

Effects of canine heartworm (*Dirofilaria immitis*) on body condition and activity of free-ranging coyotes (*Canis latrans*)

Benjamin N. Sacks and Karen M. Blejwas

Abstract: We used radiotelemetry to study relationships among canine heartworm (*Dirofilaria immitis*) infection, body condition, and activity of free-ranging coyotes (*Canis latrans*). Average body mass at death was lower for 17 coyotes in a high-intensity infected group ($\bar{x} = 33.6$ heartworms) than for 18 coyotes in a control group ($\bar{x} = 3.6$ heartworms; $p < 0.01$). Coyotes in the infected group lost body mass at an average rate of 20% per year relative to the control group ($p < 0.01$). Bone marrow fat was negatively correlated with heartworm burden ($R^2 = 0.27$; $p < 0.01$). Average body mass of coyotes at initial capture (i.e., potentially before infection) did not differ between infected and control groups ($p = 0.90$; $1 - \beta = 0.70$). Activity was negatively correlated with heartworm burden during the last 2 months of life ($R^2 = 0.30$; $p < 0.01$), but no correlation was found 2–4 months before death. Activity of the infected group ($n = 13$) declined over time ($p = 0.01$), whereas no difference in activity was observed in the control group ($n = 13$; $p = 0.50$). Our findings indicate that heartworm infection reduced body condition and activity of coyotes but that nutritional status did not significantly affect susceptibility to infection.

Résumé : Nous avons eu recours à la radiotélémetrie pour étudier la relation entre le ver du coeur canin (*Dirofilaria immitis*), la condition physique et l'activité chez des Coyotes (*Canis latrans*) en liberté. La masse totale moyenne au moment de la mort s'est avérée moins élevée chez un groupe de 17 coyotes à infections graves ($\bar{x} = 33,6$ vers du coeur) que chez un groupe témoin de 18 coyotes ($\bar{x} = 3,6$ vers du coeur; $p < 0,01$). Les pertes de masse chez les coyotes infectés se faisaient à raison de 20 % par année comparativement au groupe témoin ($p < 0,01$). Les graisses de la moelle osseuse étaient en corrélation négative avec le fardeau de vers du coeur ($R^2 = 0,27$; $p < 0,01$). La masse totale moyenne des coyotes au moment de la première capture (i.e. potentiellement avant l'infection) était la même chez les deux groupes de coyotes ($p = 0,90$; $1 - \beta = 0,70$). L'activité était en corrélation négative avec le fardeau de vers du coeur au cours des 2 derniers mois de vie ($R^2 = 0,30$; $p < 0,01$), mais aucune corrélation n'a été trouvée 2–4 mois avant la mort. Chez les coyotes du groupe infecté, l'activité a diminué graduellement ($p = 0,01$) alors qu'aucun changement dans l'activité n'a été observé chez les coyotes du groupe témoin ($n = 13$; $p = 0,50$). Nos résultats indiquent que les vers du coeur diminuent la condition physique et l'activité des coyotes mais que le statut nutritionnel n'affecte pas significativement la sensibilité au parasite.

[Traduit par la Rédaction]

Introduction

Parasites can negatively affect energy budgets of their mammalian hosts in several ways. For example, parasites can cause direct physical impairment of hosts, which in turn reduces activity, foraging efficiency, and ultimately nutritional status (Scott 1988). Less directly, parasites can reduce host digestive efficiency (Munger and Karasov 1989), increase host metabolism (Hudson and Dobson 1995), or reduce appetite (Crompton 1984). Conversely, poor nutritional status of mammals can increase their susceptibility to some parasitic infections through negative effects on immune re-

sponses (Chandra and Newberne 1977; Slater and Keymer 1986; Solomon and Scott 1994), potentially leading to synergistic relationships between parasitism and nutritional status (Chandra and Newberne 1977; Keymer and Dobson 1987). Deficiencies in protein and fat (i.e., energy sources as opposed to vitamins and minerals) may be especially influential on susceptibility to parasitic infection and disease (Slater and Keymer 1986; Lloyd 1995). Most of what is known about relationships between parasitism and host nutrition is based on humans or domestic animals (Crompton 1984; Lloyd 1995). A few studies have examined these relationships between free-ranging wildlife and parasites of relatively low pathogenicity (Munger and Karasov 1986; Booth et al. 1993; Murray et al. 1998). We investigated relationships between parasitism, host nutritional status, and activity in free-ranging coyotes (*Canis latrans*) and canine heartworm (*Dirofilaria immitis*), a potentially lethal parasite.

Canine heartworm is a filarial nematode with a 6-month life cycle requiring both mosquito and canid hosts (Otto and Jachowski 1981; Rawlings and Calvert 1995). Over the past few decades, the geographic range of heartworm has been

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expanding in North America (Otto 1972; MacKenzie and Waldie 1991; Theis et al. 1996). Northern California (U.S.A.) is one such place where heartworm has recently become established and is presently abundant (Theis et al. 1996; Sacks 1998). Until the 1970s, heartworm was thought to be a relatively rare and unimportant pathogen in wild canids (Otto 1972; Graham 1975). More recent studies, however, have found prevalence of heartworm comparable to that in sympatric domestic dogs, suggesting that heartworm also may be an important disease agent in wild canid populations (Custer and Pence 1981; Acevedo and Theis 1982; Theis et al. 1996; Sacks 1998). However, little is known about the pathogenicity of heartworm in wild canids or its relationship to host nutritional status or activity (Gier et al. 1978; Custer and Pence 1981).

Heartworm has long been recognized as a serious agent of disease in domestic dogs (Henningar and Ferguson 1957) and much is known about its pathogenicity in this host (Rawlings 1986; Sutton 1988; Rawlings and Calvert 1995). The adult parasite is large (14–31 cm; Orihel 1961) and resides in the cardiopulmonary vasculature of its canine host, potentially causing severe pathology or death (Atwell and Buoro 1988; Sutton 1988; Martin and Corcoran 1997). The severity of heartworm infection in dogs depends on the number of parasites and period of insult (Knight 1981; Atwell 1988; Sutton 1988; Rawlings and Calvert 1995; Martin and Corcoran 1997), which relate to exposure to mosquito vectors, as well as level of exercise (Knight 1981; Atwell and Buoro 1988; Dillon et al. 1995). Pathology associated with heartworm disease usually results proximally from the dog's own immune response toward non-infective stages of the parasite (Atwell 1988; Grieve 1988). Although experimental evidence suggests that dogs also may exhibit some degree of protective immunity (directed at infective-stage larvae; Wong et al. 1974; Blair et al. 1982), there is little evidence of protective immunity in naturally infected dogs (Grieve 1988).

Heartworm might be expected to affect wild canids differently than their domestic counterparts due to differences in exposure risk, amount of exercise, and the degree to which they are influenced by natural selection. One of the most important exposure factors identified for dogs in California is the amount of time spent outside (Theis et al. 1995; Walters 1995), indicating that exposure should be relatively high in wild canids. Further, unlike dogs, which typically expend little energy to obtain food, wild canids must hunt or scavenge to survive, which might be expected to aggravate effects of heartworms (Gier et al. 1978; Knight 1981; Dillon et al. 1995).

On the other hand, natural selection might result in wild canids being more resistant than domestic dogs to parasitic disease, generally. For example, necropsies of heartworm-infected coyotes have revealed relatively low inflammatory responses to microfilariae in several organs (Custer and Pence 1981), which suggests either that hypersensitivity was relatively less pronounced in these wild hosts generally or that the sample in that study was biased toward individuals with reduced hypersensitivity due to their higher survival rate (which would also suggest selection). Coyotes also might be expected to exhibit a greater protective immune response. However, a study of feral dogs in a hyperendemic region

(Capelli et al. 1996) found similar prevalence, intensity, and distributions of heartworms as in some coyote populations (Custer and Pence 1981; Sacks 1998; B.N. Sacks, unpublished data), suggesting that the degree of protective immunity may not differ substantially between wild and domestic canids.

Although postmortem examinations of wild canids have indicated that heartworm infection causes pathology in various organs, similarly to dogs but less severe (Crowell et al. 1978; Custer and Pence 1981), such studies reveal little about how heartworm infection interacts with nutritional status and behavior in free-ranging individuals. We conducted a radiotelemetry study of free-ranging coyotes with known heartworm burdens to examine relationships between heartworm infection, body mass, bone marrow fat, and activity; we used changes in parameters over time to infer causal relationships. We predicted that (i) heartworm infections would cause reduced nutritional status (indicated by body mass and bone marrow fat) and activity, owing to pathology-mediated reductions in foraging efficiency, and (ii) nutritional status would not affect intensity of infections, owing to the apparently small role of protective immunity in mediating heartworm infection.

Study area

The study was conducted at the University of California's Hopland Research and Extension Center (HREC; 39°00'N, 123°05'W), which is located in the Russian River drainage, and encompasses 21.7 km² of north coastal mountains ranging from 150 to 900 m in elevation. Primary vegetation types are oak (*Quercus* spp.) woodland, annual grassland, mixed evergreen-deciduous forest, and chaparral. Summers are hot and dry, and winters are mild and wet (Murphy and Heady 1983). The western tree-hole mosquito (*Aedes sierrensis*) is thought to be the primary heartworm vector in the area (Weinmann and Garcia 1974).

Methods

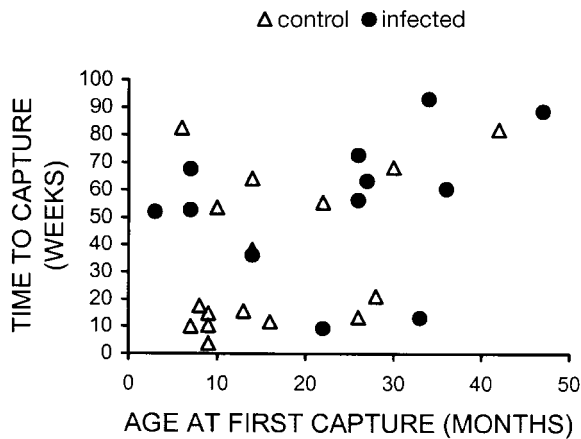
Coyote capture, radiotelemetry, and aging

Coyotes were captured during April 1993–July 1998 in No. 3 coil-spring leghold traps with padded jaws (Woodstream Corp., Littitz, Pa.) or in snares with stops to prevent strangulation. Coyotes were anesthetized with a 6.25:1 ketamine hydrochloride : xylazine hydrochloride mixture at approximately 10 mg/kg body mass, or restrained without chemicals by binding the muzzle and limbs with self-adhesive wrap. Coyotes were weighed, fitted with radio collars, and released at capture sites; after October 1995, transmitters were equipped with activity sensors (Advanced Telemetry Systems, Isanti, Minn.). Activity sensors caused transmitter pulse rates to increase in response to motion. A constant slow pulse indicated a motionless animal, a constant fast pulse indicated a highly active animal, and a variable pulse rate indicated moderate activity. Animal handling procedures were approved by the University of California at Berkeley Animal Care and Use Committee (protocol No. R190-0496).

Coyotes were monitored for activity during 1 to 3 observation periods (1 to 5 min)/day, 5 to 7 days/week. Radiotelemetry sampling was designed to maximize the number of independent locations (>6 h between locations) per coyote and represent time of day similarly among individuals. Activity data were coded as 0 (slow pulse rate), 1 (variable pulse rate), or 2 (fast pulse rate) for each observation period.

Coyotes were routinely killed by control personnel on HREC and surrounding lands, irrespective of whether they were radio-

Fig. 1. Time to recapture (by predator control personnel) versus age of coyotes in heartworm infected ($n = 12$) and control ($n = 16$) groups, illustrating that the probability of human capture was unaffected by heartworm status at the Hopland Research and Extension Center, California, August 1994 – July 1998.



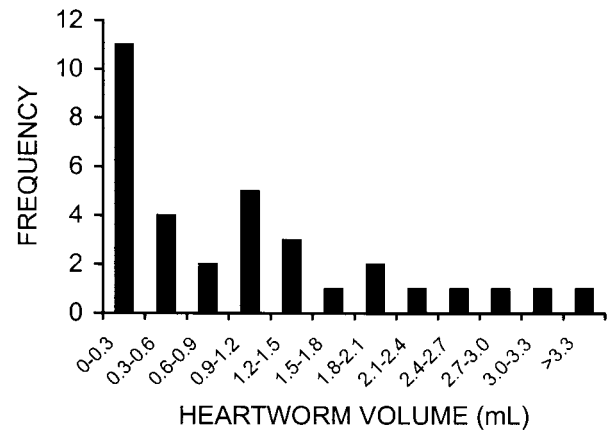
collared, to reduce predation on livestock (Sacks et al. 1999a). These coyotes composed most of the sample used in this study. Time to capture (by control personnel) of infected and uninfected radio-collared coyotes was similar, suggesting that this sampling approach was unbiased with respect to heartworm infection (Fig. 1). We estimated coyote ages to the nearest month using cementum annulus counts (Matson's Laboratory, Milltown, Mont.), using 1 April as the assumed whelping date (Sacks 1996, 1998).

Heartworms

Carcasses of coyotes were examined for adult heartworms; heartworms were counted (Sacks 1998) and their volume estimated as the difference in fluid level in a 5-mL graduated cylinder before and after submersion. We separated heartworms from each coyote into subgroups of 1 to approximately 10 individuals to standardize the volume measurement process; each subgroup was blotted firmly between sheets of absorbent paper before submersion. Voucher specimens were deposited in the U.S. National Parasite Collection (Animal Parasitology Institute, USDA Building 1180 BARC-East, Beltsville, MD 20705, U.S.A.; Accession No. 87091).

For statistical analyses, we used volume instead of number of heartworms as an index of parasite burden because volume was a function of both number and size of heartworms; heartworm size is related to the period of insult (Orihel 1961). Some analyses required that coyotes be grouped with respect to the intensity of their infections. Therefore, coyotes were divided into a control group composed of uninfected or lightly infected individuals ($\bar{x} \pm \text{SD} = 0.21 \pm 0.23$ mL, 3.6 ± 4.2 heartworms) and an infected group composed of individuals with moderate- to high-intensity infections (1.73 ± 0.73 mL, 33.6 ± 18.5 heartworms). Any choice of cutoff point for distinguishing these groups necessarily would have been somewhat arbitrary. We based our choice on the following: (i) the gap between control and infected groups (0.63–0.93 mL heartworms) was relatively large and corresponded to a natural break in our data set (Fig. 2) and (ii) although complicating factors preclude a simple dose–response relationship between heartworm burden and pathology in domestic dogs (Knight 1981), our criterion for infection was reasonable based on what is known about this relationship in dogs (Sutton 1988; Dillon et al. 1995). This breakdown also resulted in groups of approximately equal size. Comparisons between these groups were likely conservative because any non-linearity in the relationship between heartworm burden and host response would reduce statistical power.

Fig. 2. Frequency distribution of heartworm burden among 32 radio-collared coyotes, August 1994 – July 1998.



Body condition

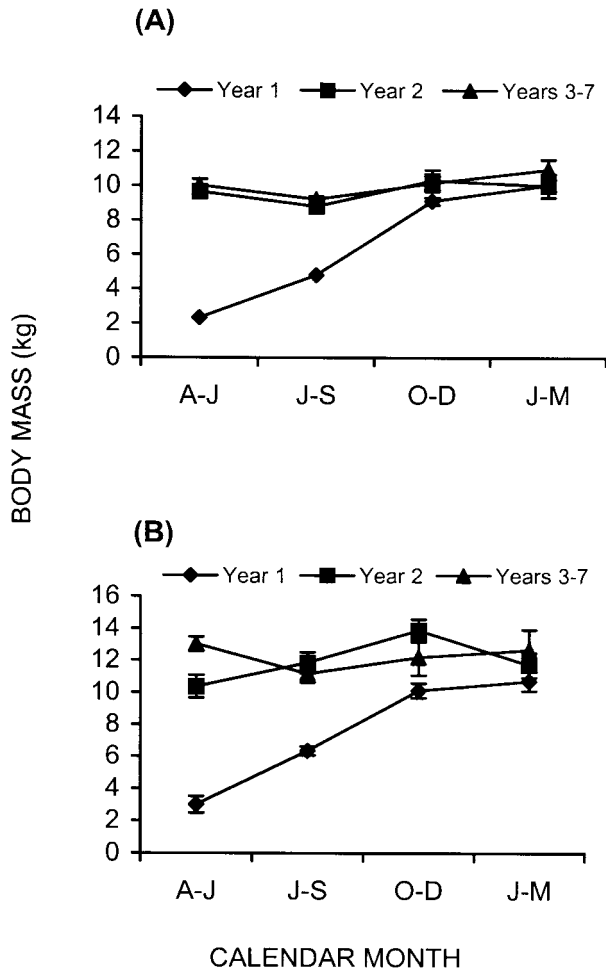
We examined two components of body condition related to protein-energy nutritional status: body mass and femur marrow fat (Gershwin et al. 1985). Although body mass involves several components (e.g., protein, water, fat, minerals), variation in body mass among individual coyotes primarily reflects differences in protein content (Huot et al. 1995). Femur marrow fat content is an indicator of body fat reserves (Huot et al. 1995). High marrow fat content does not necessarily indicate high overall body fat, but depletion of fat from bone marrow usually indicates extremely low body fat and protein-energy malnutrition (Windberg et al. 1991). Bone marrow fat was examined in coyote carcasses. Indexes of bone marrow fat were calculated as averages of color score (red = 1, pink = 2, white = 3) and consistency score (runny = 1, intermediate = 2, buttery = 3) of marrow from femurs and tibias (Anderson 1981).

To determine relationships between body mass and heartworm infection, we first needed to deal with potentially confounding effects of age and season. We used 3-month seasonal periods corresponding to the following biological periods: breeding (January–March), early pup-rearing (April–June), late pup-rearing (July–September), and prebreeding (October–December; Neale et al. 1998). Based on masses of coyotes taken at different times of the year from August 1992 to July 1998, we concluded that on average growth ceased at 12 months for females and at 15 months for males (Fig. 3). Therefore, only coyotes older than 12 (females) and 15 (males) months at the time of weighing were considered mature and used in body mass analyses.

Body mass of coyotes continued to change as a function of season (Fig. 3) and trends might have differed by sex, owing to physiological differences related to reproduction. We determined effects of season and sex on body mass using a two-way analysis of variance (ANOVA) and then multiplied body mass by correction factors to adjust for these effects. Specifically, correction factors were calculated as the ratio of the greatest group mean (season or season \times sex) to the mean for each group, so that the mean corrected body mass was equivalent among groups. Body mass is known to differ between the sexes (Bekoff 1982; Windberg et al. 1991) and was included only because of its possible interaction with season. Radio-collared coyotes and uncollared coyotes killed during removal operations were used in this analysis; multiple masses of some individuals were used.

We compared body mass at death between infected and control groups of coyotes, using a two-way ANOVA (including sex). However, lower body mass in infected than in control groups could indicate any of three possibilities: (1) that heartworm caused a loss in mass, (2) that coyotes with low mass were more susceptible to heartworm infection, or (3) that both of these effects were present.

Fig. 3. Body mass of coyotes during four seasons, August 1992 – July 1998. (A) Forty-six males weighed 64 times (average seasonal $n = 5.3$, range = 2–9). (B) Fifty females weighed 80 times (average seasonal $n = 6.7$, range = 1–16). Females were mature by the end of their first year, whereas males were not mature until July of their second year at ca. 15 months old. Although coyotes may have continued to grow after these points, seasonal changes became the primary temporal determinants of mass change. Error bars indicate 1 SE.



Therefore, to partially differentiate between these situations, we examined change in body mass over time of radio-collared coyotes. If heartworm caused a loss in mass (case 1 or 3), body mass of infected coyotes would be expected to decline over time as disease symptoms intensified, whereas if heartworm did not cause a loss in mass but coyotes with lower body mass were more susceptible to heartworm infection (case 2), no decline in body mass would be expected. We regressed the ratio of mass at death to previous live-capture masses on time between mass measurements for each group (infected and control) and then compared their slopes using Student's t distribution (Zar 1984, p. 292).

To determine whether individuals in poorer condition were more susceptible to heartworm infection (i.e., to confirm case 2 or differentiate between cases 1 and 3), we compared body mass at initial capture between infected and control groups of coyotes that were captured ≥ 2 times. We used a two-way ANOVA as described above. On average, individuals were captured for the first time 229 days (SD = 202 days) before death. We did not know the infection status of individuals at capture and it is certain that at least some of the individuals in the infected group (i.e., infected at death) were al-

ready infected during the initial capture, such a bias could serve to falsely inflate our statistical power (i.e., if heartworm caused a loss in mass). However, a negative result would imply that infection was independent of body condition.

Activity

To normalize the data, activity indexes were calculated as the square root of the average of activity observations (+0.5; Zar 1984). We first corrected activity observations for potentially confounding effects of season and time of day using a two-way ANOVA in an analogous procedure to that used above for body mass analyses. Factors included were season and diel period (04:00–09:59, 10:00–15:59, 16:00–21:59, and 22:00–03:59; Sacks 1996). Radio-collared coyotes were used in this analysis regardless of whether they were examined for heartworm (i.e., including radio-collared coyotes not killed during the study). Correction factors were then calculated accordingly for appropriate seasonal and (or) diel groups as the ratio of the greatest group mean to the mean for that group, so that mean corrected activity was equivalent among groups.

To investigate change over time in the relationship between heartworm burden and activity, we examined scatter plots of activity index on heartworm burden over each of the last 4 months of coyotes' lives. Activity indexes were also calculated for the last 2 months and previous 2 months (i.e., 2–4 months) of life for use in statistical analyses. These 2-month periods were chosen to maximize activity sample sizes and to ensure that the last period occurred close enough to the time of death for heartworm burdens to be meaningful.

We used regression analysis to determine potential effects of heartworm on activity during the last 2 months of life. Heartworm burden generally increases with age (Graham 1975; Custer and Pence 1981; Sacks 1998), which could potentially confound a relationship between burden and activity. Therefore, we included age (months) as an explanatory variable along with heartworm burden (mL) in the initial regression. Although age and burden were somewhat intercorrelated ($r^2 = 0.28$; $p = 0.008$), the magnitude of this was well within the acceptable range (Tabachnik and Fidell 1989).

We compared activity in the final 2 months of life to that in the previous 2 months to determine if activity decreased over time in infected and control groups. Similarly to body mass, if heartworm infection caused low activity, activity would be expected to decrease over time as symptoms progressed, whereas if heartworm infection did not cause low activity, but less active coyotes were more susceptible to heartworm infection, no decrease would be expected. In addition, reductions in activity observed in this analysis would necessarily have been post infection because infection occurs 6 months before adult heartworms are evident (Orihel 1961; Sutton 1988). We used one-tailed paired t tests to determine whether activity decreased over time (Zar 1984). To increase sample size, five additional coyotes that did not have activity sensors on their transmitters were used in this analysis. Activity for these individuals was recorded with the same coding system, except that values were based on amplitude fluctuation and bearing shift instead of pulse rate (Major 1983; Sacks 1996). Although this technique was subject to topographical bias, which precluded comparisons among individuals (Sacks 1996), this problem should not have affected the present analysis because increases or decreases in the activity of individuals were compared instead of absolute activity levels. Statistical significance was set at $\alpha = 0.05$ for all analyses and, unless otherwise indicated (above), the sample unit was the individual coyote.

Results

Body condition

We found no interaction between the effects of sex and season on body masses of 53 mature coyotes caught 82

Table 1. Seasonal body mass of adult coyotes and corresponding correction factors for four seasons, Hopland Research and Extension Center, California, November 1995 – July 1998.

Season	<i>n</i>	Female body mass (kg)		Male body mass (kg)		Average (kg)	Correction factor
		\bar{x}	SE	\bar{x}	SE		
Apr.–June	26	9.8	0.3	13.0	0.7	11.4	1.01
July–Sept.	18	8.9	0.3	11.4	0.5	10.1	1.14
Oct.–Dec.	16	10.2	0.3	12.9	0.4	11.6	1.00
Jan.–Mar.	22	10.6	0.5	12.1	0.4	11.4	1.02

Note: Sample size (*n*) refers to the number of measurements. The total number of individuals was 53. There was no interaction detected between season and sex, so correction factors were based on the average of male and female averages for each season.

times ($F_{[3,74]} = 1.57$, $p = 0.20$; Table 1). Main effects of sex ($F_{[1,74]} = 58.23$, $p < 0.001$) and season ($F_{[3,74]} = 3.70$, $p = 0.015$) were significant.

Body mass of coyotes at death was lower in the infected group ($\bar{x} \pm \text{SE} = 9.5 \pm 0.30$ and 12.2 ± 0.86 kg for females and males, respectively) than in the control group (10.8 ± 0.38 and 14.0 ± 0.51 kg; Fig. 4). No interaction between sex and group was detected ($F_{[1,31]} = 0.29$, $p = 0.59$). Main effects of both sex ($F_{[1,31]} = 38.7$, $p < 0.001$) and group ($F_{[1,31]} = 10.26$, $p = 0.003$) were significant. Rate of change in mass also differed between groups ($t_{[19]} = 3.05$, $p = 0.007$), yielding a net rate of loss in mass of approximately $20 \pm 11.2\%$ (95% confidence interval) per year in infected coyotes (Fig. 5A). Although individuals in the infected group tended to be older ($\bar{x} \pm \text{SE} = 36 \pm 19$ and 23 ± 8 months old for infected and control groups, respectively), age per se had no detectable effect on the change in mass (Fig. 5B). Fat content of bone marrow was negatively correlated with heartworm volume ($R^2 = 0.27$; $p = 0.009$; Fig. 6).

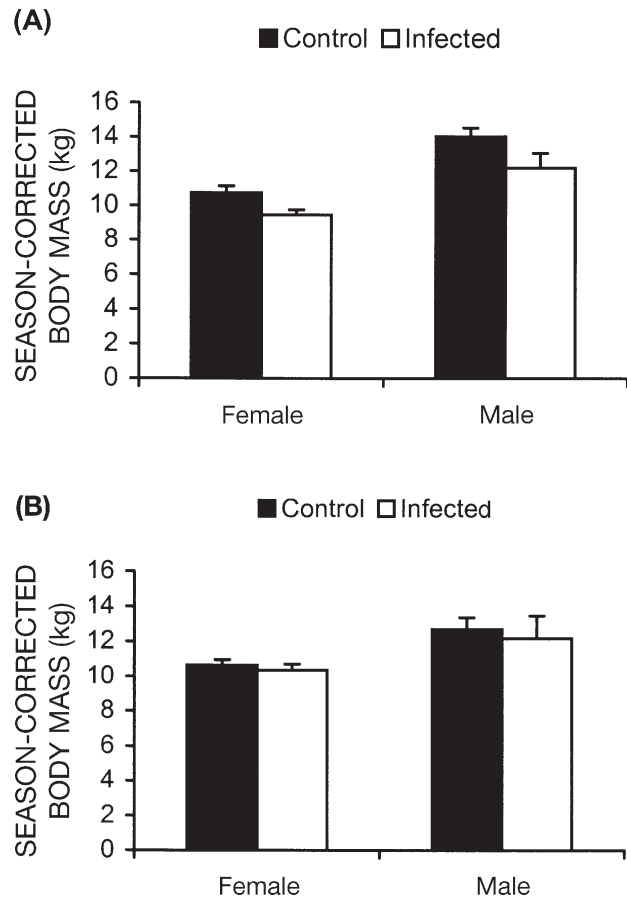
Body mass of coyotes at initial capture did not differ between the infected group (10.4 ± 0.33 and 12.2 ± 1.27 kg for females and males, respectively) and the control group (10.6 ± 0.31 and 12.1 ± 0.66 kg). No interaction between sex and group was detected ($F_{[1,28]} = 0.04$, $p = 0.84$). Main effects of sex ($F_{[1,28]} = 6.8$, $p = 0.015$), but not group ($F_{[1,28]} = 0.02$, $p = 0.90$) were significant. The statistical power of this analysis to detect a 1.5-kg difference (based on mean differences in mass at death) was 70% ($\alpha = 0.05$; Zar 1984, p. 227).

Activity

Forty-eight coyotes monitored between November 1995 and July 1998 wore radio collars containing activity sensors. We found no interaction between season and diel block on activity ($F_{[9,409]} = 1.57$, $p = 0.12$). Activity did not differ by season ($F_{[3,409]} = 0.39$, $p = 0.76$), but differed greatly by diel block ($F_{[3,409]} = 58.0$, $p < 0.001$; Table 2).

Thirty-two radio-collared coyotes were examined post mortem for heartworm (including five individuals without activity sensors and two that were not regularly monitored near death). Twenty-seven of these coyotes were killed by humans, one was killed by a mountain lion (*Felis concolor*), one was killed by a conspecific, one was killed by a blow to the chest (possibly a kick from a domestic llama), and two died of unknown causes.

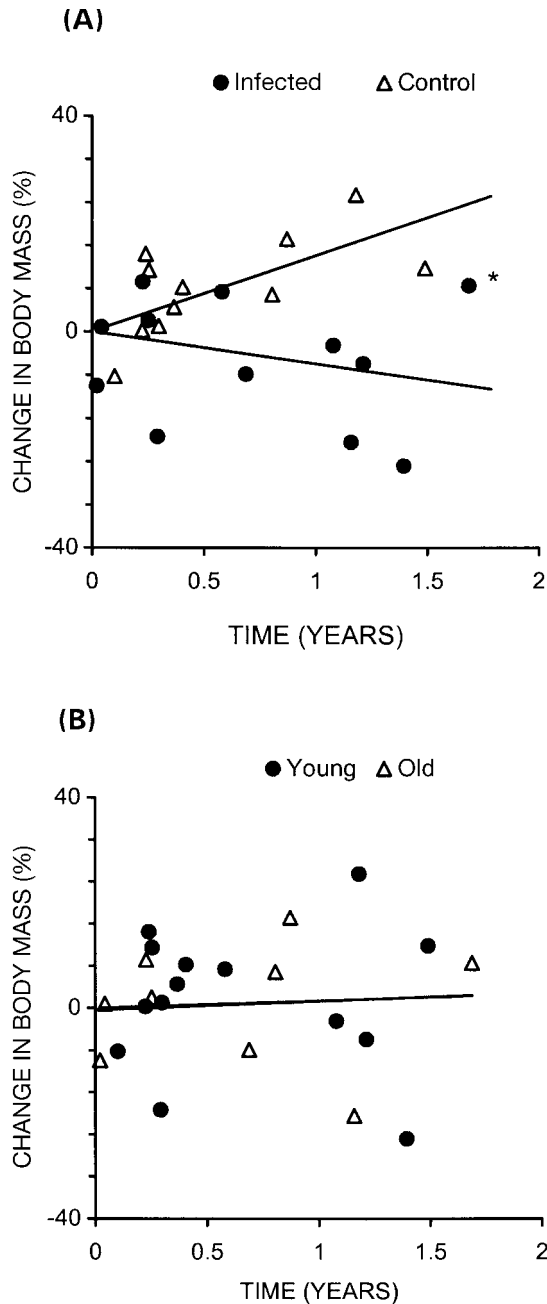
Fig. 4. Bar graphs demonstrating lower death masses of coyotes with moderate- to high-intensity heartworm infections at death than a control group infected with no or few heartworms at death (A) and no difference between initial capture masses of coyotes eventually infected versus a control group (B), August 1994 – July 1998. Sample sizes in the infected group were 12 and 10 females and 5 and 4 males (at death and before death, respectively). Sample sizes in the control group were 8 and 7 females and 10 and 8 males (at death and before death, respectively). Error bars indicate 1 SE.



An inverse relationship between activity and volume of heartworms at death was evident in each of the first 2 months before death, but not in the third or fourth months before death (Fig. 7). A regression of activity on heartworm volume and coyote age during the last 2 months of life was significant ($R^2 = 0.32$, $df = 21$, $p = 0.019$). Because only the *t* value for heartworm volume was significant ($t = -2.23$, $p = 0.036$ vs. $t = -0.66$, $p = 0.515$ for age), we regressed activity on heartworm volume alone ($R^2 = 0.30$, $df = 22$, $p = 0.005$; Fig. 8A). This model (adjusted $R^2 = 0.27$) fit at least as well as the one that included age (adjusted $R^2 = 0.25$). Although we used linear regression for simplicity, the relationship between activity and intensity of heartworm infection is probably better described by a curved function (Fig. 8). A similar relationship was evident when activity was plotted against number of heartworms, although one individual with 77 mostly immature heartworms was a clear exception (Fig. 8B).

The infected group showed a significant decline in activity ($\bar{x} = 19\%$, based on back-transformed indexes) from 2–

Fig. 5. Percent change in body mass over time of coyotes with moderate- to high-intensity heartworm infections (infected) compared with a control group infected with few or no heartworms (A) and in a younger (14–26 months old) versus an older (32–86 months old) group (B), August 1994 – July 1998. Equations in A were $y = 0.14x$ and $y = -0.06x$ for the control and infected groups, respectively. Trend lines were constrained to extend from the origin in this figure (A and B), but not in the analysis. Trend lines are identical for young and old groups (B). The asterisk indicates an individual with the lowest-intensity infection (12 heartworms, 1.0 mL) in the infected group (A).



4 months to 0–2 months before death ($t_{[12]} = -2.59$, one-tailed $p = 0.012$). The control group showed no difference in activity between these periods (0%; $t_{[12]} = -0.01$, one-tailed $p = 0.50$).

Fig. 6. Fat content in bone marrow versus intensity (volume) of heartworm infection in 24 coyotes, August 1994 – July 1998.

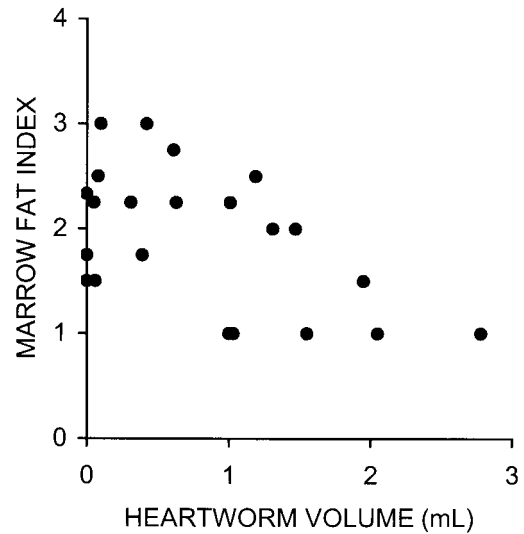


Table 2. Average activity indexes and corresponding correction factors for coyotes during four diel periods, November 1995 – July 1998.

Diel period	<i>n</i>	Average activity index ^a	SE	Correction factor
04:00–09:59	110	1.05	0.01	1.08
10:00–15:59	111	0.89	0.01	2.23
16:00–21:59	111	1.00	0.01	1.33
22:00–03:59	94	1.07	0.01	1.00

Note: Sample size (*n*) refers to the number of coyote seasons, which were distributed nearly evenly across seasons ($n = 20$ – 34 coyotes/diel block season).

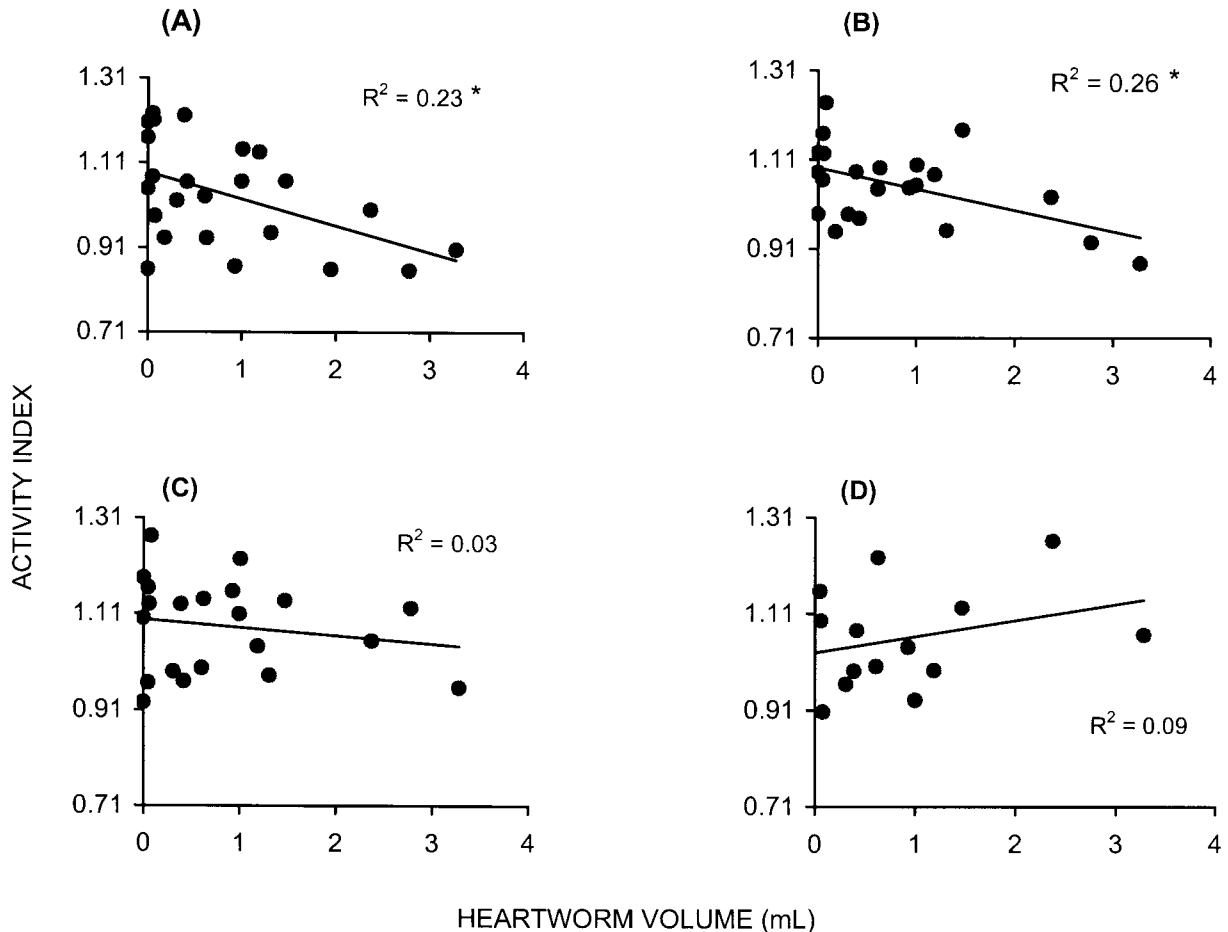
^aActivity indexes were calculated for each coyote as $(\text{average activity} + 0.5)^{1/2}$.

Discussion

Negative relationships between heartworm infection and body mass, marrow fat, and activity of coyotes at death clearly indicated an association between heartworm infection and poor condition in this study. Further, results strongly suggested that heartworm infection caused these symptoms and not the reverse. Specifically, activity of infected coyotes declined and body mass was lowest immediately before death, indicating that heartworm infection generally preceded these conditions and, therefore, these negative correlations were not due (at least solely) to increased susceptibility to infection of individuals in poorer nutritional condition. Indeed, we found no difference in body mass at initial capture of infected and control coyotes, despite the reasonably high statistical power of our test, indicating that nutritional condition had little, if any, effect on their initial susceptibility to heartworm infections.

At least two causal pathways potentially underlie our results (Fig. 9). One of these seems particularly well-supported; namely, heartworm reduced stamina through direct impairment of cardiopulmonary function, which in turn led to reduced activity and foraging efficiency. In domestic dogs, heartworm infection usually leads to some degree of cardio-

Fig. 7. Activity of coyotes versus intensity (volume) of heartworm infection at death, November 1995 – July 1998, 0–1 month (A), 1–2 month (B), and 2–3 months (C), and 3–4 months (D) premortem. Sample sizes were 24, 22, 21, and 14 coyotes in A–D, respectively. Asterisks indicate significant correlations ($p \leq 0.05$).



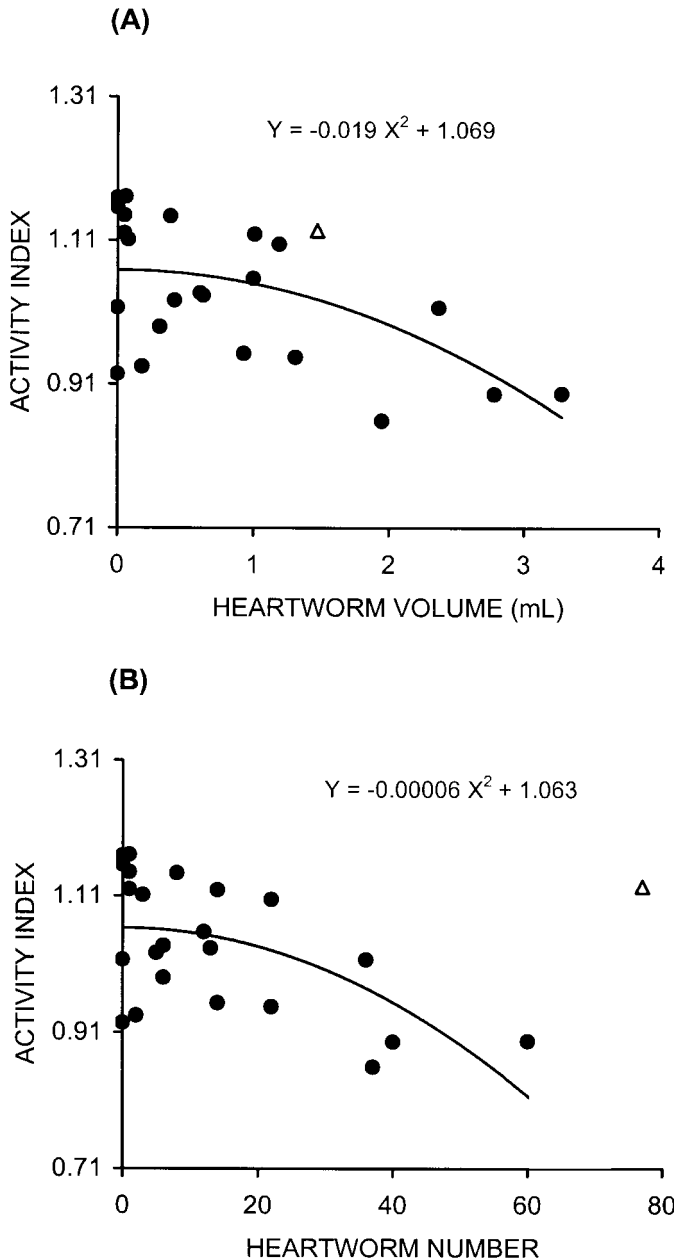
pulmonary pathology, accompanied by reduced stamina (Knight 1981; Sutton 1988; Rawlings and Calvert 1995; Martin and Corcoran 1997). Also, studies have found heartworm-associated organ pathology in coyotes to be similar to, albeit less severe than, the organ pathology in dogs (Crowell et al. 1978; Custer and Pence 1981), suggesting that coyotes may manifest similar symptoms to dogs. Furthermore, much of coyote activity (i.e., time when not resting) relates to food acquisition (Bekoff and Wells 1986; Gese et al. 1996), so that reduced activity of heartworm-infected coyotes likely translates to decreases in time spent foraging. Reduced stamina also would likely decrease ability of coyotes to capture or handle prey. Even predation on domestic sheep, which were abundant on HREC and lack many of the defenses of wild prey, requires considerable strength and endurance (Connolly et al. 1976). Indeed, two coyotes responsible for most predation on sheep during this study (Sacks et al. 1999b) had low heartworm burdens (i.e., were in the control group); interestingly, this observation contradicts previous speculation that heartworm-infected coyotes should be especially likely to prey on domestic sheep (Weinmann and Garcia 1980).

A second potential pathway is that heartworm reduced nutritional status of coyotes independently of foraging efficiency, which in turn led to reductions in activity associated

with lower energy levels (Fig. 9). Two potential mechanisms for parasite-caused reductions of nutritional status are increased metabolism (Hudson and Dobson 1995) and appetite suppression associated with infection (which is not restricted to gastrointestinal parasites; Crompton 1984). Both possibilities seem unlikely in this case because a loss in mass does not occur in domestic dogs infected with heartworm, unless they have unusually severe pathology (Rawlings 1986; Atwell 1988; Kitagawa et al. 1992; Rawlings and Calvert 1995). If typical heartworm infections substantially increase metabolism or cause considerable voluntary reductions in food intake, infected dogs would be expected to commonly exhibit at least some loss in mass even though they are typically fed ad libitum. That a loss in mass was a common symptom of heartworm infection in coyotes (i.e., in this study) but not in dogs is consistent with the first pathway (i.e., that reduced stamina led to reduced foraging efficiency in coyotes).

It is also conceivable that the loss in mass (and reduced activity) observed in this study indicated severely diseased animals, as it does in domestic dogs. A loss in mass in dogs with heartworm infections is usually associated with the terminal phase of the disease, arising from, for example, right-sided congestive heart failure or caval syndrome (Rawlings 1986; Atwell 1988; Atwell and Buoro 1988; Kitagawa et al. 1992; Rawlings and Calvert 1995). Although some individuals

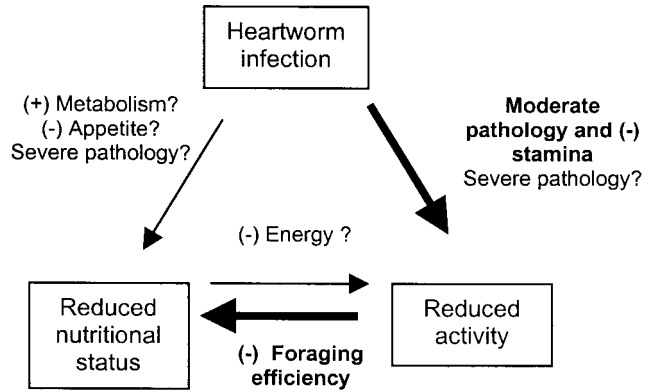
Fig. 8. Activity in the last 2 months of life versus volume of heartworms (A) and number of heartworms (B) found in 24 coyotes, November 1995 – July 1998. The open triangle in both graphs indicates an individual infected with primarily immature larvae; this outlier (in B only) was excluded for the purpose of parameterizing the equation. Note that the activity value of 0.71, as shown on this figure, is the transformed equivalent of zero activity.



in our study may have been terminally ill from heartworm disease, it seems unlikely that this would be the case for many of the heartworm-infected coyotes, particularly because a maximum of two (6%) individuals died as a direct result of heartworm disease (i.e., cause of death unknown).

Regardless of specific mechanisms underlying heartworm effects on coyote nutritional status, these effects undoubtedly have consequences for survival and reproduction of individ-

Fig. 9. Hypothetical causal pathways between heartworm infection, nutritional status, and activity of coyotes. Text shown in boldface type and thick arrows indicate relationships most likely to be primary, and thin arrows indicate relationships likely to be secondary or nonexistent.



uals. We restricted our investigation to sublethal effects (and causes) of heartworm infection on coyotes because mortality from humans was so high that few coyotes died of natural causes. However, others have speculated that heartworm is a significant mortality factor to these wild canids (Gier et al. 1978; Custer and Pence 1981), and our results do not contradict this. Indeed, there was limited evidence that heartworm infections may have been involved in four of the five natural deaths of coyotes observed during this study. First, both individuals that were killed by other carnivores had intense heartworm infections. If severely diseased coyotes had difficulty capturing prey, as suggested above, they might have been more inclined to risk death to scavenge the food of other carnivores. Previous reports of mountain lions killing coyotes often have attributed these events to the defense of food caches (Boyd and O’Gara 1985; Koehler and Hornocker 1991). Second, the two coyotes that died of unknown causes were also among the three oldest (6 and 7 years old, which is not especially old relative to coyotes in lightly exploited populations; Crabtree 1989; Windberg 1995), and pathology associated with heartworm disease in dogs intensifies with age (Sutton 1988; Martin and Corcoran 1997), suggesting that heartworm may have been a factor in their deaths, as well.

Although our prediction that nutritional status would not affect intensity of infections was supported in the present study, we cannot conclude that this prediction holds generally. Our failure to find an effect of nutrition on susceptibility could have simply indicated that coyotes generally had sufficient nourishment to be immunocompetent. Food is generally abundant on HREC (Neale 1996) and, as indicated by primary productivity, was particularly abundant during our study (Sacks 1996). It is unknown whether malnourished coyotes, which are probably more common where food is scarce, would be immunocompromised to the point where they would experience increased susceptibility to heartworm disease.

In conclusion, heartworm reduced body condition and activity of infected coyotes, and magnitude of effects increased with increasing intensity of infection. We speculate that heartworm infection reduces stamina of coyotes through direct

physical impairment, which in turn leads to reduced foraging efficiency, nutritional stress, and potentially death. Additional information is needed to determine the potential importance of synergistic effects of host nutritional status and heartworm disease under food-limited conditions.

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