

EXTREME MALE-BIASED INFECTIONS OF MASKED SHREWS BY BLADDER NEMATODES

KRYSTYNA M. COWAN, DAVE SHUTLER,* THOMAS B. HERMAN, AND DONALD T. STEWART

Department of Biology, Acadia University, Wolfville, Nova Scotia B4P 2R6, Canada

Present address of KMC: 1055 Lucknow Street, Apartment 106, Halifax, Nova Scotia B3H 2T3, Canada

Intensity of parasitic infection (number of parasites per host) can vary by date, and with host age and sex. We tested whether variation in bladder nematode (*Liniscus* [= *Capillaria*] *maseri*) intensity in Nova Scotian masked shrews (*Sorex cinereus*) was related to these variables. We collected a total of 117 shrews on 8 different sampling occasions between mid-May and mid-August. Univariate relationships suggested that intensities declined between May and August, and were higher in male shrews. In analyses simultaneously testing for relationships between intensity and date, age, and sex, the most compelling pattern was strongly male-biased parasitism.

Key words: age-biased parasitism, bladder nematodes, *Capillaria*, intensity of infection, *Liniscus*, masked shrews, sex-biased parasitism, *Sorex cinereus*

Numerous factors can affect prevalence (percent of hosts infected) and number of parasites per host (hereafter intensity of infection—Clayton and Moore 1997); thus, it can be difficult to predict patterns of parasitism (Hudson et al. 2001). In this paper, we test for correlates of bladder nematode (*Liniscus* [= *Capillaria*] *maseri* Rausch and Rausch) intensity in host masked shrews (*Sorex cinereus* Kerr). Although previous research has noted presence of this parasite in masked shrews in Nova Scotia, Canada (Downie 1986; Telfer 1984), correlates of infection have not been explored. We considered whether intensity varied by date (between mid-May and mid-August), by host age, and by host sex.

Changes in intensity of infection can be a function of many factors including changes in demography and sociality of hosts (Wilson et al. 2001). For example, many small mammal populations increase in spring after breeding; higher density of hosts may facilitate transmission of parasites and may potentially lead to increased intensity of infection (e.g., Côté and Poulin 1995). In contrast, when host densities are low, sexually reproducing parasites may be extirpated because they cannot find mates within the hosts they infect, or propagules produced by either asexual or sexual parasites may be less likely to encounter a susceptible host (Poulin 1998).

Susceptibility of individual hosts to parasites can vary with host age or sex. Younger and older hosts may have inadequately developed or deteriorating immune systems that are

unable to eliminate or reduce parasites. When hosts are unable to eliminate parasites, older hosts on average accumulate more parasites (Poulin 1998). Similarly, if host body size increases with age, older hosts may require more food and present larger surface areas, each of which could contribute to increased exposure to parasite propagules and hence more-intense infections. Differences in intensity of infection between male and female hosts can result from sex-related differences in immune systems, body size, or behavior that affect exposure and susceptibility to parasites (Folstad and Karter 1992; Folstad et al. 1989; Klein 2004; McCurdy et al. 1998; Poulin 1996; Roberts et al. 2004; Zuk and McKean 1996).

We studied a bladder nematode infecting masked shrews in Nova Scotia. The life cycle of this parasite has not yet been described, but based on other bladder nematodes in the genus *Liniscus* and the closely related *Capillaria* (Beldomenico et al. 2002; Bourque 1981; Herman 1981; Meagher and O'Connor 2001; Rausch and Rausch 1973), embryonated eggs are ingested and hatch in the gut, or juvenile nematodes are ingested directly. Juveniles or adults then migrate to the bladder where mated females shed eggs in host urine. Excreted eggs may be ingested directly, or by intermediate hosts that are subsequently eaten by definitive hosts, completing the life cycle.

Masked shrews (hereafter shrews) occur widely in North America across a broad range of habitats with diverse prey, competitors, and predators (Banfield 1974; van Zyll de Jong 1983). High metabolic rates in this and other shrew species require high rates of food consumption, increasing the likelihood that parasite propagules will be ingested. In Nova Scotia, this species generally breeds from May to September, so that population densities increase during this period, and then decline through fall and winter. On coastal islands, the breeding

* Correspondent: dave.shutler@acadiau.ca

season is extended in some years (Teferi et al. 1992), probably due to abundant food resources available in coastal wrack deposits (Stewart et al. 1989).

We tested whether the intensity of infection by bladder nematodes in shrews varied over the summer, and with host age and sex. Because we do not know the life cycle of this parasite, we had no a priori information to make predictions about the directions of these associations. For example, if the parasite has a single intermediate arthropod host that has a brief seasonal appearance in the diet, patterns of parasitism could be tightly tied to this interval. Thus, our tests were all 2-tailed; we tested only whether variation in explanatory variables was associated with variation in parasitism.

MATERIALS AND METHODS

All methods were approved by the Acadia University Animal Care Committee, and met guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). Trapping was conducted at 1 mainland and 2 island locations in southern Nova Scotia, Canada: Sandy Cove (44°48'N, 66°8'W), Long Island (44°19'N, 66°16'W), and Bon Portage Island (43°28'N, 65°45'W). Shrews were trapped on 8 separate sampling sessions, some of which occurred concomitantly in adjacent locations. Dry pitfall traps were set in late afternoon approximately 1 m apart and were dug in so that their rims were even with the ground. Cotton balls were placed in pitfall traps to protect shrews from weather, and to provide refuge from conspecifics in cases where there were multiple captures per trap. Traps were initially checked between 0600 and 0700 h every morning and at ~3-h intervals thereafter until 2100 h. Captures per trap-hour \times 24 (trap-days) were used to estimate relative abundance.

Shrews found dead in traps were immediately placed in individual plastic bags and stored on ice. Living shrews were killed by thoracic compression just before dissection. Sex was determined from the presence of testes or ovaries. Bladders were removed, compressed between 2 microscope slides, and viewed under a compound microscope to enumerate bladder nematodes. After preliminary sampling, we decided to scale intensity as 0 (no worms), 1 (1 or 2 worms), 2 (3 or 4 worms), 3 (5 or 6 worms), 4 (7–10 worms), and 5 (>10 worms). The species of bladder nematode was identified by J. M. Kinsella (HelmWest Laboratory, Missoula, Montana) as *Liniscus maseri*, although spicule lengths of males were somewhat longer and outside the range of the original description.

Carcasses were frozen at -40°C and later cleaned by dermestid beetle larvae. Shrews were assigned to 1 of 5 ordinal age categories that we could distinguish based on tooth wear (corresponding values of Rudd [1955] are given parenthetically): 1 (1–), no wear on teeth; 2 (1 and 1+), tip of metacone of P3 blunted with posterior face of unicuspid showing wear; 3 (2– and 2), wear showing on molars, terminal pigment of metacone of P3 divided and a band of wear visible along length; 4 (3– and 3), unicuspid worn over entire face with nonpigmented portions of molars strongly hollowed out and pigment on mesostyles of M1 and M2 gone or nearly so; and 5 (4– and 4) pigment gone

on M3, all unicuspid flat on occlusal surface, a slight amount of pigment sometimes remaining along metastyles and M3 sometimes only a hollowed shell. Our age class 1 comprised prebreeding, immature individuals, classes 2 and 3 comprised young adults of which some would be reproductive, and classes 4 and 5 comprised older, reproductive adults.

All statistical analyses were carried out in SAS version 9.1 (SAS Institute Inc., Cary, North Carolina). We tested for relationships between intensity and day of year, age, and sex with general linear models. We report means \pm SD.

RESULTS

A total of 117 shrews was captured. Numbers captured, numbers of trap days, and density indices (number captured per trap day), respectively, were 8, 184, and 4.3 for Long Island between 14 and 16 June 2005; 12, 108, and 11.1 for Sandy Cove between 14 and 16 June 2005; 11, 146, and 7.5 for Bon Portage shore areas between 27 and 29 June 2005; 7, 350, and 2.0 for Bon Portage inland areas between 27 and 29 June 2005; 15, 50, and 30.0 for Bon Portage shore areas between 8 and 10 August 2005; 16, 200, and 8.0 for Bon Portage inland areas between 8 and 10 August 2005; 28, 130, and 22.0 for Bon Portage shore areas between 23 and 24 May 2006; and 18, 17, and 24.0 for Bon Portage shore areas between 27 and 28 June 2006. Overall mean intensity score was 1.1 ± 1.3 . Sample sizes vary among tests because all data were not collected from all shrews.

Pooling data from 2005 and 2006, prevalences of infection (n is given in parentheses) were 59% (29) for 23–24 May, 60% (20) for 14–16 June, 65% (37) for 27–29 June, and 16% (31) for 8–10 August. Intensity of infection also declined over the course of the season and was best explained by a quadratic model ($F = 8.2$, $d.f. = 1, 109$, $P = 0.005$, $R^2 = 0.07$; Fig. 1).

In 95 shrews for which age was determined, prevalence of infection (n is given in parentheses) was 14% (7), 5% (19), 56% (55), 66% (12), and 50% (2), for ages 1–5, respectively. If uninfected individuals were included, intensity of infection was significantly lower for shrews in age class 2 compared to age classes 3 and 4 ($F = 3.2$, $d.f. = 4, 84$, $P = 0.02$, $R^2 = 0.13$; Fig. 2). However, among infected individuals, there was no difference in intensity of infection among age classes ($F < 0.1$, $d.f. = 1, 40$, $P = 0.91$, $R^2 < 0.01$).

Of all shrews captured, 64 (55%) were male. Prevalence of infection (n is given in parentheses) was 21% (53) for females and 75% (61) for males; sex was not determined for 3 individuals. Similarly, intensity scores for females (0.4 ± 0.7) were much lower than those for males (1.7 ± 1.4 ; $F = 37.8$, $d.f. = 1, 107$, $P < 0.0001$, $R^2 = 0.26$; Fig. 3). When uninfected individuals were excluded, corresponding intensity scores for females and males were 1.6 ± 0.7 and 2.3 ± 1.2 , respectively.

Changes in the demography of shrews captured over the course of the summer reduced our ability to test for multivariable associations with parasitism; for example, age distributions of shrews varied dramatically (Fig. 4). Collectively, 36% of the variation in intensity of infection was explained by year ($F = 7.9$, $d.f. = 1, 82$, $P = 0.006$), day of the year ($F = 10.7$,

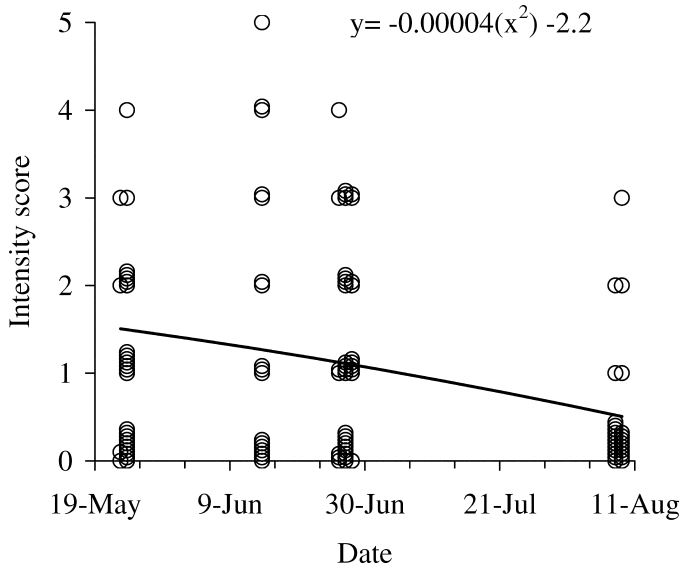


FIG. 1.—Intensities of infection by bladder nematodes (*Liniscus maseri*) declined from mid-May to mid-August in masked shrews (*Sorex cinereus*). Intensity score 0 indicates no worms, 1 indicates 1 or 2 worms, 2 indicates 3 or 4 worms, 3 indicates 5 or 6 worms, 4 indicates 7–10 worms, and 5 indicates >10 worms. Overlapping observations are shifted vertically to make them visible.

df. = 1, 82, *P* = 0.002), age (*F* = 0.7, *df.* = 1, 82, *P* = 0.39), and sex (*F* = 25.3, *df.* = 1, 82, *P* < 0.0001; overall model *F* = 11.5, *df.* = 4, 82, *P* < 0.0001, *R*² = 0.36). Thus, as revealed by the univariate analyses, intensity of infection appeared to dip in August, and was higher for males, but was not strongly

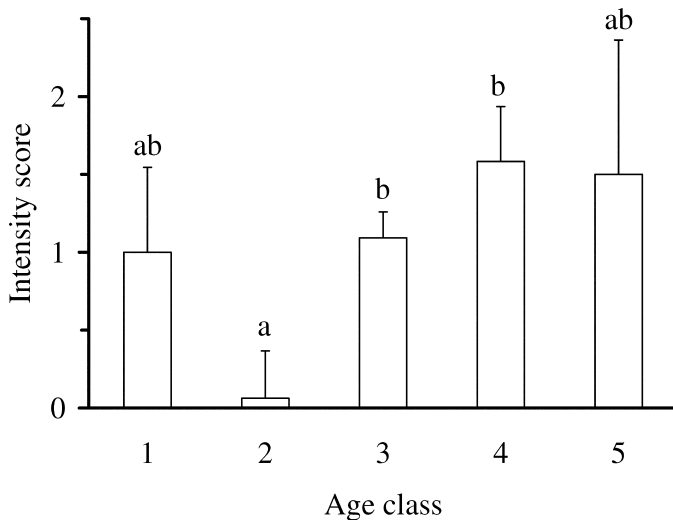


FIG. 2.—Shrews (*Sorex cinereus*) in age class 2 had the lowest intensities of infection by bladder nematodes (*Liniscus maseri*). Bars that share letters were not statistically different (*P* < 0.05; Tukey's studentized range). Age class 1 comprised prebreeding, immature individuals; classes 2 and 3 comprised young adults of which some would be reproductive; and classes 4 and 5 comprised older, reproductive adults. Intensity score 0 indicates no worms, 1 indicates 1 or 2 worms, 2 indicates 3 or 4 worms, 3 indicates 5 or 6 worms, 4 indicates 7–10 worms, and 5 indicates >10 worms.

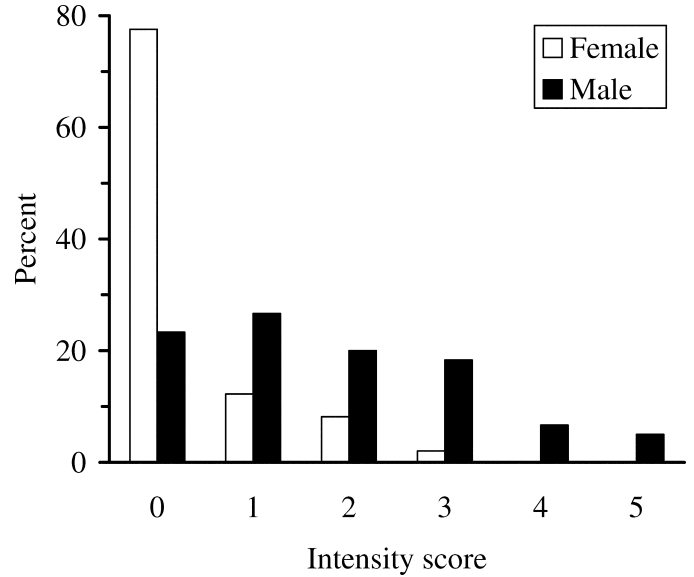


FIG. 3.—Females had lower infection intensity scores than did males. Intensity score 0 indicates no worms, 1 indicates 1 or 2 worms, 2 indicates 3 or 4 worms, 3 indicates 5 or 6 worms, 4 indicates 7–10 worms, and 5 indicates >10 worms.

associated with the age of shrews. Intensity scores were lower in 2006, although sampling intervals were not comparable. Nonetheless, we repeated analyses within years to test the robustness of the patterns detected. For 2005, day of the year (*F* = 10.1, *df.* = 1, 44, *P* = 0.003) and sex (*F* = 8.4, *df.* = 1, 44, *P* = 0.006) remained significant correlates of intensity of infection, whereas age did not (*F* = 1.0, *df.* = 1, 44, *P* = 0.34; overall model *F* = 9.4, *df.* = 3, 44, *P* < 0.0001, *R*² = 0.39). For 2006, only sex (*F* = 20.6, *df.* = 1, 35, *P* < 0.0001) remained a significant correlate of intensity (overall model *F* = 7.0, *df.* = 3, 35, *P* = 0.001, *R*² = 0.37), although there was limited variability in day of the year in this analysis. In females

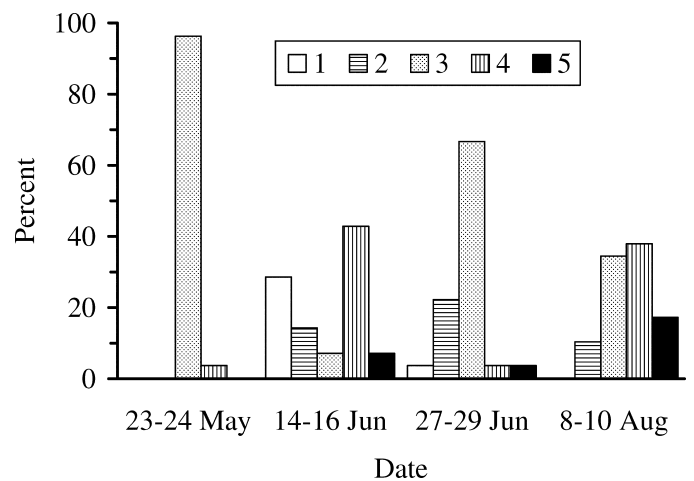


FIG. 4.—Age distributions of shrews (*Sorex cinereus*) varied among sampling dates. Age class 1 comprised prebreeding, immature individuals; classes 2 and 3 comprised young adults of which some would be reproductive; and classes 4 and 5 comprised older, reproductive adults.

(overall model $F = 0.6$, $d.f. = 2$, 41, $P = 0.19$, $R^2 = 0.08$), intensity did not vary significantly between May and August ($F = 0.6$, $d.f. = 1$, 41, $P = 0.45$) but appeared to increase with age ($F = 3.5$, $d.f. = 1$, 41, $P = 0.07$), whereas in males (overall model $F = 2.9$, $d.f. = 2$, 40, $P = 0.07$, $R^2 = 0.07$), intensity decreased in August ($F = 5.7$, $d.f. = 1$, 40, $P = 0.02$) but did not vary with age ($F < 0.1$, $d.f. = 1$, 40, $P = 0.84$).

DISCUSSION

Although intensities of bladder infections by nematodes were higher early in the year, this pattern was restricted to males and may in part be a consequence of our opportunistic sampling. We also found that older female, but not male, shrews tended to have more-intense infections. The strongest and most robust relationship we found was for extreme male-biased parasitism. Our results highlight both the complexity of interactions among the explanatory variables we considered, and the complexity of this host–parasite system in general. Because the life cycle of this parasite is not completely known, we can only speculate about the causes of these patterns of intensity of infection. This speculation is made more difficult because many aspects of the life history of masked shrews are poorly known (e.g., social structuring of populations that may significantly affect parasite transmission).

If reproduction by shrews is restricted to summer, captures of shrews in spring will be dominated by older, overwintered adults that have had extended exposure to parasite infection. Recruitment of young-of-the-year shrews, which may not yet have acquired or matured bladder nematodes, would begin in July and August. Under these circumstances one would expect a seasonal, age-driven change in parasitism. However, age distributions in our samples are not entirely consistent with this pattern, probably due to more-extended breeding seasons in island or coastal populations (Teferi et al. 1992). Therefore, changes in intensity over the summer that we observed may involve additional but unknown aspects of the life cycle of the parasite.

The most compelling pattern we found was for extreme male-biased parasitism, which is broadly consistent with the literature for male mammals (Klein 2004; Roberts et al. 2004; Schalk and Forbes 1997; see McCurdy et al. [1998] for a contrary pattern for birds). However, the magnitude of the sex-bias we observed is noteworthy; mean intensity scores (and associated nematode burdens) were roughly 5 times greater in males. Sex-biased parasitism is usually ascribed to endocrine or behavioral causes; in this case, we suspect that the magnitude of these differences in parasitism must be driven primarily by behavioral differences. For example, male mammals may be more likely to group, facilitating transmission (Klein 2004). Males of at least some soricids have home ranges that are twice as large as those of females (Cantoni 1993; Hawes 1977), which likely increases the likelihood of encountering parasite propagules. In spring, home ranges of males are 3 times as large as during other seasons and male–male interactions are more frequent than female–female interactions (Cantoni 1993). Sex-biased parasitism can also occur because higher titers of

testosterone in males can be immunosuppressive (Folstad and Karter 1992; Klein 2000, 2004). If testosterone levels increase before the mating season and decrease thereafter, sex differences in intensity of infection by bladder nematodes might vary over the year. If breeding seasons are extended on islands, and testosterone is immunosuppressive, one might expect particularly strong sex bias in infection. Hormones also could contribute to sex-biased parasitism by affecting host behavior, which may affect exposure to infection (Klein 2004).

Our study suggests several avenues for further research on this host–parasite system. In particular, further data on seasonal and age-related intensity of infection are needed. The effect of host density on both prevalence and intensity of infection should be explored. Methods should be developed to allow repeated sampling from the same individuals over time. Finally, elucidating the life cycle of this bladder nematode will help predict patterns of infection among and within populations.

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LITERATURE CITED

- BANFIELD, A. W. F. 1974. The mammals of Canada. National Museum of Natural Sciences, National Museums of Canada, University of Toronto Press, Toronto, Ontario, Canada.
- BELDOMENICO, P. M., D. HUNZICKER, J. L. TAVERNA, AND P. K. REJF. 2002. Capillariidae eggs found in the urine of a free ranging maned wolf from Argentina. *Memorias do Instituto Oswaldo Cruz* 97: 509–510.
- BOURQUE, M. 1981. The masked shrew (*Sorex cinereus*), a new host for *Capillaria plica*. *Canadian Journal of Zoology* 59:2393–2394.
- CANTONI, D. 1993. Social and spatial organization of free-ranging shrews, *Sorex coronatus* and *Neomys fodiens* (Insectivora, Mammalia). *Animal Behaviour* 45:975–995.
- CLAYTON, D. H., AND J. MOORE. 1997. Introduction. Pp. 1–6 in Host–parasite evolution: general principles and avian models (D. H. Clayton and J. Moore, eds.). Oxford University Press, Oxford, United Kingdom.
- CÔTÉ, I. M., AND R. POULIN. 1995. Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology* 6:159–165.
- DOWNIE, A. B. 1986. Spatial distributions, movements and age structure in island and mainland populations of the masked shrew (*Sorex cinereus* Kerr). B.S. Honours thesis, Acadia University, Wolfville, Nova Scotia, Canada.
- FOLSTAD, I., AND A. J. KARTER. 1992. Parasites, bright males, and the immunocompetence handicap. *American Naturalist* 139:603–622.
- FOLSTAD, I., A. C. NILSSEN, O. HALVORSEN, AND J. ANDERSEN. 1989. Why do male reindeer (*Rangifer t. tarandus*) have higher abundance of second and third instar larvae of *Hypoderma tarandi* than females? *Oikos* 55:87–92.
- GANNON, W. L., R. S. SIKES, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2007.

- Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* 88:809–823.
- HAWES, M. L. 1977. Home range, territoriality, and ecological separation in sympatric shrews, *Sorex vagrans* and *Sorex obscurus*. *Journal of Mammalogy* 58:354–367.
- HERMAN, T. B. 1981. *Capillaria hepatica* (Nematoda) in insular populations of the deer mouse *Peromyscus maniculatus*: cannibalism or competition for carcasses? *Canadian Journal of Zoology* 59:775–784.
- HUDSON, P. J., A. RIZZOLI, B. T. GRENFELL, H. HEESTERBEEK, AND A. P. DOBSON (EDS.). 2001. The ecology of wildlife diseases. Oxford University Press, Oxford, United Kingdom.
- KLEIN, S. L. 2000. The effects of hormones on sex differences in infection: from genes to behaviour. *Neuroscience and Biobehavioral Reviews* 24:627–638.
- KLEIN, S. L. 2004. Hormonal and immunological differences mediating sex differences in parasite infection. *Parasite Immunology* 26:247–264.
- MCCURDY, D. G., D. SHUTLER, A. MULLIE, AND M. R. FORBES. 1998. Sex-biased parasitism of avian hosts: relations to blood parasite taxon and mating system. *Oikos* 82:303–312.
- MEAGHER, S., AND T. P. O'CONNOR. 2001. Population variation in the metabolic response of deer mice to infection with *Capillaria hepatica* (Nematoda). *Canadian Journal of Zoology* 79:554–561.
- POULIN, R. 1996. Sexual inequalities in helminth infections: a cost of being male? *American Naturalist* 147:287–295.
- POULIN, R. 1998. Evolutionary ecology of parasites. Chapman & Hall, New York.
- RAUSCH, R. L., AND V. R. RAUSCH. 1973. *Capillaria maseri* sp. (Nematoda) from insectivores (Soricidae and Talpidae) in Oregon. *Proceedings of the Helminthological Society of Washington* 40:107–112.
- ROBERTS, M. L., K. L. BUCHANAN, AND M. R. EVANS. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. *Animal Behaviour* 68:227–239.
- RUDD, R. L. 1955. Age, sex, and weight comparisons in three species of shrews. *Journal of Mammalogy* 36:323–339.
- SCHALK, G., AND M. R. FORBES. 1997. Male biases in parasitism of mammals: effects of study type, host age, and parasite taxon. *Oikos* 78:67–74.
- STEWART, D. T., T. B. HERMAN, AND T. TEFERI. 1989. Littoral feeding in a high-density insular population of *Sorex cinereus*. *Canadian Journal of Zoology* 67:2074–2077.
- TEFERI, T., T. B. HERMAN AND D. T. STEWART. 1992. Breeding biology of an insular population of the masked shrew (*Sorex cinereus* Kerr) in Nova Scotia. *Canadian Journal of Zoology* 70:62–66.
- TELFER, K. A. 1984. Microhabitat associations, morphology, and demography of shrews (Soricidae) in Nova Scotia. B.S. Honours thesis, Acadia University, Wolfville, Nova Scotia, Canada.
- VAN ZYLL DE JONG, C. G. 1983. Handbook of Canadian mammals 1. Marsupials and insectivores. National Museum of Natural Sciences, National Museums of Canada, Ottawa, Ontario, Canada.
- WILSON, K., ET AL. 2001. Heterogeneities in macroparasite infections: patterns and processes. Pp. 6–44 in *The ecology of wildlife diseases* (P. J. Hudson, A. Rizzoli, B. T. Grenfell, H. Heesterbeek, and A. P. Dobson, eds.). Oxford University Press, Oxford, United Kingdom.
- ZUK, M., AND K. A. MCKEAN, 1996. Sex differences in parasite infections: patterns and processes. *International Journal of Parasitology* 26:1009–1024.

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