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Article

Host sex and age typically explain variation in parasitism of rock ptarmigan: implications for identifying determinants of exposure and susceptibility

Ó. K. Nielsen, A. Morrill, K. Skírnisson, U. Stenkewitz, G. R. Pálsdóttir and M. R. Forbes

Ó. K. Nielsen (<https://orcid.org/0000-0002-2855-094X>) ✉ (okn@ni.is) and U. Stenkewitz, Icelandic Inst. of Natural History, Gardabaer, Iceland. A. Morrill (<https://orcid.org/0000-0002-1938-0176>) and M. R. Forbes, Dept of Biology, Carleton Univ., Ottawa, ON, Canada. – K. Skírnisson and G. R. Pálsdóttir, Inst. for Experimental Pathology, Keldur, Univ. of Iceland, Reykjavik, Iceland. US also at: Univ. of Iceland's Research Centre at Snæfellsnes, Stykkishólmur, Iceland.

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Measures of parasitism often differ between hosts. This variation is thought due in part to age or sex differences in exposure to parasites and/or susceptibility to parasitism. We assessed how often age or sex biases in parasitism were found using a large, multi-year (2006–2017) dataset of 12 parasite species of Icelandic rock ptarmigan (*Lagopus muta*). We found host traits (i.e. age and/or sex) accounted for significant variation in abundance of 11 of the 12 parasite species. We often found increased abundance among juvenile hosts, although significant adult biases were observed for three parasite species. Additionally, higher levels of parasitism by many species were observed for female hosts, contrary to frequent male biases in parasitism reported for other vertebrates. Abundance of six parasite species was best explained by interactions between host age and sex; some degree of decrease in abundance with host age was present for both male and female hosts for four of those parasite species. We consider various host and parasite traits that could account for observed singular and repeated patterns of age and/or sex biases in parasitism (e.g. age- and sex-related grouping behaviours, age-specific mortality in relation to parasitism, acquisition of greater immunity with age). This work provides a foundation for future studies investigating age-related differences in acquired immunity and age-specific parasite-mediated mortality for males and females, as well as studies on interactions between co-infecting parasite species.

Keywords: age-biased parasitism, ectoparasitism, endoparasitism, *Lagopus muta*, rock ptarmigan, sex-biased parasitism

Introduction

Numerous studies have explored whether there are age- and/or sex-biases in parasitism within vertebrate host species. For example, sex biases in parasitism are generally reported for many parasitic organisms infecting mammals (Schalk and Forbes 1997, Moore and Wilson 2002), birds (McCurdy et al. 1998, Isomursu et al. 2006) and fish (Poulin 1996), and specific cases have been reported for reptiles and amphibians (Dare and Forbes 2009, Brown and Symondson 2014). Such biases are typically thought



to occur through two separate pathways: sex differences in either exposure or susceptibility to parasites. Sex differences in exposure and susceptibility are further thought to result from sex differences in age-specific life history traits such as growth, survivorship and reproduction (Moore and Wilson 2002). For example, males and females of vertebrate species can differentially invest in immunity, which influences susceptibility (Møller et al. 1998, Schmidt et al. 2015, Klein and Flanagan 2016). The sexes typically also differ in their foraging or breeding ecologies, which can influence traits such as home range size, feeding rates, diet and degree of sociality, thereby potentially influencing exposure to infective stages of parasites (Robinson et al. 2008, 2010).

In comparison, age biases in parasitism may arise due to differences in susceptibility or exposure between age classes. Simple adult biases in parasitism may result if infecting parasites accumulate over the host's lifetime (Wilson et al. 2002). However, changes in behaviour as hosts age may increase their exposure to parasites. For example, different patterns of dispersal by adults may increase probability of parasite encounter (e.g. host dispersal explaining age-related patterns of ectoparasitism among different rodent host species (Krasnov et al. 2006)), whereas lesser motility among juvenile hosts may limit their ability to escape parasitism or habitats with infective stages of parasites (Vale et al. 2018). Alternatively, parasites may preferentially parasitize a particular age class: adults may provide more resources and higher fitness to infecting parasites, or may produce a more intense stimulus to infective stages of parasites (e.g. fleas actively selecting adult over juvenile potential hosts in experiments, Hawlena et al. 2007).

Changes in innate or acquired immunity with age sometimes play a role in determining age biases in parasitism. Age may influence degrees of infection if juveniles are less able to recognize the infection (naïve hosts) and mount an effective immune response (Woolhouse 1998, Cattadori et al. 2005). Patterns of parasitism might even be non-linear, reflecting both accumulation of infective stages and age-related strength of immune responses to mounting infections (Woolhouse 1992, Sorci 1996). Age biases in parasitism do occur across host taxa, and among avian hosts both juvenile (Isomursu et al. 2006) and adult (Fecchio et al. 2015) age biases in parasitism have been observed, though, as mentioned above, the pattern may be embedded in an interaction between host age and host sex (Isomursu et al. 2006, Durkin et al. 2015).

When researching both sex and age biases in parasitism for a given host species, an ideal dataset would comprise large samples of each host age-by-sex combination to detect both individual host trait biases and interactive effects. As host populations are seldom infected by only a single parasite taxon, understanding the relationship between host traits and parasitism more generally would require analyses of as many of the parasite species as possible (Petney and Andrews 1998, Rigaud et al. 2010). Temporal replication is also important, ideally over a long term, to account for variation in parasitism

from year to year and to explore consistency in host trait–parasitism relations. Where host reproductive strategies impact their immunity, sampling across different seasons to account for this variation is warranted. For well-studied host taxa, some sampling should at least be taken outside of the breeding season to account for breeding-related susceptibility to parasitism for either sex. Such datasets comprising large samples of hosts infected by diverse parasites over many years are rare and invaluable resources in parasitological studies more broadly.

This study was undertaken to test whether age- and/or sex-biases in parasitism occurred for Icelandic rock ptarmigan (*Lagopus muta*, hereafter ptarmigan) sampled in October in each of 12 years, near Lake Mývatn, Iceland. In Iceland, the ptarmigan's breeding season is in May, with eggs hatching in the latter part of June. The brood is tended by the hen until the chicks become independent by August (Stenkewitz 2017). The hen therefore broods the chicks for the first month of their lives, and this social interaction affords the opportunity for vertical transmission of ectoparasites (Skírnisson and Nielsen 2019). Transmission of ectoparasites to and from hens could be further heightened by the energetic costs of brood rearing (Stenkewitz et al. 2015). The males do not take part in caring for the brood. One possibility, however, for horizontal transmission of ectoparasites is during male–male encounters associated with territoriality, approximately six months prior to sampling (Skírnisson and Nielsen 2019). Ectoparasites may also be transmitted during spring between members of a mating pair when the birds mate and while the male is actively trying to contain the female within the borders of his territory (Skírnisson and Nielsen 2019). In comparison, the endoparasites considered herein are either directly-transmitted via food contaminated with infective stages, or indirectly transmitted via an intermediate host (Stenkewitz 2017).

We tested whether age and/or sex biases in parasitism occurred among an assemblage of internal and external parasites, and then explored the extent to which statistical interactions between age and sex classes of hosts explained the degree of parasitism by particular species, controlling for the random effect of year of sampling. As our sampling always occurred outside of the breeding season, the hypothetically hormone-driven adult male biases in parasitism – often observed for many parasites of vertebrates – may not occur as expected (Poulin 1996, Zuk and Stoehr 2010). A principal objective of our study, then, was to highlight patterns where no biases in parasitism occur, where age and/or sex biases in parasitism do occur, and the extent to which these patterns may be explained by reference to the biology of either the parasite or host species. In other words, our general approach was to determine which parasite species showed similar host trait patterns first and then to search for commonalities among those parasites (e.g. transmission) in relation to aspects of host biology (e.g. sex segregation) for explaining those patterns. Our analyses were subdivided by the various parasite species, but we were asking questions about the generality

of patterns of parasitism. We did not ask questions relating to potential parasite–parasite interactions within the community more generally, which is to be treated elsewhere.

Methods

Our dataset was produced as part of a recent 12-year study (2006–2017), the first seven years of which were used to determine if parasitism related to population cycles in ptarmigan (Stenkewitz et al. 2016). This large dataset contains annual estimates of prevalence and/or abundance and intensity (or their proxies) of 16 species of parasites (a 17th parasite, *Blastocystis* sp., was recovered but not identified to species and not quantified (Stenkewitz 2017)). The study site and main methods are detailed elsewhere (Stenkewitz et al. 2016). Notwithstanding, a brief summary of the methods of bird collection and processing of parasites is provided (cf. Skirnisson et al. 2012, Stenkewitz 2017).

A total of 1140 ptarmigan were collected by shooting in autumns 2006–2017 from upland areas west, east and north of Lake Mývatn, in north–east Iceland (65°37'N, 17°00'W). 721 juvenile birds (361 females, 360 males) and 419 adult birds (150 females, 269 males) were collected during the first week of October under a license issued by the Icelandic Institute of Natural History (Skirnisson and Nielsen 2019). When removing samples from a population in a given year, one must consider the potential impacts on the population in subsequent years, both generally and within particular age and/or sex categories. However, our samples (~100 ptarmigan per year) represent a small fraction (i.e. 0.07–0.26%) of the overall estimated population within the north–east hunting zone, and are not expected to impact year-to-year population size (Sturludottir et al. 2018).

Forty parasite species have been recorded for rock ptarmigan over their geographical range (Skirnisson et al. 2012). As mentioned, sixteen parasites were identified to species. Those species included ten ectoparasites (five mite species, identifications based on Mironov et al. (2010) and Bochkov and Skirnisson (2011); three lice species, identifications based on Timmermann (1950) and Scharf and Price (1983); one fly species, identification based on Theodor and Oldroyd (1964); and one flea species, identification confirmed by Robert E. Lewis, Iowa State Univ., USA). There were six observed species of endoparasite (two coccidian species, identifications based on Skirnisson and Thorarinsdottir (2007); and four helminth species, identifications based on Madsen (1945), Wehr (1971) and McDonald (1974), and from Alexander Galkin at the Russian Academy of Sciences).

Detailed parasitological collection and quantification methods can be found in Skirnisson et al. (2012). Briefly, ectoparasites were collected either through filtered vacuuming of ptarmigan carcasses, or a combination of filtered vacuuming and direct removal of parasites from hosts. Individual vacuum filtration samples were carefully transferred to a glass jar combined with water and a small amount

(seven drops) of surfactant. After vigorously shaking and then stirring the jar, it was left to settle for several hours. Parasites were collected from the water surface and sediments under a stereomicroscope, and finally fixed to a microscope slide in Hoyer's medium for later identification. Endoparasitic helminths were extracted from the host small intestine and ceca and examined under a microscope, while coccidian oocysts were quantified from fecal material following the modified McMaster procedure (Skirnisson et al. 2012). No blood parasites have been reported for these ptarmigan (Skirnisson et al. 2012), contrary to what is found in other grouse species (Forbes et al. 1994).

As our first approach was to compare abundance of parasites with respect to age and sex of host controlling for the random factor year, we omitted from further consideration species that were rare (Results), including the flea (*Ceratophyllus garei*), and two of the helminth species, both cestodes (*Passerilepis serpentulus*, and *Mesocestoides canislagopodis* tetrathyridia). The helminths retained for further study were both nematodes. We also omitted the coccidian *Eimeria rjupa* because its high degree of aggregation prevented models based on abundance data from converging. This selection process resulted in our retaining 12 parasite species for further study (Table 1).

The habitats, life cycles and transmission biology for each parasite species could also relate to the presences or lacks of host trait biases in parasitism. These parasite traits varied among the 12 species considered (the following summary is a shortened adaptation of Table 4.1 and 4.2 in Stenkewitz 2017).

The mites *Metamicrolichus islandicus* and *Myialges borealis* both infect the skin, and are likely both indirectly-transmitted. In the case of *M. borealis*, the mite is known to form a phoretic relationship with the hippoboscid fly *Ornithomya chloropus*, and the latter then transmits infective *M. borealis* nymphs to new hosts. It is possible that this hippoboscid fly also transmits *M. islandicus* (Mironov et al. 2010). Note, however, that we found no *M. islandicus* on any of the examined *O. chloropus*. The other mites *Mironovia lagopus*, *Strelkoviacarus holoaspis* and *Tetraolichus lagopi* are directly-transmitted and parasitize the ptarmigan quills, down and vanes, respectively. The mallophagan (lice) *Amyrsidea lagopi*, *Goniodes lagopi* and *Lagopoecus affinis* all infect the plumage (in addition to the host's skin in the case of *A. lagopi*) and are directly-transmitted. The fly *O. chloropus* is directly-transmitted and infects the plumage. The coccidian *Eimeria muta* is directly-transmitted (via contaminated food) and resides in the small and large intestines, as well as the ceca, while the nematode *Capillaria caudinflata* is indirectly-transmitted via an oligochaete intermediate host and infects only the ptarmigan's small intestines and ceca. Finally, *Trichostrongylus tenuis* is a directly-transmitted nematode infecting the ceca, and is also ingested with contaminated food.

All statistical analyses were performed using R, version 3.4.4 (<www.r-project.org>). Parasite prevalence is the proportion of hosts infected by a particular parasite species;

Table 1. Parasite observation frequencies and measures of parasitism for twelve parasites of Icelandic rock ptarmigan, *Lagopus muta*, across sampled years (2006–2017). Prevalence measures are provided with Clopper–Pearson 95% confidence intervals, whereas both mean abundances and mean intensities have bias-corrected and accelerated 95% bootstrap confidence intervals. Parasites *Eimeria rjupa*, *Passerilepis serpentulus* and *Ceratophyllus garei* are omitted due to tested abundance models not converging, while *Mesocestoides canislagopodis* tetrathyridia is omitted due to its scarcity, and to only presence–absence, but not abundance, data being available.

Taxonomic group	Parasite species	Number of infected hosts (of 1140)	Number of sampled years parasite observed	Overall prevalence (95% confidence interval)	Mean abundance (95% confidence interval)	Mean intensity (95% confidence interval)
Coccidian	<i>Eimeria muta</i>	898	12	0.79 (0.76–0.81)	169.62 (129.30–250.80)	215.32 (163.48–333.35)
Nematode	<i>Capillaria caudinflata</i>	389	12	0.34 (0.31–0.37)	7.75 (6.36–9.66)	22.70 (18.90–27.91)
	<i>Trichostrongylus tenuis</i>	98	9	0.09 (0.07–0.10)	1.01 (0.59–1.91)	11.70 (7.27–21.41)
Astigmatan	<i>Tetraolichus lagopi</i>	1055	12	0.92 (0.91–0.94)	26.30 (24.49–28.60)	26.30 (24.40–28.49)
	<i>Strelkoviacarus holoaspis</i>	505	12	0.44 (0.41–0.47)	16.86 (14.39–21.18)	38.07 (32.99–46.35)
	<i>Metamicrolichus islandicus</i>	259	12	0.23 (0.20–0.25)	3.17 (2.54–4.06)	13.98 (11.49–17.85)
	<i>Myialges borealis</i>	186	12	0.16 (0.14–0.18)	0.56 (0.46–0.69)	3.42 (2.95–3.98)
Prostigmatan	<i>Mironovia lagopus</i>	74	12	0.06 (0.05–0.08)	0.07 (0.04–0.12)	2.61 (1.84–3.97)
Mallophagan	<i>Goniodes lagopi</i>	853	12	0.75 (0.72–0.77)	9.07 (8.35–9.94)	12.11 (11.23–13.11)
	<i>Lagopoecus affinis</i>	641	12	0.56 (0.53–0.59)	3.26 (2.95–3.62)	5.80 (5.37–6.40)
	<i>Amyrsidea lagopi</i>	192	12	0.17 (0.15–0.19)	1.80 (1.44–2.24)	10.72 (8.86–13.02)
Hippoboscid	<i>Ornithomya chloropus</i>	447	12	0.39 (0.36–0.42)	0.76 (0.69–0.84)	1.95 (1.83–2.11)

abundance is the number of parasites infecting any individual host, while mean intensity is the average abundance when excluding uninfected hosts (Reiczigel et al. 2019). We used generalized linear mixed models (GLMMs) modeling whether parasite abundance (negative binomial family response) was dependent on host age, sex and age:sex interaction in global models. All models include year as a random factor, as there is year-to-year variation in mean abundance and prevalence among the parasite species for unknown and perhaps myriad reasons (Stenkewitz 2017). We evaluated whether the presence of a host trait was a significant predictor of abundance of parasites in best-fitting models. Positive estimates for age indicate higher abundance in juveniles, whereas positive estimates for sex indicate higher abundance in males. The global model and all partial models were compared and sorted by AICc scores, and only those best models ($\Delta AICc < 2$; Burnham and Anderson 2002) were retained, regardless of whether they were null models or not. This series of analyses allowed us to uncover singular and repeated patterns in mean abundance of parasite species of ptarmigan in relation to host age and sex. Alpha was set to 0.05 in all analyses ($\alpha = 0.05$).

Prevalence is reported with Clopper–Pearson 95% confidence intervals. Mean abundances and mean intensities are reported with bias-corrected and accelerated bootstrap 95% confidence intervals. The degree of aggregation of parasites within samples, when provided, is calculated as the variance in abundance divided by the mean abundance (variance-to-mean ratio).

Results

As mentioned, four species were omitted from further study – this was because full models explaining parasite abundance based on host age and sex did not converge. More specifically,

the flea *Ceratophyllus garei* was too underrepresented (overall prevalence = 0.25%, 95% CI = 0.05–0.72%) to get models of mean abundance to converge, as were the two cestodes (Skírnisson et al. 2012) for which ptarmigan were rare or ancillary hosts (overall prevalence = 0.50% and 1.24% for *M. c. tetrathyridia* and *P. serpentulus*, respectively; 95% CIs = 0.18–1.08% and 0.70–2.04%). Models also converged for only one of two *Eimeria* species due to extreme aggregation of *E. rjupa* (variance-to-mean abundance ratio = 7067). Eleven of 12 species were recorded in all 12 years and one species, *T. tenuis*, was recorded in nine years. Overall prevalence ranged from 6% to 92% for the 12 species. We provide prevalence and other relevant parasitism measures for each species (Table 1).

GLMMs modeling abundances of parasites as dependent on host traits indicate several distinct patterns of host age and/or sex affecting parasitism (Table 2). These patterns are not overridden by variation in parasitism across years. Cases of both juvenile and adult age biases in parasitism were observed, as well as both male and female sex biases. Intriguingly, significant female host sex biases outnumbered male biases (seven to one; Table 3). Eight parasite species demonstrated juvenile host biases, representing the single most common type of host trait bias. All species demonstrating significant sex biases also showed significant age biases, though the inverse was not true. Age-by-sex interactions explained significant variation in abundance of six parasite species, following four broad patterns (Fig. 1, Table 3).

Capillaria caudinflata did not show any relationship with host age or sex (i.e. lacked any host trait-dependent parasitism). The remaining 11 (or > 91% of) species showed one or more patterns of host–trait bias in parasitism. Including the ‘lack’ of any pattern for *C. caudinflata*, there were a total of nine patterns of host trait-related parasitism that were not

Table 2. Generalized linear mixed model outputs and comparisons, modelling *Lagopus muta* parasite abundance as dependent on host age, sex and age:sex interaction in global models. All models include year as a random factor. Models are sorted by AICc, and only those best models ($\Delta\text{AICc} < 2$) are shown. Variable presence in the fitted model is indicated by a numeric estimate (a row with no estimates indicates a null model), while estimates in bold indicate statistical significance of the variable within the model. Positive estimates for age indicate higher abundance in juveniles, while positive estimates for sex indicate higher abundance in males. Parasites *Eimeria rjupa*, *Passerilepis serpentulus* and *Ceratophyllus garei* are omitted due to tested models not converging, while *Mesocestoides canislagopodis* tetrathyridia is omitted due to its scarcity, and to only presence-absence, but not abundance, data being available. p-values: * < 0.05, ** < 0.01, *** < 0.001.

Taxonomic group	Parasite species	Model estimates			Model AICc	Model ΔAICc
		Age	Sex	Age:Sex interaction		
Coccidian	<i>Eimeria muta</i>	0.366*	-0.286*		11534.5	
		0.233	-0.433	0.217	11536.0	1.46
Nematode	<i>Capillaria caudinflata</i>	-0.472	-0.610	0.851*	4312.6	
			-0.061		4314.4	1.78
	<i>Trichostrongylus tenuis</i>	-1.03**			1174.4	
		-1.00*	-0.327		1175.6	1.28
Astigmatan	<i>Tetraolichus lagopi</i>	-1.48*	-0.872	0.931	1176.3	1.95
		-0.222*	-1.353***	1.08***	8612.7	
	<i>Strelkoviacarus holoaspis</i>	1.37***	0.353*		5796.9	
		1.21***	0.167	0.287	5798.3	1.38
	<i>Metamicrolichus islandicus</i>	1.77***	-0.828*	0.848	2833.0	
		2.23***			2833.52	0.21
Prostigmatan	<i>Myialges borealis</i>	2.24***	-0.245		2834.1	1.09
		1.84***			1713.3	
	1.85***	0.103		1715.1	1.77	
	<i>Mironovia lagopus</i>	-3.05***	-1.77**	1.91*	372.4	
Mallophagan	<i>Goniodes lagopi</i>	1.13***	-0.952***	0.886***	6755.6	
	<i>Lagopoecus affinis</i>	1.23***	-0.819***	0.607**	4693.5	
	<i>Amyrsidea lagopi</i>	1.23**	-2.05***	2.52***	2164.7	
Hippoboscid	<i>Ornithomya chloropus</i>	0.552***	0.229	-0.479*	2677.5	

entirely mutually exclusive (Table 3). Below, we report the different patterns of infection by individual parasite species; notably, in some cases, parasites of a single taxonomic group all demonstrate similar patterns (e.g. the mallophagans), while those in other groups demonstrate a diversity of host trait-related effects (e.g. the astigmatans).

We start by considering similar age-related patterns of infection. For three mite species, *S. holoaspis*, *M. borealis*

and *M. islandicus*, age explained significant variation in mite abundance, with juveniles having more mites than adult birds, opposite to the pattern observed for quill mites (Skirnisson and Nielsen 2019). Different relationships between host sex and abundance were observed for each of these three species of mite: sex did not have a significant effect on *M. borealis* abundance, whereas *M. islandicus* showed a female bias in abundance. *S. holoaspis* was

Table 3. Patterns of host trait-related parasitism observed among species parasitizing *Lagopus muta*. Host trait biases in parasitism represent significant predictors in best-fitting GLMMs modeling abundance as dependent on host age, sex and an age:sex interaction, with sampling year as a random factor.

Host trait bias pattern	Parasites
No host trait biases	<i>Capillaria caudinflata</i>
Female sex bias	<i>Eimeria muta</i> , <i>Tetraolichus lagopi</i> , <i>Metamicrolichus islandicus</i> , <i>Mironovia lagopus</i> , <i>Goniodes lagopi</i> , <i>Lagopoecus affinis</i> , <i>Amyrsidea lagopi</i>
Male sex bias	<i>Strelkoviacarus holoaspis</i>
Juvenile age bias	<i>Eimeria muta</i> , <i>Strelkoviacarus holoaspis</i> , <i>Metamicrolichus islandicus</i> , <i>Myialges borealis</i> , <i>Goniodes lagopi</i> , <i>Lagopoecus affinis</i> , <i>Amyrsidea lagopi</i> , <i>Ornithomya chloropus</i>
Adult age bias	<i>Trichostrongylus tenuis</i> , <i>Tetraolichus lagopi</i> , <i>Mironovia lagopus</i>
Age:sex interaction 1: female bias magnified among adults	<i>Tetraolichus lagopi</i>
Age:sex interaction 2: adult bias with adult-specific female bias	<i>Mironovia lagopus</i>
Age:sex interaction 3: juvenile bias with adult-specific female bias	<i>Goniodes lagopi</i> , <i>Lagopoecus affinis</i> , <i>Amyrsidea lagopi</i>
Age:sex interaction 4: lower parasitism among adults only seen in females	<i>Ornithomya chloropus</i>

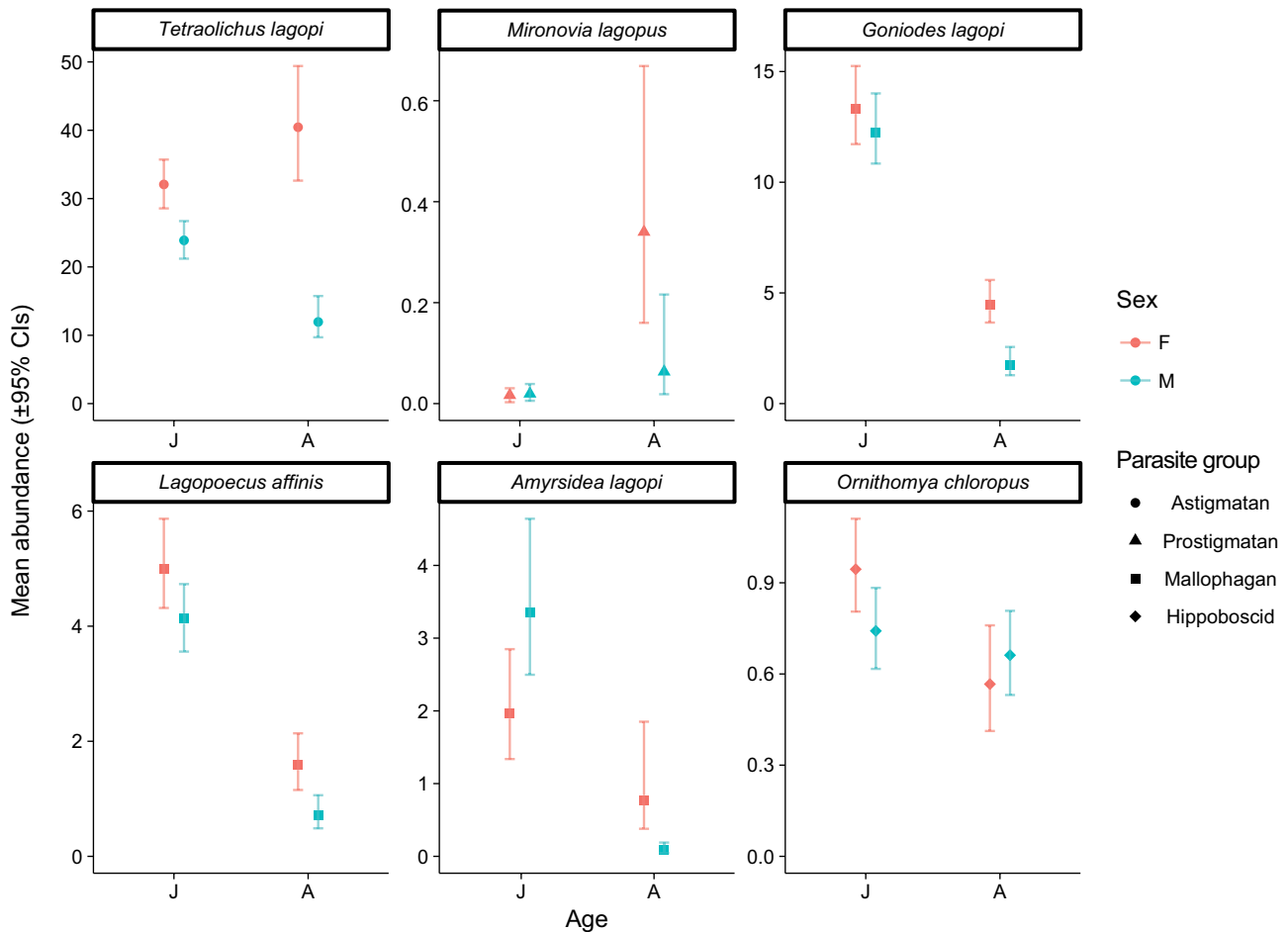


Figure 1. Age–sex comparisons of mean abundances of parasites of rock ptarmigan, *Lagopus muta*, across sampled years (2006–2017). Only parasite species whose best-fitting generalized linear mixed models included an age–sex interaction term are visualized (global model: abundance dependent on host age, sex and age:sex interaction; year included as a random factor). J=juvenile, A=adult. Confidence intervals around mean abundances are bias-corrected and accelerated 95% bootstrap confidence intervals.

the only species of 12 to show a significant male-biased parasitism.

Age was an important factor in one of the two best *E. muta* models, with juveniles having significantly more *E. muta* oocysts than adults in the best overall model. Females had more *E. muta* than did males for both best-fit models (Table 2).

While the best-fitting model explaining *C. caudinflata* abundance was the null, the second-best model included a significant age-by-sex interaction term; this likely arose due to a near-significant greater abundance among females, only observed for adult hosts. We do not consider this finding further because it was only for one model and not the best-fitting ‘null’ model.

Three best-fit models were found to explain *T. tenuis* abundance and all included age as a significant factor (adults had more worms than juveniles).

The six remaining parasite species all showed significant age-by-sex interactions in explaining variation in mean abundance, after controlling statistically for year as a

random factor (Fig. 1). These included the three lice species (*G. lagopi*, *L. affinis* and *A. lagopi*), two mite species (*T. lagopi* and *M. lagopus*) and the louse fly species (*O. chloropus*). The three lice species showed similar repeated patterns: adult males typically had fewer lice than adult females, whereas both sexes of adults generally had far fewer lice than juvenile birds. The presence or absence of a sex-biased infestation, therefore, depended on host age. There was a significant decrease in abundance between host ages for both *G. lagopi* and *L. affinis*; in comparison, for *A. lagopi*, only adult males had significantly lower abundances compared to juvenile males. Two mite species showed somewhat contrasting age-by-sex patterns of infestation. Infestations by the mite *M. lagopus* did not differ between males and females for juveniles, with mean abundances barely above zero, and only differed slightly for adults. Additionally, it appears that only females showed a significant increase in abundance of *M. lagopus* with age. The patterns of infestation by the mite *T. lagopi* were somewhat unique: females had more mites than males for juvenile birds, but this sex bias was exaggerated for adult

birds, thereby producing a significant age-by-sex interaction (adult males had significantly lower mean abundance of *T. lagopi* than juvenile males). Finally, an age-by-sex interaction explained significant variation in parasitism of ptarmigan by adults of the louse fly *O. chloropus*, where parasitism declined faster with age for females than for males.

Discussion

Large, multi-year, multi-parasite datasets, though a rarity, are invaluable resources for the study of infection patterns and dynamics. Such datasets allow the discovery of unique and repeated patterns of parasite–host associations not easily detected in smaller, single-sample and less diverse parasite datasets. Our 12-year study of the parasites of ptarmigan revealed female sex biases in parasitism among the majority (7 of 12) parasite species – a contrasting pattern to the more often observed male biases in parasitism, thought due in part to hormone-linked suppression of the immune system among breeding males (Poulin 1996, Zuk and Stoehr 2010). We also found recurrent, though not universal, juvenile age biases in parasitism. We consider both host and parasite factors in explaining such patterns of parasitism, frequently relating patterns to expected differences in summer social groupings of ptarmigan between age and sex classes, age-specific mortality in relation to parasitism, and acquisition of immunity with increasing age.

As indicated, we found a few singular and many repeated patterns of parasitism for ptarmigan. To start, the coccidian, *E. muta*, showed patterns representative of those for many other parasite species; i.e. an age bias with juveniles having more parasites (in this case, oocysts) than adults in the best-fitting model, and a sex bias with females having more oocysts than males, once the random factor of year was controlled. In our study population, *E. muta* was associated with heightened adult mortality and lower estimates of fecundity (Stenkewitz et al. 2016). It is expected this parasite is pathogenic to both adults and juveniles, but juveniles can be sampled before winter while carrying relatively high coccidian burdens. We return to the general problem of parasitism decreasing with host age below.

That parasitism by the nematode *C. caudinflata* was not dependent on age or sex of ptarmigan is a unique pattern. All other parasite species showed age and/or sex biases or an interaction between age and sex in explaining infection patterns. Holmstad and Skorpung (1998) determined that most macroparasites commonly found in willow ptarmigan (*L. lagopus*) were either male-biased or showed no bias in either infection intensity or prevalence. In their study, the prevalence of nematodes *T. tenuis* and *C. caudinflata* and intensities of cestode *Hymenolepis microps* were higher in male hosts. One other nematode (*Splendidofilaria papilloserca*) and cestode (*Paroniella urogalli*) did not exhibit any bias between sexes. Their study only collected a small sample of birds in

early fall, 1994. These results are intriguing, but patterns may vary yearly or seasonally, or not hold with larger samples.

In another study, Isomursu et al. (2006) studied helminth parasitism in three boreal grouse species, capercaillie *Tetrao urogallus*, black grouse *T. tetrix* and hazel grouse *Bonasa bonasia*. Male-biased helminth parasitism – specifically for the abundance and prevalence of the nematode *Ascaridia compar* – only occurred in the polygynous lekking grouse species that were considerably sexually dimorphic (i.e. Capercaillie where males are 2.2× heavier than females and black grouse where males are 1.3× heavier than females). The slightly size dimorphic, monogamous and territorial hazel grouse exhibited a slight male-biased parasitism in only occurrence, but not abundance, of *A. compar*. Among species, these results were more pronounced in juveniles than adults, and strongly suggest that body size and growth rates may influence the level of parasitism in these grouse species. In their study, grouse were sampled from September to October over multiple years, similar to our study.

In comparison to other studies, the lack of age or sex biases in parasitism by *C. caudinflata* for ptarmigan warrants explanation. Importantly, eggs of these nematodes are consumed incidentally with earthworms, which act as an intermediate host (Skirnisson et al. 2012). Ptarmigan chicks are known to feed on invertebrates during their first weeks of life. Presence of *C. caudinflata* in both adults and juvenile hosts suggests that adults also will ingest oligochaetes. Notably, their prevalence in juvenile ptarmigan assessed annually closely mirrors (i.e. without a lag) excess juvenile mortality, also assessed annually (Stenkewitz et al. 2016). While nematode infections are expected to accumulate with age, loss of infections is also expected among adult birds (> 15 months old), but not juveniles (ca 3 months old). Juveniles with higher worm burdens are not expected to survive the winter and enter adult cohorts the following fall. This combination of muted accumulation of worms by older birds and excess mortality of highly-infected juvenile birds might explain lack of sex or age differences in parasitism by this worm.

The nematode *T. tenuis* showed a consistent age bias, with adult hosts having more worms than juvenile birds in three models. This worm is transmitted directly and likely accumulates with age and group-living behavior. *T. tenuis* worms in this study did not even reach moderate infection intensities which can number in the hundreds or even thousands in other species (Webster et al. 2007). While this could suggest they were not pathogenic to Icelandic rock ptarmigan (Stenkewitz et al. 2016), we also acknowledge that the low prevalence of *T. tenuis* might in fact be due to virulence removing heavily-infected individuals from the population. Additionally, ptarmigan from this population may have a highly effective immune response to this nematode. The observed higher (but still low) worm intensities in adults as compared to juveniles are possibly due to accumulation with age, or increased juvenile mortality due to *T. tenuis* resulting in a biased sample.

The decline in parasitism with host age is the main (repeated) pattern seen for seven other parasite species, in addition to *E. muta* (3 mites, 3 lice, 1 fly; Table 2). However, the rate of decline is often dependent on an interaction with sex. Such age-specific differences in parasitism can be due to either acquired physiological immunity or more efficient anti-parasite behaviors (e.g. preening), or mortality falling disproportionately on birds heavily infected by one or more parasite species. One way to ascertain whether age-specific decreases in parasitism are due to acquired immunity or parasite-associated mortality is to follow individually marked birds, where parasite loads are scored repeatedly over the lifetimes of individuals. This approach was beyond the current scope of this study. If such studies are done, it will be relevant to also score condition of birds repeatedly, as birds in poor condition might be more susceptible to both parasitism and subsequent mortality. Parasite-associated mortality has been reported for wild birds (Pyrovetsi and Papazahariadou 1995, Sol et al. 2003), and acquired immunity has been reported for bird helminths (Macy 1973, Webster et al. 2011); there is no reason to exclude either of these hypotheses from explaining recurring declines in parasitism with host age.

Patterns of mite parasitism (by *M. lagopus* and *T. lagopi*) were different. In a study on quill mite infestations (*M. lagopus*) of rock ptarmigan, Skírnisson and Nielsen (2019) showed no significant inter-annual variation or consistent sex differences in prevalence or intensity of infestations, but consistent age biases with adults (at least 15 months old) having more mites than juveniles (ca 3 months old). Our modeling approach uncovers a female bias in mean abundance in addition to an adult age bias. It is unlikely that this mite species is pathogenic and accumulation with age can be potentially explained by direct reproduction on hosts. If, however, mite reproduction is influenced by body condition, it might explain an adult female bias restricted to some years because the energetic costs of brood rearing might be felt more in some years than others. Mean abundance of the wing mite, *T. lagopi*, demonstrated a particularly unique pattern. It showed a female bias in both age classes of hosts, but one that became exaggerated as birds aged. We currently have no explanation for the sex bias among juveniles, both sexes of which showed very high prevalence, as did adults (Table 1). Increases in mean abundance of adult females might be due to heightened exposure because of their group living as compared to adult males.

Generally speaking, adult biases in parasite abundance might be expected for those parasites that breed on hosts and for which their offspring also parasitize the same hosts. In this scenario, adult biases might simply reflect the longer times afforded for the population of parasites to build up. Two of the three parasites which demonstrated adult biases in abundance in this study (*M. lagopus* and *T. lagopi*) fit this criterion of offspring potentially parasitizing the same host individual that adults breed on. Such age biases might even be expected for estimates of prevalence of infection as incipient infections in juveniles might simply be difficult to detect; such a

rationale was posited recently for the quill mite (species) of ptarmigan (Skírnisson and Nielsen 2019).

The findings summarized above provide a relatively comprehensive overview of the host-related factors driving patterns of parasitism in a diverse community parasitizing a population of a widespread grouse species. Some patterns are suggestive of candidate explanations based on known biology of the ptarmigan host (e.g. differences in immunity, in group living) and its parasites (horizontal transmission). However, inferences may be of limited scope, as different parasites might show similar patterns for different reasons (e.g. an age bias due to age-specific mortality rates in relation to parasitism, versus a similar age bias due to age-associated changes in behavioral or physiological immunity). Other patterns, like the juvenile sex bias in *T. lagopi*, are less easily explained. Regardless, these findings provide direction for further observational or experimental work exploring underlying causal mechanisms.

For example, Stenkewitz et al. (2015) showed that adult females had greater spleen masses than males, suggesting that females invest more in immune defense in our study population. However, adult females were still acquiring more parasites of several species than males, compared to other related host species (above). The authors believe that the energetic task of brooding and rearing offspring (female-only tasks) may have made them more susceptible to infection during that time of year (Stenkewitz et al. 2015). This is especially the case for directly-transmitted parasites such as coccidians, lice and mites, which are more likely to accumulate in birds that were more group-living before being sampled (juveniles and adult females) compared to those that were more solitary (adult males). An interesting question is whether splenomegaly was a result of high infestations in females.

Naturally, this study does not consider an exhaustive list of all potential drivers of variation in parasitism in these rock ptarmigan, focusing, as it does, on parasite species-specific patterns. For example, parasitism may be significantly affected by interactions between coinfecting parasites, i.e. parasite species may not co-occur as independent, non-interacting entities (Karvonen et al. 2019). While such analyses are beyond the scope of the present study, one could ask how members of the parasite community interact, and whether parasites are the real drivers of observed host trait biases, or vice versa. Other questions, such as whether measures of endoparasitism and ectoparasitism are related, could also be addressed. Subsequent hypothesis generation and testing regarding drivers of patterns of parasitism in rock ptarmigan would need to consider the results described herein.

In conclusion, we show unique and repeated patterns of parasitism for ptarmigan across a range of parasite prevalence and abundances. Eleven of 12 parasite species demonstrated some degree of host-trait bias, and these biases were consistent with various aspects of host and parasite biology, in the absence of inferences from experimental infections. The female biased parasitism is consistent with energetic costs of brood rearing and group living before samples were collected, as noted elsewhere (Stenkewitz et al. 2015). The paucity of

male-biased parasitism might be explained by samples being collected outside of the breeding season or by the more solitary living of adult males. The age biases in parasitism are consistent with both acquired immunity and age-specific parasite-mediated mortality – there is evidence for the latter mechanism in this study system (Stenkewitz 2017). Researchers should sample outside of breeding seasons of hosts to uncover the range of host trait biases in parasitism, and also need to assess host trait biases in examining real or apparent interactions between parasite species.

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Data availability statement

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.02427>> (Nielsen et al. 2020).

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