1	64	6	5	53
Woodland 2	42	8	1	33
Total Woodland	106 (100%)	14 (13%)	6 (6%)	86 (81%)
Total Ecotone	100 (100%)	14 (14%)	5 (5%)	81 (81%)
Overall total	206	28	11	167

TABLE 2. Daytime versus nighttime predation on terrestrial lizard-like clay models used in the field experiment.

| Time of day	Mammal predator	Bird predator	Undetermined predator/ missing	Total
Day	15	7	6	28
Night	6	1	4	11
Total	21	8	10	39

of the models were not attacked at random when their surface was taken into consideration ($\chi^2 = 6.255$, df = 2, $P = 0.044$). In particular, the head was attacked twice more than expected by chance (49% observed vs 23% of expected attacks; Fig. 3).

Based on all attacks pooled, there was a highly significant difference (binomial test: two-tail $P = 0.007$) between the total number of models attacked during the day (28/39) versus the night (11/39). The predator type (i.e., bird, mammal, or undetermined) did not differ between daytime and nighttime (Table 2; $\chi^2 = 1.663$, df = 2, $P = 0.435$), and a similar outcome was obtained when considering only the attacks attributed to mammals and birds (Fisher's exact test, $P = 0.635$).

During the daytime, the camera trap placed in the woodland video-taped a Feral Cat (*Felis domesticus*), a Roe Deer (*Capreolus capreolus*), and a pigeon-sized bird. During the nighttime, a fox and a Stone Marten (*Marte foina*) were recorded. No recordings were made by the camera in the ecotone, which was inactive and may have experienced a battery failure.

There were no significant differences between woodland sites ($\chi^2 = 3.174$, df = 2, $P = 0.205$) and between habitat types in the observed predation patterns (Table 1; $\chi^2 = 0.067$, df = 2, $P = 0.968$). The overall observed predation rate was 18% (39/206) over the 3-d experimental period for all sites pooled. During this 3-d trial, we observed a non-significant decrease in the observed number of attacks: 16, 12, and 11 models were attacked in the first, second, and third day, respectively ($\chi^2 = 1.077$, df = 2, $P = 0.584$).

There were no differences in the proportions of models not attacked versus attacked during the day or the night between the two woodland sites ($\chi^2 = 3.174$, df = 2, $P = 0.205$; Table 1) and therefore the data were pooled in subsequent analyses. Based on all attack data pooled among sites and habitats, however, there was a significant difference (binomial test: two-tail $P = 0.007$) between the total number of models attacked during the day (28/39) and the night (11/39). Finally, the type of predator (i.e., bird, mammal, or undetermined) did not differ between daytime and nighttime (Table 2; $\chi^2 = 1.663$, df = 2, $P = 0.435$). A similar result was obtained when considering only the attacks attributed to mammals and birds (Fisher's exact test, $P = 0.635$).
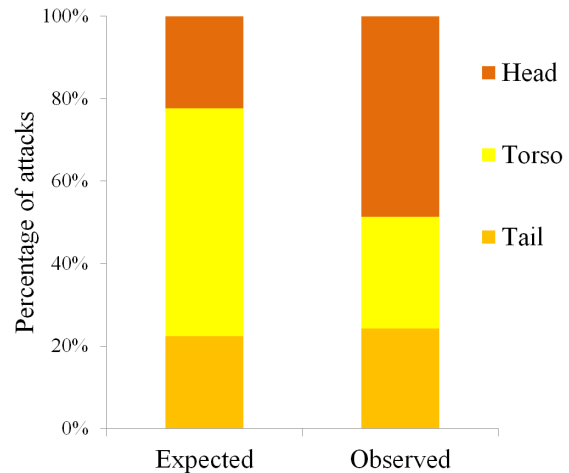
FIGURE 3. Percentages of the expected and observed attacks on the lizard-like clay model body parts. The expected percentages were calculated on the basis of the surfaces of the differents model parts (see text).

DISCUSSION

The main finding of our study was that the potential predation pressure on lizard models during the day was more than twice that observed during nighttime. This result was expected based on the available literature (Seifert et al. 2018) and the specific ecology of the local Common Wall lizard, which is active only during daytime (Biaggini et al. 2011). There were no differences between the two habitat types (woodland and ecotone), suggesting that they were perceived as a continuous habitat, at least by the predators that attacked the lizard-like models. Additionally, predators selectively attacked the heads of the lizard-like models, providing evidence that they perceived clay models as real prey items and were aiming to attack the most vulnerable body part of their target organism. This is consistent with the behavior of predators observed in other studies (Wüster et al. 2004; Worthington and Gill 2019). This outcome is a prerequisite for any reliable application of techniques that use artificial animal replicas in experiments on predator-prey systems (Kuchta 2005; Bateman et al. 2017).

Although it is usually assumed that mammals are primarily nocturnal and use odor rather than visual cues when searching for prey, we found a similar frequency of models attacked by mammals during daytime and nighttime (e.g., Hughes et al. 2010). Therefore, the assumption that mammals, particularly rodents, do not prey upon diurnal prey, particularly terrestrial lizards (e.g., Diego-Rasilla 2003; Oversby et al. 2018), should be validated on a case by case basis. Our experiment with lizard-shaped clay models indicated that, at least in the study area, rodents are active during daytime and

should therefore be evaluated as potential predators on the strictly diurnal and terrestrial lizards. For this reason, excluding mammals as predators of diurnal lizard models may produce spurious results. For example, it may inflate the relative frequency of other predatory animals or reduce the overall predation rate.

Our findings suggest that potential predation rate on clay model replicas is highly context-dependent and that it should be opportune to validate more carefully the occurrence of predation events. This is especially the case of experiments lasting several days or even weeks and, in particular, in cases when models are checked only once at the end of the trial. For example, we recommend a pilot study evaluating the timing of attacks, the animals responsible for them, and the influence of weather conditions. This could be also achieved by checking the models in the field every morning and evening to obtain more reliable timing of the observed predation events (Nibaken and Mappes, 2005; Seifert et al. 2018). This procedure, however, is costly in terms of personnel and may not be feasible in isolated or difficult to reach experimental locations. In addition, the use of camera traps that record the exact time of the attacks should be useful (e.g., Sato et al. 2014), although this last solution appears technically challenging and bears high costs, especially in the case of experiments that display in the field dozens of models at the same time.

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