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# Tardigrade Abundance in Relation to Urbanisation and Highly Anthropogenic Substrates

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Research article

# Tardigrade abundance in relation to urbanisation and highly anthropogenic substrates

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## Abstract

Animals colonising urban environments are exposed to a series of novel stressors and ecological challenges, which can result in adaptations to alternative urban niches. Tardigrades are cosmopolitan invertebrates present in all types of ecosystems, including urban environments; and they can survive under extreme conditions, including periods of desiccation, thus allowing them to colonise novel harsh habitats. Tardigrades are thus a promising model to investigate the challenges and opportunities encountered by urban colonisers. Our aims were 1, to determine if tardigrade abundance in natural substrates (moss, lichen and leaf litter) differs between rural and urban sites and 2, to assess if tardigrades have successfully colonised urban substrates that are highly anthropogenic (road sediment, and material accumulated under cars and in wall crevices). Among natural substrates, we found fewer tardigrades in Cork city than in rural sites. However, in urban sites we found no differences between the number of tardigrades present in natural and anthropogenic substrates. In fact, the highest tardigrade abundances in urban samples were found in abiotic material accumulated in wall crevices. We conclude that even though urbanisation may restrict tardigrade abundance, this group of organisms can successfully colonise alternative urban substrates. More research is needed on the ability of tardigrades and other taxa to inhabit highly unusual and disturbed urban substrates effectively, and the adaptations that may take place when animals colonise such substrates.

Key words: abundance, Tardigrada, tardigrades, urbanisation, urban ecology, novel habitat

## Introduction

Over 50% of the world population already lives in cities and by 2030 that figure is estimated to approach 70% (Secretariat of the Convention on Biological Diversity 2012). This fact combined with the accelerating human population growth will lead to a significant expansion of urban environments worldwide (Ellis and Ramankutty 2008; Grimm et al. 2008; McPhearson et al. 2016). Loss of habitat and the spread of human populations will force an increasing number of species into these expanding urban environments (Secretariat of the Convention on Biological Diversity 2012). In fact, cities can sustain a relatively high number of threatened species and can thus be important areas when planning and managing their conservation (Ives et al. 2016). Animals colonising urban environments are exposed to a series of novel stressors and ecological challenges, including air pollution, endocrine-disrupting chemicals, light pollution, and complex and changing habitats (Sattler et al. 2010; Gaston, Visser, and Hölker 2015), possibly resulting in novel adaptations and usage of alternative urban niches (McDonnell and Hahs 2015; Johnson and Munshi-South 2017; Winchell et al. 2018).

Tardigrades are a very interesting group for investigating the challenges and opportunities encountered by urban colonisers. They are cosmopolitan and present in all types of ecosystems, including urban environments (Nelson 2002; Mitchell, Miller, and Davis 2009; Meyer, Hinton, and Samletzka 2013). Tardigrades are small invertebrates (200–500 μm length on average) and can survive under extreme conditions, such as extremely low and high temperatures, lack of oxygen, lack of water, exposure to radiation levels that would kill most other organisms and extreme high pressure (Jönsson et al. 2008; Schill 2019). Despite their ability to survive extreme conditions, tardigrade diversity is consistently lower in urban sites than in rural sites (Johansson et al. 2011; Meyer, Hinton, and Samletzka 2013; Rocha et al. 2016; delBarco-Trillo 2019). The fewer species found in cities may be impacted by pollution and other urban stressors (Steiner 1994a,b, 1995; Hohl, Miller, and Nelson 2001; Vargha, Otvös, and Tuba 2002; Moly de Peluffo et al. 2006; Šatkauskienė 2012), so it is possible that those species exist in low numbers within urban environments (Meininger, Uetz, and Snider 1985). However, available studies present contradictory results regarding tardigrade densities in urban sites compared to rural sites (Morgan 1977; Meyer, Hinton, and Samletzka 2013; Rocha et al. 2016). For example, tardigrades in urban areas have been found to be as abundant (Johansson et al. 2011; Meyer, Hinton, and Samletzka 2013) or more abundant (Morgan 1977; González-Reyes et al. 2016; Rocha et al. 2016) compared to rural areas.

Terrestrial tardigrades can be found in substrates that retain a film of water, such as bryophytes, lichen crusts, tree bark, leaf litter and soil (Glime 2013), and they should be able to colonise similar types of substrates within urban environments. Tardigrades can also survive periods of desiccation through cryptobiosis (Jönsson 2005; Boothby et al. 2017). This may allow tardigrades to take advantage of artificial substrates that undergo periods of desiccation (Séméria 2002). For example, tardigrades have been found within sediment accumulated on the sides of roads in the city of Chetumal, Mexico (Pérez-Pech et al. 2017).

In this study, we quantified and compared tardigrade abundances in natural substrates (moss, lichen and leaf litter) in both rural and urban sites. Based on previous studies, we predicted similar tardigrade abundances in rural and urban areas. Within urban sites, we also quantified tardigrade abundances in artificial substrates (road sediment, and material accumulated under cars and in wall crevices). We predicted that within a city, artificial substrates will harbour fewer tardigrades than natural substrates.

## Methods

### Study areas and samples

Sampling was conducted from October to December in 2018 in Cork County, Ireland. Four rural sites were chosen across the West Cork region: 1, a 2-km stretch of local road near the local village of Rossmore (51.668 N, 9.013 W); 2, a mixed woodland in Castlefreke (51.573 N, 8.970 W); 3, a small section of local road at Toe Head, a coastal headland near Skibbereen (51.489 N, 9.222 W); and 4, a mixed woodland within the area of Manch, close to the village of Balineen (51.730 N, 8.999 W). Within these rural sites, the types of samples that were collected included moss and lichens from deciduous and coniferous trees, moss on rocks, leaf litter around deciduous and coniferous trees, and soil sediment. We collected 15 samples from each of the four sites (i.e. $N = 60$ rural samples). Across sites, most of the samples were moss (6–9 samples), followed by lichens (3–6 samples), leaf litter (2 samples per site) and soil sediment (1–2 samples). The local roads were single lane, small back roads with very infrequent traffic. The Rossmore road was flanked by earthen banks, fences surrounding fields and gates opening into fields. The Toe Head road was much more exposed to wind, with the road length sampled being on top of a headland by the coast. In these sites, all samples were taken from substrates that were around 1.5 m from the road.

We also collected 60 urban samples within Cork city: 15 samples from natural substrates (moss and lichen on deciduous trees, moss on rocks, leaf litter around deciduous and coniferous trees, and soil sediment) in Fitzgerald park (51. 895951 N, 8.4958170 W), and 45 samples from artificial substrates from the Ballymacthomas area and the University College Cork main campus area. These artificial substrates included road sediment ($N = 15$), built-up sediment on the underside of cars directly over the wheels ($N = 15$), and built-up sediment from small cavities in walls and corners from windowsills not protruding from walls ($N = 15$). All samples were placed into a separate paper bag and stored until analysis.

We did not identify the tree species on which moss and lichen samples were collected. The lichen samples were all common greenshield lichen (*Flavoparmelia caperata*). The moss species in rural areas were hypnum moss (*Hypnum cupressiforme*) and wood bristle-moss (*Orthotrichum affine*), whereas in urban areas it was rock cap moss (*Dicranum* sp.). We collected a similar volume for each sample (e.g. approximately 2 cm in length and width and 1 cm in depth for moss and soil samples; and 3–4 cm in diameter for lichen samples). Soil samples were collected from the top 2 cm soil layer directly with the fingers and placed in a paper bag. Soil samples were moist when collected.

### Sample analysis

Each sample was placed in a 250-ml beaker and sprayed with tap water for rehydration. After 15 min, the beaker was filled up halfway with tap water. After an additional 15 min (i.e. there was a total of 30 min of rehydration), the contents in the beaker were poured over two sieves, a top brass sieve with a mesh size of 250 μm (Endecotts, London), and a purposely built bottom sieve with a mesh size of 41 μm. Tap water was run over the two sieves for 10 s. The bottom sieve was tilted and running tap water was used to accumulate any remaining material at one end of the sieve. This material was suspended in 5 ml of water, and 1 ml of this suspension was transferred with a pipette to a 1-ml plastic Sedgewick Rafter counting cell (model S50, Pyser Optics, UK). Tardigrades in each sample were counted using a stereomicroscope Nikon SMZ1000. We did not find tardigrades in any soil samples, possibly due to the small volumes of soil that we sampled, as well as not using a specialised extraction method (Czerneková et al. 2018), so we did not include soil samples in our analyses.

### Statistical analyses

We conducted our statistical analyses using R, version 3.4.2 (R Core Team 2017). Statistical significance was set at $P < 0.05$, and results are reported as mean ± standard errors. We fitted a generalised linear mixed model (GLMM) with a negative-binomial distribution and log link function using the glmer.nb function in the package lme4 to determine if the number of tardigrades differed among rural substrates; with the number of tardigrades being the response, type of substrate (moss, lichen and leaf litter) being the fixed factor and site being the random factor. We fitted another negative-binomial GLMM including type of site (rural road or woodland) as a second fixed factor. We used a Mann–Whitney $U$ test to determine if the number of tardigrades in natural substrates (moss, lichen and leaf litter) differed between rural and urban samples. To determine if the number of tardigrades differed among urban substrates, we implemented a Kruskal–Wallis test. In the last two cases, we did not fit a GLMM as we did not have a random effect.

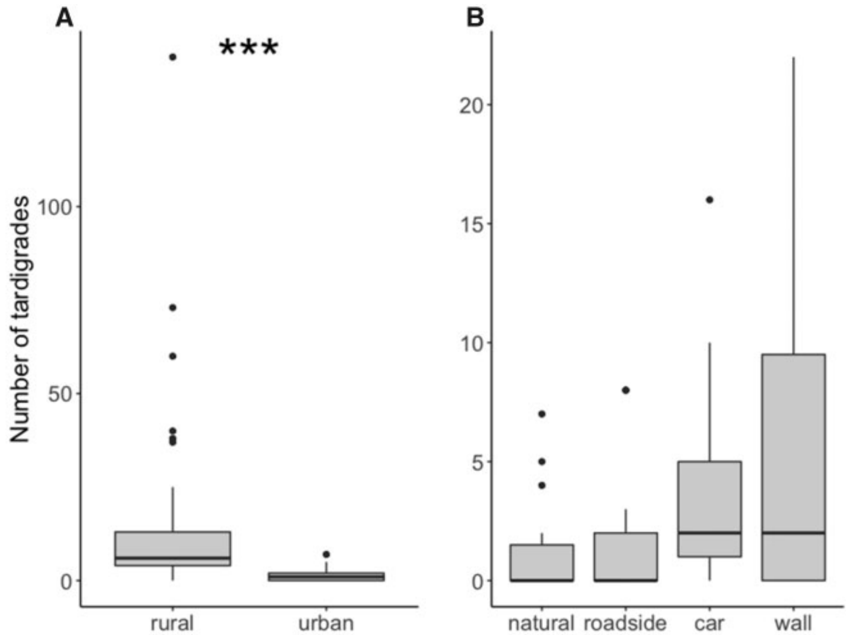


**Figure 1:** Number of tardigrades in (A) natural substrates (moss, lichen and leaf litter) in rural and urban sites, and in (B) different urban substrates (natural substrates in a park, roadside substrates, underside of a car and wall crevices). For each boxplot, the bar within each box represents the median, each box represents the range between the 25th and 75th percentiles, the two whiskers represent the maximum values that are within 1.5 * IQR (inter-quartile range), and points beyond the whiskers represent outliers. ***$P < 0.0005$.

## Results

In total, we counted 940 tardigrades, 751 from rural samples and 189 from urban samples. Tardigrades were present in 51 of the 60 rural samples taken (85%; being absent in the 5 soil samples, 3 moss samples and 1 lichen sample), whereas they were present in only 36 of the 60 urban samples (60%).

Across rural sites, we found a similar number of tardigrades in moss samples (mean ± standard deviation: 16.5 ± 4.95 tardigrades), lichen samples (11.35 ± 4.2 tardigrades) and leaf litter samples (7.89 ± 2.62 tardigrades; GLMM: $P = 0.28$). We also found a similar number of tardigrades in both types of rural sites (rural roads: 13.07 ± 2.76 tardigrades; woodland: 14.21 ± 5.36 tardigrades; GLMM: $P = 0.65$).

The number of tardigrades found in natural substrates (moss, lichen and leaf litter) was higher in rural samples (13.65 ± 3.02 tardigrades) than in the equivalent urban samples (1.62 ± 0.64 tardigrades; Mann–Whitney *U* test: $W = 615.5$, $P < 0.0001$; Fig. 1A).

When comparing the different types of urban substrates, we found a statistically similar number of tardigrades in natural substrates (1.4 ± 0.57 tardigrades per sample, with tardigrades being present in 7 out of 13 samples, i.e. 53.85% of samples), roadside substrates (1.73 ± 0.71 tardigrades per sample, and present in 7 out of 15 samples, i.e. 46.67% of samples), car undersides (3.8 ± 1.15 tardigrades per sample, and present in 12 out of 15 samples, i.e. 80% of samples) and wall crevices (5.67 ± 1.83 tardigrades per sample, and present in 10 out of 15 samples, i.e. in 66.67% of samples; Kruskal–Wallis test: $\chi^2 = 6.15$, df $= 3$, $P = 0.1$; Fig. 1B).

## Discussion

We found fewer tardigrades in natural substrates in Cork city than in nearby rural sites, which suggests that tardigrades are negatively impacted by urban stressors. However, within the urban environment we did not find any significant differences in the number of tardigrades present in natural or highly artificial substrates. This latter result highlights the ability of tardigrades to colonise alternative urban habitats; and it is consistent with the possibility that only a few species of tardigrades are colonising urban areas and that these species are equally able to use natural and artificial substrates. Future studies should determine which individual species or set of species are most successful at colonising artificial substrates, and whether any adaptation events have taken place during those colonisation processes.

In many groups of organisms, urbanisation leads to a decline in species richness, although the few successful urban species can be very abundant (Grimm et al. 2008). Such a pattern of increased abundance in urban species is not clearly supported by the available studies in the case of tardigrades (Morgan 1977; Johansson et al. 2011; Meyer, Hinton, and Samletzka 2013; González-Reyes et al. 2016; Rocha et al. 2016). In the present study, we found a lower abundance of tardigrades in Cork city compared to rural sites, but other studies in other cities report similar tardigrade abundances between rural and urban habitats (Johansson et al. 2011; Meyer, Hinton, and Samletzka 2013); or even very high densities in urban samples, e.g. in roof mosses in Swansea, UK (Morgan 1977) and in lichen and moss growing on trees in the city of Salta, Argentina (González-Reyes et al. 2016; Rocha et al. 2016). Such differences across cities deserve further investigation.

The literature indeed indicates that the number of tardigrade species declines in cities compared to rural environments (reviewed in delBarco-Trillo 2019). However, our results showing that tardigrades can be relatively abundant in previously unstudied substrates raises the possibility that some urban species may have gone undetected in previous studies due to a sampling focus on natural substrates (Meyer, Hinton, and Samletzka 2013; Pérez-Pech et al. 2017; Yankova 2018). We also suggest that tardigrades may be able to colonise many other alternative substrates within cities not considered in this study. Such colonisation of artificial substrates may be driven by the ability of tardigrades to survive under extreme conditions, and the ensuing benefit of avoiding predators, which may be unable to sustain such extreme conditions in those artificial substrates. Future studies should sample anthropogenic landscapes more broadly, considering a combination of natural and artificial substrates.

Although there was no significant difference in abundance of tardigrades between natural and artificial substrates in urban sites, there was a trend towards higher abundance in artificial substrates. In fact, the lowest average number of tardigrades per sample (1.4 tardigrades per sample) and the lowest maximum number of tardigrades (7 tardigrades present in a lichen sample) were found in natural substrates, whereas the highest average (5.67 tardigrades per sample) and the highest maximum number of tardigrades (22 tardigrades) were found in material accumulated in wall crevices, a type of substrate investigated here for the first time in regards to tardigrades. The second highest average abundance (3.8 tardigrades per sample) was found in samples collected from the underside of cars, which is the type of substrate in which tardigrades were found more regularly (80% of samples), reinforcing the fact that tardigrades can consistently colonise artificial substrates. In summary, a statistically non-significant pattern emerges in which tardigrade abundance is higher in anthropogenic than in natural substrates. That statistically non-significance may have been due to the zero-inflated nature of our data, with tardigrades not being detected in a relatively high number of samples.

The time of year within which the tardigrades were sampled could have influenced the observed abundances. In the centre of Zürich, tardigrade abundances were noticeably lower during the months of October–December (Steiner 1994c), which is the period during which we conducted our sampling. Future studies should assess tardigrade densities in artificial substrates throughout the year and determine the relative effect of seasonality on the ability of tardigrades to thrive in these types of substrates.

In conclusion, the present study offers an insight into how prevalent tardigrades may be in urban microhabitats that have not been previously studied or even considered. Future studies should consider the possibility that tardigrades and other groups of animals can inhabit unusual and highly anthropogenic niches effectively. That is, some animals may not only survive in cities by utilising those habitats that closely resemble their natural ones, but also by exploiting completely new types of substrates and environmental conditions.

## Supplementary data

Supplementary data are available at *JUECOL* online.

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## Data availability

The datasets used in the analyses are available as Supplementary Material (.xlsx file).

*Conflict of interest statement*. None declared.

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