Infracommunity crowding as an individual measure of interactive-isolationist degree of parasite communities: disclosing the effects of extrinsic and host factors

Nicola Ferrari
Università degli Studi di Milano - Dipartimento di Scienze Veterinarie e Sanità Pubblica
E-mail: nicola.ferrari@unimi.it

Carlo Vittorio Citterio
Istituto Zooprofilattico Sperimentale delle Venezie – SCT2 Belluno – Italy
E-mail: ccitterio@izsvenezie.it

Paolo Lanfranchi
Università degli Studi di Milano - Dipartimento di Scienze Veterinarie e Sanità Pubblica
E-mail: paolo.lanfranchi@unimi.it

Corresponding author:

Nicola Ferrari
Università degli Studi di Milano
Dipartimento di Scienze Veterinarie e Sanità Pubblica
Via Celoria 10
20133 Milano, Italy
Tel: +39 02 503 18094
Fax: +39 02 503 18095
E-mail: nicola.ferrari@unimi.it

Abstract

Background: Interactions between parasite species within a host play a fundamental role in shaping parasite communities and parasite communities have been classified as interactive or isolationist. Interactive communities are principally structured by interactions between parasite species, while isolationist communities are structured by processes independent of the presence of other parasite species. Assessing whether, and to what extent, parasite communities exist along this continuum has been challenging due to a lack of an index that quantifies the degree of interactivity. Moreover, the absence of an index at the individual host level has made it unfeasible to identify host and extrinsic factors that may influence the degree of interactivity of a parasite community.

Methods: Here we propose an infracommunity crowding index that can reflect the degree of interactivity of a parasite community within each individual. This index quantifies the mean number of parasites that the average parasite within a community is exposed to, including the different aspects of parasite communities important in determining the level of interactivity, *i.e.* total abundance, species richness and evenness.

We applied this analytical approach to the abomasal parasite community of three alpine ruminant species that are traditionally viewed as harbouring isolationist parasite communities.

Results: The application of our index to abomasal parasite communities shows that the majority of parasites live in highly crowded communities, suggesting that these host species harbour interactive parasite communities. In addition, the infracommunity crowding was highly variable, being influenced by the host species, as well as by the timing of sampling and by host age and sex.

Conclusions: Despite increasing evidence on the influence of interactions between parasite species in shaping infections, an analytical measure to quantify the degree of interactivity of parasite community is lacking. Here we present a new analytical approach which, when

applied to parasite communities, appears to be sensitive to both extrinsic and host factors, highlighting that the degree of interactivity is not a static and specific feature of host species, but rather a dynamical process that keeps evolving during the host's life. This new index opens to new investigations aimed at revealing the determinants of parasite interactivity.

Keywords: co-infection, Interactivity Isolationism, Parasite interaction, infracommunity structure, ruminants, abomasal infection.

Background

Animals are frequently infected by multiple parasite species and thus in each individual host a community, referred to as an infracommunity [1, 2], may be established. Parasitologists have investigated the origin and evolution of the structure of these communities and, among the different proposed hypotheses, Holmes and Price [3] focused on the role of interactions between parasite species, formulating the interactive vs. isolationist classification. According to their hypothesis, communities can be assigned into two mutually exclusive groups, depending on whether parasite interactions have an evolutionary role and play a structuring role or whether these roles can be considered as negligible. Holmes and Price [3] suggested that the key points to identify a community as interactive include the presence of many parasite species with high infections rates, a high number of co-infections and large infection niche overlap, as these characteristics may promote a high potential for inter-specific interactions. Conversely, the key elements proposed for an isolationist parasite community are low numbers of parasite species and low infection rates, leading to small infrapopulations and few co-infections. These features lead to a low potential for interspecific interactions and thus the community structure is shaped by the individual infection rates of each single parasite species rather than by their interactions. The classification of parasite communities by Holmes and Price [3], although influential, presents some practical problems for its application. The two extremes of the interactive vs isolationist communities can be easily identified if all the features classifying them as either isolationist or interactive are present. However, in natural systems, a continuum between the extremes is likely to exist [4 – 9]. As such, a dichotomous classification may be limiting, not allowing for a quantification of the degree of interaction that may occur between parasite communities. Therefore, the assignment of communities to one of these two extreme classes has mostly involved a purely qualitative assessment, or has

been achieved through analytical approaches based on a restricted number of the features included in Holmes and Price's definitions [10]. On the one hand, these diversified approaches hinder comparisons between studies and, on the other hand, they make it difficult to quantify the effects of extrinsic and host factors promoting either isolationism or interactivity.

Dove [11], and then Poulin and Luque [12], proposed interactivity indices in orderto quantify the degree of interactivity/isolationism of parasite communities. These indices are based on accumulation curves of the number of species identified on the whole number of hosts sampled, giving a single mean value of the interactivity/isolationism for the entire host population. However, parasite infections are known to greatly vary between individuals and several extrinsic and host factors have been identified as determinants of these heterogeneities [13]. In particular, the definition of the factors affecting parasite abundance, species richness and their evenness [4 -9, 13-14] is possible thanks to the fact that all these parameters assume values that are quantifiable at an individual level.

In the absence of a measure which can quantify the degree of interactivity/isolationism within each single individual host, analyses of the comparative effect of host and extrinsic factors on parasite community structure are difficult. To address this issue, we developed an index called “infracommunity crowding” by extending the concept of crowding previously proposed by Lloyd [15] which measures the number of other individuals experienced by a single individual. The Lloyd's measure measures the group size perceived by a group member as opposed to the group size measured from an outsider's viewpoint(e.g. intensity of infection or population density) and is referred exclusively to intraspecific interactions [16]. Here, we translate Loyd's concept to interspecific interactions occurring within a community, obtaining an index which represents the number of individuals of other species that the average parasite individual of that community may establish a relationship with. Thus, following the Bush and Lotz [17] statement that “not all the competitive interactions involve crowding but all

crowding events involve competition", if we condense the crowding that the community experiences into a single number we can use it as an index of interactivity/isolationism.

The aim of this paper is twofold. Firstly, we present a new infracommunity crowding index, describing its logical basis, the details of its computation and presenting some of its properties. We then apply this index to the infracommunities of three mountain ruminant species which, through analyses on a restricted number of different characteristics, had been previously viewed as harbouring isolationist infracommunities [18-20]. Using our dataset, we finally quantify infracommunity crowding and analyse the influence of host (species, age, sex) and extrinsic factors (year, month) on infracommunity crowding.

Methods

The infracommunity crowding index

The infracommunity crowding index (hereafter ICr) is calculated by averaging the crowding each species experiences from other species over the total number of individual parasites within the infracommunity. Essentially, the crowding each species experiences is the number of all possible interactions its individuals may have with all the individuals of any other species, excluding conspecifics (Fig 1).

Figure 1 Here

Thus, if we define x_a, x_b and x_c as the number of individuals of species A, B and C respectively, each individual of species A may establish interspecific interactions with x_b+x_c

136 and the whole crowding of species A can be expressed by $x_a*(x_b+x_c)$.

137 Averaging the crowding of all species over the total parasite abundance we obtain:

138 $$ICr = \frac{2 * \sum_{j=1}^{S-1} \left(x_j * \sum_{i=j+1}^{S} x_i \right)}{N} \qquad \text{Eq: 1}$$

139 where x_j represents the abundance of the j-th parasite species, S the total number of parasite
140 species (hereafter species richness) within the infracommunity and N the total parasite
141 abundance (hereafter total abundance).

142 To demonstrate how to calculate and how we developed the infracommunity crowding index,
143 we do so for the hypothetical parasite community in figure 1, with a richness (S) of 3 species
144 and single species abundances x_j respectively of 2, 4 and 3 individuals, leading to a total
145 abundance (N) of 9 parasites. As such, each parasite of the species A experiences a crowding
146 by 4 parasites of species B and 3 of species C. Hence, the overall crowding suffered by
147 parasites of species A is 2*(4+3)=14. Similarly, the overall crowding experienced by parasites
148 of species B and C is 4*(2+3)=20 and 3*(4+2)=18, respectively. The total infracommunity
149 crowding can be obtained by summing crowding values for the 3 species and therefore will be
150 (2*(4+3))+(4*(2+3))+(3*(4+2)) which can be simplified in: 2*(2*3+2*4+3*4) and finally
151 rearranged in 2*(2*(4+3)+4*3).

152 In order to obtain the infracommunity crowding experienced by the average parasite, the total
153 infracommunity crowding must be averaged on the total parasite abundance, thus producing
154 the ICr formula generalised in equation 1:

155

156 $$ICr = \frac{2 * [2 * (4+3) + 4 * 3]}{9}$$

157 Hence, for this hypothetical infracommunity ICr results in 5.77 parasites/parasite, meaning
158 that the average parasite interacts with a mean number of 5.77 other parasites.

Despite the calculations, we assume that when the richness is smaller than 2 parasite species, the infracommunity crowding index has a value of 0 since we are not dealing with a community.

The infracommunity crowding measures the opportunity for the average parasite individual to interact with parasite individuals of other species. Interspecific interactions increase when infracommunities have either more parasites (i.e. higher total abundance) and/or more parasite species (i.e. higher species richness). Moreover, interspecific interactions should even increase when parasite individuals are more evenly distributed among species. For example, a community with a richness of four species and a total abundance of 100 parasites will provide more opportunities for interspecific interaction when all four species hold 25 parasites each, compared to the case where one species holds 97 parasites and the other three have just one individual each.

The infracommunity crowding index includes all these factors since its computation is based on total abundance (N), species richness (S) and different assemblages of species accounting for single species intensities (x_j).

Data on the abomasal parasite communities of alpine ungulates

The infracommunity crowding was calculated using data on parasite infracommunities of 261 chamois (*Rupicapra rupicapra*), 126 roe deer (*Capreolus capreolus*) and 58 alpine ibex (*Capra ibex*). Alpine ibex were collected from Graubunden (South Switzerland), chamois and roe deer from hunting districts of the Lecco province (North Italy). The data were collected during hunting seasons (ie. September-December) of 1989-1990 and 2007 for Graubunden and 1998-2005 for Lecco province. Data for chamois comes from the same database analysed

183 by Citterio et al. [20].

184 The total abomasal parasite community of these three host species is composed of 11 species
185 of *Trichostrongylidae* parasites, that can be considered as a guild of species since they use the
186 host resources in a similar way [1, 21].

187 Nematodes were identified by morphological criteria according to [22-25]. The following
188 parasite morphologies were considered to represent a single species:

189 - *Teladorsagia circumcincta*/*T. trifurcata*/*T. davtiani* (*T. pinnata*) as *T. circumcincta*
190 complex, [26],
191 - *Marshallagia marshalli*/ *M. occidentalis* as *M. marshalli* complex [27],
192 - *Spiculopteragia spiculoptera*/*Rinadia mathevossiani* as *S. spiculoptera* complex [28],
193 - *Ostertagia leptospicularis*/*O. Kolchida* as *O. leptospicularis* complex [29],
194 - *Osteragia lyrata*/*O. Ostertagi* as *O. ostertagi* complex [30].

195 For each host individual we recorded species, sex, age, month and year of sampling. For each
196 infracommunity we recorded parasite abundance, species richness and evenness (according to
197 [1]). Since the morphological criteria apply only to male nematodes, the abundance of each
198 parasite species has been calculated as twice the number of male helminths collected,
199 assuming a 1:1 sex ratio [20]. The total abundance of parasites within the parasite community
200 was calculated as the sum of all nematodes from all species. Species richness corresponds to
201 the number of species recovered in each host individual. Evenness has been calculated using
202 the Brillouin index, as this represents the most appropriate measure for fully censused
203 communities [31]. The Brillouin index ranges from 1, when all the species are equally
204 abundant, to 0 when a single species dominates the community. These epidemiological indices
205 for each parasite species are summarised in table 1.

206

207 ***Statistical analyses***

208 For each host individual, infracommunity crowding was calculated using equation 1.
209 To investigate ecological sources of community variability, we fitted generalised linear
210 models to explore the effect of host species, sex, age (continuous) and the extrinsic factors
211 month and year of sampling (both considered as discrete) on the following dependent
212 variables: infracommunity crowding (ICr), total abundance, species richness and evenness.
213 Models initially included all first order interactions between the explanatory variables. Terms
214 not significantly contributing to explain the observed variability of the response variable were
215 removed in a stepwise manner, using a likelihood ratio test until we obtained the minimal
216 adequate model [32].
217 The error distributions producing the best model fits were the Poisson distribution for species
218 richness and negative binomial distribution for ICr, total abundance and evenness.
219 For the minimal adequate model on infracommunity crowding, we present estimates of the
220 effects of all explanatory variables. On the other hand, analyses on total abundance, species
221 richness and evenness were mainly run to identify the main factors affecting these parameters,
222 in order to subsequently compare whether host and extrinsic factors had an analogous
223 influence on crowding or not. Therefore, the composition of these minimal adequate models is
224 presented discharging details on the effects of these factors.
225 All analyses were undertaken in R 3.2.2 [33], using MASS package for models with negative
226 binomial distributions. Descriptive statistics of the dependent variables are presented as
227 means ± standard error of the mean, while for prevalence 95% confidence intervals are used.
228

Results

Composition and structure of the nematode communities

The abomasal parasite communities of the whole sample totalled 11 nematode species, all belonging to the *Trichostrongylidae* family. The total numbers of parasite species detected in the three host species were similar, ranging from 7 to 10 helminth species each. Conversely, the infracommunities were more diversified, showing variability both between and within host species (Table 1).

Table 1 Here

The infracommunity structure was strongly affected by host species (Table 2). In particular, richness was significantly higher in alpine ibex and roe deer, which harboured respectively 2.8 (±0.12 S.E.M.) and 2.4 (±0.08 S.E.M.) species/individual, and lower in chamois with 1.8 (±0.08 S.E.M.) species/individual (Table 1). Mean abundance was higher in ibex with 1875.3 (±164.4 S.E.M.) parasites/individual, whereas roe deer and chamois harboured 867.2 (± 86.8 S.E.M.) and 214.2 (±23.0 S.E.M.) parasites/individual, respectively (Table 1). The evenness was higher in ibex and roe deer, showing values of 0.64 (±0.03 S.E.M.) and 0.62 (±0.03 S.E.M.), respectively, and lower in chamois with a value of 0.43 (±0.02 S.E.M.) (Table 1). Additionally, the effect of host species on total abundance varied with sampling year and with host age (Table 2), whereas its effect on evenness varied with host sex and sampling month (Table 2). Sex influenced directly only richness and evenness while total abundance was affected through interactions with sampling year and month (Table 2). Host age did not affect directly any of these parameters (Table 2). Finally, temporal variability played a great role with direct effects of month and sampling year. In particular, richness, total abundance and evenness differed between years and the latter two parameters varied even with sampling months. Additionally, the monthly variability of evenness differed between sampling years

and with host age (Table 2).

Patterns of infracommunity crowding.

The infracommunity crowding of all the three examined species showed an aggregated distribution with a significant fit to the negative binomial distribution (Deviance= 1329, df= 5779, p=1, maximum likelihood estimate of k=0.23). This distribution implies that most parasites live in a crowded community: 67% of all the sampled parasite individuals were indeed recovered in the hosts harbouring the top 20% most crowded communities.

Among the factors affecting the infracommunity crowding, host species was highly influential with ibex harbouring the most crowded communities with 611.0 (±61.8 S.E.M.) parasites/parasite and chamois the least crowded with 60.3 (±6.3 S.E.M.) parasites/parasite. Roe deer showed an intermediate value of 266.8 (±36.0 S.E.M.) parasites/parasite (Table 3). Host age had a different effect depending on the host species: in chamois the communities tended to be less crowded with increasing age, whereas in ibex there was a slight increase with age and in roe deer the crowding sharply increased with age (Fig. 2). Finally, the infracommunity crowding was influenced by the timing of sampling with direct and indirect effects of month and year. In particular, host species, sex and month effects showed different responses in different years.

Table 3 and Figure 2 Here

Discussion

We propose infracommunity crowding index (ICr) as a measure to quantify the

isolationist/interactivity degree of parasite infracommunities. This measure expresses the number of parasite individuals of other species that the average individual of a parasite community can establish an interaction with. Moreover, since the index accounts for parameters which reflect other aspects of the isolationism/interactivity degree (i.e. total parasite abundance, number of species and their evenness), it also provides a measure to quantify this continuum.

The application of this index to a dataset of abomasal parasite communities characterised by high variability of total abundance, species richness and evenness, revealed a high variability in infracommunity crowding. This variability was principally due to host species, timing of sampling but also, to a lesser extent, to host age and sex. These results emphasise how the interactive nature of a community should not be viewed as a static characteristic but rather as a dynamic feature evolving and shifting through time and between host individuals.

Studies about interactions among parasite species have fostered our understanding of the role that such interactions may play in influencing parasite infections and shaping communities [34 - 38]. However, the lack of adequate measures to quantify parasite interactions at the community level has hampered these investigations so that most recent studies approached these topics through pairwise analyses between parasite species [35 - 38]. Although pairwise analyses can provide robust results [39], this analytical approach shifts the attention from the community level to a population ecology point of view [40] where each set of interactions between pairs of species is analysed singularly, leading thus to potentially miss the emerging properties rising from multiple interactions which characterise the communities [40]. Infracommunity crowding can provide this measure at the community level.

On the other hand, a limit of community level measures resides in their difficulty to shed light on presently occurring mechanisms: for example, in our case, observed infracommunity

302 crowding may be the result of previous events without informing on the ongoing interactions.
303 In these instances, null models can predict results from hypothesised conditions, thus giving
304 baseline values for comparison with observed results [41]. The development of this approach
305 for infracommunity crowding would supply expected values for the opposite condition of
306 interactivity or isolationism, allowing a comparison of the observed values.
307 Besides the development of null models, another future step to improve the interpretation of
308 infracommunity crowding will be a deep sensitivity analysis, aimed at elucidating the relative
309 contribution of abundance, richness and evenness on crowding. Preliminary analyses on
310 simulated data, showed that infracommunity crowding increases with increasing values of
311 abundance, richness and evenness; however, more comprehensive analyses would allow to
312 disclose the relative contribution of these epidemiological characteristics and their synergic
313 effects.
314
315 Compared to previous measures based on accumulation curves [11, 12], infracommunity
316 crowding allows an extensive analysis of the effect of extrinsic and host factors, since it is
317 computed for each host individual. Moreover, since infracommunity crowding represents the
318 number of individuals of other species that the average individual within a community
319 experiences, it represents an absolute measure thus allowing for direct and biologically
320 meaningful comparisons between species, samples, and sites from different studies.
321
322 Parasite interactions may be based on different mechanisms, such as direct interference,
323 competition for resources or host-mediated processes, such as those mediated by the immune
324 system [36, 42 - 44]. Interactions may thus be established between parasites sharing the same
325 organ and anatomical systems [35], but also between parasites living in different locations
326 (*e.g.* stomach and skin, [45, 46]) and even between parasite species with very distant

327 taxonomic relationships (e.g. helminths and ticks or protozoa and viruses, [46 - 48]. Even if
328 infracommunity crowding can be visualised more intuitively as a direct contact between
329 parasites, its computation is not based on the biological mechanisms of interaction between
330 parasites. Therefore, this index can be easily computed for several forms of parasite
331 community, from those limited to a specific organ to those including the whole host organism
332 and composed of any taxonomical mix of parasites. It must be noticed that, in its present
333 form, infracommunity crowding presents the limit of being calculated based on parasite
334 abundances (n° parasite/host), thus excluding those parasite where counting is not feasible or
335 meaningful, such as microparasites, haemoprotozoa, or cestoda. In particular, regarding
336 cestoda, parasite burden is better evaluated through parasite biomass rather than abundance
337 (49, 50): in this case, calculation of infracommunity crowding would not be biologically
338 sound, but it would feasible to calculate the index by scaling parasite abundances to their
339 biomasses through an appropriate correction coefficient.
340
341 The application of infracommunity crowding to abomasal parasite community of alpine
342 ungulates showed an aggregated distribution with a small proportion of hosts harbouring the
343 most crowded parasite communities. This implies that the vast majority of parasites live in
344 highly crowded communities and can establish interactions with other species, suggesting
345 that, in abomasal parasite communities of ruminants, parasite interactions may have a
346 prominent evolutionary and structuring role and that these communities can be viewed as
347 interactive. This interpretation contrasts with previous studies that classified parasite
348 communities of alpine ruminants as being “typically” isolationist [18 - 20]. In particular,
349 conclusions by [20] were drawn on a subsample of animals (i.e. chamois) that is actually
350 included in the present dataset, but that had been previously analysed using a limited number
351 of community parameters and in a host health management rather than a parasite community

ecology perspective. This points out that infracommunity crowding, by including a greater number of community features, can provide a more exhaustive picture and take into account the variability along the continuum between extreme values of isolationism and interactivity.

The abomasal parasite infracommunities of the three alpine ruminants were characterised by a high variability of parasite species richness, total abundances and evenness. However, while a wide set of host and extrinsic factors has been identified as influencing these three parameters, the infracommunity crowding was found to be affected mainly by host species and by the timing of the sampling, with a lesser effect of host age and sex. Thus, although each host species seems to hold distinct parasite communities with respect to their degree of crowding, the effect of time and host factors indicates that interactivity is not a fixed host species characteristic [3, 18, 20], but it should be viewed as a variable and dynamical process evolving throughout the host's life. This latter result thus implies that the classification of the interactivity degree should not be viewed as an host species-specific feature and, at the same time, it leads to reconsider the evolutionary role of interactivity in structuring parasite communities [3], depending on its temporal occurrence and variability between host individuals .

Conclusions

In the present study we propose the use of infracommunity crowding (ICr) as a new measure to evaluate the degree of isolationism/interactivity of parasite communities. This measure takes simultaneously into account different features of parasite communities, *i.e.* total abundance, species richness and evenness, that are known to be important in determining the level of isolationism/interactivity. As a further step, we need to elucidate the relative contribution of these features to infracommunity crowding and formulate null models against

which to test isolationist or interactivity conditions. Contrasting to previous studies, the application of infracommunity crowding to a field dataset of parasite communities in alpine ruminants suggests, contrasting to previous results, that such communities have high levels of interactivity and that their former classification as isolationist communities, although functional for analyses on health impact of parasites, should be overcome. Moreover, the influence of host species, age and sex and sampling time suggests that interactivity, rather than being a host species-specific feature, is a dynamical process that evolves during the host's life. In this sense, rather than simply investigating whether a community is interactive or isolationist, new questions may regard the "degree of interactivity" of the communities, which can be scaled along a continuum and, even more importantly, offers an absolute measure that can be compared to indices of other communities. This study is a step forward in our investigations on the role of parasite interactions as structuring forces in infracommunities since it provides a new analytical approach which opens to a broader overview on the extent of interactivity and on the factors promoting it.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NF and CVC originally formulated the idea and hypotheses, NF developed the mathematical index and analysed the data, NF, CVC and PL interpreted the output and wrote the manuscript.

All authors read and approved the final version of the manuscript

Acknowledgements

We thank Joanne Lello, Laura Stancampiano, Sarah Perkins and Claudia Romeo for their valuable contributions on previous versions of this manuscript. This study was carried out within the PRIN project 2010–2011 (project n. 2010P7LFW4).

404 # References

405 1. Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own
406 terms: Margolis et al. revisited. J Parasitol. 1997; 83:575-583.
407 2. Petney TN, Andrews RH. Multiparasite communities in animals and humans:
408 frequency, structure and pathogenic significance. Int J Parasitol. 1998; 28: 377-393.
409 3. Holmes JC, Price PW. Community of Parasites. In: Kikkawa J, Anderson DJ, editors.
410 Community Ecology: Patterns and Processes. Melburne: Blackwell Scientific
411 Publications; 1986. pp. 187-213.
412 4. Gregory RD, Keymer AE, Harvey PH. Helminth parasite richness among vertebrates.
413 Biodivers Conserv. 1996; 5: 985-997.
414 5. Poulin R. Patterns in the evenness of gastrointestinal helminth communities. Int J
415 Parasitol. 1996; 26:181-186.
416 6. Feliu C, Renaud F, Catzeflis F, Hugot JP, Durand P, Morand S. A comparative analysis
417 of parasite species richness of Iberian rodents. Parasitology. 1997; 115:453-466.
418 7. Shaw DJ, Grenfell BT, Dobson AP. Patterns of macroparasite aggregation in wildlife
419 host populations. Parasitology. 1998; 117: 597-610.
420 8. de Bellocq JG, Morand S, Feliu C. Patterns of parasite species richness of Western
421 Palaeartic micro-mammals: island effects. Ecography. 2002; 25: 173-183.
422 9. Krasnov BR, Shenbrot GI, Khokhlova IS, Allan Degen A. Relationship between host
423 diversity and parasite diversity: flea assemblages on small mammals. J Biogeogr.
424 2004; 31: 1857-1866.
425 10. Sousa WP. Patterns and processes in communities of helminth parasites. Trends Ecol
426 Evol. 1994; 9: 52-57.
427 11. Dove ADM. A new index of interactivity in parasite communities. Int J Parasitol.

428 1994; 29: 915-920.
429 12. Poulin R, Luque JL. A general test of the interactive-isolationist continuum in
430 gastrointestinal parasite communities of fish. Int J Parasitol. 2003; 33: 1623-1630.
431 13. Wilson K, Bjørnstad ON, Dobson AP, Merler S, Poglayen G, Randolph SE, et al.
432 Heterogeneities in Macroparasite Infections: Patterns and Processes. In: Hudson PJ,
433 Rizzoli A, Grenfell BT, Heesterbeek H, Dobson AP, editors. The Ecology of Wildlife
434 Disease, Oxford: Oxford University Press; 2002. p. 6–44.
435 14. Perkins SE, Cattadori IM, Tagliapietra V, Rizzoli A, Hudson PJ. Empirical evidence
436 for key hosts in persistence of a tick-borne disease. Int J Parasitol. 2003; 33: 909–917.
437 15. Lloyd M. Mean Crowding. J Anim Ecol. 1967;36: 1-30.
438 16. Reiczigel J, Lang Z, Rózsa L, Tóthmérész B. Measures of sociality two different
439 views of group size. Anim Behav. 2008; 75: 715-721.
440 17. Bush AO, Lotz JM. The ecology of crowding. J Parasitol. 2000; 86: 212–213.
441 18. Genchi C, Manfredi MT, Rizzoli AP, Madonna M, Zaffaroni E. Comunità elmintiche
442 in popolazioni di caprioli (*Capreolus capreolus*). Parassitologia. 1992; 34: Suppl 1:
443 74–75.
444 19. Zaffaroni E, Fraquelli C, Manfredi MT, Siboni A, Lanfranchi P, Sartori E, Partel P G.
445 Abomasal helminth communities in eastern alpine sympatric roe deer (*Capreolus*
446 *capreolus*) and chamois (*Rupicapra rupicapra*) populations. Suppl Ric Biol
447 Selvaggina. 1996; 24: 53–68.
448 20. Citterio CV, Caslini C, Milani F, Sala M, Ferrari N, Lanfranchi P. Abomasal nematode
449 community in an alpine chamois (*Rupicapra r. rupicapra*) population before and after
450 a die-off. J Parasitol. 2006; 92: 918-927.
451 21. Zaffaroni E, Manfredi M, Citterio C, Sala M, Piccolo G, Lanfranchi P. Host specificity
452 of abomasal nematodes in free ranging alpine ruminants. Vet Parasitol. 2000; 90: 221-

230.

22. Skryabin KI, Shikhobalova NP, Schulz RS, Popova TI, Boev SN, Delyamure SL. Key to parasitic nematodes: Vol. 3, Strongylata. Israel Program for Scientific Translation, Jerusalem. Leiden: E. J. Brill Publishing Company; 1961.
23. Drözdz J. Studies on helminths and helminthiases in Cervidae. I. Revision of the subfamily Ostertaginae (Sarwar, 1956) and an attempt to explain the phylogenesis of its representatives. Acta Parasit Pol. 1965; 13: 445–481.
24. Durette-Desset MC. Sur les divisions génériques des Nématodes Ostertagiinae (*Trichostrongylidae*). Ann Parasitol Hum Comp. 1982; 64: 375–381.
25. Cabaret J, Morales S, Durette-Desset MC. Caractérisation de *Teladorsagia circumcincta* et *T. trifurcata*. II. Aspects morphologiques. Ann Parasitol Hum Comp. 1986; 61: 55–64.
26. Grillo V, Craig BH, Wimmer B, Gilleard JS. Microsatellite genotyping supports the hypothesis that *Teladorsagia davtiani* and *Teladorsagia trifurcata* are morphotypes of *Teladorsagia circumcincta*. Mol Biochem Parasit. 2008; 159: 59-63.
27. Dallas J, Irvine R, Halvorsen O. DNA evidence that *Marshallagia marshalli* Ransom, 1907 and *M. occidentalis* Ransom, 1907 (Nematoda: Ostertagiinae) from Svalbard reindeer are conspecific. Syst Parasitol. 2001; 50: 101-103.
28. Liénard E, Depaquit J, Ferté H. *Spiculopteragia mathevossiani* Ruchliadev, 1948 is the minor morph of *Spiculopteragia spiculoptera* (Gushanskaya, 1931): molecular evidence. Vet Res. 2006; 37: 683-694.
29. Manfredi MT, Di Cerbo AR, Tranquillo V, Nassuato C, Pedrotti L, Piccolo G. Abomasal nematodes of the red deer *Cervus elaphus* in North-Eastern Italy. J Helminthol. 2007; 81: 247-253.
30. Zarlenga DS, Hoberg EP, Stringfellow F, Lichtenfels JR. Comparisons of two

478 polymorphic species of *Ostertagia* and phylogenetic relationships within the
479 Ostertagiinae (Nematoda: Trichostrongyloidea) inferred from ribosomal DNA repeat
480 and mitochondrial DNA sequences. J Parasitol. 1998; 84: 806-812.
481 31. Magurran AE. Measuring biological diversity. Oxford: Blackwell Publishing; 2004.
482 32. Crawley M. The R book. Chichester: Wiley; 2007.
483 33. R Development Core Team 3.2.2. R: a language and environment for statistical
484 computing. Vienna, Austria: R Foundation for Statistical Computing; 2015. ISBN 3-
485 900051-07-0, URL http://www.R-project.org, accessed 14/08/2015.
486 34. Stock TM, Holmes JC. Functional relationships and microhabitat distributions of
487 enteric helminths of grebes (*Podicipedidae*): the evidence for interactive communities.
488 J Parasitol. 1988; 74: 214-227.
489 35. Lello J, Boag B, Fenton A, Stevenson IR, Hudson PJ. Competition and mutualism
490 among the gut helminths of a mammalian host. Nature. 2004; 428: 840-844.
491 36. Maizels RM, Balic A, Gomez- Escobar N, Nair M, Taylor MD, Allen JE. Helminth
492 parasites – masters of regulation. Immunol Rev. 2004; 201: 89-116.
493 37. Lafferty KD. Interacting Parasite. Science. 2010; 330: 187-188.
494 38. Telfer S, Lambin X, Birtles RJ, Beldomenico PM, Burthe S, Paterson S, Begon M.
495 Species interactions in a parasite community drive infection risk in a wildlife
496 population. Science. 2010; 330: 243-246.
497 39. Fenton A, Viney ME, Lello J. Detecting interspecific macroparasite interactions from
498 ecological data: patterns and process. Ecol Lett. 2010; 13: 606-615.
499 40. Begon M, Townsend CR, Harper JL. Ecology. from individuals to ecosystems.
500 Oxford: Blackwell; 2005.
501 41. Gotelli N J, Graves GR. Null Models in Ecology. Washington: Smithsonian Inst Press;
502 1996.

42. Behnke JM, Bajer A, Sinski E, Wakelin D. Interactions involving intestinal nematodes of rodents: experimental and field studies. Parasitology. 2001; Suppl 1: S39-S49.
43. Poulin R. Interactions between species and the structure of helminth communities. Parasitology. 2001; Suppl 1: S3-S11.
44. Graham AL, Cattadori IM, Lloyd-Smith JO, Ferrari MJ, Bjørnstad ON. Transmission consequences of coinfection: cytokines writ large? Trends Parasitol. 2007; 23: 284-291.
45. Balestrieri A, Remonti L, Ferrari N, Ferrari A, Valvo TL, Robetto S, Orusa R. Sarcoptic mange in wild carnivores and its co-occurrence with parasitic helminths in the Western Italian Alps. Eur J Wildl Res. 2006; 52: 196-201.
46. Ferrari N, Cattadori IM, Rizzoli A, Hudson PJ. *Heligmosomoides polygyrus* reduces infestation of *Ixodes ricinus* in free-living yellow-necked mice, *Apodemus flavicollis*. Parasitology. 2009; 136: 305-316.
47. Hartgers FC, Yazdanbakhsh M. Co-infection of helminths and malaria: modulation of the immune responses to malaria. Parasite Immunol. 2006; 28: 497-506.
48. Cattadori IM, Boag B, Hudson PJ. Parasite co-infection and interaction as drivers of host heterogeneity. Int J Parasitol. 2008; 38: 371-380.
49. Moore J, Simberloff D. Gastrointestinal helminth communities of bobwhite quail. Ecology. 1990; 71: 344–359.
50. Poulin R, Giari L, Simoni E, Dezfuli B. Effects of conspecifics and heterospecifics on individual worm mass in four helminth species parasitic in fish. Par res. 2003; 90:143–147.

Figure legends

Figure 1. Representation of the hypothetical total number of interactions the species A may have with individuals of species B and C.

Figure 2. Model predicted effect of host age on the infracommunity crowding in the chamois, roe deer and alpine ibex abomasal helminth communities (The other explanatory variables were held as: sex=Male, year=2000, month=November).