Flea and tick infestation of a wild rodent in an anthropogenic landscape of the semi-arid Chile

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Abstract

Background: A series of abiotic and biotic modifications occur in an anthropogenic landscape which influence the ecology and physiology of wild hosts and their parasites. How these changes affect the parasitism is highly context-dependent, based on the host-parasite system and the type of perturbation. We aimed to investigate the differences in infestation with fleas and ticks of the native rodent *Phyllotis darwini* in three sites with different anthropogenic impacts and to evaluate environmental and host factors associated with parasite richness and load.

Methods: We studied wild, rural and peri-urban sites of the semiarid region of Chile in the spring and summer seasons between 2018 and 2020. We applied a multi-level analysis to assess environmental and host variables at the individual and microhabitat level. We used Generalized Linear Mixed Models and Generalized Linear Models to test factor effects on the richness and load of ectoparasites of adult rodents.

Results: Overall, out of 310 rodents trapped, 71% were flea-infested and 22% showed tick infestation. We identified six flea genera, being *Hectopsylla* sp. and *Neotyphloceras* sp. the most abundant fleas across sites. Flea richness was significantly higher in male rodents and during the spring and was positively affected by the micromammal species richness. The flea load was also higher in spring, in males, and positively associated to tick load. *Hectopsylla* sp., one of the most abundant fleas, showed a higher load in the peri-urban site and at grids with lower vegetation index values (NDVI), and *Neotyphloceras* sp. fleas were associated with tick load and lower temperatures. All ticks were larvae or nymphs of *Ixodes* sp. and showed a higher load in summer months and higher temperatures.

Conclusions: We discuss possible environmental requirements of fleas and ticks, the association between their loads and male-biased parasitism. Further experimental investigations are needed to seasonally precise microclimate thresholds required for the survival of these ectoparasites.

Background

Anthropogenic land use change is considered one of the main causes for the emergence of infectious diseases because impact on the structure and dynamics of wild hosts, vectors, and parasites at several ecological scales [1]. The potential mechanisms by which these changes may alter the transmission of parasites are: (i) the modification of the host, vector and/or parasite niche; (ii) the changes in the community composition; (iii) the behavioural changes of hosts and/or vectors and (iv) the physiological changes in nutritional status, stress, and immunity [2]. Since all these changes are highly interconnected, occurring from within-host to among-host or landscape scales, with diverse effects on host-parasites systems and, therefore, highly context-dependent [1 – 4]. For some host species, intrinsic determinants such as sex, age, and body condition can be more relevant causes than environmental factors to explain the host physiological status (e.g., immunity) and host-parasite relationship. In other species, extrinsic

environmental factors and/or the parasite community could strongly shape the host parasitism which also presents seasonal variations [5].

The most intense environmental changes and perturbations occur in the old core of cities and their effects gradually decline when moving outward from it into residential suburbs, lightly developed rural areas and finally reaching undeveloped wild areas [6]. At local level, land use conversion modifies the microclimatic conditions of niches affecting temperature/humidity, composition, configuration, and structure of the vegetation and, therefore, trophic resources [7]. All of this comprises an environmental filter that strongly influences the diversity and abundance of hosts and their parasites [8–11]. The host community structure varies according to ecological and life-history traits (e.g., dispersal ability, behavioural flexibility, diet) and adaptability to new environmental conditions (e.g., stress tolerance) [12,13]. In turn, the structure of the parasite communities depends on the environment represented by their hosts which provide parasites with food and living space, and the external environment represented by abiotic variables [14].

Wild rodents and their ectoparasites (macroparasites such as fleas and ticks) are a good ecological model to study how human-induced land-use changes affect the host-parasite community across landscapes. Rodents have a large distribution range, are abundant, allowing the study of different environments independently due to small home ranges and host several species of ectoparasites. On the other hand, ectoparasites such as fleas and ticks that develop part of their life cycle outside their host, are greatly affected by environmental factors [11] [15].

For instance, vegetation configuration and the microclimatic conditions generated near the ground surface greatly affect tick survival when they are off host [7,16].

Fleas show variable responses to temperature and humidity according to the species and development stage [17,18]. Usually, the larvae and pupal stage of fleas are completely of-host (in the host burrow), and the humidity plays a paramount role [17,19]. Low relative humidity is detrimental for fleas' larvae, especially under high temperatures for some species [17,18]. Humidity and temperature are correlated with precipitation and the amount of green vegetation, therefore, low rainfall regions may limit the distribution of many flea species and, for this reason, the number of fleas adapted to dry conditions is smaller [20].

The behaviour and physiology of the rodent host at a moment of its life are relevant to define a given pattern of parasitism. For instance, male-biased parasitism has been a commonly found in many host-parasite systems [8,21–24]. Altered landscapes tend to reduce the availability of food resources, increasing host population densities and competition, and affecting nutritional, immunological status, and the stress level of hosts [1,2,25]. A host poor condition increases the infection probability, triggering a vicious mechanism of a positive feedback loop that leaves the host prone to carry more parasites (increasing load or intensity) [25–28]. In turn, the changes in the physiological condition of the host, as well as the exposure to novel parasites, can modify the composition, interactions, and dispersion (i.e., transmission) of parasites [29–31]. Within this host environment, parasites interact with each other

('bottom-up' processes) and with the host immune system ('top-down' processes) [29–31]. Finally, the sum of all interactions between host-environment (off individual) and parasite-host environment (within individual) shapes the community of parasites that we could observe in a host.

Knowing the structure and dynamics of wild host-parasite systems is essential to understand the transmission and emergence of diseases in human-dominated landscapes. In some areas, the environmental conditions may favour higher spatiotemporal overlap between species (wild-domestic-human) with a consequent risk of multi-directional transmission of zoonotic or potentially zoonotic parasites [32,33]. Evidence of how wild host species and their parasites respond to the changes that occur in anthropized landscapes has been studied mainly in Europe and North American ecosystems [2,34–37]. In Chile, disease ecology research is growing, and some investigations have advanced in knowing how wild species and their parasites are distributed across different areas [38–41]. However, little is known about the environmental and intrinsic host factors that may or not favour the transmission of parasites across human-land uses. This is especially important to address in the north-central ecosystems of Chile which present multi-century history of anthropogenic impacts by urban and agricultural expansion, intensification of productive activities, in a semiarid area at risk of suffering the negative effects of climate change [42,43].

This study aimed to assess the effects of the anthropogenic changes through different land-uses on the dynamics of parasite infestation in a wild small mammal. We studied differences in infestation with fleas and ticks in *Phyllotis darwini* (Cricetidae), evaluating environmental and host factors in wild, rural, and peri-urban sites of a landscape in Northern Chile. Thus, we assessed whether the site, season, and host factors (sex, body condition, and parasitism) affected the ectoparasite infestation at the individual level. We also assessed whether environmental factors [i.e., temperature, relative humidity and an index of vegetation (NDVI)] influenced the flea and tick infestation at the microhabitat level. We predicted that if environmental changes increase from the wild to the more urbanized zone, the parasitism pattern of *P. darwini* could differ between sites. We expected to find higher ectoparasite diversity in sites with less human perturbation, but higher ectoparasite loads as environmental disturbance increases.

Materials And Methods

Study area and design

The study area was in the Coquimbo Region in north-central Chile (71° 12' to 71° 40' W, 29° 58' to 30° 39' S; Fig. 1). This is a semiarid Mediterranean climate with a mean annual precipitation of 126.8 mm. The 90% of the rain is concentrated during winter months (May-September) whereas summer months (December–March) are warm and dry [42, 47]. The mean temperature range 12–18°C at 2 meters above ground near the coast and the relative air humidity can approach saturation (90–100%) [42].

The landscape comprised a natural mosaic of vegetation formations and human-dominated land uses [43]. Three sites with different anthropogenic pressures across the study area were sampled: (1) Wild, (2)

Rural and (3) Peri-urban (Fig. 1). The wild site comprised a xeric and mesic environment (e.g., thorn scrub and scrub with cacti and other succulents) in the Bosque Fray Jorge National Park (BFJNP). The rural site was an agropastoral community ("Tangue ranch") with a land matrix of mixed native and exotic vegetation formations. The peri-urban site involved suburban land-parceling near 'Tongoy town", a land subdivision that is the outcome of the increasing demand for recreational housing and the tourism of this coastal area. Vegetation is strongly perturbed with a dominance of exotic plantations of *Atriplex* sp. or ruderal native species.

Within each site, four trapping grids were deployed and separated ≥ 1km from each other. Each grid consisted of a total of 200 Sherman like traps using a specific trap arrangement (200 x 100 m, 2 ha) [48]. This trap arrangement considers the minimum home range of the potential rodent species to be trapped in the study area. Traps were activated on four consecutive nights, during the austral spring and summer seasons for two consecutive years: spring 2018 (henceforth Spr1), summer 2019 (Sum1), spring 2019 (Spr2) and summer 2020 (Sum2). A trapping grid of the peri-urban site (i.e., PU1) was relocated in the second year (i.e., Spr2 and Sum2) due to logistical restraints and an unsuccessful capture rate of rodents (Fig. 1).

Trapping And Ectoparasites Collection

Sherman traps of each trapping grid were baited with oat flakes and vanilla essence and activated at sunset (7 pm) and checked early the next morning (7 am). All captured individuals were moved to a central processing tent in the site and the rodents were separated by species and checked for their general condition. Captured *P. darwini* individuals were first placed in a 500mL volume induction chamber containing a cotton embedded with 1mL of Iso- or Sevoflurane. Then, the rodents were weighed using a digital scale (Pesamatic Newton Series®, Model EJ1500; ±0.1 gr SD) and injected intramuscularly with a Ketamine (0.011 mg/g) and Xylazine (0.003 mg/g) combination.

After recumbency, the animals were combed thoroughly for 5 minutes. We used fine tweezers to remove the ectoparasites adhered to the skin and carefully placed on a white plate with bi-distilled water for counting. Ticks and fleas were then placed into cryotubes with 70% ethanol for taxonomic analyses. All *P. darwini* individuals underwent morphometric measurements such as body and hind-foot length using a digital caliper (Uberman®, precision 0,01 mm). The sex and reproductive status of each individual was recorded, then marked with a numbered ear-tag (National Band & Tag Company®, New Port, USA) for seasonal tracking, and then released in the same trap of capture. Recaptured individuals within the four-night trapping period were sampled only once.

Determination Of Ectoparasite Infestation Parameters

Fleas and ticks were taxonomically classified up to genus level by direct microscopy examination, following conventional keys [44–46, 49–52]. We considered two infestation parameters at individual

level: (1) ectoparasite richness, as the number of flea or tick genera recorded on each animal, and (2) load of fleas or ticks, as the total number of fleas or ticks collected on each individual. We also calculated these parameters at the grid level, being (1) the ectoparasite richness, as the total number of genera identified during the four days in each grid, and (2) the mean of ectoparasites load, as the sum of the individual loads divided by the total captured rodents.

Microhabitat Characterization

We determined microhabitat parameters at trapping grid level, in each site and season. We registered temperature and humidity by a HOBO data logger (MX2300 Series, Onset Corporation, Bourne, Massachusetts) installed for three consecutive days in each trapping grid. The HOBO device was positioned at three random points within each grid at one meter above the ground. It was programmed to record maximum (max) and minimum (min) daily temperature and relative humidity (%) per hour (24 data per day), with a sampling interval of 10 minutes within every hour. We then computed means of max and min temperatures and humidity in each trapping grid.

We also calculated the normalized difference vegetation index (NDVI) on each buffered trapping grid per season employing satellite images obtained from a dockerised version of the R-package 'sen2R' [53] (see Appendix *S*1 for method details). As the NDVI values increase, it indicates a greater vigor of the vegetation (photosynthetic activity). We considered NDVI values as a fine-scale proxy of food availability and vegetation cover (biomass) for rodents in each trapping grid during the years of study [54–57].

Finally, to represent the biotic community we calculated the total number of captured micromammals (all species) and the rodent species richness during the four trapping days in each grid.

Determination Of Host Factors

We recorded the sex, age, and body condition of *P. darwini*. The sexing of individuals was carried out by common evaluation of the distance between the anus and the genitals, which is greater in males than in females. Animals were classified into age classes as adults, and juveniles, according to body weight per sex criterion [58]. For individuals captured during the first year, males ≥ 40 g and females ≥ 35 g were classified as adults [58]. This criterion was differentiated for the first and second years of study because the distribution of the weight data for both sexes was significantly different between the two years of survey. Thus, we decided that in the second year, males ≥ 35 g and females ≥ 30 g were classified as adults. To avoid bias associated with weight variations due to physiological growth, juveniles were excluded from analyses (e.g. [59]). Finally, we calculated a body condition index, the Scaled Mass Index (SMI) [60], which standardizes body mass based on a linear body measurement estimated from the scaling relationship between mass and length.

Statistical analysis

Assessment of land-use and seasonality variation of environmental factors

Minimum and maximum temperatures and humidity were statistically compared between sites and season, after checking normality and homoscedasticity of variances, with linear mixed models (LMM) using grid as random effect to control the 3 consecutive days of HOBO records in each grid. The analysis was conducted using R-package lm4 [61] in R software. We compared the NDVIs using two-way ANOVA and conducted the TukeyHSD post-hoc test to evaluate differences among sites and seasons.

Determination of factors associated with flea and tick infestation

We assessed factors affecting infestation parameters at two hierarchical levels [62], at individual and microhabitat (grid) scopes (e.g. [63]). At the individual level, we analysed the effects of host and environmental factors that could influence ectoparasites richness and load using Generalized Mixed Linear Models (GLMM) with a Poisson error for ectoparasites richness and negative binomial for ectoparasites load due to the overdispersion of the former parameter. Host fixed factors were sex, SMI, and the counterpart parasite load (tick load for flea load models and flea load for tick load models). The fixed factors representing the environment were the site (i.e., peri-urban, rural, wild) and season of the year. The 'trapping grid' was left as a random effect for all models. The models were built from a full model (all factors), simplifying them through step-by-step elimination of non-significant covariates and avoiding collinearity among factors.

At the microhabitat level, we evaluated what environmental variables at the grid could affect the ectoparasite richness and the mean ectoparasites load. We modelled fleas and ticks load by GLM and GLMM with Poisson error for ectoparasites richness and negative binomial responses for loads. The explanatory variables were the site, the mean of min and max temperatures and humidity and the NDVI. To better numeric convergence, NDVI values were scaled (i.e., index value x 100). We also tested separately the total number of captured micromammals and the species richness. For GLMM models the 'trapping grid' was left as a random effect. We constructed these models by combining environmental variables avoiding collinearity among them using the Variance Inflation Factor (VIF) and Pearson correlation matrix in R-package MASS [62]. We found no VIF ≥ 10 for any variable or correlation coefficients exceeding 0.7 thresholds between predictors [64]. We ranked models using Akaike's Information Criterion for small samples (AICc) and considered models with a ΔAICc of ≤ 2 as competing [65] using R-package MuMIn [66]. We reported the incidence rate ratio (IRR) and 95% confidents intervals (95% CI) for each predictor variable of the most plausible models (i.e., top-ranked models) with the lower ΔAICc and highest model weight.

Results

A total of 310 captures of adults *P. darwini* were achieved across the two years of the study with a trapping effort of 33,840 traps/night (Table 1). Overall, 48% of the captures corresponded to the wild, 25% to the rural and 27% to the peri-urban site; 73% were achieved during the first year of sampling and most trapped rodents were males (58%).

Table 1
Total number of sampled rodents and infection with ticks and fleas, according to sampling season and sex of the host at each site. The percentages of infested rodents are shown in parentheses.

Variable	Wild		Rural		Peri-urban		Total	
	Ticks	Fleas	Ticks	Fleas	Ticks	Fleas	Ticks	Fleas
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Season								
Spr1	72 (11)	67 (78)	28 (0)	28 (75)	34 (21)	34 (88)	*134 (11)*	*129 (80)*
Sum1	48 (29)	33 (70)	20 (40)	19 (32)	24 (33)	17 (59)	*92 (33)*	*69 (57)*
Spr2	21 (33)	16 (100)	24 (17)	24 (96)	14 (14)	14 (79)	*59 (22)*	*54 (93)*
Sum2	7 (71)	7 (29)	6 (50)	6 (17)	12 (8)	12 (25)	*25 (36)*	*25 (24)*
Sex								
Males	92 (25)	77 (87)	45 (18)	44 (68)	44 (27)	42 (69)	*181 (24)*	*163 (77)*
Females	56 (20)	46 (57)	33 (21)	33 (64)	40 (15)	35 (71)	*129 (19)*	*114 (63)*
Total	*148 (23)*	*123 (76)*	*78 (19)*	*77 (66)*	*84 (21)*	*77 (70)*	310 (22)	277 (71)

Spr1 = Spring 2018; **Sum1** = Summer 2019; **Spr2** = Spring 2019; **Sum2** = Summer 2020

Microhabitat Characterization

The min and max temperatures and the relative humidity (RH%) differed significantly between sites and seasons (see Appendix *S2* for results). In the rural site, the min and max temperatures were on average 2.6 and 2.8 ºC, respectively higher than those of the wild site, while the peri-urban site had both temperatures 2ºC higher than those recorded in the wild site (Fig. *S2* and Table *S2*.1). The min and max RH of the rural site were 5.5% and 7% lower than those of the wild and peri-urban sites, respectively (Fig. *S2* and Table *S2*.1). Both, temperature and RH data, characterized the rural area as the warmer and dryer

site in study the area. Temperatures increased from Spr1 to Sum2 (Table *S*2.2). Also, a significant decrease in RH between the first and second years of the study was observed (Table *S*2.2).

NDVI values also yielded significant differences between sites and seasons (see Appendix *S*3). The NDVI values were significantly lower in the rural compared to the wild site, and also lower in the peri-urban comparing with the rural site. Also, significant differences between Spr1 and all following seasons were observed (Fig. *S*3 and Table *S*3). Considering assessed microhabitat parameters (i.e., temperature, RH, and NDVI), the sampling seasons from the last sampling period (Spr2 and Sum2) were drier and warmer than the first sampling period (Spr1 and Sum1), which is coincident with published rainfall records (CEAZA, 2021), leading to a reduction in mean NDVI from 0.24 to 0.18.

Genera of fleas and ticks detected in P. darwini

A total of 277 adults of *P. darwini* were examined for fleas and overall, 71% of them were fleas- infested (Table 1). The proportion of flea-infested rodents was 76%, 66% and 70% in wild, rural, and peri-urban sites, respectively. Overall, we found six genera of fleas belonging to four families: *Tetrapsyllus* sp., *Delostichus* sp. and *Polygenis* sp. (Rhopalopsyllidae), *Hectopsylla* sp. (Pulicidae), *Neotyphloceras* sp. (Ctenophthalmidae) and *Sphinctopsylla* sp. (Stephanocircidae) (Fig. 2a). Specimens of the Rhopalopsyllidae family of unidentified genera were considered as a separate group. Most of the animals presented two fleas genera (n = 140 rodents) and 48 individuals showed up to 3 flea genera. In the wild and rural sites, all genera were observed while *Polygenis* sp. and *Sphinctopsylla* sp. were no detected in the peri-urban site (Fig. 2a). *Hectopsylla* sp. and *Neotyphloceras* sp. were the most abundant fleas found in all sites. Finally, all adult rodents were examined for ticks and 22% resulted infested (Table 1). All ticks belonged to the genus *Ixodes* sp.

Factors Associated With Flea And Tick Infestation At The Individual Level

At individual level, the flea richness was affected by season and rodent sex, being significantly higher in Spr2 and lower in Sum2, and in male than female rodents (Table 2). On the other hand, the flea load was significantly affected by the season, sex, and tick load of the host (Table 3). The Spr2 showed a significant positive effect while sum2 had a negative effect on flea load, and it was significantly higher in males, being 1.89 times the incident rate for the females (Table 3 and Fig. 2b). On the other hand, the tick load (mean load ± SD = 0.66 ± 2.01) was influenced by season, showing a higher load in both sum1 and sum2 (Table 4).

Table 2

Top-ranked generalized mixed model testing host and environmental factors on flea richness on adults P. *darwini*. Estimates and incidence ratios (IRR) of predictor variables are shown. Significant fixed effects are highlighted with asterisks (n_{obs}=277).

Fixed factors[a]	Estimate (SE)	IRR	IRR 95% CI	z-value	Pr(>|z|)	
(Intercept)	-0.07 (0.12)	0.94	0.74−1.19	-0.55	0.59	
Sum1	-0.24 (0.15)	0.78	0.58−1.06	-1.58	0.11	
Spr2	0.37 (0.14)	1.45	1.10−1.90	2.64	0.01	**
Sum2	-1.27 (0.37)	0.28	0.14−0.57	-3.48	0.00	***
Male	0.27 (0.12)	1.31	1.03−1.66	2.19	0.03	*
Spr1 = Spring 2018; **Sum1** = Summer 2019; **Spr2** = Spring 2019; **Sum2** = Summer 2020						
[a] Spr1 and females were defined as reference; * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001.						

Table 3
Top-ranked negative binomial mixed models testing host and environmental factors on flea loads of adults *P. darwini*. Estimates and incidence ratios (IRR) of predictor variables are shown. Significant fixed effects are highlighted with asterisks. (n_{obs}=277).

Fixed factors[a]	Estimate (SE)	IRR	IRR 95% CI	z-value	Pr(>|z|)	
Total flea load						
(Intercept)	0.42 (0.19)	1.52	1.04−2.21	2.18	0.03	*
Sum1	0.05 (0.18)	1.05	0.74−1.49	0.27	0.79	
Spr2	0.64 (0.19)	1.90	1.32−2.76	3.41	0.00	***
Sum2	-1.46 (0.33)	0.23	0.12−0.44	-4.49	0.00	***
Male	0.64 (0.15)	1.89	1.41−2.53	4.29	0.00	***
Tick load	0.13 (0.04)	1.14	1.06−1.23	3.60	0.00	***
Hectopsylla sp. load						
(Intercept)	-1.94 (0.58)	0.14	0.05−0.45	-3.32	0.00	***
Rural	-0.23 (0.78)	0.79	0.17−3.63	-0.30	0.77	
Peri-urban	1.74 (0.78)	5.68	1.22−26.41	2.21	0.03	*
Sum1	1.01 (0.34)	2.75	1.43−5.32	3.02	0.00	**
Spr2	1.84 (0.40)	6.29	2.89−13.69	4.64	0.00	***
Sum2	-0.06 (0.47)	0.94	0.37−2.39	-0.12	0.90	
Male	0.81 (0.27)	2.25	1.32−3.84	2.99	0.00	**
Neotyphloceras sp. load						
(Intercept)	0.01 (0.19)	1.01	0.70−1.46	0.04	0.97	
Sum1	-0.68 (0.23)	0.51	0.32−0.79	-3.01	0.00	**
Spr2	0.26 (0.21)	1.29	0.85−1.96	1.20	0.23	
Sum2	-2.83 (0.64)	0.06	0.02−0.21	-4.40	0.00	***
Male	0.49 (0.18)	1.63	1.14−2.31	2.70	0.01	**
Tick load	0.16 (0.04)	1.17	1.09−1.26	4.31	0.00	***
Spr1 = Spring 2018; **Sum1** = Summer 2019; **Spr2** = Spring 2019; **Sum2** = Summer 2020						
[a] Wild site, Spr1 and females were defined as reference; * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001.						

Table 4

Top-ranked negative binomial mixed model testing host and environmental factors on tick load of adults *P. darwini.* Estimates and incidence ratios (IRR) of predictor variables are shown. Significant fix effects are highlighted with asterisks (n_{obs}=277)

Fixed factors[a]	Estimate (SE)	IRR	IRR 95% CI	z-value	Pr(>|z|)	
(Intercept)	-1.90 (0.42)	0.15	0.07−0.34	-4.49	0.00	***
Sum1	1.44 (0.42)	4.20	1.83−9.64	3.39	0.00	***
Spr2	0.64 (0.49)	1.89	0.72−4.95	1.29	0.20	
Sum2	2.15 (0.57)	8.55	2.81−26.01	3.78	0.00	***
Flea load	0.07 (0.04)	1.07	1.00−1.15	1.88	0.06	
[a] **Spr1** = Spring 2018 defined as reference; **Sum1** = Summer 2019; **Spr2** = Spring 2019; **Sum2** = Summer 2020						
[a] Spr1 defined as reference; *** p-value < 0.001.						

In addition, we decided to test factors affecting the most abundant fleas. The load of *Hectopsylla* sp. was significantly and positively affected by the peri-urban site, the Sum1 and Spr2 seasons, and the male sex (Table 3 and Fig. 2c). The *Neotyphloceras* sp. load was negatively affected by both summer seasons (Fig. 2d) and positively by male sex and tick load of the rodents.

Factors Associated With Flea And Tick Infestation At The Microhabitat Level

At the microhabitat level, the flea richness was positively influenced by the micromammal species richness (IRR = 1.17, 95% CI = 1.08, 1.27; see Table S8 in Appendix S8 for competing models). The min temperature was a predictive factor for the flea load in the top model, but its effect was no supported. The mean load of *Hectopsylla* sp. was negatively associated by NDVI (IRR = 0.85, 95% CI = 0.75, 0.94) (Fig. 3a). The min temperature was negatively associated with the *Neotyphloceras* sp. mean load [IRR = 0.88, 95% CI = 0.78, 1] (Fig. 3b). Finally, the maximum temperature was positively associated with tick load (IRR = 1.25, 95% CI = 1.06, 1.250), increasing by 0.22 the number of ticks for every 1ºC rise in temperature (Fig. 3c).

Discussion

The multi-level approach applied in this study, allowed us to identify a strong seasonal and host effect (individual level) as well as an environmental influence (microhabitat level) on the evaluated infestation parameters. Overall, individuals were more parasitized by fleas during spring while in the summer they were more parasitized by ticks. It is highly likely that this seasonal contrast is produced by the species-

specific requirements of these ectoparasites, mainly due to the life cycle of each ectoparasites where immature stages depend on microclimatic conditions [17,67,68]. The results provided by our microhabitat analysis support this seasonality since the NDVI and temperatures were the main factors that affected flea and tick loads, and notably changed among seasons.

Flea richness had a seasonal influence, with higher genera found in spring of the second period of sampling (Spr2 2019). Different preferences for air temperature and humidity have been reported in different flea species and could explain this pattern [17,18,69]. For instance, larvae of the *Xenopsylla cheopis* and *Synopsyllus fonquerniei* appear both to survive longer at higher RH, the former, independently of the air temperature, while the latter also combined with high temperatures [18]. Parasite community depends on the community of hosts in the ecosystem [19,20,70,71], and it has been proposed that areas with intermediate levels of human pressure could keep higher diversity of main and alternative hosts for some parasites [12]. We certainly found a positive relationship between the diversity of parasites and micromammals in line with this hypothesis. We captured a total of nine small mammal species in the wild, seven in rural and six in the peri-urban site and the number of trapped individuals was significantly lower in the peri-urban site. Accordingly, in the wild and rural sites, we found six flea genera, but only four were detected in the peri-urban site, not detecting *Polygenis* nor *Sphinctopsylla* genera. The two most frequent flea genera identified in this study, *Neotyphloceras* sp. and *Hectopsylla* sp., are common for *P. darwini* and many other members of micromammals in the semi-arid Coquimbo region [46]. In contrast, in our study area, the *Polygenis* genus has been described only in *Thylamys elegans* [44], marsupial species that was captured only on a few sampling occasions in the peri-urban site. Likewise, *Sphinctopsylla* sp. has been detected in *Abrothrix longipilis* in Fray Jorge National Park [46], rodent not captured in the peri-urban site, and also in *Abrothrix olivaceus, Oligoryzomys longicaudatus, Spalacopus cyanus, Thylamys elegans and Rattus rattus* in others zones of Chile [38,44,51]. Therefore, the distribution of these genera of parasites appears to be affected by their host distribution but also by different microhabitat conditions of the land-uses of each site [20].

The higher flea load in spring when compared to summer suggests that high humidity and lower temperatures could be appropriate for the development of most flea genera. The load of *Neotyphloceras* sp. followed this general pattern. Interestingly, the load of *Hectopsylla* sp. was higher in peri-urban site and during the first summer and the second spring and with lower NDVI values. *Hectopsylla* species (Pulicidae), also known as 'sticktight flea', are characterized having a sessile mode of life and inhabiting sandy terrain of warm dry climates [72,73]. Peri-urban sites from our study comply with the environment described for this flea. The low NDVI values found at peri-urban sites represent poor vegetation vigour and even areas without vegetation cover (see Table *S*3 and Figure *S*5), and probably, that areas offer optimal conditions for these fleas.

Our results also showed a male-biased flea infection as several studies, same as other previous reports elsewhere [15,21–23,76–78]. Differences between male-female parasitism could be explained by two non-mutually exclusive hypothesis based on host encounter with parasites ('encounter filter', e.g., mobility and spatial behavior differences) and compatibility for parasites ('encounter filter', e.g. mobility and

spatial behavior differences) and compatibility for parasites ('compatibility filter', e.g. immunocompetence [79,80]). The first hypothesis explains the bias by the higher mobility of males, which usually present larger and more broadly overlapped home ranges than females facilitating encounters with more parasitic infectious stages of parasites [76,80,81]. This phenomenon could be occurring in *Hectopsylla* sp. fleas have, while female fleas remain fixed on the host (around the muzzle or vibrissae of the animal), males fleas move on the body of the host and the ground [74,75]. This sessile performance suggests that *Hectopsylla* sp. greatly depends on the within-host environment. Moreover, the phenomenon could have a seasonal pattern, like the one seen in this study, linked to the immunosuppressive effects of increasing androgens levels [80]. During the reproductive period, *P. darwini* males commonly make large trips showing an average home range of 592 m2 (range 135.3-1154 m2) [82–84]. We captured the highest number of active adult individuals (pregnant females and scrotal males) during spring surveys; therefore, we cannot discard a hormonal/immunological influence for the observed male-biased parasitism. In addition, highly abundant fleas can also inhibit the immune response of the hosts [85,86], enhancing host susceptibility to more parasites [87].

We found a positive relationship between tick load and total flea load, especially for *Neotyphloceras* sp. This association could be linked to the generalist condition of this flea across different hosts, such as ticks and to individual differences in ectoparasite exposition. On the other hand, *Neotyphloceras* sp. fleas and ticks showed a different seasonal pattern, with lower flea loads and higher tick loads in summer. Co-infection is of increasing interest to disease ecology and further studies are needed to assess the factors driving the co infection with fleas and ticks in this species [29,88–91]. However, a potential explanation to this pattern is plausible, since a parasite load may be suppressed (antagonist interactions) or enhanced (synergic interactions) by another parasite [90,92–94]. For example, in the South African sengis (*Elephantulus myurus*) the dominant parasite, a tick (*Rhipicephalus* spp.), competes with mites and strongly with lice since tick removal resulted in increases in the abundance of chiggers and lice [92]. Other cases show notable stability of the parasite communities, such as those reported by [88] who removed helminths in wild mice and only one parasite species responded to deworming but temporarily. In-situ manipulation experiments are necessary to explore our ectoparasite association and to allow further understanding of the dynamic and stability of the host-parasite systems, physiological responses, and parasitism impact on host fitness.

The higher tick load found during the summer and at high maximum temperatures, is closely related to essential features described for tick survival: warm temperatures (always >7°C), high RH (>85%) and the high abundance of many potential hosts [16,95]. The role of the RH for survival as well as the tolerance to temperature extremes and desiccation varies depending on the species, developmental stage, sex, and physiological condition of ticks. Some tick species have shown to survive only when RH does not drop below 80% (e.g. *Ixodes Ricinus* and *Dermacentor variabilis*), others resist high temperatures but with high RH (e.g., *Rhipicephalus sanguineus*) or are adapted to warm and dry conditions (e.g., *Rhipicephalus zambesiensis* [16]).

Ectoparasites benefit from the vegetation composition that provides various small-scale climatic conditions near the ground where they develop, which may protect them from the extreme environmental fluctuations and desiccation [7,15,16,96,97]. Indeed, it has been shown that more shielded habitats and dense understories provide a more constant microclimate that benefits the development and the establishment of tick populations [16]. For example, habitats with greater shrub cover, denser understories, and greater canopy cover have shown higher tick abundances for *Amblyomma triguttatum* [98], *Ixodes scapularis* [99], and *Ixodes ricinus* [100]. In our study, we found a higher flea richness and load of fleas and ticks in the wild site which presented the highest proportion of the native 'scrub and succulents' land cover and values of vegetation vigor (i.e., NDVI) compared to rural and peri-urban sites. This higher vegetational coverage and amount of green vegetation (biomass), could provide greater availability of refuges, suitable food resources and microclimate conditions to rodent hosts and their parasites.

The study of factors affecting ectoparasites abundance is of public health and wildlife conservation concern since ticks and fleas are vectors of many pathogens that affect human and animal health [38,97,101–103]. In Chile, recent studies have compared infection patterns with micro and macroparasites in wild rodents in anthropized and protected areas finding for example that the Andes Hantavirus infection risk is higher in rodents inhabiting native forests compared to those in surrounding pine plantations (*Pinus radiata*), mainly because the reservoir, *Oligoryzomys longicaudatus*, was further associated with the former habitat type [41]. In the same area, helminth prevalence varied among seasons and host species, but not among habitat types [39] and the prevalence of mite *Ornithonyssus* sp. was higher in plantations [40]. Also, a recent study detected DNA of *Rickettsia* spp. in 15 flea species of micromammals in several areas of Chile, and areas with lower human density, villages and natural sites, showed the highest prevalence of *Rickettsia* with zoonotic potential [38]. These studies demonstrated that parasitism depends on a combination of environmental and host factors that differ through the different landscapes, which must be clarified and monitored to prevent and control the emergence of diseases.

In conclusion, this study contributes to the knowledge of flea and tick infection pattern in an anthropized semi-arid environment of central Chile. Further studies might be necessary to precise microclimate conditions thresholds required for the survival of these ectoparasites. In addition, further studies are underway to understand the microbiome these ectoparasites harbour and whether the higher rate of vector-borne pathogens is maintained in areas close to human habitations and thus help to predict the emergence of infectious diseases in an ecosystem highly influenced by climate change.

Abbreviations

BFJNP: Bosque Fray Jorge National Park; Spr1: Spring months year 1 (September to November 2018); Sum1: Summer months year 1 (January to March 2019); Spr2: Spring months year 2 (September to November 2019); Sum2: Summer months year 2 (January to March 2020); Max: Maximum; Min: Minimum; RH: relative humidity; NDVI: Normalized Difference Vegetation Index.

Declarations

Ethics approval and consent to participate

Captures were approved by the Agricultural Service and Livestock of Chile (SAG permit number 2670/2018 and 7838/2019), National Forest Corporation (CONAF permit number 31/2018). Also, this survey was included under the ethical license for the Fondecyt Regular Project and Doctoral Project approved by the Bioethics Committee for 'Use of Animal in Research' of the Universidad Austral de Chile (permit number 315/2018 and 357/2019). The protocol follows guidelines from the American Association of Veterinary Medicine (AVMA) and the American Society of Mammologists (ASM) for the use of wild mammals in research and education.

Consent for publication

Not applicable.

Availability of data and materials

All data generated during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

GA conceived and designed the study and also analyzed the data. EB conducted fieldwork, collected and taxonomically classified samples, performed data gathering, statistical analysis and led the writing of the manuscript. DG performed specific taxonomic analysis to confirm ectoparasites genera and contributed with ideas to write the manuscript. PB supported in interpretation of results. All authors contributed critically to the drafts, read and approved the final version for publication.

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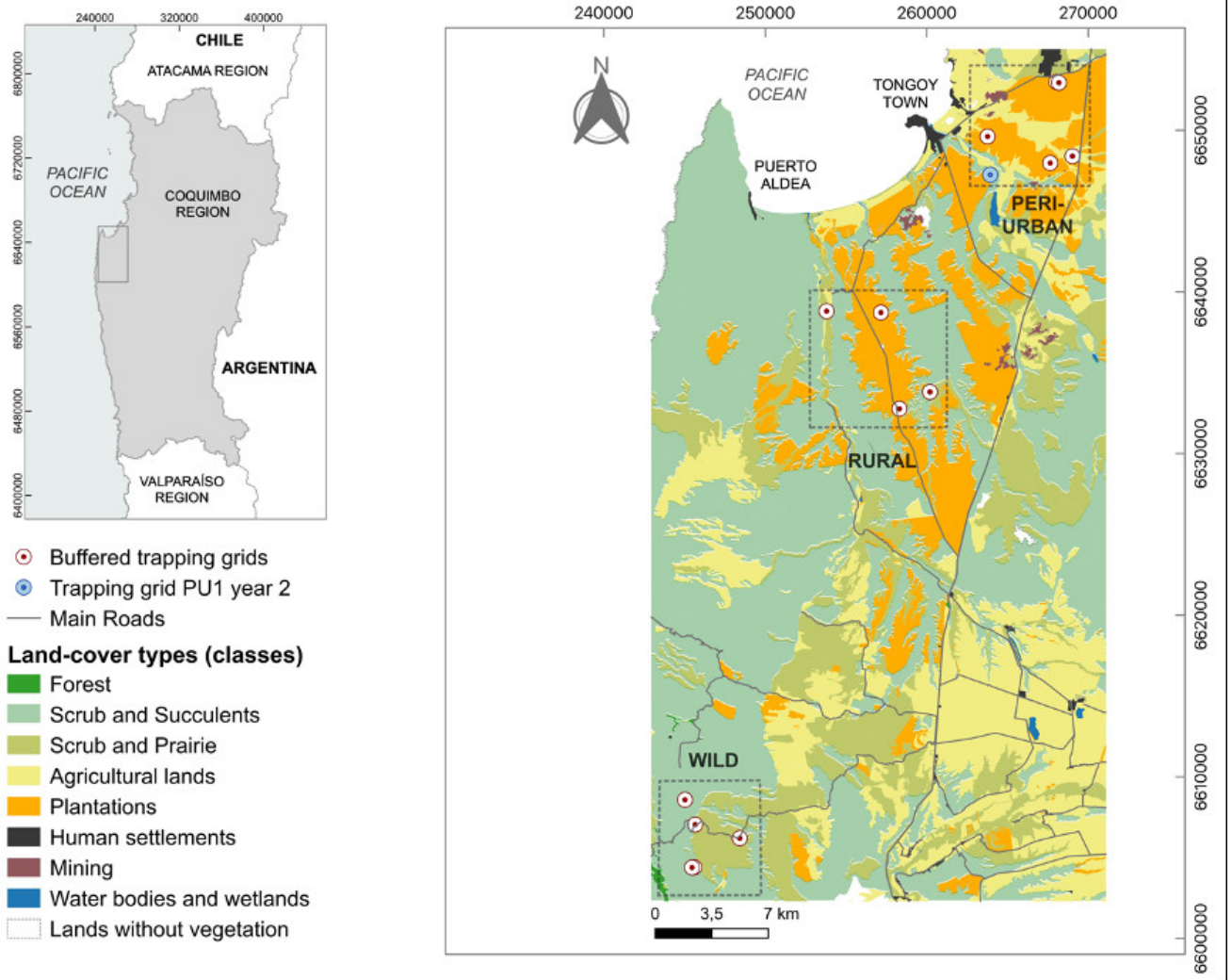
Figures

Figure 1

Landscape and trapping grids of the study area in the Coquimbo Region of Chile (2018-2020). The main land-cover types identified across our study area are shown. The wild site includes the Bosque Fray Jorge National Park, the rural site corresponds to the agropastoralism area El Tangue ranch and the peri-urban site comprises the suburban land-parceling next to Tongoy town. The four trapping grids in each site are highlighted, including relocation of one PU trapping grid in the second year of the study.
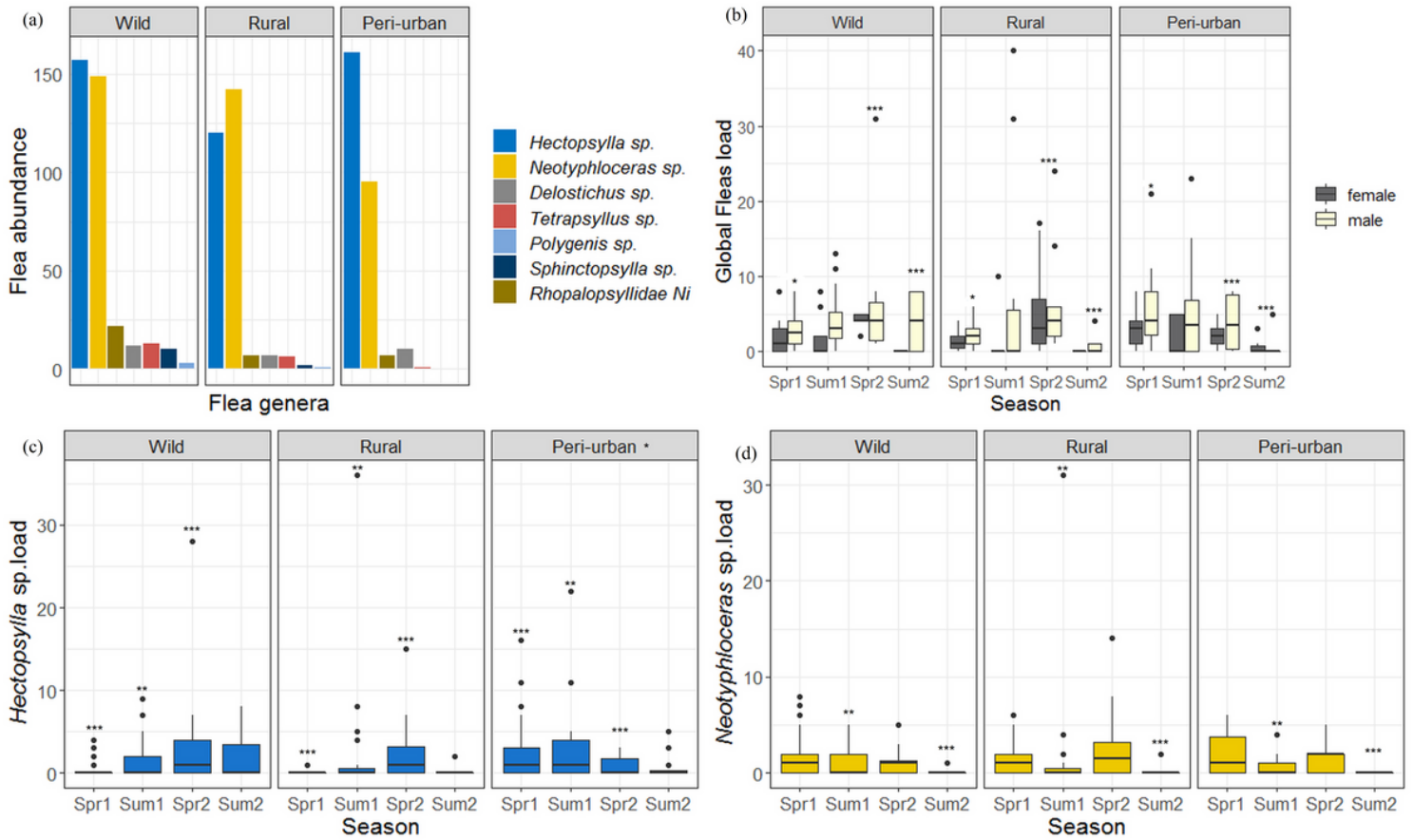
Figure 2

Flea genera and loads per site and seasons. Panel (a) shows abundance of flea genera in each site. Panel (b) shows global flea load for males and females across seasons per site. Panel (c) represent the load of *Hectopsylla* sp. and (d) the load of *Neotyphlocer*as sp. across seasons in each site. Significant effects are highlighted with asterisks.
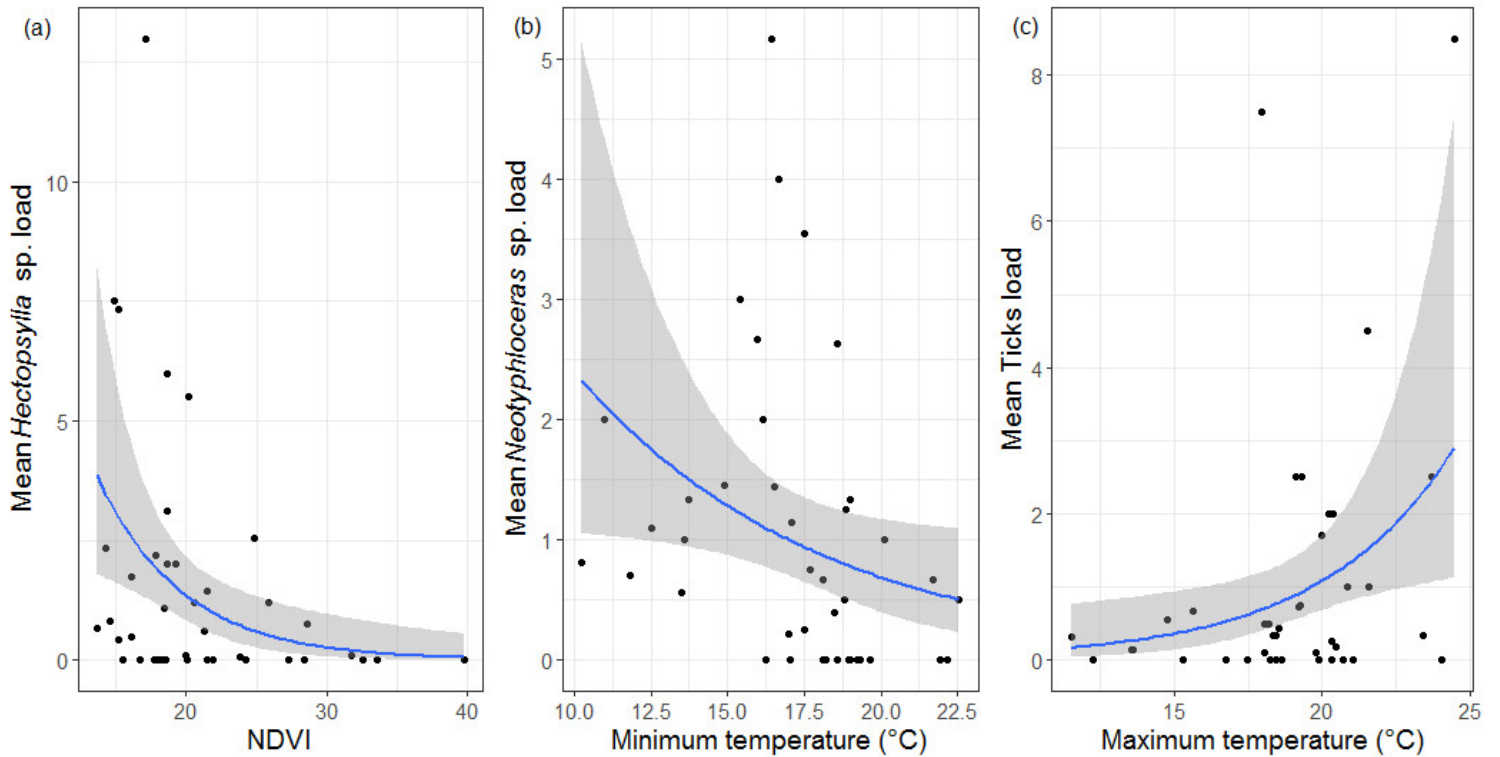
Figure 3

Mean ectoparasites load as predicted by top-ranked microhabitat-level models (GLM). (a) Relationship between the mean *Hectopsylla* sp. load and NDVI values; (b) Relationship between the mean *Neotyphloceras* sp. load and minimum temperature; (c) Effect of the maximum temperature on the mean tick load.

Supplementary Files

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