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Tardigrades in the city: a review of diversity patterns in response to urbanization

Javier delBarco-Trillo [1,*]

[1] *School of Biological, Earth and Environmental Sciences; University College Cork; Cork, Ireland*

* Corresponding author: Javier delBarco-Trillo; delbarcotrillo@gmail.com

Running title: Urban tardigrades

ABSTRACT

In different taxonomical groups, the number of species found in urban environments tends to decline compared to adjacent non-urban environments. It is unclear whether tardigrades also conform to this pattern of diversity decline in cities. Tardigrades are microscopic invertebrates which have been understudied, despite the fact that they are cosmopolitan and found in all types of habitats. Due to their capability to withstand extreme conditions, tardigrades should be able to successfully thrive in urban environments. Here, all available information about tardigrade diversity in cities was compiled. It was quantitatively determined that tardigrade diversity declines in urban areas compared to adjacent rural areas. Geographically closer cities are also likely to harbor a more similar set of tardigrade species. In comparison to other groups like mammals and birds, there are no tardigrade species consistently found in most studied cities. In fact, most urban tardigrades have only been found in one single city. Ultimately, the species of tardigrades found in a given city will normally depend on the set of species already living in the adjacent native environments. One question that deserves further investigation is why only a subset of such native species is able to colonize the new environmental niches available in cities.

Keywords: Tardigrada, tardigrades, diversity, urbanization, urban ecology

INTRODUCTION

In most groups of organisms, urbanization leads to a decline in diversity, although the few successful urban species can be very abundant (Grimm *et al.* 2008). In contrast, some other groups may experience a heightened diversity in urban environments, as it can be the case with bees (Fortel *et al.* 2014). The diversity pattern for tardigrades in urban environments remains unclear. Tardigrades are microscopic invertebrates, which are cosmopolitan and present in all types of ecosystems, including urban environments (Nelson 2002). Terrestrial tardigrades can potentially be found in any sample of moss or lichen, and they are known for surviving under extreme conditions (e.g. 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) (Schill 2019). Consequently, tardigrades should a priori be unaffected by urban stressors, and thus species richness should be similar in urban areas and in neighboring rural areas. However, in the studies in which tardigrade diversity has been investigated in both rural and urban sites, the pattern seems to be for the number of species to be lower in urban sites (de Peluffo *et al.* 2006; Johansson *et al.* 2011; Meyer *et al.* 2013; Rocha *et al.* 2016).

A possible decline in tardigrade diversity in cities could involve pollution as a main explanatory factor (Roberts & Zimmer 1990; Steiner 1994). For example, in Zürich, the number of tardigrade species decreased with increasing levels of air SO_2 (Steiner 1994). The negative effect of pollution on tardigrade diversity seems further supported by studies reporting fewer tardigrade species in polluted sites (Hohl *et al.* 2001; Vargha *et al.* 2002) or in response to experimental exposure to pollutants (Steiner 1995). The lower pH and lower humidity normally found in cities have also been used to explain lower tardigrade diversity in urban environments (Meininger *et al.* 1985), although at least in one city pH levels could not explain differences in tardigrade diversity between rural and urban areas (Johansson *et al.* 2011).

Tardigrades remain a very understudied group, and this is particularly the case in the context of urban ecology (Rocha *et al.* 2016). However, tardigrades can be a very powerful model to investigate the challenges and opportunities encountered by urban colonisers. Tardigrades are found in cities worldwide, they are easy to sample in large numbers in short periods of time, and they can easily be transported across countries. Consequently, tardigrades can be used to understand worldwide patterns of colonisation and adaptation to urban environments. It is thus important to have a preliminary understanding of the effect of urbanization on tardigrade diversity as a first step to guide future studies.

Here I compiled all available information to date on tardigrade diversity in cities to quantitatively answer two main questions: (i) whether there is a consistent decline in the number of tardigrade species in urban sites compared to non-urban sites across cities; and (ii) whether the similarities between cities in their urban tardigrade communities can be explained by the geographical distance separating those cities.

METHODS

I made a comprehensive search in Web of Science on 2 April 2017, compiling results from several searches using the terms "tardigrad*" or "water bear" plus "urban" or "city". After a preliminary filtering, I considered a total of 73 publications. From these 73 publications, only those that reported the number of tardigrade species within a city were considered in the analyses (most of those 73 publications did not include any urban samples). A few publications in which tardigrades were not identified at the species level (e.g. Pérez-Pech *et al.* 2016) were also excluded. This selective process resulted in 10 relevant publications (Table 1). From these 10 publications, the following information was extracted: the total number of species in urban sites (using information only present in figures when necessary), the total number of species in rural sites (when available), and the number of samples analyzed in each habitat type.

In all studies, urban sampling took place across the whole city, including highly urban sites. Although samples mostly consisted of mosses and lichens, there were considerable differences among studies: in General Pico, Santa Rosa, Cincinnati, and Salta, moss and lichen samples were collected from trees; in Belfast, samples consisted of lichen exclusively on lime trees; in Lake Charles, samples included mosses, lichens, plants, and leaf litter; in Zürich, samples were exclusively mosses on walls; in Fresno and Tokyo, lichen and moss samples were collected from several substrates, including trees, rocks, concrete and soil; in Nice, lichen and moss samples were complemented with samples from artificial substrates like pavement. Extraction of tardigrades from samples in most studies involved rehydration with water and collection of tardigrades from the suspension. Only two studies used extraction methods involving a funnel and movement of tardigrades along a gradient (Meininger *et al*. 1985; Steiner 1994).

All statistical tests were implemented in R (R Core Team 2014). Values are reported as mean ± SD. Significance level (α) was set at 0.05. Differences in tardigrade richness between urban and rural sites were determined using paired *t*-tests, considering either the total number of species in each type of habitat or the number of species divided by the number of samples analysed. A Mantel test was used to calculate the relationship between a matrix of similarities between cities based on the occurrence of tardigrade species and a matrix of geographical distances between cities. Diversity similarities were calculated as Jaccard distances between cities using the binary data in Table 2. Lower distance values indicated cities with similar tardigrade communities (e.g. General Pico and Santa Rosa, in Argentina). The geographical distances between each two cities were obtained from www.distancecalculator.net. The function mantel (package vegan) was used to run the Mantel tests, selecting 9999 permutations.

RESULTS

The mean number of tardigrade species found in cities was 6.52 ± 2.5 (range = 2-10 species), when considering all available studies (Table 1). When comparing rural and urban areas from studies in which both habitat types were sampled, tardigrade diversity was significantly lower in urban areas (7.2 ± 1.81 species) than in rural areas (13.03 ± 4.83 species; paired *t*-test: t_5 = -4.57, p = 0.006; Fig. 1). Species richness was also lower in urban sites than in rural sites after controlling for the different sampling effort in both habitats (paired *t*-test: t_5 = -3.89, p = 0.01). The decline in tardigrade diversity in urban sites compared to rural sites (where

0% decline would indicate the same number of species in rural and urban sites, and 50% decline would indicate that the total number of species in urban sites was half than that in rural sites) ranged from 32.6% to 52.94%. That is, in all cities investigated to date there is a substantial decline in tardigrade diversity compared to rural sites (Table 1).

There was a positive association between diversity similarities and geographical distances between cities, i.e. geographically closer cities tended to have more species in common (Mantel test: $r = 0.53$, $p = 0.003$).

DISCUSSION

Species richness of tardigrades was lower in urban sites than in adjoining rural sites. This was the case for all available studies making a direct comparison between urban and rural sites. However, it must be noted that the number of available studies is very low, especially since the considered cities are distributed worldwide. The low sample sizes prevented considering the effect of confounding variables like sampling effort, types of substrates sampled, extraction methodologies, and ecological differences between cities. Despite the low statistical power of this study, the overall result is consistent and offers interesting research venues for future studies.

There are several factors that have been used to explain the decline in tardigrade diversity in urban areas, including increased pollution, lower humidity and lower pH in cities (Hohl *et al.* 2001; Meininger *et al.* 1985; Vargha *et al.* 2002). It is still unclear, however, which ones of these factors may determine the set of species that can be found in any given city. It must be noted that each species may be affected differently by one or more of these factors. In particular, pollution seems an obvious candidate to explain declines in diversity, despite the fact that tardigrades have remarkable abilities to sustain all sorts of environmental stressors. For example, in Zürich, the number of tardigrade species decreased with increasing levels of air SO_2 (Steiner 1994) and the abundances of two tardigrades were significantly correlated with air pollution (Steiner 1994). In contrast, in

Lithuania the same set of tardigrade species were found in lichens beside highways and in unpolluted sites (Šatkauskienė 2012).

It is apparent that not all tardigrade species are able to colonize cities to the same extent. What remains to be explained is why in different cities we find a different community of tardigrades (Johansson *et al.* 2011). Only a few tardigrade species were found in several cities (although not in all of them), and most urban tardigrades have so far been identified in only one city. It would not seem that any eusynanthropic (completely adapted to the urban environment) tardigrade exists (Luniak 2004), i.e. a tardigrade equivalent to the Norway rat or the feral pigeon. The set of species that can be found in a given city may thus be explained by an impoverishment of the higher diversity that exists in the rural matrix where the city is sited. Only in one study, conducted in Fresno, CA, USA, were most of the urban species not found in the adjoining rural areas (Johansson *et al.* 2011). In all other cases, most of the urban species were also found in the surrounding rural areas (Meininger *et al.* 1985; Meyer *et al.* 2013; Rocha *et al.* 2016; Séméria 1981, 2002). If the tardigrade species found in a city depend on the species already established in the territory around the city, we would expect that closer cities will have more urban species in common than distant cities. Indeed, a statistically significant relationship was found between the similarity in the species found in any two given cities and the geographical distance between them.

It is possible that the low levels of tardigrade diversity reported for urban environments reflects insufficient sampling effort. Some tardigrade species found in rural areas may also be present in urban areas but in such low numbers that the relatively low sampling effort performed in some previous studies were not able to detect such species in urban sites. Furthermore, some tardigrade species living in urban areas may do so in different habitats than moss and lichen, which are the habitats that are normally sampled in tardigrade studies (Séméria 2002). Any tardigrade species thriving in alternative urban habitats but not in natural habitats within a city may have thus gone undetected in previous studies. For example, in the Mexican city of Chetumal some tardigrade species that were not found in moss samples were however present in road sediment, including the recently discovered species *Doryphoribius chetumalensis*, so far only found in this type of habitat (Pérez-Pech *et al.* 2016, 2017a).

Which native species are able to colonize a city may depend on the particular nature of that city, including the set of pollutants being produced and accumulated and the environmental conditions in its geographical area (e.g. yearlong extreme temperatures and rain patterns). That is, not all cities may provide conditions that are optimal for the same tardigrade species. In Zürich, for example, Steiner (1994, 1995) described *Macrobiotus persimilis* as being able to endure high levels of pollution, whereas he considered *Macrobiotus hufelandi* to be adversely affected by air pollution (Steiner 1994). However, *M. persimilis* has not been found in any other city, whereas *M. hufelandi* is present in most cities investigated so far.

Meyer *et al.* (2013) pointed out that most urban species described at the time were eutardigrades. Terrestrial tardigrades are divided into the two Classes Eutardigrada and Heterotardigrada (Bertolani *et al.* 2014). The data compiled in this study confirm that all urban tardigrades, except those in the genus *Echiniscus*, belong to the Class Eutardigrada. Similar results have been found in studies characterizing tardigrades at the genus level (Pérez-Pech *et al.* 2017b). The Eutardigrada is the largest Class of tardigrades, but this fact alone cannot explain the much higher success of eutardigrades in urban environments.

Tardigrade abundance in a given sample is normally similar in rural and urban sites (Meyer *et al.* 2013). In fact, in some cities tardigrade abundance can be higher in urban sites than in rural sites (Rocha *et al.* 2016). Therefore, urban environments may not be particularly inhospitable to those tardigrade species that are able to colonize and get established in cities. However, it is unclear whether the success of urban tardigrades relies on morphological or physiological adaptations to the urban environment; or whether the transition from rural areas to urban areas does not require the involvement of any genetic adaptation or phenotypic plasticity in those species that successfully colonize cities. More research is definitely needed on the establishment of tardigrade species in cities worldwide. As some of the differences between previous studies may have arisen due to variation in sampling effort, collection and extraction methodology, equipment used, or taxonomical expertise, I suggest that the optimal approach to understand how different types and intensities of urbanization affect tardigrade diversity and abundance may require the same research group to survey in different cities and across urban gradients while using the same sampling methodology. Unfortunately, across-city replication is still rare in urban ecology studies (Bonier 2012).

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FIGURE LEGENDS

Fig. 1 Tardigrade biodiversity in relation to urbanization. Each line connects the number of species in rural and urban sites in the same city.

Table 1. Available data on tardigrade richness in urban environments. Studies are ordered by population size in ascending order.

City	Population	# samples (% samples containing tardigrades)	# Urban species	# Rural species	% Urban decline in richness	References
General Pico, Argentina	52,000	56 (98.2 %)	5			(de Peluffo *et al.* 2006)
Lake Charles, LA, USA	72,000	40 (68%	8	17	52.94	(Meyer *et al.* 2013)
Santa Rosa, Argentina	100,000	157 (80.9 %)	5			(Peluffo *et al.* 2007)
Cincinnati, OH, USA	385,409	5[1]	5	8	37.5	(Meininger *et al.* 1985)
Nice, France	400,000	88 (61.4 %)	8	16	50	(Séméria 1981, 1982, 2002)
Belfast, UK	487,417		2			(Roberts & Zimmer 1990)
Fresno, CA, USA	502,000	73 (38.3 %)	10	19	47.37	(Johansson *et al.* 2011)
Salta, Argentina	535,303	144	6	9	33.33	(Rocha *et al.* 2016)

Zürich, Switzerland	836,284	80	6.2	9.2	32.61	(Steiner 1994)
Tokyo, Japan	30,303,794	191[2]	10			(Utsugi 1985)

Human population size offers a crude proxy for city size. I used the population sizes stated in the publications; otherwise I found the population size for the time the study was conducted in worldpopulationreview.com. [1] 5 sites across the city but unclear how many samples per site. [2] 191 'locales' across the 23 wards of Tokyo, but unclear how many samples per site.

Table 2. Tardigrade species found in cities.

Species	Class	Order	Family	GP	LC	SR	C	B	Z	N	F	S	T	Total
Milnesium reticulatum	Eutardigrada	Apochela	Milnesiidae		X									1
Milnesium tardigradum	Eutardigrada	Apochela	Milnesiidae	X		X		X		X	X		X	6
Astatumen bartosi	Eutardigrada	Parachaela	Hypsibiidae				X							1
Diphascon oculatum	Eutardigrada	Parachaela	Hypsibiidae								X			1
Diphascon scoticum	Eutardigrada	Parachaela	Hypsibiidae				X							1
Eremobiotus alicatai	Eutardigrada	Parachaela	Hypsibiidae								X			1
Hypsibius canadensis	Eutardigrada	Parachaela	Hypsibiidae										X	1
Hypsibius convergens	Eutardigrada	Parachaela	Hypsibiidae						X					1
Hypsibius dujardini	Eutardigrada	Parachaela	Hypsibiidae		X					X				2
Hypsibius pallidus	Eutardigrada	Parachaela	Hypsibiidae							X				1
Isohypsibius granulifer	Eutardigrada	Parachaela	Hypsibiidae								X			1
Isohypsibius marcellinoi	Eutardigrada	Parachaela	Hypsibiidae								X			1
Isohypsibius prosostomus	Eutardigrada	Parachaela	Hypsibiidae						X					1
Isohypsibius silvicola	Eutardigrada	Parachaela	Hypsibiidae								X			1
Isohypsibius sismicus	Eutardigrada	Parachaela	Hypsibiidae								X			1
Ramazzottius anomalus	Eutardigrada	Parachaela	Hypsibiidae								X			1
Ramazzottius oberhaeuseri	Eutardigrada	Parachaela	Hypsibiidae	X		X				X	X		X	5
Macrobiotus echinogenitus	Eutardigrada	Parachaela	Macrobiotidae		X									1

Macrobiotus harmsworthi	Eutardigrada	Parachaela	Macrobiotidae		X								X	2
Macrobiotus hibiscus	Eutardigrada	Parachaela	Macrobiotidae				X							1
Macrobiotus hufelandi	Eutardigrada	Parachaela	Macrobiotidae				X	X	X	X		X	X	6
Macrobiotus persimilis	Eutardigrada	Parachaela	Macrobiotidae						X					1
Macrobiotus recens	Eutardigrada	Parachaela	Macrobiotidae										X	1
Minibiotus acadianus	Eutardigrada	Parachaela	Macrobiotidae		X									1
Minibiotus hufelandioides	Eutardigrada	Parachaela	Macrobiotidae										X	1
Minibiotus intermedius	Eutardigrada	Parachaela	Macrobiotidae				X				X		X	3
Paramacrobiotus areolatus	Eutardigrada	Parachaela	Macrobiotidae	X	X	X				X		X		5
Paramacrobiotus richtersi	Eutardigrada	Parachaela	Macrobiotidae		X					X				2
Echiniscus arctomis	Heterotardigrada	Echiniscoidea	Echiniscidae										X	1
Echiniscus japonicus	Heterotardigrada	Echiniscoidea	Echiniscidae										X	1
Echiniscus rufoviridis	Heterotardigrada	Echiniscoidea	Echiniscidae	X		X						X		3
Echiniscus testudo	Heterotardigrada	Echiniscoidea	Echiniscidae							X				1

Cities are ordered by population size, being smallest in General Pico and largest in Tokyo. Species are listed by class, then by order, then by family, and then in alphabetical order. Only identified species are listed, and thus the number of species listed under a city may be lower than the number indicated in Table 1. GP: General Pico, Argentina (de Peluffo *et al.* 2006); LC: Lake Charles, LA, USA (Meyer *et al.* 2013); SR: Santa Rosa, Argentina (Peluffo *et al.* 2007); C: Cincinnati, OH, USA (Meininger *et al.* 1985); B: Belfast, UK (Roberts & Zimmer 1990); Z: Zürich, Switzerland (Steiner 1994); N: Nice, France (Séméria 1981, 2002); F: Fresno, CA, USA (Johansson *et al.* 2011); S: Salta, Argentina (Rocha *et al.* 2016); T: Tokyo, Japan (Utsugi 1985).