INVASION NOTE



Biodiversity in a box: three non-native invertebrates preferentially find refugia in green space management infrastructure across urban Los Angeles

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Abstract In Southern California, irrigation infrastructure is a prerequisite for urban green space management, and valve boxes are installed widely to manage water flow. These below-ground, plastic boxes protect valves and manifolds, create space for connecting pipes, and present a scarce ecological resource—elevated humidity and shelter from potential predators. We provide the first systematic survey of the biodiversity of valve boxes and evaluate their

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Department of Ecology and Evolutionary Biology, University of California, Los Angeles, Los Angeles, CA 90095, USA role in the establishment of non-native species. We conducted comprehensive surveys of slug and spider refugial habitat elements, including leaf litter, crevices, decaying logs and other cover objects, and valve boxes, across urban Greater Los Angeles and adjacent wildland areas. We found that valve boxes comprised nearly all of the surveyed habitat for three common non-native species, including two slugs in the genus Ambigolimax and a spider in the genus Steatoda. At 83 of 85 sites, we detected these species only in valve boxes and not in any other habitat elements. While valve boxes were significantly more frequent in urban than wildland areas, detections in wildland areas were also largely restricted to valve boxes. All of these species share a preference for elevated levels of humidity, and we speculate that introduced slug and spider taxa within irrigation infrastructure may be a general feature of many urban areas, especially in relatively xeric climates with locally high densities of valve boxes. Under these conditions, irrigation infrastructure likely facilitates the establishment and persistence of non-native species requiring high humidity throughout the urbanized world, and could contribute to their cosmopolitan distribution.

Keywords Risk management · Human-mediated dispersal · Urbanization · Nature-based solutions · Urban green infrastructure · Connectivity

Introduction

Urban green spaces are indispensable for maintaining ecosystem services in cities (Bowler et al. 2010; Larondelle and Haase 2013) as well as high levels of biodiversity and connectivity within the urban habitat matrix (Beninde et al. 2015). Grass lawns are a ubiquitous element of urban green spaces worldwide (Ignatieva et al. 2020) and their management requires watering, especially in regions receiving little or highly seasonal precipitation. Southern California receives modest annual rainfall, drought years are common (Okin et al. 2018), and irrigation infrastructure is a prerequisite for any urban green space, particularly those containing lawns.

Valve boxes (also referred to as sprinkler boxes) are a standard component of irrigation infrastructure that provide protected, below-ground access to the valves used to manage water flow and are installed widely throughout urban green spaces in Southern California (Fig. 1A). These closed, plastic boxes represent a scarce ecological resource: a high-humidity, protected shelter that is not easily accessible to many potential predators. Our field observations indicate that soil within boxes is usually moist, in contrast to drier soil in the immediate surroundings outside of boxes, and condensation frequently occurs on the inside walls of boxes. While they are a "closed" environment, valve boxes necessarily have small, below-ground entry points, where water lines enter and leave. Boxes are closed-off with covers, which usually contain small openings, generally < 1 cm in size, and human interference is infrequent. Valve boxes therefore present a unique, relatively ubiquitous microhabitat for small fossorial or secretive species requiring elevated levels of humidity. Such habitats are naturally extremely restricted in Southern California, and native species that require them are also rare. For example, there are 17 native species of terrestrial slugs in California (Mc Donnell et al. 2009), including the iconic banana slugs of the genus *Ariolimax*. However, these and other native land-living slugs are most common in wetter parts of central and northern California, as demonstrated by more than 15,000 iNaturalist observations (as of April 2022).

One of the challenges faced by urban biodiversity surveys is the high proportion of available open space that is privately owned and therefore inaccessible to most researchers. Community or citizen science is a solution to this problem that has been extremely successful in Los Angeles and many other urban centers. Projects initiated and hosted by the Natural History Museum of Los Angeles County (NHMLA), such as Snails and slugs Living in Metropolitan Environments (SLIME) and the Spider Survey (Ballard et al. 2017), have elucidated fine-scale distribution patterns of many species in Greater Los Angeles. As part of these efforts, more than 1200 records of slugs of the genus Ambigolimax have been reported from Los Angeles County (Vendetti et al. 2019). Native to the Iberian Peninsula and mostly recorded from temperate regions, some Ambigolimax species have been introduced globally and have cosmopolitan distributions that include the tropics (Yap and Tan 2021). Ambigolimax valentianus (Férussac, 1821) was first recorded in California over 100 years ago (Gregg 1944). In contrast, Ambigolimax parvipenis Hutchinson, Reise, and Schlitt, 2022, formerly classified as A. nyctelius (Bourguignat, 1861; Hutchinson et al.,

Fig. 1 The inside of a valve box, with the cover removed (A), and an inverted valve box cover (B), containing (I) *Ambigolimax* spp. slugs, (II) *Oxychilus* sp. non-native snails, and (III) the egg sac of a false widow spider, *Steatoda grossa* (white, attached to the rim in the top right)



2022) was collected and reported for the first time in California as part of SLIME's sampling efforts (Vendetti et al. 2019). Unfortunately, the two species are indistinguishable based on external morphology, and the distribution of *A. parvipenis* may well be under-documented (Vendetti et al. 2019). Information on dispersal, habitat requirements, and resource use by both species is limited, and their morphological near-identity requires either molecular or anatomical dissections for unambiguous species identification. It is likely that both slug species were accidental introductions via horticulture (Cowie et al. 2008).

Like A. valentianus, the False widow spider, Steatoda grossa (Koch, 1838) has a cosmopolitan range, and is widely distributed across Europe and North America. Steatoda grossa was first reported from Los Angeles County in the 1970's, and has recently emerged as the spider species most commonly detected in the Los Angeles County Spider Survey, with nearly 600 records (Kempf et al. 2021).

We here report on the association between valve boxes and the presence of these three non-native species in the greater Los Angeles ecosystem. All three are small, secretive, and share a preference for elevated levels of humidity (Harvey 2021). We quantify their presence in different habitats across multiple survey sites, compare their frequencies in urban versus wildland areas, and identify habitat elements that are most strongly associated with their presence. Identifying such associations is the first step in developing management strategies for these non-native taxa across the urban Los Angeles landscape.

Material and methods

Field work was conducted in the Southern California counties of Los Angeles, Orange, Riverside, San Bernardino and Ventura, from March through August 2019. The study area covers roughly 7,800 km² and was designed to include large parts of the Greater Los Angeles urban region, especially the heavily urbanized city of Los Angeles and the surrounding mountainous areas (Fig. 2). These wildland mountains retain large areas of native vegetation, predominantly California Coastal Sage Scrub, and transition



Fig. 2 190 Sampling sites within the study extent encompassing large parts of the Greater Los Angeles metropolitan area (grey=urban areas; green: wildland areas). Inset shows the study extent with respect to County boundaries and California

steeply into urban ecosystems. Our study design specifically contrasted adjacent urban and wildland habitats, which were identified following the US Census Bureau (2018) designations.

Our field surveys were part of a larger effort to obtain genetic samples for a multispecies landscape genomics project, which focuses on widely distributed, common species (the UCLA Los Angeles Genomics Project). Subsequent to many drought years in the region, there was an exceptionally high level of precipitation in the first half of 2019, prior to and during the sampling season, which increased habitat suitability for our three focal species and increased the detectability of slug species. Sites were searched systematically, with a focus on suitable habitat elements for slugs and spiders. We targeted five habitat types where spiders and slugs are often encountered: (i) under small-to-medium sized, flippable rocks (~5-20 cm); (ii) in and among logs/ deadwood; (iii) within crevices between buildings/ structures/rocks; (iv) in valve boxes; and (v) under leaf litter. Two additional habitat elements indicate consistently high moisture levels and were chosen specifically to describe suitable slug habitat: (vi) decaying vegetation; and (vii) puddles. Presence of puddles indicated recent rainfall and increased slug detectability. When encountered, habitat elements (i)-(vi) were searched thoroughly, by turning logs, combing through leaf litter, or lifting covers of valve boxes. We focused on plastic valve boxes, as these are mostly used for water management and their covers are easily lifted. They are most frequent either with rectangular covers (30 cm x 50 cm and a depth averaging 30 cm) or with round covers (about 20 cm in diameter and 30 cm average depth). We did not search concrete boxes, which are commonly used for electric, telephone, CATV, and broadband infrastructure, and are also widespread in Greater Los Angeles.

In urban areas, we generally sampled urban green or open spaces; in wildland areas, sites were frequently located along trails or in public parks. To quantify our sampling efforts, we noted the total amount of time spent at each site, the proportion of that time spent searching for the three focal taxa (since other taxa were also being sampled at some sites), and the area searched at each site. The total area searched was estimated using Google Earth at the end of each field day. In addition, the availability of the seven habitat elements described above was quantified at over half of all visited sites, on a scale from 0 to 3. This scale was based on the approximate distance between habitat elements: a mean distance between habitat elements < 50 m was scored "3"; a mean distance between 50 and 500 m was scored "2"; a mean distance > 500 m was scored "1"; and no habitat elements was scored as "0". When any of our three focal species were detected, we recorded the associated habitat element in which it was found. We analysed the availability of suitable habitat elements jointly for slugs and spiders and compared their distribution between urban and wildland sites using Wilcoxon rank sum tests. We also collected and genetically barcoded (using mtDNA CO1 sequence data) a variable number of slugs from nine sites to distinguish A. parvipenis from A. valentianus, following Vendetti et al. (2019).

To quantify the presence of valve boxes in urban areas more generally, we counted the number of valve boxes at two urban green spaces on the campus of the University of California Los Angeles (UCLA) and along a segment of Santa Monica Boulevard in the cities of Los Angeles and Santa Monica in May and June 2021.

Results

Sampling intensity

We visited 190 sites, of which 144 were situated in urban and 46 in wildland areas. A total of 217.5 person-hours were spent in the field, of which 72.75 were dedicated entirely to detecting the focal slug and spider species. 50.5 h (69%) of detection search hours were spent in urban areas, and 22.5 h (31%) in wildland areas. Field work was conducted at varying times of the day, including 29.25 search hours between sunset and sunrise when spiders and slugs are most active. Of these night search hours, 18.75 h (64%), were conducted in urban areas and 10.5 h (36%), in wildland areas. The proportions of search effort dedicated to urban and wildland areas closely match the spatial extents of both habitat types within the study region, composed of 65% urban areas and 35% wildland areas.

Differences in suitable habitat elements between urban and wildland sites

Habitat elements were quantified for 121/190 (63%) of all sites. All habitat elements except valve boxes and leaf litter showed higher availability in wild-land areas, although this difference was only significant for flippable rocks. Leaf litter availability was very similar between urban and wildland sites, while valve boxes showed the strongest difference in mean availability between habitat types, and were more than twice as common in urban as in wildland sites (Table 1).

Species detected in valve boxes

The spider *S. grossa* was detected at 64 sites: 53 urban and 11 wildland. Of the 11 occupied wildland sites, five were within 150 m of urban areas, five were in managed areas with high human frequency (parking lots and picnic areas of state or regional parks), and one was in an area that recently transitioned from wildland to urban land use. All individuals of *S. grossa* were detected within valve boxes with the exception of a single individual that was found beneath a log in the Simi Hills in Ventura County, CA.

Table 1 Mean differences (±standard deviation) in avail-
ability score of suitable habitat elements for slugs and spi-
ders, compared between urban and wildland sampling sites
(* denotes significance). The numbers are the average score
across sites, where a mean distance between habitat elements

at a site of <50 m was scored "3"; a mean distance between 50 and 500 m was scored "2"; a mean distance >500 m was scored "1"; no habitat elements was scored as "0". Scores were based on the approximate density as estimated by a single observer (JB) across all sites

Habitat element	urban	wildland	Wilcoxon W	<i>p</i> -value
Flippable rocks	0.28 ± 0.47	0.51 ± 0.56	1389.00	0.018*
Decaying trees	0.34 ± 0.58	0.65 ± 1.01	1571.50	0.212
Crevices	0.48 ± 0.73	0.57 ± 0.83	1690.50	0.618
Valve boxes	0.83 ± 0.83	0.38 ± 0.79	2399.00	0.001*
Leaf litter	0.26 ± 0.57	0.24 ± 0.6	1812.50	0.796
Puddles	0.11 ± 0.35	0.16 ± 0.44	1718.50	0.602
Decaying vegetation	0.09 ± 0.33	0.11 ± 0.39	1777.50	0.992

The two species of *Ambigolimax* slugs were detected at 21 sites, 19 urban and 2 wildland. One of these wildland sites recently transitioned from wildland to urban land-use and the other was an urban-adjacent, managed green space. All specimens of *Ambigolimax* were detected within valve boxes, except for one individual that was detected in a pile of decaying vegetation along the Santa Ana River in Orange County, CA.

Sixty-one individuals of *Ambigolimax* spp. from nine sites were sequenced for the CO1 mtDNA barcoding gene; 38 individuals were identified as *A. parvipenis* and 23 individuals were *A. valentianus*. At five of these nine sites, we sequenced more than one individual (an average of 11 individuals), and both species were detected at three of the sites.

At 11 out of the 21 sites where *Ambigolimax* spp. were found, the spider *S. grossa* was also detected, frequently within the same valve box (Fig. 1B).

Other gastropod species found in valve boxes include introduced species in the slug genus *Deroceras* (at 4 sites), the land snail genus *Oxychilus* (3 sites), and individuals of an unidentified pale white species of slug, probably also in the genus *Deroceras* (1 site). Native land snails of the genus *Helminthoglypta*, were found at six sites, two wildland and four wildland-adjacent urban sites, but never in valve boxes.

Density of valve boxes

We measured the density of valve boxes in representative urban green spaces on the UCLA campus at two sites, and along a partially-vegetated 4–8 lane street in West Los Angeles and Santa Monica. The UCLA green spaces had valve box densities of 2.55/ha and 1.67/ha, with a mean straight-line distance between boxes of 56 m (\pm 27 m SD) and 74 m (\pm 42 m SD), respectively. The 5.9 km segment along the northern sidewalk of Santa Monica Blvd had 2.1 valve boxes/100m, with a mean distance of 48 m (\pm 111 m SD) between neighbouring boxes.

Discussion

Our field surveys demonstrate a strong association of two non-native species of Ambigolimax slugs and the non-native False widow spider, S. grossa, with both urban habitats and valve boxes. These valve boxes are a ubiquitous element of urban green space management infrastructure in Greater Los Angeles and are significantly less frequent in wildland areas. In combination, one or more of our three target species were detected in valve boxes at 83 out of 85 sites, while only two observations came from other apparently suitable habitat elements. Barcode sequencing of Ambigolimax individuals confirmed the widespread presence of both A. parvipenis at 5/9 sites, and A. valentianus, at 7/9 sites. Both species of slug were present at three of these nine sites as indicated by our sequencing results.

Valve boxes create a microclimate that is scarce in Southern California, providing elevated humidity and shelter from larger-bodied competitors and predators. To our knowledge, this is the first study to systematically quantify the species present in the unique habitat provided by valve boxes and evaluate their potential role in the establishment of non-native species. We found only two other studies focusing on valve boxes as potential habitat, one evaluating their suitability as artificial dens for the federally endangered San Joaquin kit fox (Cypher et al. 2021) and the other exploring their use as traps in an effort to locally control California Ground Squirrel density (Ellis et al. 2006).

The strength of association to valve boxes is noteworthy, especially for wildland areas where other suitable habitat elements greatly outnumber valve boxes. With all three of our focal species predominantly restricted to valve boxes and urban areas, this could indicate a low risk of ecological spill-over effects deeper into wildland areas (Spear et al. 2018). Our finding that these slug and spider species are still largely confined to valve boxes in urban-adjacent wildland sites further suggests that their establishment/persistence success is currently limited in wildland habitats without green space management infrastructure. However, we also note that elevation could be a confounding factor. Abundance-elevation associations are not well established in any of our three study species, and it remains possible that the lower prevalence of the study species in wildland areas may reflect the higher elevation of wildlands in the Los Angeles ecosystem. Our surveys showed that when sites transition from wildland to suburban, they can be colonized quickly, as demonstrated by detections from a site that was developed only a few years prior to field work (first bulldozed seven years prior) and where we detected a high number of both Ambigolimax spp. slugs and S. grossa spiders in valve boxes. Human-mediated dispersal of these taxa presumably facilitates rapid colonization of emerging suitable habitat throughout this urban region, as has been shown for land snails (Bergey 2019), and could easily occur by the movement of slug egg-containing soil from nurseries (Cowie et al. 2008). Landscape connectivity for these species may therefore be high in their urban, introduced range, potentially comparable to, or even higher than, within their native range. Given the overall availability and density of valve boxes throughout metropolitan Los Angeles County, and especially along roadways like Santa Monica Boulevard, the distribution of these species is presumably extensive and relatively continuous.

It remains unknown whether valve boxes offer protection from predators, provide food or breeding resources, or are simply safe, high-humidity daytime retreats, and they may be a combination of these factors. It is also possible that other urban habitat factors are important in the establishment and persistence of these species. Other research has demonstrated that waste food is a readily utilized resource by arthropods in urban environments (Youngsteadt et al. 2015). Fermenting bread dough is known to attract A. valentianus, greatly increasing collecting probability, which may indicate that discarded or waste bread, which is widely available in urban settings, facilitates the persistence of this species (Veasey et al. 2021). A comparison of sites with varying ground beetle presence, a natural slug predator, showed negative correlations of ground beetle and slug abundance, although too few individuals of A. valentianus were detected to statistically confirm this trend (Scaccini et al. 2020). The association of slugs with urban valve-boxes may be further mediated by an increased availability of additional food resources in urban areas and/or increased predation rates in wildland areas (Table 1).

All three focal species are commonly observed on iNaturalist, also within this study's survey area, and are frequently recorded from other microhabitats. While the photos associated with iNaturalist observations do not always indicate precisely where individuals were found, non-native gastropods are especially common in private yards that provide supplemental water for gardening (Bergey 2019), and *Ambigolimax* sp. were observed under flower pots within the study area (pers. observation J. Beninde). Such microhabitats are seldomly found in the public spaces that we surveyed, and their importance in the overall distribution of our focal species require further investigation.

For those studying the urban natural history of small invertebrates such as spiders, slugs, centipedes, and earthworms, valve boxes provide an important, and seemingly underappreciated sampling habitat. As far as we are aware, little has been published about the value of these structures for species inventories and specimen collecting. Also, because many of the species that take refuge within valve boxes are nocturnal, it benefits diurnal field work and thorough surveys of biodiversity to include these microhabitats as analogues to cover boards, pitfall traps, and funnel traps. We speculate that our demonstration of introduced slug and spider taxa within irrigation valve boxes may indicate the importance of this ubiquitous microhabitat for other non-native taxa, in Los Angeles and other regions. Particularly in relatively xeric climates, we predict that non-native species assemblages are regular inhabitants of valve boxes. As such, these boxes may facilitate the establishment of species requiring higher humidity throughout the urbanized world, and could contribute to their cosmopolitan distribution.

Author contributions JB and HBS designed the study, JB carried out the field work, advised by JEV. JEV did molecular work. JB performed analyses and wrote a first version of this manuscript, with substantial input from JEV and HBS.

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Data availability Survey data is available in the supplementary materials.

Code availability Research did not involve code.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval Research did not involve experiments or invasive sampling of vertebrate animals.

Consent to participate Research did not involve human participants.

Consent for publication All authors approved the final version of the manuscript.

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