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Habitat Selection and Overwintering Survival of the Introduced Wall Lizard,

Podarcis muralis

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Habitat Selection and Overwintering Survival of the Introduced Wall Lizard,  
*Podarcis muralis*

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## **Abstract**

Habitat choice can be an important challenge for invasive species as they disperse to a novel environment. Habitat selection is especially important to ectotherms for temperature regulation, but overwintering habitat choice has not been well studied compared to habitat choice during the breeding season. The invasive common wall lizard, *Podarcis muralis*, was introduced to Cincinnati, Ohio in the 1950s from Italy and has since spread throughout parts of southwest Ohio, Kentucky, and Indiana. Previous studies suggested that they are limited to limestone walls in urbanized areas, however, *P. muralis* could use railroad beds as dispersal corridors. We tested the hypothesis that wall lizards prefer habitats that facilitate overwinter survival. We predicted that *P. muralis* would choose habitats that were more buffered against freezing temperatures, and they would favor the limestone walls where they are most commonly found. Manipulative field experiments, designed to test habitat selection between rock, wall, and log substrates, showed that the small rock piles similar to railroad beds buffer best against cold weather. *P. muralis* did not prefer any particular substrate, nor did they prefer warmer individual piles, but overwinter survival of lizards was positively associated with habitat piles that stayed warmer during the coldest temperatures. These findings suggest that habitat choice is not a key factor in the ability of *P. muralis* to survive the colder winters of their introduced range. In light of the apparent risk associated with freezing, selection for other mechanisms to tolerate freezing may be occurring. Contrary to expectations, wall lizards do not appear to be restricted to rock walls, and further spread and increased contact with native species is likely.



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## **Introduction**

Invasive species are a global concern, and they often adversely affect native species (Allendorf and Lundquist 2003; Sakai et al. 2000). Habitat selection may be critical for many invasive species because they need to find appropriate places to breed and survive as they disperse or are introduced in novel environments (Mooney and Cleveland 2001). Alien species that select habitats with characteristics better suited for survival can result in rapid expansion and negatively affect native species (Bontar et al. 2009). Knowledge about features of the habitat that are crucial for survival of invasive species increases the ability to predict what type of habitat certain species will use in novel locations or in response to human habitat alterations, as well as their ultimate distributions as they expand their range (Harvey and Weatherhead 2006). Human facilitated translocations, both accidental and deliberate, are likely contributing to the spread of alien species (Allan et al. 2006). Human structural features within natural environments may also contribute to the spread of invasives by creating novel habitats and empty niches in which they can persist (Bax et al. 2002; Brown et al. 1995; Bulleri et al. 2006; Burke and Deichsel 2008). For invasive species to use human altered habitats, the landscape must harbor physical characteristics that meet the requirements for survival. Understanding the patterns of habitat selection can help predict where invasives will establish and spread in novel areas, and the impact they will have on native species (Alldredge and Griswold 2006; DeGrandchamp et al. 2008).

Habitat selection has important consequences for an animal's life history (Edge et al. 2010), physiology and ecology (Manly, McDonald and Thomas 1993). The ecological performance of an animal includes the behavior, morphology, and physiology of an animal, as well as the environment in which it lives (Huey 1982). The environment of a species and its physiological interactions with the environment will affect its ecological performance, and thus

both are important considerations during habitat selection, especially for ectotherms (Huey 1982; Huey 1991; Martin and Huey 2008; Kingsolver and Huey 2008). Ectotherms must rely on their environment for thermoregulation, and effective choice of habitat is a critical factor for ectotherm growth, performance and survival (Christian and Tracy 1981; Cowles and Bogert 1944; Huey and Kingsolver 1989).

Ectotherms can maintain relatively constant body temperatures by selecting microhabitats with thermal properties that assist in keeping their body temperature within a preferred range (Cowles and Bogert 1944; Porter et al. 1973; Sabo 2003). Habitat requirements are likely to be particularly limiting for invasive terrestrial ectotherms in temperate climate zones, because of the highly variable thermal environment (McConnachie et al. 2007). Lizards should select habitats that optimize thermal properties above ground for basking, below ground within burrows, and within hibernacula (Huey 1991; Porter and Gates 1969; Swoap et al. 1993). Ectotherms typically select habitats based on availability of prey (McConnachie et al. 2007), predator avoidance (Amo et al. 2003; Blomberg and Shine 2000; Downes and Shine 1998), thermal regulation (Huey 1982; Row and Blouin-Demers 2006), hibernation sites (Edge et al. 2010; Huey et al. 1989), breeding sites and reproduction (Dubois et al. 2009), and territory and home ranges (Brown et al. 1995).

Whereas breeding behavior and thermal regulation of temperate zone lizards have been well-studied, less research has been done on the winter behavior and habitat choice of temperate zone ectotherms (Huey 1991; Mathies and Martin 2008). Some reptiles aggregate within hibernacula during winter, which is attributed to either limited over-wintering areas (Vitt 1974) or thermoregulatory requirements (Boersma 1982; Boykin and Zucker 1993). Because ectotherms rely on their environment for maintaining body temperatures, lizards must find hibernacula with appropriate thermal properties, which can be exceptionally important for



survival during long periods of freezing in temperate or montane habitats (McConnichae et al. 2007).

The invasive Common Wall lizard, *Podarcis muralis*, was introduced to Cincinnati, Ohio in 1951 from Lago Garda, Italy and has since spread throughout parts of Ohio, Kentucky, and Indiana (Deichsel and Gist 2001). Ten *P. muralis* were released outside a house with stone walls, which are similar to those they inhabit in their native range (Burke and Deichsel 2008). In the years since the introduction, they have been primarily found in and near habitat with unmortared limestone walls (Deichsel and Gist 2001). More recently, Burke and Deichsel (2008) found *P. muralis* in woody habitats (railroad ties, piles of cut wood) and others have suggested stones of railway beds may also be suitable habitat (Hedeen and Hedeen 1999). Because rock walls are common near their point of introduction, it is not known whether *P. muralis* is restricted to rock wall habitats with occasional dispersal to other types of habitat, or whether they can thrive away from rock walls. The use of a wider range of habitats and substrates would imply a greater likelihood of encountering and affecting native species in Ohio and Kentucky as they expand their range, including *Sceloporus undulatus*, *Plestiodon fasciatus*, *Plestiodon laticeps*, and *Scincella lateralis*. *Podarcis muralis* are suspected of competing with other lizards in Cincinnati (Deichsel and Gist 2001) and in their native range (Arnold and Ovenden 2002), but their effect on native species remains unknown.

We suspect dispersal of *P. muralis* and overwinter survival may be limited by suitable habitat, because the temperatures in Cincinnati are routinely below freezing for several days (Weather Underground, Inc., 2010). *Podarcis muralis* is one of the first vertebrate species shown to have adaptations for cold-hardiness, including supercooling and freeze-tolerance (Weigman 1929). Supercooling allows the body fluids to remain unfrozen at temperatures below

freezing, whereas freeze-tolerance involves the freezing of body fluids, followed by complete recovery (Costanzo et al. 1995). *Podarcis muralis* are considered only moderately freeze tolerant (Weigman 1929; Claussen et al. 1990) compared to some other temperate lizards. They can supercool at body temperatures as low as  $-5.9^{\circ}\text{C}$ , but only up to 26 hours, and they can withstand some freezing of their tissues for a maximum of about two hours. Because they can only freeze for short periods of time, *P. muralis* are vulnerable to extended periods of subfreezing temperatures, and must use other mechanisms to avoid dying (Claussen et al. 1990), such as finding a suitable hibernacula.

We used a manipulative field experiment to test the general hypothesis that *P. muralis* will choose habitats that increase winter survivorship. We chose manipulative field experiments because they offer opportunities for controlled manipulation and replication in a natural setting, which is important for eliciting natural behavior. We tested for preference between three types of habitat: rock walls, railroad bedding rock and treefalls. We hypothesized that lizards would prefer rock walls because walls are so commonly used in their native and introduced range (Deischel and Burke 2008) and because lizards generally take advantage of the thermal properties of rocks for thermoregulation (Huey 1989). We measured lizard substrate preference, activity patterns, and overwinter survival to determine the consequences of habitat choice. We measured the thermal properties of each type of substrate as well as each particular replicate pile to determine if lizard habitat choice or survival was affected by the traits of individual overwintering locations.

## Materials and Methods

### *Study Species*

The European Common Wall lizard, *Podarcis muralis* is an insectivorous lizard that grows to about 15-20 cm. as an adult. They are diurnal and typically active on sunny days and found most commonly on rock walls (Conant and Collins 1991). Breeding season is from April-June and hatchlings are usually seen in August and September depending on the ambient temperature. They are oviparous and lay up to three or four clutches a year of about 5 eggs each (Kwiat and Gist 1987). There are three abdominal scale color morphs in the Cincinnati region population: white, yellow, and red. *P. muralis* are typically shades of brown and green and have blue spots along both sides of their bellies, which are usually more dominant in males than females (Deichsel and Gist 2001).

### *Study Site*

We conducted two manipulative field experiments during the winters of 2008 and 2009. Both experiments were set up along south facing slopes at a local airport in Lunken, Ohio (latitude/longitude: 39/84 degrees, elevation: 483 ft.), which is located about a half mile away from the Little Miami State and National Scenic River and within their introduced range. The enclosures were constructed in a grassy field on a sloped, elevated plateau created by rubble from a building demolition overlain with soil. We observed abundant food for *P. muralis*, including a variety of crickets, grasshoppers, slugs, worms, moths, beetles, and other arthropods. Habitat piles were composed of either stacked flat limestone rocks to mimic rock walls, chipped rock (#2 aggregate) to mimic railroad bedding, or tree trunks to mimic a treefall (Fig. 1). Each

habitat pile was ~1.5 m. wide x 1 m. deep x 0.75 m. high and was backed by an equal volume of soil covered with mulch.

### *Data Collection*

Historical mean daily low ambient temperatures of Verona, Italy (Latitude/Longitude: 45/11 degrees, elevation: 223 ft.) and Cincinnati, Ohio from November to March 1947-1995 are presented in Figure 2. Verona is approximately 20 km. away from Lago Garda, the native source of *P. muralis*. Daily minimum air temperatures were obtained from a weather station maintained by Lunken airport in Cincinnati, OH from Oct. 1, 2009 to March 31, 2010 to investigate the air temperatures at which *P. muralis* was active during our experiments. Monte Scuro (Latitude/Longitude: 39/16 degrees, elevation: 1710 ft.), Italy is approximately 15 km. from Lago Garda, the native source of *P. muralis*, so air temperature was also obtained from a weather station maintained by Monte Scuro airport to compare daily low temperatures.

Temperature data for year one were obtained by placing data recorders (ibutton DS1990A; Maxim Integrated Products, Inc., Sunnyvale, CA) in three replicate piles outside of the enclosure. The sample rate was every 15 minutes from Dec. 30, 2008 to Jan. 18, 2009. We used this data to determine the best ibutton placement and concluded that the center of the pile stayed the warmest. We set up our main temperature experiment in year two based on the conclusions from the pilot experiment and placed one ibutton in the middle of each habitat pile. Each ibutton was placed in a small ziplock bag, within a waterproof capsule with drierite, and inserted through a pvc pipe that was located in the back-center of each pile, approximately ten cm. above the ground and at the interface of the substrate and soil (Figure 1b). The capsule was placed where the dirt and substrate intersected and the pvc pipe was closed off with a clear

plastic tube and several sponge seals to protect from precipitation and to insulate the core from external temperature. The sample rate was every 15 minutes from Oct. 1 to Dec. 5, 2009 and again from Jan. 5 to March 30, 2010. Throughout this paper we refer to cold periods as the average daily minimum temperatures less than ten degrees Celsius, which were obtained because the focus of our experiment is to investigate which substrate buffers best against cold winter temperatures. The average daily maximum temperatures within habitat piles were obtained in order to determine the number of consecutive hours that each substrate maintained a temperature of less than zero degrees Celsius, and thus the duration that each substrate was buffering against freezing temperatures.

Lizards were caught; toe clipped for permanent individual identification; sexed by color, head size, and the presence or absence of hemipenes; weighed to the nearest 0.1 g.; snout-vent length (SVL) was measured to the nearest 0.5 mm.; and were temporarily marked with paint. Lizards were monitored periodically in thirty minute increments approximately 10:00-3:30pm on mostly sunny days to determine habitat use. During the 30-minute scan, the observable lizards (and IDs if possible), and habitat pile on which they were located, were recorded using binoculars and a video camera. Observations of lizard activity began on Oct. 7, 2008 and Oct. 1, 2009 for year one and two, respectively. Habitat choice was determined based on lizard activity, which we calculated as the average number of lizards visible at any 30 minute time period per sunny day.

### *Year 1 Experiment*

Year one was a pilot experiment to determine if lizards could survive in artificial enclosures and to determine if individual identification could be accomplished non-invasively. The experimental

design for year one was comprised of one 12x12 m. enclosure with nine habitat piles (3 rock, 3 wall, 3 log) and 20 lizards: 5 males, 6 females, and 10 subadults (Figure 3a). During year one, drift fence and pvc pipe was used to construct a barrier 1 m. high and was buried 6" into the ground and supported by metal posts. Lizards were collected from four locations within Cincinnati (Lunken, Klotter, Eden Park, and Delhi Avenue). We could not easily identify individuals during the thirty minute scan and we did not have good exclusion of neighboring lizards as a result of our fence design. We believe that our lizards were contained, but we did not anticipate the large lizard population located outside of our enclosure and their ability to climb up the fence wall and into our enclosure. Our fence design for year one controlled for preventing lizards from escaping, but it did not control for lizards coming into the enclosure, and as a result there was an influx of lizards in the spring. Two additional lizards were caught, identified, measured, and added to the experiment on Oct. 31 and Nov. 2. Six out of the 22 total lizards were identified at the end of June when we dismantled the experiment, and only these lizards verified in the experiment before winter were included in our data set of survivors. Survival was estimated by recapture and individual identification using permanent toe clippings and shoulder spots at the conclusion of the experiment on June 24, 2009.

### *Year 2 Experiment*

For the main experiment in year two, we controlled for edge effects, added sixfold replication, and evaluated individual habitat choices. The experimental design was six 23 ft. wide octagonal enclosures, each with three different substrates (1 rock, 1 wall, 1 log), and six lizards placed in each enclosure (Figure 3b). Habitat piles were placed at equal distances from the fence edge and from the other two piles, and oriented towards the south where the observer stood. The

placement of different substrates varied systematically within each enclosure to balance for position effects (See Figure 2b). During year two, plastic sheeting was used to construct a barrier 1m. high and buried 6-10" into the ground and supported by metal posts and PVC tubing. Lizards were collected from four different locations in Cincinnati, OH (Lunken Airport, Fairview Park, Klotter Ave., and Shattuc Ave.). In September 2009, 14 males, 13 females, and nine subadults were sorted into six groups of similar composition and distributed randomly into one of six enclosures so that at least two males were present in each. Individual lizards were initially marked over their dorsal area with one of six colors of paint (Testors enamel paint) to help temporarily identify them from a distance. Photos and video footage were taken of unique spotting patterns and scales above the forelimb to aid in individual identification throughout the observation period. There was low activity observed in enclosure three and very low activity in enclosures five and six during early November of year two. On Nov. 9, 2009, we replaced these "missing" lizards with one, three, and three lizards, respectively. Lizards were introduced to the enclosures by Oct. 1, but we started habitat choice observations on Oct. 5 because we believe this would allow them a few days to adjust to their new surroundings and explore the available habitats and select one accordingly.

Overwinter habitat choice was determined by the last place the lizard was observed in the fall before the onset of freezing temperatures for 31 of 36 lizards. For 22 of these lizards, the last pile observed matched the habitat type most frequented by the lizard in the fall. For the remaining 5 of 36 lizards, the habitat pile where the lizard was seen most in the fall was used because the last sighting was the sole observation on that pile and the last observation did not occur just before the onset of freezing weather. Survival was monitored by individual identification of emerging adults during the first warming periods of the spring and confirmed by

recapture during March 2010. We were able to observe fall and winter lizard activity as separate categories during year one, but had to combine fall and winter activity into one category during year two because lizards ceased being active much earlier than in year one.

### *Statistical Analysis*

A one-way ANOVA was conducted to determine which substrate buffered best against the coldest temperatures as well as to compare the maximum number of consecutive hours that each substrate stayed below 0° C. For both tests, we used the average temperature for each of 18 habitat piles and six replicates of each pile type, when air temperature was below freezing. The year one activity and habitat use was tested with chi square analysis with the number of lizards active per time period. Year two activity and habitat use was compared with a one-way ANOVA with three levels representing the three substrate types, and with a fourth level representing non-substrate use (“other”: grass or fence walls). We used simple correlations to compare the relationship between temperature within habitat piles and fall activity or final habitat choice. We used a t-test to analyze the relationship between surviving lizards and the number of days that each lizard was observed during fall, as well as the relationships between survival and temperature within piles, SVL, and weight. Associations with survival and sex was determined with a chi square analysis.

To test for aggregation in year two, we compared the maximum number of lizards seen in every time period any pile to the expected maximum number of lizards for any pile regardless of substrate. The average deviation of observed and expected was calculated for each enclosure and these six values were tested against the expected no difference (0) using a one sample t-test.



Expected values were generated for each category of total lizards (N=2 to N=6) and expected maxima were calculated using 1000 iterations using MS Excel.

## Results

### *Temperature Data*

The mean daily minimum temperature in January for Cincinnati, Ohio was colder than Verona, Italy (~20 km. from native source) on average by more than four degrees Celsius (Fig. 2). The daily low temperatures of Monte Scuro (~15 km. from native source) and Cincinnati show that Cincinnati reached colder temperatures than Monte Scuro by 6.1 °C in year one and 3.4 °C during year two. The historical average lows from Verona illustrate the temperature pattern for 48 years, and mean monthly daily lows from Monte Scuro during the past two years of our experiments verify that the introduced range is colder than the source location.

During year two, average daily minimum temperatures during cold periods (<10 °C) illustrate that the piles of small rocks buffered best against the cold (1.43 °C), followed by rock wall (0.39 °C), then logs (-0.08 °C) and these differences were significant (Fig. 4;  $N=18$ ;  $F_{2,6}=40.56$ ;  $P=0.0001$ ). All substrates experienced multiple days in a row where the daily maximum temperature never exceeded 0° C, ranging from three days in two small rock piles to 34 days in one log pile. Substrates differed in the maximum number of consecutive hours spent below 0° C (Fig. 5;  $N=18$ ;  $F_{2,6}=6.44$ ;  $P=0.001$ ). Small rock piles stayed below freezing for shorter periods than rock walls, while logs stayed below freezing the longest (Fig. 5; Pairwise Tukey tests:  $P < 0.05$  for log and small rock).

### *Lizard Activity and Habitat Choice*

During year one the lizards were active year round including when air temperatures were below freezing. On average, 20% of the lizards were visible at any time period per day during winter compared to the 30% and 29% in fall and spring, respectively (Table 1). Lizards were observed in winter more often on logs opposed to small rock and rock wall than expected by chance (Fig.6;  $N=146$ ,  $X^2 = 246$ ,  $P < 0.0001$ ). Most lizards were observed on the center log pile, which may have reflected a tendency to aggregate. For instance, ten out of 11 lizard sightings occurred on the center log pile on November 23. On sunny days when lizards were active between November 18 and March 4, groups on the center log pile ranged from three to 11 lizards. The first daily minimum air temperature below freezing occurred on October 23 and was followed by sporadic days that reached below freezing, and then a longer period for multiple days in a row from November 17 to November 24. This suggests that aggregation may have played a role in lizard survival, or the center pile was otherwise unusually preferable perhaps because of its central location. We found evidence of burrowing during the pilot study as two lizards were observed crawling into a hole in the ground below a log pile.

For year two we calculated the proportion of lizards active, or visible during each 30 min time period, from October to the end of November when lizards ceased activity due to cold temperatures (Fig. 7). The overall proportion of lizards seen on rock (0.332), wall (0.327), and log (0.337) were very similar and there was no significant difference in habitat use between rock, wall, and log ( $N=18$ ;  $F_{2,6} = 0.008$ ;  $P=0.992$ ). Although the lizards had no preference between the three types of habitat, they preferred to occupy substrates rather than remain in the grass ( $N=24$ ;  $F_{3,6} = 10.93$ ;  $P=0.0002$ ).

Four of the enclosures had four or five lizards out of the six possible per enclosure choosing the same final habitat pile, which suggested possible winter aggregation as in year one. However, the test for aggregation using final habitat choice did not show dramatic evidence of aggregation and only three out of six enclosures showed signs of aggregation that were greater than expected by chance. Average maximum aggregation was actually less than expected by chance which indicated no aggregation (paired t-test;  $df=10$ ;  $t=0.02$ ;  $P=0.98$ ). Considering all cases where two to six lizards were seen in an enclosure at the same time before winter, only 71 of 156 observations (46%) showed an excess of lizards at a single time period, further suggesting that at these experimental densities there was no evidence for aggregation. There was no correlation between the mean daily minimum temperature of each habitat pile during cold periods and observed lizard occupancy during fall ( $r=0.14$ ,  $P=0.59$ ) or final habitat choice ( $r=0.08$ ,  $P=0.76$ ).

#### *Overwinter Survival*

During year one, five out of 21 (24%) lizards survived including two juveniles, two males, and one female. Twenty-one out of thirty-six lizards (58%) survived the winter in year two. Surviving lizards chose all three types of substrates (Fig. 8a.), and two of six enclosures (replicates 3 and 5) had no survivors. There was no association between survival and type of substrate chosen before the onset of winter ( $N=36$ ,  $X^2=2.47$ ,  $P=0.29$ ). However, surviving lizards tended to occupy habitat piles with significantly warmer temperatures (Fig. 8;  $N=36$ ;  $t=2.06$ ;  $P=0.05$ ), and these habitats were warmer than those of non survivors by approximately 0.49 °C. During year two surviving lizards were not observed more days during the time period from October 5, when lizards were adjusted to the artificial enclosures, to November 28, just before

the onset of freezing temperatures compared to non surviving lizards (N=36;  $t=1.75$ ;  $P=0.09$ ).

Survival was also unrelated to sex (N= 36;  $\chi^2= 0.047$ ;  $P=0.97$ ), mass (N= 36;  $t= 0.76$ ;  $P=0.45$ ), or SVL (N= 36;  $t= 0.42$ ;  $P=0.68$ ).

## **Discussion**

Temperature records and our manipulative field experiments suggest that introduced *P. muralis* in Cincinnati experience colder winters than lizards from their native location in northern Italy. We found freezing temperatures cause high mortality, substrates differ in their ability to buffer against extreme cold, and yet lizards do not appear to select specific substrates or warmer habitats. *P. muralis* have limited cold-hardiness strategies and are vulnerable to extended periods of freezing. Temperature data show that a lizard seeking refuge within a habitat pile would have endured extended periods of time below freezing over several days, and because they aren't choosing between habitats, other mechanisms are likely keeping them alive. Lizards most likely burrow below the substrate into the soil to survive, and our results clearly show that less digging would be required to escape freezing with some substrates over others, and survival was higher for lizards in warmer habitat piles. We found some evidence suggesting aggregation may occur at higher densities, but not at lower densities. Selection pressure to avoid winter freezing appears to be present, but may be manifested in ways other than habitat choice. Invasive *P. muralis* do not appear to be restricted to urbanized areas with rock walls as had been suggested in earlier field studies (Burke and Deichsel 2008).

Cincinnati is colder than much of the native range and source population of *P. muralis*, which presents a challenge for overwinter survival. The year one pilot experiment showed that artificial enclosures were feasible, they allowed lizards to endure freezing temperatures, and they

also were suitable for breeding. At the end of year one, all piles were dismantled, two clutches of eggs were found and one egg hatched in the lab. Year two incorporated a better design with sixfold replication and reduced edge effects within enclosures, and winter mortality decreased (73% in year one to 42% in year two).

### *Habitat Selection*

A considerable amount of literature has shown how ectotherms select microhabitats to increase their physiological performance (Huey 1982; Huey et al. 1989; Martin and Huey 2008; Row and Demers 2006; Seebacher 2009), and lizards generally avoid habitats that decrease their performance (Amo et al. 2004; Amo et al. 2007; Croak et al. 2008). We expected *P. muralis* to follow this pattern, however, our results show that they did not choose habitats that provided better thermoregulatory conditions that could potentially maximize their survival during winter. Choosing warmer habitats would increase survival, yet *P. muralis* do not choose warmer habitats. Small rock piles buffered best against the coldest temperatures, yet *P. muralis* showed no preference for rock piles.

The habitat selection during overwintering and breeding season involve different sets of environmental cues that are likely to affect an animal's behavior or choice of habitat (Lutterschmidt et al. 2006; Mathies and Martin 2008). Two broad mechanisms for lizards to choose between different habitats are the use of a cue or sampling and comparing each habitat. If lizards in our experiment were using a physical cue, then we would have expected them to favor rock walls, where they commonly occur. Most lizards in year two sampled all three substrates by moving between habitat piles in the fall. However, movement activity decreased as winter approached, and it is unlikely that lizards were capable of sampling new habitats when the

temperatures were near freezing. We presume that habitat choice would have to occur at a time before the onset of extreme cold. Our finding that lizards did not choose habitats that helped them to survive the winter suggests that lizards may not be capable of assessing future value of overwinter habitat because of physical, physiological, or cognitive limitations.

### *Selection Pressures for Winter Survival?*

If *P. muralis* are not selecting particular habitats and there is high mortality associated with freezing, then there is the potential for natural selection to act on other traits, for example, burrowing, winter aggregation, or physiological adaptations to avoid or tolerate freezing. Increased burrowing in response to freezing temperatures may reduce mortality (Mathies and Martin 2008) and it may be either innate or learned. We speculate that the potential for learning is minimal because lizards may not have the ability to adjust their strategies during periods of very cold temperatures because they are well below their functional temperature range at these times.

Aggregation or huddling is known in ectotherms that experience low temperatures (Elfstrom and Zucker 1999; Bishop and Echternacht 2004; Gregory 1984; Shah et al. 2003). The decision to aggregate can be affected by available habitat or the presence of conspecifics (Graves and Duvall 1995). At some time point during the winter season many lizards become less aggressive and lose their territorial behavior, which could allow for aggregations to form within hibernacula (Ruby 1978; Stamps and Crew 1976; Marler and Moore 1988). Aggregations of several lizards in a hibernaculum may retain more heat during cold spells (Elfstrom and Zucker 1999), or aggregations may be a sign of communal digging and less energy expended per individual to create a deeper burrow. Our results are equivocal with regard to aggregation in *P.*

*muralis*, and suggest that if aggregation occurs it is only at higher densities. High densities may foster more aggregation behavior, and *P. muralis* attains locally very high densities, up to ~1500 individuals per acre (Kwiat and Gist 1987). Further studies of aggregating behavior in *P. muralis* and its role in overwinter survival are necessary.

If *P. muralis* are not actively choosing habitats with beneficial thermal properties, selection for physiological adaptations to tolerate freezing temperatures may be more pronounced (Les et al. 2009). Evolution for increased freeze tolerance would require genetic differences among individuals on which selection could act, as has been shown in deer mice (Webert and Hoekstra 2009). Temperature data during year two show that the substrates stay below zero degrees Celsius for days at a time (Fig. 5). The most recent experiments on *P. muralis* involving cold-hardiness strategies were conducted 15 years ago (Costanzo et al. 1995), and it would be interesting to see if either supercooling or freeze tolerance has evolved in response to the colder climate in the introduced range.

### *Prospects for Invasion*

In their introduced range, *P. muralis* seem to prefer rock walls because this is where they are most commonly found. Our results suggest that *P. muralis* are not limited to urbanized areas and can survive in other habitats, which will help them to expand their range. *Podarcis muralis* are sometimes seen around raised railroad beds with good southern exposure and less often on south facing forested slopes, but these habitats are not plentiful where *P. muralis* were initially introduced. The range expansion of *P. muralis* is dependent upon the availability of habitat, the time since introduction, and the mode of spread. The most common mode of spread is via humans and *P. muralis* are more likely to be introduced into a garden rather than a sloped area

with treefall (Lescano & Petren ms). The original introduction from Italy to Cincinnati and a large portion of the populations of *P. muralis* in Ohio, Kentucky, and Indiana have been established as a result of anthropogenic dispersal. We anticipate *P. muralis* will expand rapidly to outlying points in urbanized habitat anthropogenically, and then they will expand more slowly by natural means into natural areas where they will encounter larger numbers of native lizards. Further studies on the possible impact of *P. muralis*, on native species are therefore warranted.

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## Tables

Table 1. Lizard seasonal activity and ambient temperature during year one. Lizards were active year round including air temperatures below freezing. N period=number of 30 minute scanning periods; N days= total number of days lizards were observed.

	<b>FALL</b>	<b>WINTER</b>	<b>SPRING</b>
Start Date	10/7/2009	11/18/2009	3/3/2009
End Date	11/6/2009	2/24/2009	3/23/2009
N Periods	62	53	46
N Days	18	15	10
Mean number out per 30 min.	5.9	3.9	5.7
Percentage out per 30 min.	30%	20%	29%
Max Lizards Seen	17	10	9
<b>Mean Daily Temperature (Celsius)</b>			
High	21.8	8.5	18.3
Low	4.2	-3.1	1.8
Average	12.8	2.8	9.9

## Figure Legends

- Fig. 1. a. Frontal view of habitat treatment piles. Substrates used were rock, wall, log, respectively. Dimensions: 1.5 m wide x 1 m deep x .75 m high.
- b. Side view of habitat substrate piles. PVC pipe for ibuttons and temperature data was placed in the middle of each pile.
- Fig. 2. Mean daily low temperatures of Verona and Cincinnati from Nov. to March 1947-1995. Cincinnati was approximately four degrees colder than Verona on average. Verona is about 20 km. from Lago Garda, the native source of *P. muralis*.
- Fig. 3. Experimental design of the pilot experiment during year one (a) and the main experiment during year two (b).
- Fig. 4. Mean minimum temperatures during cold periods ( $< 10^{\circ}\text{C}$ ) per habitat pile for year two; the x-axis is the average of all 18 ibuttons and rock is significantly greater than wall, which is significantly greater than log at buffering against cold temperatures.
- Fig. 5. The average number of consecutive hours that each substrate stayed below  $0^{\circ}\text{C}$  was calculated to estimate the length of time that each substrate was buffering against freezing temperatures.

Fig. 6. Year one winter activity. Average number of lizards seen per day on each substrate; Other =fence or ground; Lizards were observed more on logs than other substrates during winter.

Fig. 7. Year two winter activity. Mean proportion of lizards observed in year two; Other= ground; Lizards used all substrates but had no preference between rock, wall, or log.

Fig. 8. Upper panel: Proportion of lizard activity within each enclosure. Each circle represents an individual lizard. The solid and hollow circles represent whether the lizards were or were not verified after winter, respectively. Grey circles signify that the lizard was not seen past November. The placement of circles is on individual lizard's last preferred habitat before winter. There was no association between survival and preferred substrate. Lower panel: Mean minimum temperature during cold periods ( $< 10^{\circ}\text{C}$ ) recorded per habitat pile, divided by enclosure. Lizards did not occupy warmer piles based on fall activity or final habitat choice. Survivors occupied warmer habitats.

## Figures

a.



b.



Fig. 1.



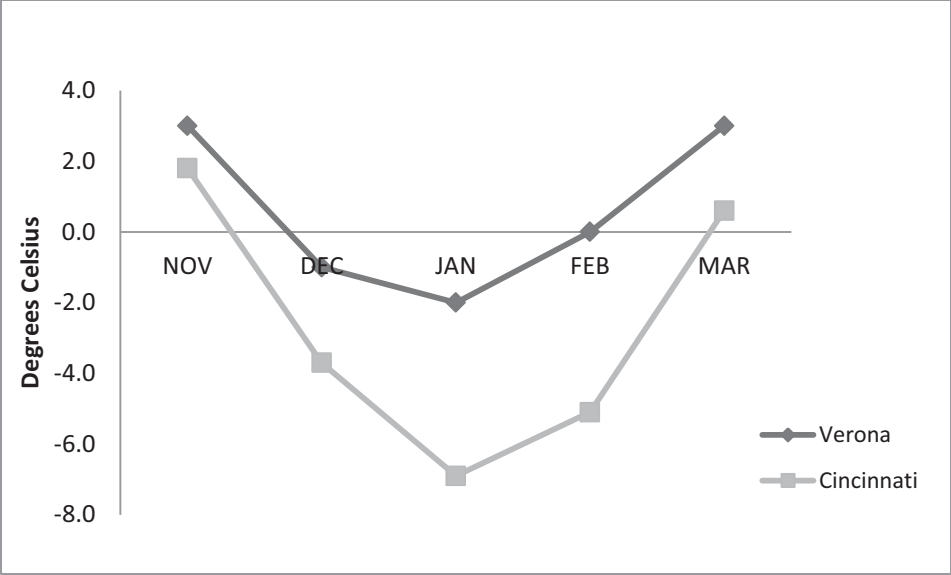
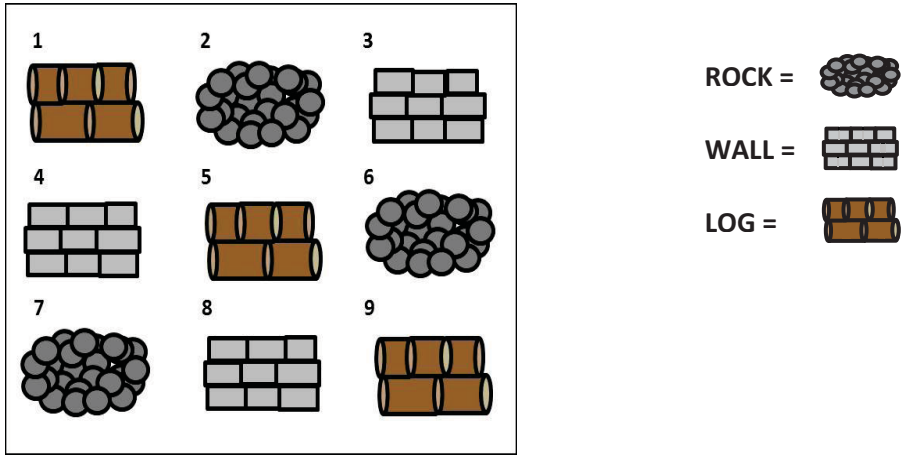


Fig. 2.

a.



b.

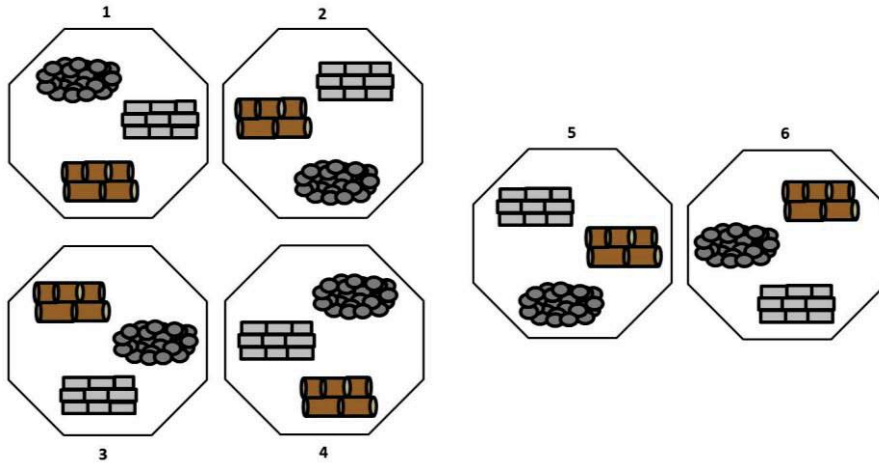


Fig. 3.

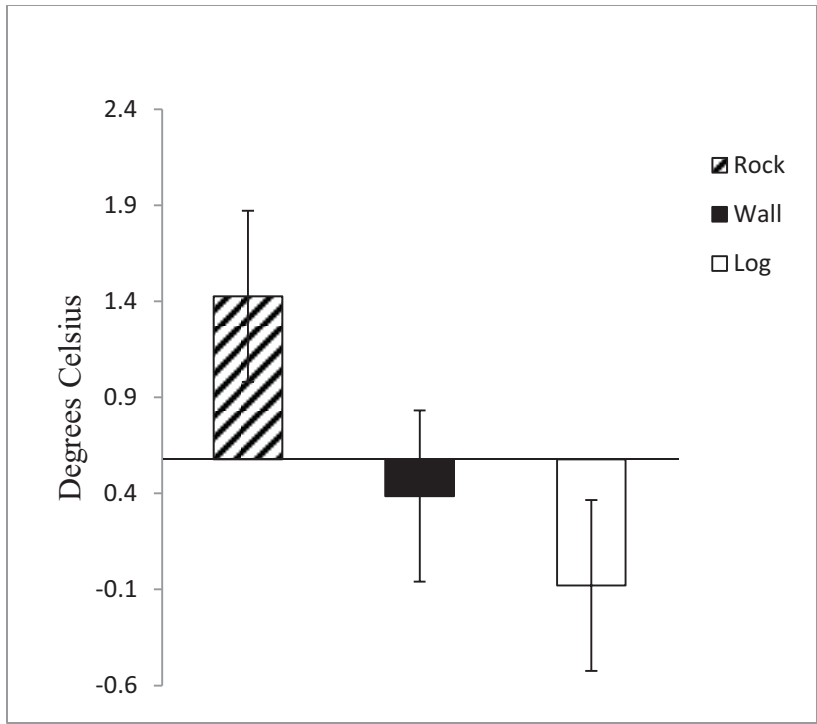


Fig. 4.

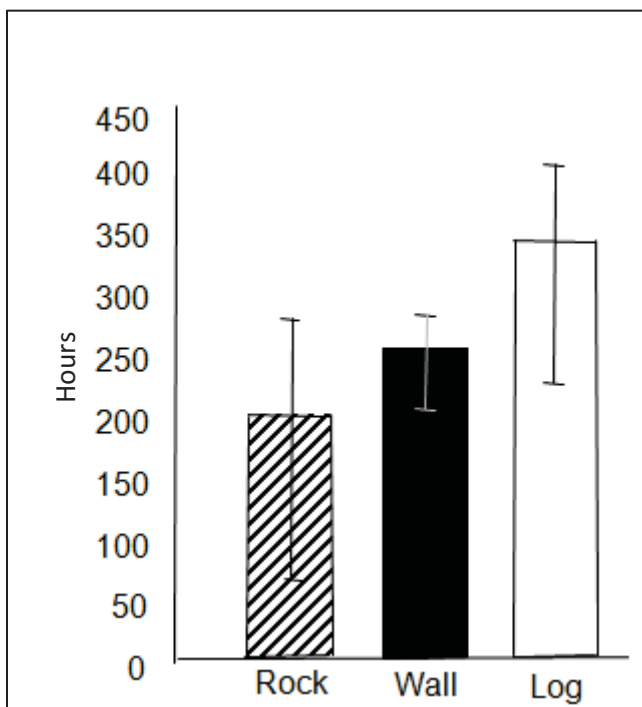


Fig. 5.

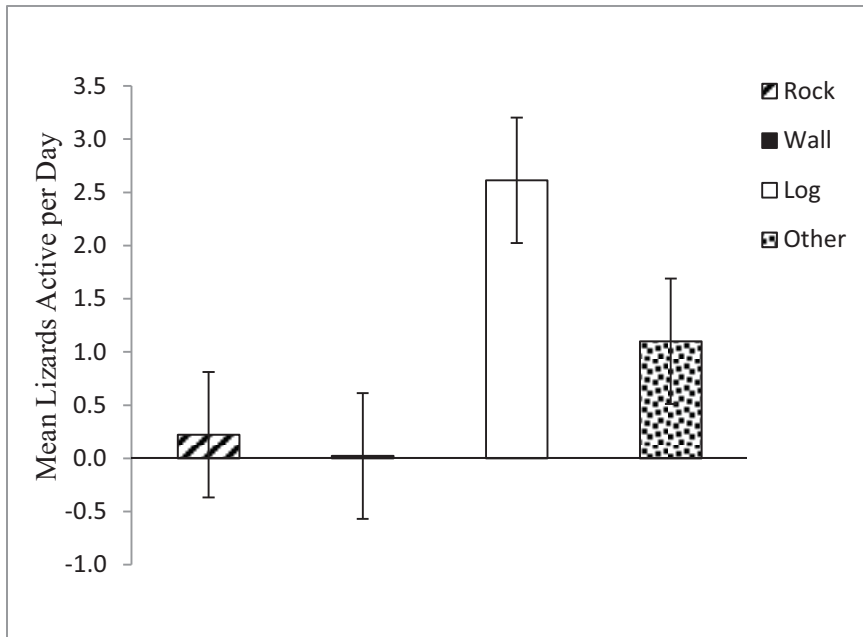


Fig. 6.

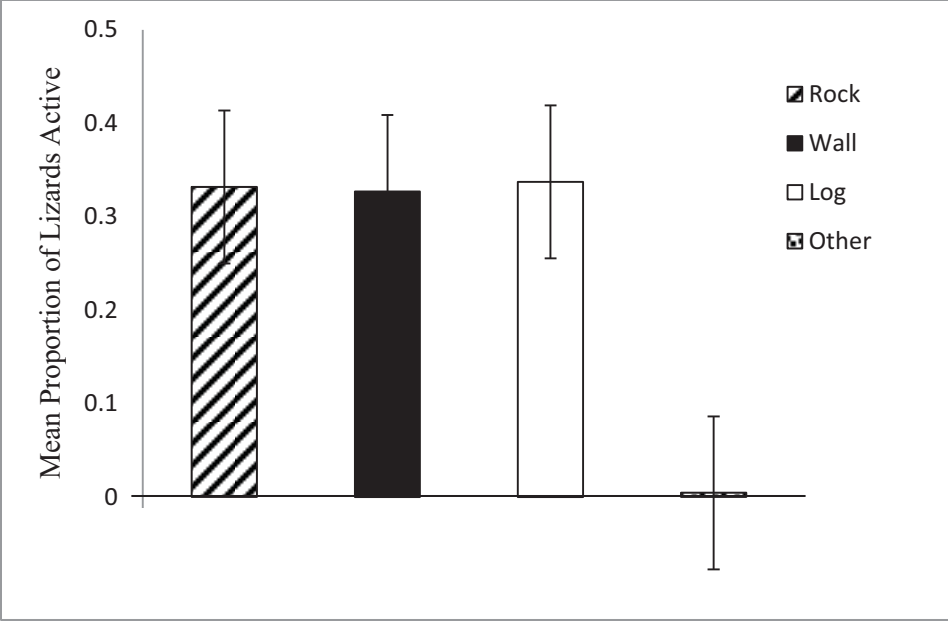


Fig. 7.

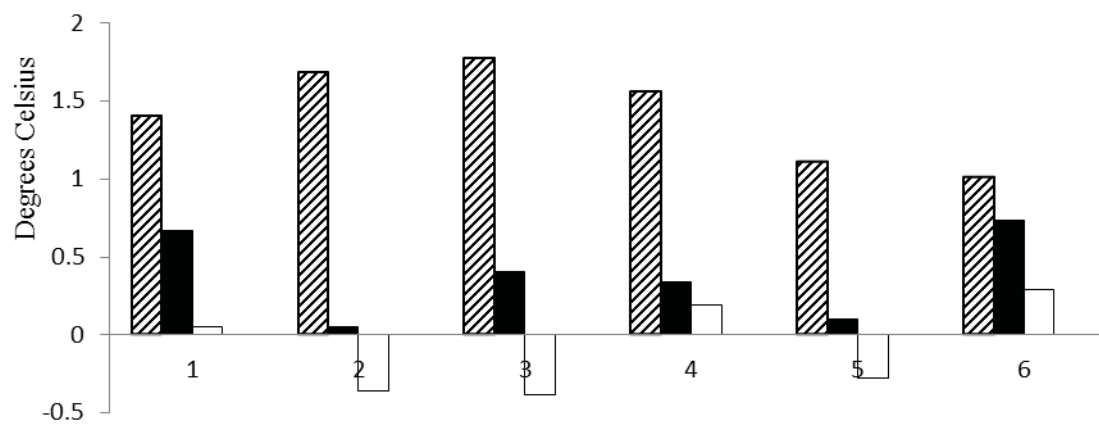
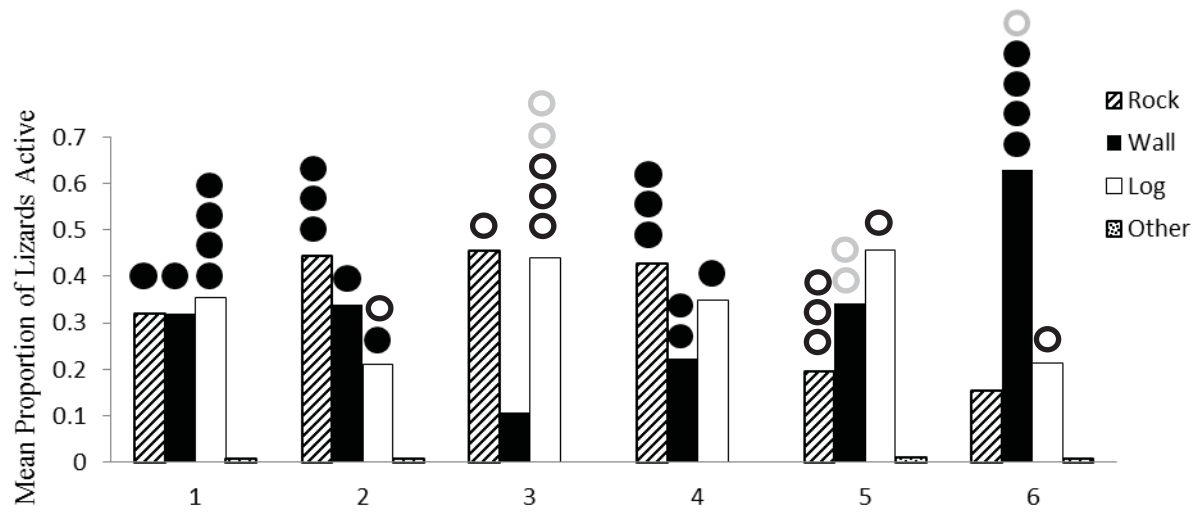


Fig. 8.