

A community analysis approach to parasite transmission in multi-host systems: Assemblages of small mammal prey and *Echinococcus multilocularis* in an urban area in North America

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ABSTRACT

Background: *Echinococcus multilocularis* (*Em*) is a parasite with a complex life cycle whose transmission involves a predator-prey interaction. Accidental ingestion of *Em* eggs by humans may cause alveolar echinococcosis, a potentially fatal disease. Although previous research suggested that the composition of the assemblage of prey species may play a key role in the transmission, the relation between *Em* presence and the prey assemblages has never been analyzed. Herein, we propose a community analysis approach, based on assemblage similarity statistics, clustering, non-metric dimensional scaling and GLM modelling to analyze the relationships between small mammal assemblages, environmental variables, and the prevalence of *Em* in intermediate and definitive hosts in an urban area.

Results: In our study areas within the City of Calgary, Alberta (Canada), we identified three main small mammal assemblages associated with different prevalence of *Em*, characterized by a different proportion of species known to be good intermediate hosts for *Em*. As expected, assemblages with higher proportion of species susceptible to *Em* were observed with higher prevalence of parasite, whereas the total abundance *per se* of small mammals was not a predictor of transmission likely due to dilution effect. Furthermore, these assemblages were also predicted by simple environmental proxies such as land cover and terrain.

Conclusions: Our results indicated that the use of a community analysis approach allows for robust characterization of these complex and multivariate relationships, and may offer a promising tool for further understanding of parasite epidemiology in complex multi-host systems. In addition, this analysis indicates that it is possible to predict potential foci of disease risk within urban areas using environmental data commonly available to city planners and land managers.

1. Background

Echinococcus multilocularis (*Em*) is a parasitic tapeworm that can cause human alveolar echinococcosis (AE), currently considered among the most serious zoonotic diseases outside of the tropics (Massolo et al., 2014). The parasite is endemic across the northern hemisphere, and its distribution is expanding (Davidson et al., 2012; Massolo et al., 2014). The disease has high fatality rate (i.e. > 90%) if not treated, and often requires life-long treatments (Craig, 2003). In 2010, it was estimated that globally there were 18,235 human cases of AE annually (Torgerson et al., 2010) with an increasing trend. Only few cases were reported in North America outside of Alaska, but there are indications that the risk of AE may be increasing (Massolo et al., 2014).

Echinococcus multilocularis is a trophically transmitted parasite with a complex life cycle that involves two different hosts and a free-living stage. The parasite typically infects canid predators such as foxes *Vulpes* spp. and coyotes *Canis latrans* (but also domestic dogs) as definitive hosts (DH). The adult parasite, in the DH intestine, produces embryonated eggs which are released in the environment with feces. More than 40 small mammal species (usually rodents) act as intermediate hosts (IHs) by accidentally ingesting these eggs (Liccioli et al., 2013; Vuitton et al., 2003) and developing the final infectious larval stage in the target organ (often the liver). The life cycle is completed when infectious IHs are predated by DHs. Although climate conditions likely determine the limit of the parasite distribution at the global scale, at more local scales the presence and relative abundance of the IH species

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plays a key role in the parasite distribution and transmission intensity (Giraudoux et al., 2004; Liccioli et al., 2014; Romig et al., 2017). In the southern edge of its European distribution, for example, *Em* spread was deemed to be limited by the presence of single species of small mammal IH (Guerra et al., 2014). Landscape and environmental characteristics that define the distribution of small mammals (e.g., the proportion of the landscape composed by optimal habitat for the susceptible small mammal species) can be important predictors of where the intensity of the parasite transmission is high (Giraudoux et al., 2004; Raoul et al., 2015).

However, the influence of susceptible small mammal species on the transmission of *Em* is made complex by interactions among small mammal species and between predator and prey. Higher population density of DH is expected to increase the transmission rate (Raoul et al., 2015). Even in an area inhabited by susceptible small mammal species, parasite transmission is unlikely if their relative abundance within the prey ensemble is low. The presence of other species that are not susceptible to the parasite but preferred as prey by DHs will reduce the probability of *Em* transmission (Baudrot et al., 2016; Guerra et al., 2014). Our previous research on the distribution of small mammals and *Em* in the City of Calgary suggested that the proportion of susceptible species within the small mammal community may be a key factor in determining the prevalence of the parasite (Liccioli et al., 2014).

Despite these recent findings, so far researchers have only analyzed the effects of single intermediate species variations on transmission of *Em*, and speculated on or modelled the effects of the small mammal assemblages as a whole. Following up on our previous study (Liccioli et al., 2014), we wanted to explore in more detail the association between small mammal assemblages and *Em* transmission using an analytical approach typical of community ecology. In addition, we analyzed environmental features associated with the prevalence of the parasite, which may allow us to predict areas of high risks.

In particular, we aimed to

- A. characterize the composition and structure of the various types of small mammal prey assemblages in the study area;
- B. explore the association between the various types of prey assemblages and *Em* infection in both definitive and intermediate hosts;
- C. identify the environmental proxies that are associated with the various assemblages, and the environment where *Em* transmission is more likely to occur, using geographical data commonly available for city planners.

2. Methods

2.1. Study area and data description

The samples were collected in the City of Calgary (AB, Canada; 51°5'N, 114°5'W; Fig. 1), located in the southeastern region of Alberta in the foothills of the Canadian Rocky Mountains, from June 2012 to July 2013. The city encompasses an area of 848 km² and has a population of 1,235,171 (The City of Calgary, 2016). The city ranges in elevation from 965 to 1304 m a.s.l., and encompasses many streams and water bodies with riparian habitats that are often designated as parks and natural areas. The climate is relatively dry (annual precipitation of 412.6 mm) and cold, with an average annual high temperature of 10.5 °C and low temperature of −2.4 °C (Statistics Canada, 2017). Common habitats in parks and natural areas are grasslands in dry areas, aspen forests in moderately well-drained areas, and willow shrublands in imperfectly drained areas (The City of Calgary, 2014).

Common mammals in the city area are snowshoe hares (*Lepus americanus*), white-tailed jack rabbit (*Lepus townsendii*), Richardson's ground squirrels (*Urocyon richardsonii*), gray squirrels (*Sciurus carolinensis*), southern red-backed and meadow vole (*Myodes gapperi*; *Microtus pennsylvanicus*), deer mouse (*Peromyscus maniculatus*), muskrat (*Ondatra zibethicus*), coyote, beaver (*Castor canadensis*), mule deer

(*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and less commonly red fox (*Vulpes vulpes*; The City of Calgary, 2014). Of these, only southern red-backed vole, meadow vole, deer mouse, beaver and muskrat are currently described as IHs for *Em* (Liccioli et al., 2013), but beaver and muskrats were rarely reported in the diet of urban coyotes in the City of Calgary (Liccioli et al., 2015).

We used data on small mammal relative abundance per site collected for the study of Liccioli et al. (2014) between June 2012 and July 2013 in sites within Calgary urban parks and natural areas. Specifically, these sites were located in Nose Hill Park (site NHP1 ~ NHP3), Bowmont (BM1 ~ BM3), Weaselhead (WSH1 ~ WSH3), Southland lowland (SL1 & SL2), and Fish Creek Provincial Park (FCPP1 ~ FCPP3; Fig. 1). Samples collected in June and July 2013 were not used in the study by Liccioli et al. (2014) because of their interest in seasonal pattern, but were included in this study in order to increase the sample size, whereas the first trapping session in June 2012 included in Liccioli et al. (2014) was removed from this study because it was conducted with a different protocol (i.e. trappings were conducted for 4 nights in row instead of 3 due to weather condition causing most traps to misfire on the first night). The small mammals sampled within these sessions totaled 1223 small mammals of 9 different species (Liccioli et al., 2014).

We used DH prevalence estimated from 385 coyote feces collected by Liccioli et al. (2014) in the same five areas between May 2012 and July 2013 (Table 1: Liccioli et al., 2014). For more details on the small mammal and fecal collection methods and data analysis methods, see Liccioli et al. (2014).

2.2. Assemblage analysis

The trap catch-rate of small mammals caught at each site were standardized by aggregating all captures for each site and then divided by number of trap-nights, not counting misfires and traps that caught other species (e.g. a trap that caught a deer mouse could not have caught a meadow vole that same night) to represent the relative abundances of each species (Table 1). Differences between species composition at each trap site were measured using the Bray-Curtis statistic (Bray and Curtis, 1957), treating each trap site as statistical unit and the relative abundance for each species as variable. The relative abundances were log-transformed ($\log(x+1)$) before calculation of the similarity matrix (Beals, 1984).

The Bray-Curtis similarity was visualized through hierarchical agglomerative clustering dendrogram, using group average algorithm to calculate the distance between clusters (Field et al., 1982). To test the robustness of the cluster structures, clustering with single-linkage and complete-linkage algorithms were also performed. In addition, hierarchical agglomerative clustering was performed on data transformed to percentage of each species before calculation of Bray-Curtis similarity, again using group average, single-linkage, and complete-linkage. Resulting cluster structures were compared for consistency. Significance of the clusters were tested using similarity profile (SIMPROF) tests (Clarke et al., 2008). The association between clusters and presence of *Em* infected small mammals were statistically tested using Fisher's exact test (Sokal and Rohlf, 1995).

To identify the general characteristics of each cluster type, we performed Canonical Correlation Analysis on the principal coordinates (CAP) procedure (Anderson and Willis, 2003). This procedure displays cloud of multivariate points with reference to a hypothesis set a priori by finding axes that maximize the difference among groups. The procedure also tests the significance in the difference among groups using permutation tests and “trace” statistics, equivalent to Pillai's trace statistics in traditional multivariate analysis of variance test. Pearson correlation coefficients between the abundance and proportion of each species to the CAP axes were calculated and their vectors overlaid on the plot.

A pooled *Em* prevalence was calculated for each small mammal assemblage for each site as the number of infected animals divided by the total number of small mammals caught. This pooled prevalence was

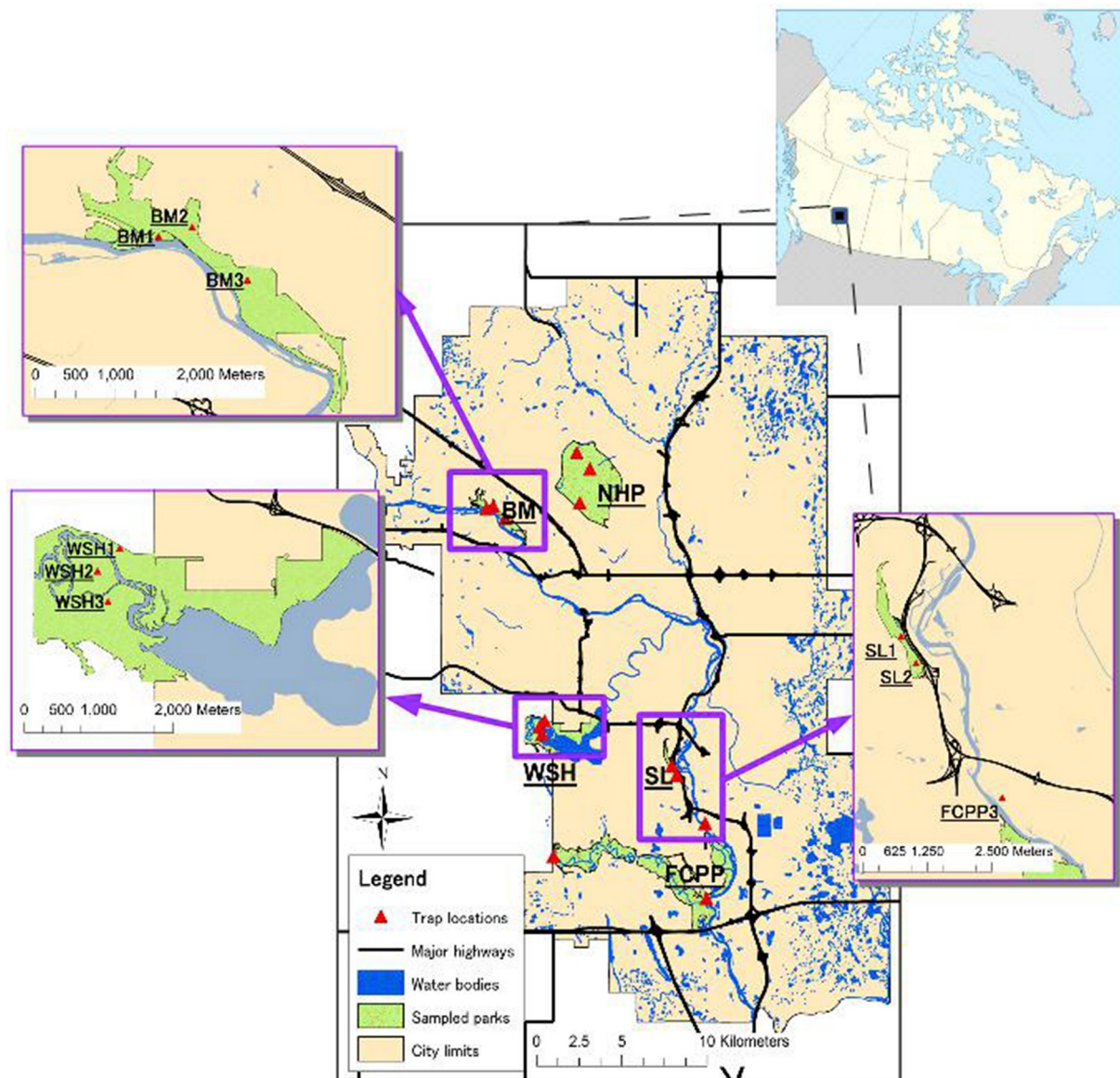


Fig. 1. Study sites for the characterization of the small mammal assemblages in urban Calgary, AB, Canada in 2012–2013, showing the location of five areas in Urban Calgary and detailed map of Bowmont, Southland Lowlands, and Weaselhead. Bowmont (BM), Fishcreek Provincial Park (FCPP), Nose Hill Park (NHP), Southland Lowlands (SL), and Weaselhead (WSH).

a simple estimate of the likelihood for a coyote to become infected by preying on a specific assemblage.

We associated the DH prevalence estimates for the five areas to the trap sites in each area, which is a reasonable assumption considering the distance between each area and territoriality of coyotes. The possible exception was the FCPP3 site which was close to SL. However, because SL and FCPP had similar estimate of DH prevalence, FCPP3 could be either associated with DH of FCPP or SL with little difference.

2.3. Environmental analysis

The environmental proxies surrounding each trap site were identified using ArcGIS Desktop (Release 11. Redlands, CA: Environmental Systems Research Institute). We hypothesized that combination of land cover types, distance to water, and terrain features would allow us to identify habitats associated with small mammal assemblages. Land cover types and distance to water were obtained from a Land Cover map (updated at 2014) with 5 m resolution (Fiera Biological Consulting Ltd., 2014). Terrain features (the average aspect, slope, and

“ruggedness” or the standard deviation of the slope) of each trap site were calculated from a digital elevation model with resolution of 0.75 arc-second, or approximately 18 m (Natural Resources Canada, 2012).

A multinomial logistic regression (MLR) model (Fox, 2008; Hosmer et al., 2013) associating the environmental variables to assemblage types was developed. We built a set of alternative models based on what we considered biologically relevant combinations, such as land cover types of forest, grassland, and shrub lands and terrain features. We used focal statistics with circle of 200 m radius to standardize the way we measure surrounding environment, assuming that areas within 200 m were sufficient for identifying the habitats influencing the small mammals based on their home ranges while also approximating the areas covered by trap grids (Madison, 1980; Madison et al., 1984). We used total numbers of raster cells within 200 m radius for each land cover type as predictor variables. We used the mean value of the cells within 200 m radius for the terrain variables after resampling each terrain raster to 5 m resolution. The models were then compared using the corrected AICc scores and weights (Burnham and Anderson, 2002). The best performing MLR model was then applied to develop a

Table 1

This table summarizes the data on small mammal assemblages composition extracted from (Liccioli et al., 2014) collected in 2012–2013 in city parks and natural areas in the City of Calgary (Alberta, Canada). In table are reported the catch rate for each species (indicated by genus names) for every hundred trap-nights, the presence of *Echinococcus multilocularis* (1 = present, 0 = absent) and prevalence (%) among intermediate hosts (IH), and prevalence among definitive hosts (DH) at each site. The species of small mammals are: Deer mouse (*Peromyscus maniculatus*), Meadow vole (*Microtus pennsylvanicus*), Shrews (*Sorex* sp.), Southern red-backed vole (*Myodes gapperi*), Western jumping mouse (*Zapus princeps*), Thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*), Least chipmunk (*Tamias minimus*), Northern pocket gopher (*Thomomys talpoides*), and House mouse (*Mus musculus*).

Site	Peromyscus	Microtus	Sorex	Myodes	Zapus	Thomomys	Spermophilus	Tamias	Mus	IH presence (Prevalence %)	DH Prevalence (%)
BM1	2.9530	0.1030	0.1545	0.1545	0	0	0	0	0.1545	1 (1.43)	63.07
BM2	1.2318	0.0462	0.2304	0	0.3682	0	0	0	0	1 (2.44)	
BM3	0.8246	0.0462	0.5969	0	0.0923	0	0	0	0	0	
FCPP1	0.8836	3.8810	0.7804	0.0524	0	0	0	0	0	0	6.23
FCPP2	0.1284	0.7653	0.5963	0	0.2138	0	0	0	0	0	
FCPP3	0	0.3745	0.8696	0	0	0	0	0	0.0626	0	
NHP1	2.0658	3.2526	1.6213	0	0	0	0	0	0	1 (0.93)	17.28
NHP2	1.4470	2.4053	0.8836	0	0.4178	0	0	0	0	1 (1.00)	
NHP3	1.5603	1.9081	0.2874	0	0.4304	0	0	0	0	0	
SL1	1.6618	4.0874	3.4752	0	0	0.0367	0	0	0.0367	1 (0.38)	5.42
SL2	0.4288	1.0230	2.4780	0	0	0.0861	0	0	0	0	
WSH1	0.9620	0.7610	0.6098	0.1021	0.2041	0	0.3058	0	0	0	6.22
WSH2	2.0045	1.1976	0.9752	2.5111	0.9009	0	0	0.1513	0	0	
WSH3	2.0101	3.2258	1.5980	2.6622	0.3407	0	0	0.1706	0	0	

predicted distribution map of the small mammal assemblage for the entire area of the City. Because we did not sample small mammals from agricultural areas, and because the agricultural areas were at the periphery of the city, we removed agricultural areas from the final map. Similarly, because small mammals are known to avoid mowed grass (Bowers and Dooley, 1993), and because in one experimental trapping we found no small mammal in a field of mowed grass adjacent to a naturally wooded area, we also removed areas classified as manicured grass. Final map was resampled to 50 m resolution to be used for an agent-based simulation of the transmission of *Em* in future study.

All the statistical analysis, except for the MLR were performed using software Primer ver.6 with PERMANOVA + add-on (Anderson et al., 2008). MLR was performed using SPSS ver.24 (IBM Corps. 2016).

3. Results

3.1. Assemblage analysis

To reduce the noise on the community analysis, least chipmunk, northern pocket gopher, and house mice were removed from relative abundance data prior to the calculation of the Bray-Curtis similarity matrix because of their minimal abundance in the data. The hierarchical clustering of the small mammal assemblage identified three major assemblage types using an arbitrary cut-off line of 45% similarity, although SIMPROF test failed to detect significance ($p = 0.678$, Fig. 2a).

Cluster 1 consisted of three BM sites, where Liccioli et al. (2014) found two IHs positive for *Em*, and estimated highest prevalence among DH. Cluster 2 consisted of three NHP sites, FCPP1, SL1, WSH2 and WSH3. Liccioli et al. (2014) found positive IHs in three sites and estimated moderately high prevalence among DH in NHP. The remaining sites WSH1, SL2, FCPP2, and FCPP3 constituted cluster 3. Liccioli et al. (2014) found no positive IH in these sites and estimated low prevalence of DH in these areas (Table 1). Fisher's exact test on the *Em* positive cases of small mammals and the three clusters could not detect any significant difference ($p = 0.136$).

Clustering with complete-linkage algorithm also grouped trap sites into the same three clusters ($p = 0.695$, Fig. 2b). The same pattern was not observed with single-linkage, where trap sites successively joined groups instead of grouping into distinct clusters (not shown, $p = 0.718$). Similar, but slightly different cluster patterns were observed when group-average and complete-linkage clustering algorithms were performed on percentage of species ($p = 0.144$ and 0.136 respectively, result of group-average shown in Fig. 2c). With percentage of species, cluster 3 was smaller and consisted of only two sites, but BM still formed a single

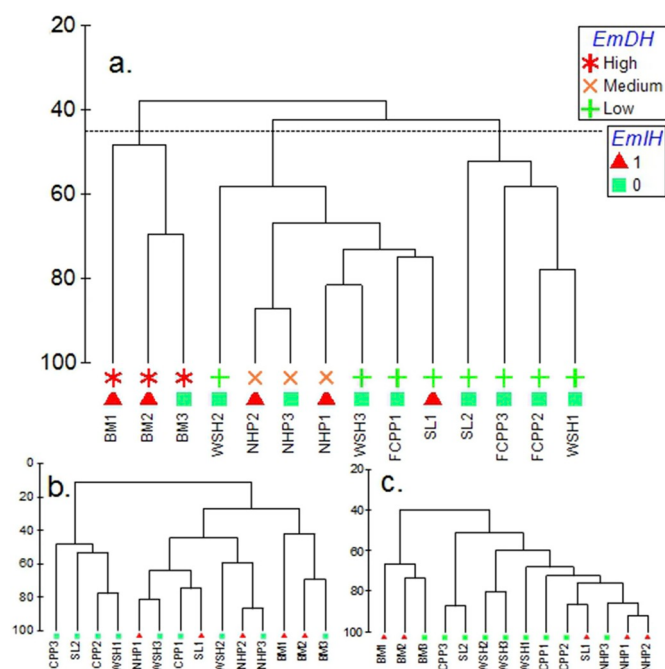


Fig. 2. Dendrograms derived from the Bray-Curtis similarity of small mammal assemblages in five parks and natural areas in urban Calgary, AB, Canada, 2012–2013. a) Dendrogram using abundance data and group-average clustering algorithm. The dashed line indicates the cluster cut-off line of 45% similarity. Symbols for each site indicate the prevalence of definitive hosts (*EmDH*) and presence (1) or absence (0) of infected small mammals (*EmIH*). b) Dendrogram using abundance data and complete-linkage clustering algorithm. Note how it is similar to the dendrogram using group-average algorithm. c) Dendrogram using proportion data and group-average clustering algorithm. Note how all BM sites are in single cluster and all NHP sites and most sites are in another cluster, similar to the dendrogram using abundance data.

cluster. Clustering percentage data with single-linkage algorithm showed less distinct a pattern, but BM sites still grouped into a single cluster ($p = 0.127$). All the following analyses are based on relative-abundance data and clusters based on group-average algorithm.

Conversely, CAP procedure of the small mammal assemblages, using the three clusters identified as grouping factors (Fig. 3), highlighted the significant difference between the three clusters (trace statistics 1.6536, $p = 0.001$). Vectors representing the correlation between the CAP axes

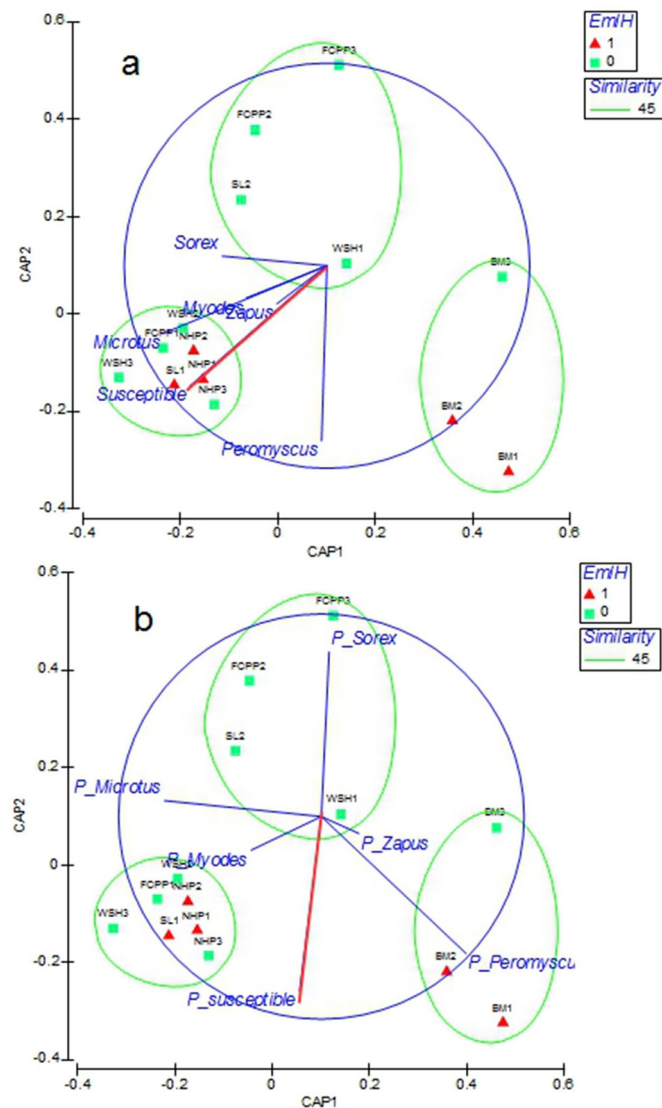


Fig. 3. Plot of the Canonical Correspondence Analysis on the principal coordinates (CAP) of small mammal assemblages in five parks and natural areas in urban Calgary, AB, Canada, 2012–2013, using the cluster as a grouping factor. The cluster 1 is on the lower right corner, cluster 2 is on the lower left corner, and cluster 3 is on the center to the top of the plot. The 45% similarity contour line is drawn based on the dendrogram in Fig. 2a. a) Vectors were overlaid showing the correlation with the abundances of each species to each cluster. The red vector shows the correlation with the abundance of all the susceptible species combined. b) Vectors were overlaid showing the correlation with the proportion of each species to each cluster. The red vector shows the correlation with the proportion of all the susceptible species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and the abundances of each species overlaid in Fig. 3a indicated that all the species were more abundant in cluster 2. Vectors indicating the correlation of the CAP axes to the proportion of each species and (Fig. 3b) indicated that cluster 1 assemblages (particularly BM 1 and BM2) were dominated by deer mice, whereas cluster 3 sites (SL2 and FCPP2) were characterized by shrews.

3.2. Environmental proxies for assemblages

The best MLR model selected by AICc used north-south aspect and ruggedness as predictor variables, with AICc of 18.722 and classified 13 out of 14 trap sites correctly (Table 2). When this model was applied to the natural areas of the City of Calgary, 1209 ha (6.96%) were predicted

to be habitat for cluster 1, 8164 ha (47.02%) for cluster 2, and 7991 ha (46.02%) for cluster 3 (Fig. 4; see supplementary material for parameter estimates).

4. Discussion

Using a community ecology analytical approach, we identified three assemblage types of small preys of coyotes, the main carnivore species in urban settings in Calgary, Alberta (Canada). More importantly, we highlighted an association between these assemblages and the presence of *Echinococcus multilocularis* in wild hosts. Finally, we identified potential environmental proxies calculated from land use data readily available for urban settings that could be associated with parasite within this sylvatic cycle. Our results provided evidence to support recent theoretical models that emphasized the role of complexity of host community in transmission of trophically transmitted parasites (Baudrot et al., 2016).

4.1. Small prey assemblages and *Echinococcus multilocularis*

Our clustering analysis using Bray-Curtis similarity identified three clusters. Although the clusters structures were not statistically significant by SIMPROF test possibly due to small sample size, similar structure were observed in both relative abundances and percentage of species, and with different clustering algorithms, providing some confidence in classifying the small mammal assemblages to the three assemblage types. Assemblage 1 consisting of BM sites seemed to be highly susceptible to the transmission of *Em*, while assemblage 3 seemed to be least susceptible to *Em* transmission.

In our study area, the three small mammal species of most interest due to their known susceptibility to *Em* are deer mice, meadow voles, and southern red-backed voles (Liccioli et al., 2013). Particularly interesting was the correlation of proportion of deer mice to assemblage 1, where the prevalence among DH was high (Fig. 3b). This is consistent with conclusions drawn by Liccioli et al. (2014) that proportion of susceptible species is the key factor in the transmission of the parasite. However, even though assemblage 1 and 2 had about same proportion of susceptible species, assemblage 1 was associated with higher prevalence in both DH and IH. Compared to assemblage 1, assemblage 2 had higher abundance of most species of small mammals (Fig. 3a). Therefore we suspect that higher abundance of small mammals in general, both susceptible and non-susceptible, led to dilution of exposure and reduced transmission (Baudrot et al., 2016).

Any inference drawn from this cluster analysis is limited by the samples size - an issue associated with the low prevalence at which the parasite occurs in intermediate hosts, which likely caused the lack of statistical significance for the Fisher's exact test on the assemblage types and the presence of positive IHs. However, the patterns observed were in agreement with the theoretical studies (Baudrot et al., 2016; Raoul et al., 2015).

4.2. The urban small mammal assemblages and their landscape proxies

In our study, the terrain features (north-facing aspect and ruggedness) turned out to be better predictors of assemblage types than land cover types and were selected for the best MLR model. This was probably because the land cover classification was too coarse for the habitats of small mammals. While land cover types were good indicator of where the natural land covers were (because we collected samples only from natural areas), terrain features were probably better indicators of subtle differences in habitats (Franklin, 1995). For example, assemblage 2 seemed to be associated positively with north-facing aspect and negatively with ruggedness (supplementary Table). These terrain features may be better predictors of vegetation types that prefer moist environment. Distance to water was not selected in the model, probably because most sites were close to water, and the only site that differed for this variable (NHP3) had species composition resembling other sites.

Table 2

Comparison of multinomial logistic regression models predicting various types of assemblages of small mammals developed from data collected in 2012–2013 in city parks and natural areas in the City of Calgary, (Alberta, Canada). The values k , Δ_i , and w_i indicate number of parameters estimated, difference in AICc, and relative weights of AICc, respectively. Although the model with north-south aspect and ruggedness performed best in terms of AICc, N-S aspect and slope model, N-S aspect, slope, and ruggedness model performed comparably well.

Model	k	$-2 * \log$ likelihood	Classification accuracy (%)	AICc	Δ_i	w_i
N-S Aspect & Ruggedness	3	4.322	92.9	18.722	0	0.4857
N-S Aspect & Slope	3	5.616	92.9	20.016	1.2940	0.2543
N-S Aspect & Ruggedness & Slope	4	0	100	20.4444	1.7224	0.2053
N-S Aspect only	2	14.06	78.6	23.1509	4.4289	0.0530
Slope only	2	22.887	57.1	31.9779	13.2559	0.0006
Ruggedness & Slope	3	18.586	64.3	32.9860	14.2640	0.0004
Intercept only	1	29.805	50	33.3023	14.5803	0.0003
Ruggedness only	2	24.532	50	33.6229	14.9009	0.0003

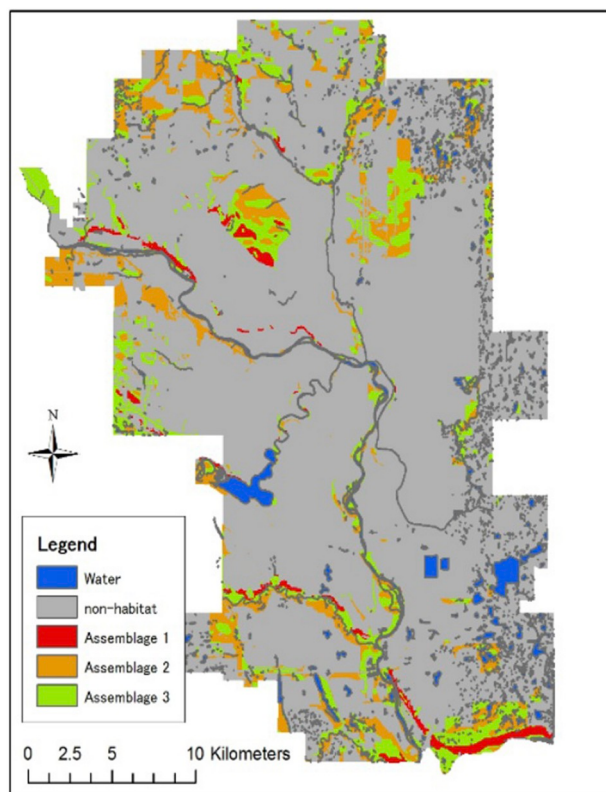


Fig. 4. Map showing the geographic distribution of three small mammal assemblage types predicted for the City of Calgary area by a multinomial logistic regression (MLR) model associating the environmental variables to assemblage types, developed from data collected in 2012 and 2013 (Liccioli et al., 2014). Note how large portion of BWM and NHP were classified as assemblage 1 as expected, but also large portion of FCPP, where it was not expected.

Interestingly, the predicted distribution of the small mammal assemblage type 1 was characterizing most of BM and a fair portion of NHP (Fig. 4). The prevalence of *Em* in these two areas were higher than the other three areas (Liccioli et al., 2014) and in agreement with our inference that assemblage 1 contributed most to the transmission of the parasite, and possibly explains why NHP had higher prevalence even though all three NHP sites were in assemblage 2. However, the predicted distribution of assemblage 1 also covered large area of FCPP, where the prevalence of *Em* was estimated to be low, both in DH and IH (Liccioli et al., 2014).

Another possible explanation for the observed pattern of *Em* prevalence would be the availability to coyotes of food sources other than small mammals, which was not quantified in our study. Coyote diet in our study area includes deer and lagomorphs, fruits and vegetable matters, and anthropogenic food sources (Liccioli et al., 2015). Abundance of

deer in the area is expected to be particularly important in winter, when they are more frequently consumed and the parasite prevalence in IH is highest (Liccioli et al., 2014, 2015). Large and/or connected parks such as NHP, FCPP, and WSH would likely be used by deer more frequently than smaller, less connected parks such as BM and SL.

The feeding and marking behavior of coyotes (i.e., DHs) can also be important for the transmission of the parasite. Although the small mammal assemblages and environmental proxies in and around each site may provide some clues on the parasite transmission, coyotes are known to have wide home ranges and readily travel through urban areas (Gehrt, 2007; Lamy, 2015). To estimate transmission of parasites and their spatial patterns, analysis of small mammal assemblages alone is not sufficient. Studies on spatial behavior of coyotes, using simulations such as agent-based models, would provide further understanding of the spatial patterns of *Em* transmission. Such studies would also allow testing if changes in small mammal assemblages could exert significant effects on parasite transmission.

Declaration of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2019.03.012>.

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