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Alteration Effects of Ornamental Whitewashing of Rocks on the Soil Properties and Body Condition of Fossorial Amphisbaenians that Live Under Them

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***Abstract.*—The ecology and conservation state of fossorial herpetofauna is poorly understood but they may be at particular risk from soil disturbance. However, their low conspicuousness may lead conservationist researchers and managers to overlook potential problems. Amphisbaenians are a group of fossorial reptiles but, because of their secretive habits, there are few data on their ecology and potential conservation problems. Here, we examined the effects of an anthropogenic factors (alteration of rocks by whitewashing), which affects chemical properties of the soil, on body condition of fossorial *Trogonophis wiegmanni* amphisbaenians. Results indicated that the increased amounts of inorganic carbonates under whitewashed rocks may negatively affect the body condition of amphisbaenians, their fitness, and the health of the populations. We suggest potential management actions to solve this problem and emphasize the need for periodic surveys of subterranean herpetofauna to prevent future conservation problems.**

Key Words.—amphisbaenians; fossorial reptiles; North Africa; soil alterations; *Trogonophis*

Introduction

Several groups of reptiles and amphibians, as high as 20%, nearly 3,000 species of the herpetofauna of the world, are fossorial (Measey 2006). However, as occurs with other fossorial animals, their ecology and conservation status is much less well understood than that of their epigeal relatives (Copley 2000; Wolters 2001; Böhm et al. 2013). This may have unfortunate hidden consequences for conservation because anthropogenic soil disturbance may greatly affect fossorial species, and local extinction of populations may be occurring unnoticed (How and Shine 1999; Decaens et al. 2006; Measey 2006; Measey et al. 2009).

Amphisbaenians are reptiles with important morphological, functional, and ecological adaptations to fossorial life (e.g., Gans 1978; Papenfuss 1982; Martín et al. 1991; López et al. 1998; Webb et al. 2000). However, because of their secretive habits, there is very little information on their ecological requirements and there is a chronic lack of data on their potential conservation problems (Böhm et al. 2013). The amphisbaenian *Trogonophis wiegmanni* (Fig. 1a) is a North African species (Bons and Geniez 1996; Gans 2005) that lives buried in the soil and it is usually found thermoregulating or foraging under rocks (López et al. 2002; Civantos et al. 2003; Martín et al. 2011a, 2013a,b). This species is listed as of Least Concern by the International Union for Conservation of Nature (IUCN) in view of its wide distribution and presumed large population (Mateo et al. 2009). However, the potential threats to this species have been little studied (Martín et al. 2011b, 2015), in spite that soil alterations that may have a profound impact on the health and conservation of this species and other fossorial reptiles.

Urbanization has induced modifications of physical, chemical, and biological properties of soils (Craul 1992). Anthropic activities have led to profound modifications of the original soil horizons and the addition of organic and artificial residues (Craul 1992). In addition, in Mediterranean countries, walls and rocks are often traditionally white-washed for ornamental purposes, for example for marking paths or boundaries (Fig. 1b). Whitewash, or lime paint, is a type of paint made mainly from slaked lime (calcium hydroxide, $Ca[OH]_2$) and chalk (whiting calcium carbonate, $CaCO_3$) mixed with water. Whitewash cures through a carbonatation reaction with carbon dioxide in the atmosphere to form calcium carbonate in the form of calcite. This is a very cheap and easy way to create an effective white paint. Moreover, in the past (now it is discontinued) people sometimes added white lead paint, which is based on lead carbonate ($PbCO_3$), to obtain brighter whites and increase durability. However, the paint on rocks washes down into the soil over the course of time potentially affecting chemical properties of the soil. These soils are,

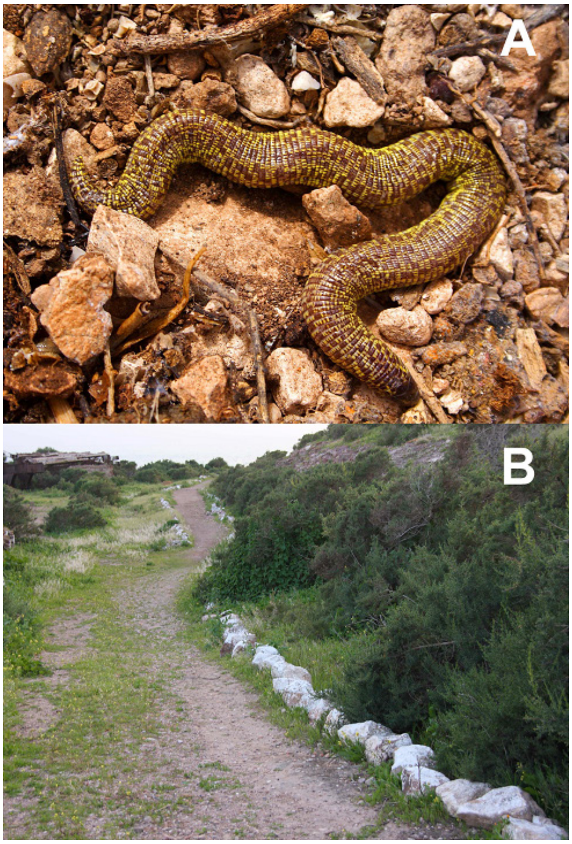

Figure 1. A) Adult *Trogonophis wiegmanni* amphisbaenian found under a rock. (Photographed by José Martín). B) Example of path marked with lined whitewashed rocks, under which amphisbaenians can be found, at the Chafarinas Islands (NW Africa). (Photographed by José Martín).

however, often used by fossorial animals, but alteration of the soil chemical properties and the possible presence of contaminants such as lead (Markus and McBratney 2000; Sparling et al. 2010) might affect amphisbaenians directly or indirectly; for example, through effects on their invertebrate prey.

We hypothesized that the potential alteration of chemical properties of the soils under whitewashed rocks may affect the body condition of *T. wiegmanni* amphisbaenians that used these soils. Body condition is an important measure of fitness because it reflects the relative amount of energy stores (Green 2001; Schulte-Hostedde et al. 2005) that can be allocated to maintenance, growth, or reproduction (Perrin and Sibly 1993; Heino and Kaitala 1999; Madsen and Shine 2002). A change in body condition can affect population dynamics.

Materials and Methods

We conducted field work at the Chafarinas Islands (Spain) during two weeks in March 2011. This is a small archipelago located in the southwestern area of the Mediterranean Sea (35°11'N, 02°25'W), 2.5 nautical miles off the northern Moroccan coast (Ras el Ma, Morocco) and 27 nautical miles to the east of the Spanish city of Melilla. It consists of three small islands: Congreso (25.6 ha), Isabel II (15.1 ha) and Rey Francisco (13.9 ha). The Chafarinas Islands are designated as a nature reserve dependent of the National Parks Spanish Organism. We made the study in Isabel II, which is the only island currently inhabited temporally by a small human population (fewer than 50 people). However, the island supported a greater population until the first half of the 20th Century. Many buildings (some in ruins), often with whitewashed walls, and paved streets cover 34% of the island surface (García 2005). Vegetation of natural areas is dominated by woody bushes (*Salsola*, *Suaeda*, *Lycium*, and *Atriplex*) adapted to salinity and drought (García et al. 2002; Martín et al. 2013a). Soils are poorly developed and immature and are characterized by a thin A horizon, rich in organic matter, which is underlain by the original volcanic rock (García 2005; García et al. 2007). Several sand or paved paths running throughout the island are often marked with lined whitewashed or non-painted rocks, both taken from the nearby habitat where they are abundant. Whitewashed rocks were not necessarily linked to soils with other anthropogenic alterations (e.g., compaction or addition of residues), as these paths often cross natural habitats far from human buildings. Amphisbaenians are abundant and often found under rocks in the paths and surrounding areas.

We searched all the available types of habitats (natural and altered; García 2005). We found amphisbaenians by lifting rocks on warm sunny days and between 0700 and 1800 (GMT). We captured amphisbaenians by hand and noted whether the rocks where they were found were whitewashed or non-painted. We gathered morphological measurements in situ and released amphisbaenians at their exact point of capture in < 5 min. We measured total length (TL; from the tip of the snout to the tip of the tail) with a metallic ruler (to the nearest 1 mm) and body mass with a Pesola spring scale (to the nearest 0.01 g). To avoid confounding effects, we measured individuals with empty stomachs. Amphisbaenians usually expulsed most gastrointestinal contents when handled, but we also compressed gently their vents to force the expulsion of faeces. We used as a body condition index the residuals of an ordinary least squares linear regression of log-transformed mass against log-transformed total length (r = 0.97, $F_{1,38}$ = 645.4, P < 0.001). Such residuals have been considered to provide the cleanest way to separate the effects of condition from the effects of body size (Bonnet and Naulleau 1994; Jakob et al. 1996; see reviews in Green 2001; Schulte-Hostedde et al. 2005). Body condition indexes are used as proxies of health state in reptiles and

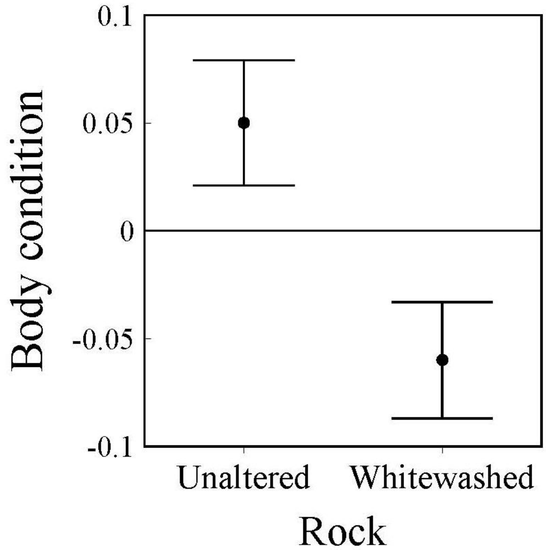

Figure 2. Comparison of body condition (mean; SE) of amphisbaenians found under unaltered rocks or rocks that had been whitewashed by men.

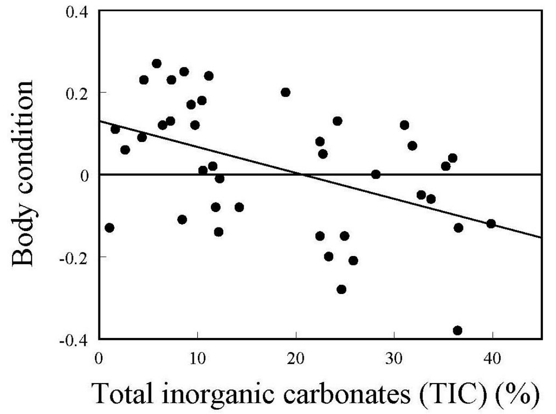

Figure 3. Relationship between proportion of total inorganic carbonates (TIC) in the soil under rocks and body condition of amphisbaenians found in those soils.

many other animals (Amo et al. 2006, 2007; Wikelski and Cooke 2006; Brischoux et al. 2012).

Then, to examine whether whitewashed and non-painted rocks where we found amphisbaenians were located in similar microhabitats, we estimated visually the percentage cover of grass, rocks, bare soil, and bushes in a circular area of 2 m diameter around the rock (Martín et al. 2013a). Subsequently, to test for the potential effects of whitewashing on soil characteristics, we took a bulked soil sample (around 300 g) under the rock, between the surface and 10 cm depth (or less if the soil was less deep), coinciding with the soil layers more used by amphisbaenians (pers. obs.). In the laboratory, soil samples were air-dried, crushed, and sieved (< 2 mm) to analyze chemical characteristics. We measured soil total inorganic carbonates (TIC) using a pressure-calcimeter and pH with a combined electrode in soil paste with either water (H_2O) or KCl (1:2.5). The availability of Pb was determined using an ICP-OES device, after extraction with a neutral 0.05 M-EDTA solution (for details of chemical analyses see Sparks 1996).

We treated all observations of amphisbaenian as independent given their high density (Martín et al. 2011b,c) and because we avoided sampling the same area twice. Thus, the probability of repeated sampling of the same individual was extremely low. To test for overall differences between sites occupied by amphisbaenians under whitewashed or non-painted rocks, we used General linear Models (GLMs), multivariate analyses of variance (MANOVAs), and linear correlations (Sokal and Rohlf 1995). Variables expressed as percentages were subjected to angular transformation; the remainder, except pH, were logarithmically transformed to ensure normality. In all cases, after transformation, data were normally distributed (tested with Shapiro-Wilk's tests).

Results

We found amphisbaenians both under whitewashed rocks that marked the margins of some tracks, gardens, etc., and under non-painted rocks in the same paths or the nearby surroundings. Whitewashed and non-painted rocks where we found amphisbaenians were located in similar microhabitats (MANOVA for the four microhabitat cover variables, Wilks' $\lambda = 0.93$, $F_{4,35} = 0.614$, $P = 0.655$). Considering only the rocks used by amphisbaenians, soils under the whitewashed rocks had a significantly higher proportion of total inorganic carbonates (TIC) than soils under non-painted rocks (mean ± SE, 29.3 ± 2.4% vs. 12.1 ± 1.6%, respectively; $F_{1,38} = 36.71$, $P < 0.001$). Soils under whitewashed rocks also had higher values of pH (pH_{H2O}: 8.47 ± 0.16 vs. 7.72 ± 0.76, respectively; $F_{1,38} = 11.25$, $P < 0.001$; pH_{KCl}: 7.63 ± 0.16 vs. 7.29 ± 0.62, respectively; $F_{1,38} = 3.47$, $P = 0.070$). In addition, Pb concentration in soils under whitewashed rocks was significantly greater than under non-painted rocks (33.6 ± 4.3 mg/kg soil vs. 16.0 ± 8.7 mg/kg soil, respectively; $F_{1,38} = 13.64$, $P < 0.001$).

Amphisbaenians were similarly abundant under non-painted or whitewashed rocks and the body condition of amphisbaenians under whitewashing rocks was not significantly different than under rocks that had not been whitewashed ($F_{1,38} = 3.25$, $P = 0.079$; Fig. 2). Despite non-significance, the body condition of amphisbaenians decreased significantly when the proportion of inorganic carbonates in soil increased ($r = -0.47$, $F_{1,38} = 10.78$, $P = 0.002$; Fig. 3). However, body condition was not significantly related with pH values (pH_{H2O}: $r = 0.01$, $F_{1,38} = 0.001$, $P = 0.975$; pH_{KCl}: $r = -0.05$, $F_{1,38} = 0.114$,

$P = 0.737$) or with concentration of Pb in the soil ($r = 0.14$, $F_{1,38} = 0.732$, $P = 0.398$).

Discussion

Our results indicated that the anthropogenic disturbance of whitewashing rocks for ornamental purposes induced important changes in soil chemical properties. Moreover, we found that body condition of fossorial amphisbaenians *T. wiegmanni*, although not significant, tended to be lower under these whitewashed rocks in comparison with amphisbaenians found in similar habitats under non-painted rocks. The pavement of surfaces and the construction of buildings directly eliminate the habitat available for soil animals (Craul 1992). However, our study suggests that, in addition to these major alterations, even apparently minor anthropogenic indirect alteration of soil properties may be a threat to fossorial animals. This is because amphisbaenians are still abundant in the seminatural areas close to buildings, found under unaltered and whitewashed rocks, but also below anthropogenic materials such as bricks, tiles, or concrete roof tiles. In addition, the low mobility rates of individual amphisbaenians in the soil (José Martín, unpubl. data) may make them more vulnerable to soil alterations in the small areas where they live, as they would not be able to disperse too far from the suboptimal areas.

Artificially whitewashed rocks that are common in these Mediterranean areas, among other regions, resulted in increased levels of inorganic carbonates and lead and increased pH in the soil below them. Amphisbaenians living under whitewashed rocks tended to have lower body conditions, which probably resulted from the high levels of soil carbonates, which are negatively related to body condition in all the population. This observation contrasts with the overall selection by this amphisbaenian species of soils with naturally higher pH values (Martín et al. 2013a). However, this general pattern of soil selection may simply result of the avoidance of the more acid saline soils, which detrimental effects are higher (Martín et al. 2015).

The potential physiological effects of inorganic carbonates in amphisbaenians are unknown, but the calcium hydroxide used to paint rocks has well known biocide and antiseptic properties that can kill microrganisms and invertebrate parasites. For example, sometimes, whitewashing is used to prevent ants from climbing tree trunks. Also, whitewashing increases soil pH levels, which reduce availability of micronutrients, phosphorus, and nitrogen in the soil. Thus, it is likely that whitewashing has detrimental effects on invertebrates of the soil and that refuge under rocks, reducing availability of prey for amphisbaenians.

Contamination by lead observed under whitewashed rocks can come from paint chips containing lead (i.e., white lead paint), but lead in soil can also come from water dissolution of old lead pipes or from incineration of garbage (Markus and McBratney 2000). Exposure to lead may cause adverse effects to human and animal health (Carrington and Bolger 1992; Sparling et al. 2010). Lead paint is hazardous, it can cause nervous system damage, stunted growth, kidney damage, and delayed development (Duggan and Inskip 1985; Lidsky and Schneider 2003). However, we have not found a direct effect of lead in soil on body condition of amphisbaenians, which may be explained by the relatively low levels of lead contamination in our study site. Nevertheless, higher levels of contamination by lead may be more detrimental and, also, there could be other hidden adverse effects of lead on other physiological parameters of amphisbaenians not considered here but that should be examined (Sparling et al. 2010).

It might be argued that the location of many whitewashed rocks close to or in areas with anthropic disturbed soil might also explain, rather than the rock whitewash per se, the observed differences in body condition of amphisbaenians. This is because, for example, anthropogenic altered soils are more compact and body condition of amphisbaenians is lower in the most compact soils (Martín et al. 2015). However, amphisbaenians were found both under many unaltered rocks that were also located on anthropogenic disturbed soils and under many whitewashed rocks that were located in relatively undisturbed natural areas. In fact, our results showed that characteristics of microhabitats surrounding whitewashed and non-painted rocks used by amphisbaenians were similar. Nevertheless, the interaction of the effects of whitewashing with additional multiple effects may explain that the observed decrease in body condition of amphisbaenians under whitewashed rocks was not larger and of significance.

We conclude that apparently minor anthropogenic alterations of the rocks used by anphisbaenians deeply affect soil properties and that these changes in the soils might affect body condition of *T. wiegmanni* amphisbaenians. We suggest avoiding whitewashing of rocks and soils in areas used by amphisbaenians, which may help to mitigate negative consequences of soil alteration on amphisbaenians. Finally, our study emphasizes that, to prevent future conservation problems of fossorial animals, we need to perform periodic surveys to collect baseline data on the health state of the little known subterranean herpetofauna.

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