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Original Article

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~~s~~ to biodiversity and ecosystem function~~s~~. Parasites are of particular interest, as they directly impact animal health, and can ~~sometimes~~ 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. ~~By looking at the trends and life histories of closely related helminth species, it allows us to better elucidate the causes of observed changes in prevalence and abundance.~~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. However, it remains important to analyze the entire helminth community, as intraspecific interactions and the effect that ecosystem modification has on them may help determine what species persist. ~~Furthermore, examining helminths residing within the same location of the intestine, we found there may be an effect of interaction in addition to ecosystem modification. Therefore, the entire helminth community of a host must be investigated in order to fully understand the effects of ecosystem modification.~~

Keywords: Ecosystem modification, Urbanization, Helminth community, *Apodemus speciosus*

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 as the rate of urbanization in particular, is occurring at an astonishing rate with more than 1.5 million km^2 of land predicted to be added to cities between the years 2011 and~~by~~ 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 ~~is~~has become of particular interest as they directly affect wildlife health, and can ~~sometimes~~ 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~~s~~ and others a decrease~~s~~ [3,9–16]. Although ~~a few~~ studies have investigated the response of helminth communities to ecosystem modification, namely of frogs [12–14], birds

[5,15,16], and ~~rodents~~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 ~~knowledge in regards to how parasites are affected in a more general sense,~~ability to more broadly understand how helminths as a group respond to ecological disturbance~~thus preventing the identification of broad scale trends~~. Furthermore, changes in the community structure of plants and animals are rarely unidirectional, with a complex interaction between species~~, where some increase and some decrease in abundance~~ [22]. Because many parasites depend on multiple hosts and can modify the interactions between them, the effects of ecosystem modification on parasites should be even more complex. ~~Additionally, while previous~~Although some studies have looked at~~investigated~~ the parasite 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~~Furthermore~~, ~~while~~ some studies have ~~compared urban parasites communities to those in~~utilized rural areas as their control [18,31–33]~~.,~~ However, ~~using rural areas as a reference is~~ this is likely inadequate as a reference, as such areas~~they~~ have already been modified for various human uses. Without a comparison to relatively undisturbed natural areas, few insights can be gained on how parasite 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 parasite species per host including uninfected hosts) of each parasitic helminth species in each type of ecosystem (i.e. natural, rural, and urban). We then compared all parasite 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 parasite 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 site~~s~~; Supplementary Material 1) in order to determine if differing types of modified environments affect the parasite 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 42º57'47.42" to 42º58'33.24 N and 143º17'53.88" to 143º19'26.80 E). The hills themselves are a patchwork of logging sites with planted Japanese larch (*Larix kaempferi*) and ~~undisturbed habitat.~~ 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 ~~undisturbed~~ secondary~~deciduous~~ forest, at least 1km 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 10km south of~~-~~ Obihiro (between 42º47'52.42" to 42º48'39.12" N and 143º5'43.49" to 143º6'39.41" 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 (42º55'11.40" N, 143º7'32.80" E)~~, a park~~located within Obihiro, which 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 meters. Specific site coordinates and, number of traps nights, and the number of *A. speciousus* that were captured -can be found in Supplementary Material 1B. The number of *Apodemus argenteus* captures was also included in Supplementary Material 1B, as 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 found, it is not discussed within the present study., andLastly, additional information on the rural and urban sites is available from past studies [40,41].

2.3 Sampling of hosts and parasites

The capturing of rodents was confined to early summer (June 15th to July 17th, 2016) to avoid seasonal variation in the parasite community. Sherman traps baited with Oatmeal were used to capture the animals. At each site, approximately 40 traps spaced 10 meters 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 -20C until laboratory examination. The digestive tract was removed and carefully checked for helminths under a stereo microscope (model: Olympus SZX10),. and aAll parasites 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] [40] and Haukisalmi et al. [44] [41] 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] ~~[40]~~. Cestodes were also identified genetically using 28S nuclear rDNA as described in Haukisalmi et al. [44]Ha ~~[41]~~. All cestodes were grouped together for statistical analysis due to the low number found and their similar life histories.

2.4 Statistical analyses

Parasite richness, the number of distinct types of parasites found in a habitat, was estimated for each type of ecosystem. Parasite diversity was estimated using the Shannon-Wiener diversity index (H') where the larger the number indicates higher diversity [45]. For each species of parasite 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]. ~~The difference in P~~parasite abundance compared between ecosystem type was first tested for using a GLM with poisson distribution where a~~A~~bundance 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 with the same variables. The negative binomial and poisson GLMs were then compared using a log-likelihood ratio test in the R package “lmtest”. In addition, due to the large number of zero’s typically seen in parasite 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.~~zero-inflated model with negative binomial distribution (ZINB) using the R package “pscl” after checking for overdispersion of the GLM equivalent model using the R package “AER”~~. ~~Abundance was the response variable, with ecosystem type, sex, and host weight as explanatory variables. A GLM with negative binomial distribution was used to compare the abundance of *Heligmonoides speciosus* due to nearly 100% prevalence, and therefore a ZINB was not appropriate. All statistical analyses were run separately for each parasite species. *Heligmosomoides kurilensis* was omitted from abundance analysis, as no individuals were found in the natural area. The prevalence of *H. speciosus* could not be analyzed with the binomial model due to all but two host individuals being infected. Both prevalence and abundance of *Syphacia agraria* could not be compared between the natural and rural sites due to no individuals being found in the rural area.~~ Acanthocephalans and ~~*Heligmosomoides desportesi*~~*Syphacia emileromani* were~~ also~~ omitted from the prevalence and abundance models due to only one individual being infected with each.~~, as well as the trematode *Brachylaima asakawai*, due to only 1 individual in each ecosystem type being infected.~~

3. Results

3.1 Parasites 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~~7~~ species of Nematoda were identified (*Heterakis spumosa*, *Syphacia emileromani*, *Syphacia agraria*, *Heligmosomoides kurilensis*,~~ *Heligmosomoides desportesi*,~~ and *Heligmonoides speciosus*), 1 species of Trematoda (*Brachylaima asakawai*.), 1 species of Acanthocephala (*Moniliformies* sp.), and 2 species of Cestoda~~cestodes~~ (*Microsomacanthus sp.* and *Catenotenia sp.*) (Table 1).

3.2 Diversity

Intestinal helminth richness was ~~nearly identical among the three~~lower in both types of modified ecosystems, with it being lowest in the urban park (natural = 8, rural = 7~~10~~, and urban = 5~~8~~ species, Table 2). Shannon-Wiener diversity was most altered within the urban area (H'=0.9339~~1.023~~) where it was twice as high as compared to the natural (i.e. reference) area (H'=0.4614~~81~~), and only moderately higher in the rural area (H'=0.6743~~724~~) as we expected, though 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, ~~diversity~~it was re-analyzed while omitting this species.

Subsequently, H’ became nearly equal in all three ecosystem types with 0.7412~~906~~, 0.8756~~1.269~~, and 0.7224~~983~~ at the natural, rural, 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 ~~for~~explaining the prevalence of two~~three~~ (*H. spumosa*~~,~~ ~~*S. emileromani*,~~ 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 ~~*S. emileromani*~~*H. spumosa* and *H. speciosus* (Table 3). While ~~H~~host sex~~,~~ ~~however,~~ was ~~only~~ a significant factor in explaining the prevalence of ~~cestodes~~*H. kurilensis*, Cestoda spp., *Microsomacanthus*, and *B. asakawai*, it failed to predict abundance in all helminths except *H. spumosa*. ~~, whereas it was significant for the abundance of *S. emileromani*, *H. kurilensis*, and *H. speciosus*~~ (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 (Table 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, Table 1 and 3). However, while the prevalence of Cestoda spp. decreased in the modified ecosystems, *H. kurilensis* increased (Fig. 2A, Table 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, Table 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, Table 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, Table 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, Table 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, Table 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, Table 1 and 3). While the Cestode *Microsomacanthus sp.* showed the same trend when analyzed separately, it was not significant (Table 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, Table 1 and 3). The trematode

B. asakawai was the only helminth with very little change in abundance within either modified environment (Table 1 and 3).

For the comparison of three different ecosystems, overall results were consistent to our initial prediction with some mixed results (Fig. 2, Tables 1 and 3). In terms of prevalence, three parasites (*S. agraria*, *H. kurilensis*, and cestodes) supported our prediction that prevalence would be most significantly altered at the urban site (Fig. 2, Tables 1 and 3). *H. kurilensis* was the only species to increase in prevalence in both modified ecosystems, while the other two decreased. For abundance, *S. agraria*, and *H. speciosus* followed our expectation of being the most altered within the urban site (Fig. 2, Tables 1 and 3). *H. speciosus* showed a particularly clear pattern with mean abundance decreasing by 75 percent at the urban site as compared to the natural area. Similar to prevalence, *H. kurilensis* is the only species that had higher abundance in both modified ecosystems, with the highest in the urban site (Fig. 2 and Table 1), although it could not be tested using our statistical models. *S. emileromani* and cestodes, however, had the highest amount of alteration of abundance in the rural area as opposed to the urban site (Fig. 2, Table 1 and 3).

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 was decreased in both modified ecosystems, with it being lowest in the urban park. This trend can partially be explained by the reduction of cestodes, as 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 ~~are~~ highly susceptible to local extirpation when biodiversity of free-living species decreases [50,51], as is common in modified environments [4,7]. As the loss of suitable habitat is highest within urban areas [52], it increases the potential loss of a necessary intermediate host, thereby ~~. The loss of a single one of its hosts will~~ preventing the helminths persistence within the ~~environment~~ecosystem [50,51]. ~~Because the loss of suitable habitat is highest within urban areas [51], it increases the potential loss of a necessary intermediate hosts.~~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. ~~unchanged, and species diversity was highest in the urban area. We also found species richness to be highest within the rural forest fragments imbedded within an area predominately composed of agricultural crop fields, similar to previous parasitological studies of rodents [13,45]. This may indicate that anthropogenic modification affects parasite communities differently than animals due to complex host-parasite interactions. Also, higher parasite diversity may not necessarily be a good indicator of ecosystem health, because our reference site had the lowest species diversity when compared to both modified environments. The diversity index, however, was affected significantly by a single~~

species of nematode, *H. speciosus*. Therefore, analysis of species richness and diversity was insufficient within this study.

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 parasites 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 Routine population crashes of many small rodents [51], including *Apodemus spp.* in Hokkaido [52], can lead to local extirpation of obligate parasites within forest fragments, as the uninfected hosts may be the ones to persist. Furthermore, forest fragmentaion, as it greatly reduces dispersal of small rodents *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, as there was only a single urban park in which we captured the host *A. speciosus*.Therefore, more generalist parasites such as *S. emileromani* should have a higher chance to survive in a modified habitat, as they can reside in alternative hosts.

Taking prevalence and abundance of each helminth species into account, we were able to see how the intestinal parasite community is altered in modified ecosystems. Contrary to the increase of species diversity in the urban area, the prevalence of *S. agraria*, and cestodes was significantly lower, of which the latter is particularly reasonable. Parasites with complex lifecycles requiring more than one host such as cestodes, are highly susceptible to local extirpation when biodiversity of free-living species decreases [46]. The loss of a single one of its hosts will prevent persistence within the environment [46]. Because the loss of suitable habitat is highest within urban areas [47], it increases the potential loss of a necessary intermediate hosts. Although we combined all cestodes into a single group for analysis, this logic holds true for all species due to their similar life histories that depend on arthropod intermediate hosts. Therefore, the results would likely remain unchanged when separated into individual species, especially with only a single host being infected within the urban park.

Interestingly, both species of the genus *Syphacia* had highly altered prevalence and abundance in both modified ecosystems, but in opposite ways: *S. agraria* decreased in both prevalence and abundance, whereas *S. emileromani* increased. Because *S. agraria* occurred almost exclusively within the natural area, these differences could be explained by forest fragmentation. Importantly, *A. speciosus* is the main host for *S. agraria* in Hokkaido [48,49], with this helminth rarely being reported from *Apodemus argenteus* and *Apoodemus peninsulae* [50]. *S. emileromani*, however, is known to infect multiple sympatric *Apodemus* species [49]. Routine population crashes of many small rodents [51], including *Apodemus spp.* in Hokkaido [52], can lead to local extirpation of obligate parasites within forest fragments, as the uninfected hosts may be the ones to persist. Furthermore, forest fragmentaion greatly reduces dispersal of small rodents [53,54], preventing the reintroduction of helminths. Therefore, more generalist parasites such as *S. emileromani* should have a higher chance to survive in a modified habitat, as they can reside in alternative hosts.

A possibility explaining the opposite trends in generalist helminth species is interspecific competitionNot 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 parasites with limited space and resources [56]. , which may partially explain the trend seen in *H. speciosus* and *H. kurilensis* in the present study. Both are generalists of *Apodemus* spp., and reside within the same location of the small intestine. Although prevalence of *H. speciosus* remained high in the rural, and urban ecosystems, the lower it's abundance, the higher *H. kurilensis* became in both prevalence and abundance, most noticeably in the urban site (Fig. 2 and Tables 1 and 3). Despite *H. speciosus'* small size (1.5 to 3.5mm 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, as 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. ~~By looking at the trend of multiple helminth species within the same host, particularly those that reside within the same location, it can increase our ability to determine potential causes leading to their observed alteration within modified ecosystems for which future studies can investigate.~~

The observed increase in diversity going from the natural area, to the agricultural forest fragments, to the city park is particularly interesting, as 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 mentioned 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 parasites 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. ~~In the present study, we found the Shannon-Weiner diversity index to be an inadequate indicator of overall change of the intestinal helminth community of a single host species in modified ecosystems. Comparison of prevalence and abundance, however, is important in elucidating the causes behind altered parasite communities. This is particularly true when looking at closely related species or those that reside within the same location of the intestine, as they can interact with one another or respond differently due to slight differences in life histories. Therefore~~Furthermore, it is ~~essential~~ important to consider the entire helminth community when trying to understand the response of a single species to ecosystem modification, as interspecific competition may influence the outcome. ~~Additionally,~~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~~se~~ reference sites that are as close to undisturbed as possible~~, rather than rural areas,~~ when investigating the role that anthropogenic ecosystem modification has in altering parasite communities. ~~In this way, we can begin to develop generalized knowledge on how human activity affects parasites that can then be applied to individual situations or species of conservation or public health concern.~~

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Figure Legends

Fig. 1: Line graph depicting Shannon-Wie~~ei~~ner Diversity index estimates for intestinal helminths in all three ecosystem types. Solid line includes all species while dashed line omits *H. speciosus*.

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

Supplementary Material 1: a) Satellite image of the city of Obihiro and surrounding areas with collection sites marked by colored pins, and b) a table with GPS coordinates, trap nights, and number of individuals captured for each site.