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Comparison of the intestinal helminth community of the large Japanese field mouse (*Apodemus speciosus*) between urban, rural, and natural sites in Hokkaido, Japan

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ABSTRACT

Anthropogenic ecosystem modification has affected over 80% of the global land cover. Interest in its effects on wildlife has been growing over the past several decades, specifically in regard to biodiversity and ecosystem function. Parasites are of particular interest, because they directly impact animal health, and can be transmitted to humans through the process of zoonosis. However, most studies so far tended to focus on only one or two parasites with few looking at the entire community, thereby limiting our understanding of the effects of ecosystem modification on parasitic organisms. In this study, we estimated the intestinal helminth diversity and species richness of the large Japanese field mouse (*Apodemus speciosus*), as well as the prevalence and abundance of each species in two modified ecosystems, a rural agricultural area and an urban park. We then compared them to a natural area to see how they have been altered. We found that diversity, prevalence, and abundance were all highly altered within both modified ecosystems, but generally to a greater degree within the urban park. However, there was great variation in the direction and degree of response of each helminth species, suggesting that generalized trends may be difficult to ascertain. Furthermore, it is important to analyze the entire helminth community, because interspecific interactions and the effect that ecosystem modification has on them may help determine what species persist.

1. Introduction

Anthropogenic modification of ecosystems has affected over 80% of global land cover, with the two most common forms being the conversion to agriculture and urbanization [1]. This is likely to increase even further, because the rate of urbanization in particular is occurring at an astonishing rate with > 1.5 million km^2 of land predicted to be added to cities between the years 2011 and 2030 [2]. Therefore, interest in understanding how this trend affects various aspects of ecosystems has been growing over the past several decades [3–5]. Studies so far have shown that both agriculture and urbanization significantly simplify and alter ecosystems, cause a general decrease in biodiversity, disrupt ecosystem functions, and can detrimentally affect wildlife health [6,7].

The effects of ecosystem modification on parasites has become of particular interest, because they directly affect wildlife health, and can be transmitted to humans through zoonotic events [3,8,9]. Both urbanization and agricultural practices alter parasite prevalence (% of host population infected) and intensity (number of parasites infecting a single host) of individual species, although there is no consistency between studies, where some have reported an increase and others a decrease [3,9–16]. Although studies have investigated the response of helminth communities to ecosystem modification, namely of frogs [12–14], birds [5,15,16], and mammals [11,17–20], most focused on only one or two species usually of particular importance for conservation or public health [3,18,21]. While such case studies are necessary to understand how particular species of immediate concern are affected for the sake of management and control it severely limits our ability to more broadly understand how helminths as a group respond to ecological disturbance. Furthermore, changes in the community structure of

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plants and animals are rarely unidirectional, with a complex interaction between species [22]. Because many parasitic helminths depend on multiple hosts and can modify the interactions between them, the effects of ecosystem modification on helminths should be even more complex. Although some studies have looked at the intestinal helminth community of rodents within urban areas [11,17,23–25], most notably in black and brown rats (*Rattus rattus* and *R. norvegicus* respectively) [17,26–30], only one provided a comparison to a natural area [11]. Instead, some studies have utilized rural areas as their control [18,31–33]. However, this is likely inadequate as a reference, because such areas have already been modified for various human uses. Without a comparison to relatively undisturbed natural areas, few insights can be gained on how helminth communities are altered by urbanization.

In this study we used the Large Japanese field mouse (*Apodemus speciosus*) as the focal host to determine how its intestinal helminth community is altered in two anthropogenic modified ecosystems, a rural agricultural area and an urbanized city, as compared to a more undisturbed natural area (i.e. a reference site). Rodents are an ideal host organism for such studies due to their limited dispersal [34,35], allowing us to assume with near certainty that they experience the pressures of a single ecosystem type, unlike birds, the most common taxonomic group used in urban ecology studies [5]. We estimated helminth diversity and species richness, as well as prevalence and abundance (number of individuals of a single helminth species per host including uninfected hosts) of each intestinal helminth species in each type of ecosystem (i.e. natural, rural, and urban). We then compared all helminth community metrics in the modified ecosystems to the natural area using statistical models. We expected that the intestinal helminth community would be highly altered within both modified ecosystems. Urban areas are considered the most heavily modified ecosystems [36], causing a higher degree of forest fragmentation [37], altered trophic interactions [3,9] and others, all of which can affect helminth transmission and survival. Therefore, we expected the largest degree of alteration to occur within the urbanized city, so that each species would exhibit an increasing or decreasing trend in both prevalence and abundance going from natural to urban.

2. Materials and methods

2.1. Host species

The Large Japanese field mouse (*A. speciosus*) is a common small rodent throughout Japan. It is primarily a forest dweller in Hokkaido, but also commonly found in fields. The field mouse is an omnivore that consumes a wide variety of food, although primarily insects in summer, and plants and seeds in autumn and winter [38]. Females of this species are territorial and solitary, but males are not [39].

2.2. Study sites

This study was conducted in the Tokachi region on the island of Hokkaido, northern Japan. Animals were collected from three distinct ecological categories (6 natural, 3 rural, and 1 urban sites; Supplementary Fig. 1) in order to determine if differing types of modified environments affect the intestinal helminth community to different degrees. The natural sites were located in the hills 9 km north east of the city of Obihiro and situated next to the pristine protected area of Osarushinai Forest (between 42°57′47.42″ to 42°58′33.24N and 143°17′53.88″ to 143°19′26.80 E). The hills themselves are a patchwork of logging sites with planted Japanese larch (*Larix kaempferi*) and secondary forest. However, logging has almost entirely ceased, and most previously logged patches have been recolonized by native trees and plants. The collection sites were located within the secondary forest, at least 1 km from any inhabited areas or agricultural fields, and up in elevation as compared to any locations of potential run-off of pollutants. The rural sites were forest fragments surrounded by agricultural fields about 10 km south of Obihiro (between 42°47′52.42″ to 42°48′39.12″ N and 143°5′43.49″ to 143°6′39.41″ E), where Chinese yam, onions, daikon radish, and wheat are primarily grown 6 months out of the year, but agricultural practices cease during the long and cold winters. The urban animals were collected from Tsuda Park (42°55′11.40″ N, 143°7′32.80″ E) located within Obihiro, and is surrounded by major roads and a high density of buildings with very little if any vegetation outsides the park limits. The park itself consists mostly of a managed forest with multiple pathways running through it, and a small area in the south west corner that has been cleared of trees with basic structures for lounging. There is also a small baseball field in the north east corner. The forests at all sites where the mice were collected were primarily composed of deciduous trees, with the underbrush largely consisting of dwarf bamboo (*Sasa kurilensis*) and leaf litter, and their elevation below 500 m. Specific site coordinates, number of traps nights, and the number of *A. speciousus* that were captured can be found in Supplementary Fig. 1B. The number of *Apodemus argenteus* captured is also included in Supplementary Fig. 1B, because it is a closely related species that often resides within the same habitat patches as our focal host. However, due to the low number of individuals captured (five in total), it is not discussed within the present study. Lastly, additional information on the rural and urban sites is available from past studies [40,41].

2.3. Sampling of hosts and intestinal helminths

The capturing of rodents was confined to early summer (June 15th to July 17th, 2016) to avoid seasonal variation in the helminth community. Sherman traps baited with Oatmeal were used to capture the animals. At each site, approximately 40 traps spaced 10 m apart in a 4 by 10 grid pattern were set for an average of 3 days, except for one natural site which had 80 traps and one other with 50 due to low population density. Traps were checked twice a day (i.e. early morning and evening), and those with a captured animal were replaced with a fresh trap.

Upon capture, each individual was identified, euthanized by cervical dislocation, sexed, weighed for a rough estimation of age [42], given an ID, and frozen at −20 °C until laboratory examination. The digestive tract was removed and carefully checked for helminths under a stereo microscope (model: Olympus SZX10), and all helminths were preserved in 70% ethanol. For morphological identification, nematodes and acanthocephalans were cleared with creosote, and examined under an Olympus BX50 microscope. Trematodes were stained with acetocarmine and cestodes were stained with alum carmine before examination as described in Nakao et al. [43] and Haukisalmi et al. [44] respectively. Additionally, trematodes were analyzed genetically using nuclear 28S ribosomal DNA (rDNA) and mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) as described in Nakao et al. [43]. Cestodes were also identified genetically using 28S nuclear rDNA as described in Haukisalmi et al. [44]. All cestodes were grouped together for statistical analysis due to the low number found and their similar life histories.

2.4. Statistical analyses

Helmith richness, the number of distinct types of helminths found in a habitat, was estimated for each type of ecosystem. Helminth diversity was estimated using the Shannon-Wiener diversity index (H′) where the larger the number indicates higher diversity [45]. For each species of helminth found, prevalence and mean abundance were estimated for each type of ecosystem.

All statistical models were analyzed using R version 3.4.3 (The R Foundation for Statistical Computing 2017; available at www.R-project.org). All figures were created with the R package “ggplot2”. Difference in prevalence between ecosystem type (i.e. natural, rural, and urban) was first tested using a generalized linear model (GLM) with binomial distribution with a logit link using the R package “lme4”. Infection status was the response variable, and ecosystem type, sex, and host weight the explanatory variables. The helminth species that could not be analyzed using the standard GLM due to perfect separation of the coefficients as determined using the R package “safeBinaryRegression” were re-analyzed using Firth's bias-reduced logistic regression with the same variables [46]. Helminth abundance compared between ecosystem type was first tested for using a GLM with Poisson distribution where abundance was the response variable, with ecosystem type, sex, and host weight as explanatory variables. After checking for overdispersion of the model using the R package “AER”, abundance was re-analyzed using a GLM with negative binomial distribution using the same variables. The Poisson and negative binomial GLMs were then compared using a log-likelihood ratio test using the R package “lmtest”. In addition, due to the large number of zero's typically seen in intestinal helminth abundance data, both a zero-inflated Poisson (ZIP) and a zero-inflated negative binomial (ZINB) model were used to re-analyze the abundance of each species. The ZIP and ZINB models were compared using a log-likelihood ratio test, as well as compared to the GLM equivalents using the vuong test. The best model was then selected based on the comparative statistics. Lastly, those helminth species for which abundance could not be analyzed using these models due to perfect separation of the coefficients were analyzed using a Man-Whitney *U* test. However, this prevented us from including host sex or weight in the analysis. Acanthocephalans and *Syphacia emileromani* were omitted from the prevalence and abundance models due to only one individual being infected with each.

3. Results

3.1. Intestinal helminths detected

A total of 67 *A. speciosus* were examined in this study consisting of 20, 23, and 24 mice from the natural, rural, and urban areas respectively. Within the intestine, 5 species of Nematoda were identified (*Heterakis spumosa*, *Syphacia emileromani*, *Syphacia agraria*, *Heligmosomoides kurilensis,* and *Heligmonoides speciosus*), 1 species of Trematoda (*Brachylaima asakawai*.), 1 species of Acanthocephala (*Moniliformis* sp.), and 2 species of Cestoda (*Microsomacanthus* sp. and *Catenotenia* sp.) (Table 1).

3.2. Diversity

Table 2
Estimations of species richness and Shannon-Wiener diversity of intestinal helminths in three types of ecosystems.

		Shannon-Wiener diversity	
Ecosystem	Richness	All helminths	Omitting H. speciosus
Natural	8	0.4614	0.7412
Rural	7	0.6743	0.8756
Urban	5	0.9339	0.7224

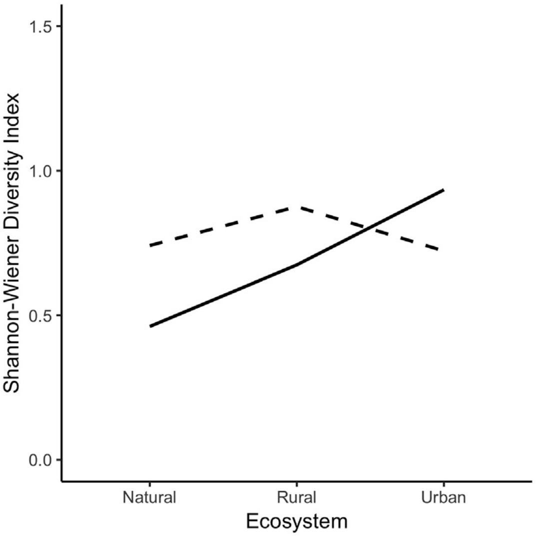

Fig. 1. Line graph depicting Shannon-Wiener diversity index estimates for intestinal helminths in all three ecosystem types. Solid line includes all species while dashed line omits *H. speciosus*.

Table 1
Prevalence and abundance for each species of intestinal helminth within each type of ecosystem.

	Natural			Rural			Urban		
Helminth Species	Prevalence (%)	Abundance		Prevalence (%)	Abundance		Prevalence (%)	Abundance	
		Mean ± S.E.	Range		Mean ± S.E.	Range		Mean ± S.E.	Range
Nematoda									
Heterakis spumosa	65	12.7 ± 4.3	0–68	56.52	25.7 ± 6.1	0–87	58.33	19.4 ± 6.4	0–117
Syphacia emileromani	5	0.15 ± 0.15	0–3	0	0 ± 0	0	0	0 ± 0	0
Syphacia agraria	45	33.7 ± 18.6	0–341	0	0 ± 0	0	0	0 ± 0	0
Heligmosomoides kurilensis	0	0 ± 0	0	56.52	16.7 ± 6.9	0–131	83.33	29 ± 7.5	0–114
Heligmonoides speciosus	100	342.9 ± 62.3	6–1230	91.3	178.4 ± 37.7	0–593	100	80.9 ± 13.6	5–279
Trematoda									
Brachylaima asakawai	5	0.4 ± 0.4	0–8	4.35	0.04 ± 0.04	0–1	4.17	0.3 ± 0.3	0–6
Cestoda									
Cestoda spp. (combined)	35	0.9 ± 0.3	0–4	26.09	1.7 ± 0.8	0–14	4.17	0.2 ± 0.2	0–4
Microsomacanthus sp.	5	0.1 ± 0.1	0–2	21.74	1.3 ± 0.7	0–14	0	0 ± 0	0
Catenotaenia sp.	5	0.1 ± 0.1	0–2	0	0 ± 0	0	0	0 ± 0	0
Cestoda spp. (unidentified)	25	0.7 ± 0.3	0–4	4.35	0.4 ± 0.4	0–10	4.17	0.2 ± 0.2	0–4
Acanthocephala									
Moniliformis sp.	0	0 ± 0	0	0.043	0.3 ± 0.3	0–8	0	0 ± 0	0

Intestinal helminth richness was lower in both types of modified ecosystems, with it being lowest in the urban park (natural = 8, rural = 7, and urban = 5 species, Table 2). Shannon-Wiener diversity was most altered within the urban area (H′ = 0.9339) where it was twice as high as compared to the natural (i.e. reference) area (H′ = 0.4614), and only moderately higher in the rural area (H′ = 0.6743) as we expected, although the trend was opposite to richness (Fig. 1 and Table 2). Due to an extremely high abundance of *H. speciosus* relative to other species in both the natural and rural areas, thereby biasing the diversity estimate, it was re-analyzed while omitting this species. Subsequently, H′ became nearly equal in all three ecosystem types with 0.7412, 0.8756, and 0.7224 at the natural, rural,

Table 3
Statistical comparison of differences in prevalence and abundance between a natural area and both modified ecosystems (i.e. rural and urban). The letter corresponds to statistical test used for analysis as listed below.

		Prevalence			Abundance		
Helminth species	Comparison	Est.	SE	p	Est.	SE	p
Heterakis spumosa[a,d]	Natural – Rural	1.949	1.602	0.248	0.775	0.383	0.043
	Natural – Urban	3.256	1.753	0.063	−0.015	0.369	0.967
	Host Weight	0.267	0.077	0.001	0.106	0.019	< 0.001
	Sex	−1.039	1.038	0.317	−0.958	0.303	0.002
Syphacia agraria[b,e]	Natural – Rural	−3.784	1.481	< 0.001	U = 333	NA	< 0.001
	Natural – Urban	−3.839	1.478	< 0.001	U = 348	NA	< 0.001
	Host Weight	−0.029	0.036	0.429	NA	NA	NA
	Sex	−0.381	0.896	0.677	NA	NA	NA
Heligmosomoides kurilensis[b,c]	Natural – Rural	6.902	2.103	< 0.001	U = 100	NA	< 0.001
	Natural – Urban	10.206	2.652	< 0.001	U = 40	NA	< 0.001
	Host Weight	0.224	0.073	< 0.001	NA	NA	NA
	Sex	−1.044	1.371	< 0.001	NA	NA	NA
Heligmonoides speciosus[b,c]	Natural – Rural	−0.61	1.62	0.731	−0.738	0.248	0.003
	Natural – Urban	0.841	1.92	0.687	−0.87	0.246	< 0.001
	Host Weight	0.086	0.054	0.103	0.077	0.008	< 0.001
	Sex	1.132	1.268	0.431	−0.109	0.21	0.602
Cestoda spp. (combined)[a,d]	Natural – Rural	−0.513	0.771	0.506	1.602	0.509	0.002
	Natural – Urban	−2.915	1.222	0.017	−2.187	1.25	0.08
	Host Weight	0.047	0.031	0.124	−0.076	0.033	0.022
	Sex	−1.617	0.741	0.029	0.931	0.566	0.099
Microsomacanthus sp.[b,e]	Natural – Rural	1.58	1.084	0.118	U = 191	NA	0.12
	Natural – Urban	−1.903	1.796	0.248	U = 252	NA	0.294
	Host Weight	0.118	0.064	0.019	NA	NA	NA
	Sex	−2.238	1.128	0.034	NA	NA	NA
Brachylaima asakawai[b,e]	Natural – Rural	−0.357	1.229	0.778	U = 232	NA	0.92
	Natural – Urban	−0.44	1.219	0.723	U = 242	NA	0.896
	Host Weight	0.005	0.044	0.921	NA	NA	NA
	Sex	−2.259	1.302	0.069	NA	NA	NA

[a] Prevalence models: Generalized linear model with binomial distribution.
[b] Prevalence models: Firths's bias-reduced penalized-likelihood logistic regression.
[c] Abundance models: Generalized linear model with negative binomial distribution.
[d] Abundance models: Zero-inflated model with negative binomial distribution.
[e] Abundance models: Man-Whitney *U* test.

and urban sites respectively, though it was highest within the rural sites, (Fig. 1 and Table 2).

3.3. Prevalence and abundance

Although host weight and sex are not the main concern in this study, we found some significant effects on prevalence and abundance (Table 3). Host weight, and by proxy age, was a significant factor explaining the prevalence of two (*H. spumosa* and *H. kurilensis*) of the five nematode species, as well as the cestode *Microsomacanthus* sp., but not cestodes in general (Table 3). It was also significant in explaining the abundance of cestodes, as well as the nematodes *H. spumosa* and *H. speciosus* (Table 3). While host sex was a significant factor in explaining the prevalence of *H. kurilensis*, Cestoda spp., *Microsomacanthus*, and *B. asakawai*, it failed to predict abundance in all helminths except *H. spumosa*. (Table 3).

We found the effect of ecosystem type on helminth prevalence varied greatly between species (Fig. 2A, Tables 1 and 3). Both species of *Syphacia* as well as *Catenotaenia* sp. showed an equal decrease in both the rural and urban areas due to being present solely within the natural area, significantly so only for *S. agraria*, partially agreeing with our hypothesis (Tables 1 and 3). The nematode *H. kurilensis* and Cestoda spp. were the only helminths to follow our prediction with both showing significant differences in prevalence (Fig. 2A, Tables 1 and 3). However, while the prevalence of Cestoda spp. decreased in the modified ecosystems, *H. kurilensis* increased (Fig. 2A, Tables 1 and 3). While *H. spumosa* slightly decreased in prevalence in both modified ecosystems, it was lowest in the rural agricultural area partially disagreeing with the expected trend (Fig. 2A, Tables 1 and 3). The cestode *Microsomacanthus* sp. was the only helminth to have highest prevalence within the rural area and lowest within the urban park, completely disagreeing with our hypothesis, although this comparison was not statistically tested (Fig. 2A, Tables 1 and 3). *H. speciosus* was the only species that exhibited almost no change, with all but two hosts in this study being infected regardless of ecosystem type (Fig. 2A, Tables 1 and 3).

The change in helminth abundances within the modified ecosystems were found to be just as variable, with the abundance of many species mirroring the change in their prevalence (Fig. 2, Tables 1 and 3). The abundance of both species of *Syphacia* as well as *Catenotaenia* sp., similar to their prevalence, exhibited equal changes within both modified ecosystems due to no individuals being found in either the rural or urban habitats (Table 1). However, the change in *S. agraria* was the only statistically significant comparison (Table 3). The nematodes *H. kurilensis* and *H. speciosus* had the largest changes within the urban area and intermediate in the rural area, but in opposite directions with *H. kurilensis* increasing and *H. speciosus* decreasing in abundance (Fig. 2B, Tables 1 and 3). Cestoda spp. exhibited significantly increased abundance in the rural area as compared to the natural forest and a nearly significant decrease in the urban park, opposite of what was expected (Fig. 2B, Tables 1 and 3). While the cestode *Microsomacanthus* sp. showed the same trend when analyzed separately, it was not significant (Tables 1 and 3). *H. spumosa* abundance was slightly but significantly higher in the rural area as compared to the natural forest, but not in the urban park (Fig. 2B, Tables 1 and 3). The trematode *B. asakawai* was the only helminth with very little change in abundance within either modified environment (Tables 1 and 3).

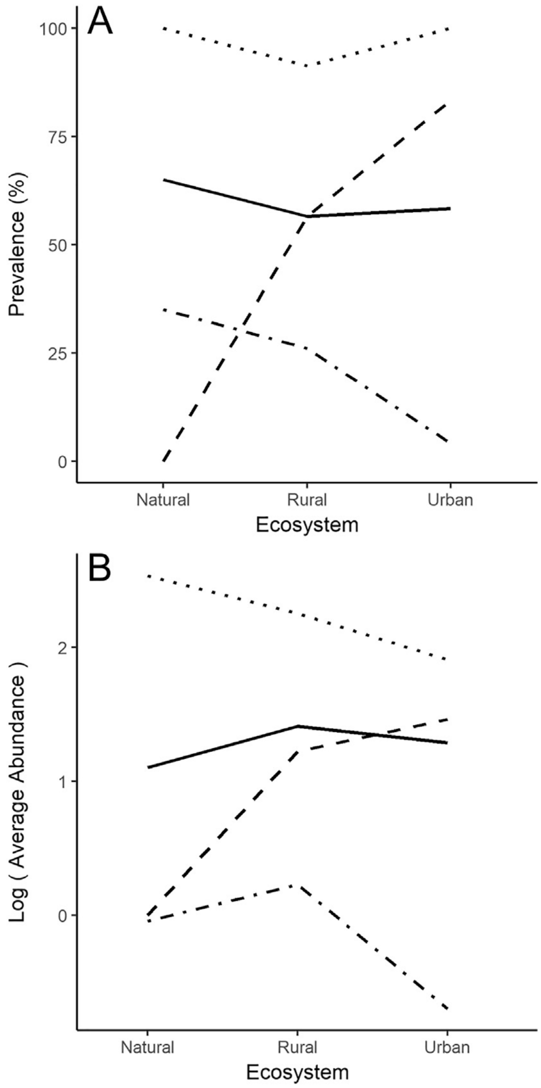

Fig. 2. Line graph depicting a) prevalence and b) log transformed average abundance of four species of intestinal helminths in all three ecosystem types. The solid line is *H. spumosa,* dashed is *H. kurilensis*, dot dash is Cestoda spp. (combined), and dotted is *H. speciosus*.

4. Discussion

While anthropogenic modification of ecosystems generally decreases the diversity of animals [4,7], species richness of intestinal helminths in mammals has been shown to increase [[13,46,47] but see Marcogliese [48]]. This is particularly true in response to forest fragmentation, logging, and agricultural practices [11,47,48], though urban areas remain understudied [11]. However, in the present study we found that species richness of intestinal helminths decreased in both modified ecosystems with it being lowest in the urban park. This trend can partially be explained by the reduction of cestodes, because they have a complex life cycle that requires one or more intermediate hosts, typically an arthropod. Helminths with complex life cycles are known to be highly susceptible to local extirpation when biodiversity of free-living species decreases [50,51], as is common in modified environments [4,7]. Because the loss of suitable habitat is highest within urban areas [52], it increases the potential loss of a necessary intermediate host, thereby preventing the helminths persistence within the ecosystem [50,51]. However, this trend does not hold true for all helminths with complex life cycles, as the trematode *B. asakawai* which utilizes rodents as the final host [53] changed little in either prevalence or abundance within this study. *B. asakawai* was described using individuals collected from the small snail *Discus pauper*, largely from urban parks [53]. Because *D. pauper* serves as both the first and second intermediate host and is likely resistant to anthropogenic influences to a certain degree, it allows *B. asakawai* to persist.

Nematodes were not entirely exempt from disappearance within the modified environments, most notably both species of *Syphacia* despite having a simple life cycle in which transmission occurs through the oral-fecal route. Because *S. emileromani* primarily parasitizes the closely related small Japanese field mouse (*Apodemus argenteus*) [54], the single individual *A. speciosus* infected within the natural forest was likely accidental. On the other hand, *S. agraria* commonly parasitizes *A. speciosus* and may have been negatively affected by pollutants through direct application of pesticides and herbicides for agricultural practices or park management, or indirectly through runoff [12,49,51]. While studies have shown highly variable responses of helminths to pollutants [49], those of the genus *Syphacia* that parasitize rodents were found to be negatively affected by herbicides [19] and petrochemicals [20], presumably through direct exposure of the eggs within the environment or consumption by the host [[49,51, but see Tersago et al. 55]]. The effect of these chemicals may be further compounded by forest fragmentaion, because it greatly reduces dispersal of *A. speciosus* [34,35], preventing the reintroduction of helminths. However, it must be noted that the lack of *S. agraria* in the urban area in this study may be due to chance, because there was only a single urban park in which we captured the host *A. speciosus*.

Not all helminths within this study were negatively affected by ecosystem modification, however, as the abundance of *H. spumosa* slightly increased within the rural forest fragments, and both the prevalence and abundance of *H. kurilensis* increased with it highest in the urban park. Because the abundance of *H. speciosus* decreased dramatically in both the urban and rural sites, and it resides within the same location of the small intestine as *H. kurilensis*, interspecific competition or lack thereof may explain the apparent dominance of *H. kurilensis*. A single host could be considered a micro ecosystem for helminths with limited space and resources [56]. Despite *H. speciosus'* small size (1.5 to 3.5 mm in length), when hundreds of individuals are present as seen in our study, it severely limits available space for the significantly larger *H. kurilensis* (10.6 to 13.8 mm in length). Furthermore, *Heligmosomoides polygyrus*, a species closely related to *H. kurilensis* has been shown to thrive in environments subjected to pollutants such as heavy metals [55]. Therefore, it is plausible that *H. kurilensis* has a competitive advantage within polluted environments such as cities and agricultural areas. There may even be a synergistic effect if *H. speciousus* is negatively affected by the same pollutants or other factors associated with ecosystem modification, although no study has been done. Therefore, ecosystem modification may not only directly impact intestinal helminths, but also indirectly by altering interspecific competition dynamics. However, the fact we did not find a single host parasitized by *H. kurilensis* within the natural area is an oddity, because this helminth commonly infects *A. speciosus* [57]. Therefore, any interpretation of the effects of ecosystem modification on this species must be done with caution until further research is conducted.

The observed increase in diversity going from the natural area, to the agricultural forest fragments, to the city park is particularly interesting, because it is opposite of what we found for species richness. However, this increasing trend was the result of a single species of helminth (i.e. *H. speciosus*) dramatically decreasing in abundance, causing an increase in the evenness among all species. Therefore, when it was removed from the diversity analysis, diversity estimates remained relatively similar within all three ecosystem types, although this is still counter to our estimates of species richness. As discussed above, the reduced richness within the modified environments was caused by the reduced number of cestodes found, and an accidental infection by a nematode species only occurring in the natural forest, all of which are rare events. If our sample size of the host species were to have been larger, we may very well have found more individuals infected with cestodes, thereby increasing species richness. However, prevalence would still be significantly less.

Although we found clear but opposite trends in the response of helminth species richness and diversity to anthropogenic ecosystem modification, prevalence and abundance showed a much more varied and complex response. Because ecologists often only think of parasites, particularly endoparasitic helminths, as a source of disease with detrimental effects on wildlife, they fail to consider them as animals in their own right [58]. Such a view has been continuously reinforced due to most studies so far having focused on species of conservation (of their host) or public health concern [59]. However, when helminths are viewed as animals, it should come as no surprise that while many species disappear from or become rare within anthropogenic modified ecosystems as seen in this study, others are able to exploit the new environment and thrive, similar to their hosts. Furthermore, it is important to consider the entire helminth community when trying to understand the response of a single species to ecosystem modification, because interspecific competition may influence the outcome. Finally, despite the rural agricultural and urban areas undergoing different forms of ecosystem modification, individual helminth species tended to respond to both in a similar fashion (e.g. increasing or decreasing in prevalence in both), though to a greater degree within the urban park. Future studies should utilize reference sites that are as close to undisturbed as possible when investigating the role that anthropogenic ecosystem modification has in altering helminth communities.

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