UNIVERSITY OF CALIFORNIA

Santa Barbara

The Ecology and Transmission of the Raccoon Roundworm, *Baylisascaris procyonis.*

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution and Marine Biology

by

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ACKNOWLEDGEMENTS

I would like to thank undergraduates Adam Lee Lota, Amanda Tokuyama, Angela Bustamante, Antonia Sowunmi, Bex Girton, Camille Lake, Christopher Wessel, Elim Yee, Emily Lum, Gabriel Ruelas, Gabriela Dunn, Helena Montin, Jacey Van Wert, Jenna Rydz, Judy Ngo, Jon Francis Mendez, Michelle Armijo, Mona Luo, Rachel Lee, Savannah Perez, Scott Hannah, Tucker Grigsby, Willis Wong, and Yasmine Hays for assistance in the lab and field; Ecological Parasitology lab members John McLaughlin, Dana Morton, Ana Elisa Garcia Vedrenne, Clark Marino, Jasmine Childress, Alan Wood, Ian Cook, Julia Buck and Ryan Hechinger for support and discussion; fellow grad students Nate Emery, Mark Wilber, Andrew MacDonald, Sarah Sapp and Lauren Camp for discussions, collaborations and encouragement; Vasyl Tkach and Mike Kinsella for assistance with parasite identifications; Paul Collins at the Santa Barbara Museum of Natural History and Phillip Kahn at the Museum of Vertebrate Zoology for assistance with mammal specimen preparation; Cathi Arnold and Terry Marchiando for facilitating research; Robert Jacobs for the equipment to outfit the “raccoon cave”; Animal & Insect Pest Management, Inc. and Santa Barbara County Animal Control for help with specimen collection; the volunteers that provided blood samples and Pacific Diagnostic Laboratories, Cottage Health, and the Centers for Disease Control and Prevention for facilitating the human serology study; Coal Oil Point Reserve and Cristina Sandoval for facilitating fieldwork; financial support from the National Science Foundation Graduate Research Fellowships Program, the University of California Santa Barbara, and the America Society of Parasitologists; the incredible support of my family; and, of course, the years of patient mentoring from my advisers Kevin Lafferty and Armand Kuris and committee members Cheryl Briggs and Hillary Young.

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ABSTRACT

The Ecology and Transmission of the Raccoon Roundworm, *Baylisascaris procyonis.*

by

Sara Beth Weinstein

Emerging infections in both humans and wildlife can often be traced back to human mediated changes in host densities, host communities, and the environment. In Chapter 1, I reviewed how humans affect wildlife nematodes. Nematode responses to human actions vary, thus knowing host and parasite natural history, and the mechanisms underlying disease dynamics are critical for predicting parasite responses and managing disease.

Among wildlife nematodes, raccoon roundworm (*Baylisascaris procyonis*) is infamous for its ability to cause fatal disease in both humans and wildlife. This parasite infects millions of raccoons in North America. Although adult worms cause little pathology in raccoons, larval worms undergo extensive tissue migrations in other hosts, often causing neurological damage. Disease risk is driven by environmental egg contamination, which increases with raccoon density, worm intensity, and worm prevalence. In Chapter 2, I examined 189 raccoons from southern California to investigate how host age and season affect parasite abundance, demography, and fecundity. Roundworm infected 90% of Santa Barbara County raccoons, juveniles hosted more worms than adults, and more heavily infected raccoons released more eggs.

In Chapter 3, I investigated whether animals can avoid raccoon roundworm contaminated sites, and if such avoidance balances disease costs and foraging preferences. Using wildlife cameras, I monitored animal behavior at raccoon latrines — sites that concentrate both seeds and pathogenic parasite eggs, and found that latrine contact rates reflected background activity, diet preferences and disease risk. Disease-tolerant raccoons

and rats displayed significant site attraction, while susceptible birds and small mammals avoided these high-risk sites.

The introduced black rat, *Rattus rattus*, occurs throughout the native range of the raccoon roundworm, *Baylisascaris procyonis*, and frequently forages in latrines. In Chapter 4, I examined the role of these rats and other California rodents in *B. procyonis* transmission. I surveyed wild rodents for *B. procyonis* and found that *B. procyonis* infected *R. rattus* at intensities more than 100 times greater than loads in co-occurring native *Reithrodotomys megalotis* and *Peromyscus maniculatus*. I also conducted scavenger trials using motion activated cameras and found that rodent carcasses were scavenged by opossums, skunks and raccoons, suggesting that these infected rodents, particularly *R. rattus*, contribute to *B. procyonis* transmission in this coastal California ecosystem.

Raccoon roundworm infects both rodents and raccoons in southern California, but we know little about infection risk for other species. In Chapter 5, I used information on animal time allocation and behavior to build a model for predicting community-wide exposure risk for raccoon roundworm. This model suggests that larval worms are likely widespread in the animal community and provides a non-invasive method for identifying "at-risk" species.

Raccoon roundworm infection can cause devastating pathology in humans, but there is growing evidence that subclinical cases also occur. As there is limited information on the frequency of these subclinical human infections, in Chapter 6 I surveyed 150 adults from California for *B. procyonis* antibodies. Eleven participants were seropositive suggesting that subclinical infection does occur and that previously undetectable infections warrant further study.

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1. How do humans affect wildlife nematodes?

Abstract

Human actions can affect wildlife and their nematode parasites. Species introductions and human-facilitated range expansions can create new host-parasite interactions. Novel hosts can introduce parasites and have the potential to both amplify and dilute nematode transmission. Furthermore, humans can alter existing nematode dynamics by changing host densities and the abiotic conditions that affect larval parasite survival. Human impacts to wildlife might impair parasites by reducing the abundance of their hosts; however, domestic animal production and complex life cycles can maintain transmission even when wildlife becomes rare. Although wildlife nematodes have many possible responses to human actions, knowing host and parasite natural history, and the mechanisms behind the changing disease dynamics might improve disease control in the few cases where nematode parasitism impacts wildlife.

Humans alter infectious processes

Humans have long battled nematodes, and we have fared well, reducing soil-transmitted helminth prevalence and almost eradicating the human Guinea worm (*Dracunculus medinensis*) through intensive intervention (de Silva *et al.* 2003; Hopkins & Ruiz-Tiben 2011). While deliberate anti-parasite campaigns have reduced the human disease burden, introduced species, changing agricultural practices, and habitat degradation have unintentionally affected wildlife diseases. These effects come through two routes. First, humans create new host-parasite dynamics through host and parasite introductions. Second, humans alter existing transmission dynamics by changing host density, parasite survival, and

host-parasite contact rates. When such changes increase disease in wildlife or humans, parasitologists have the challenge of developing interventions that work in wildlife. Despite concern for human-mediated increases in wildlife disease, that is not the whole story; human activities can also unintentionally decrease parasitism.

In contrast to the 115 described human nematodes, the ~5,000 wildlife nematodes are still little known (Ashford & Crewe 2003; Hodda 2011). What we know well comes from work with domestic animals and a few natural host-parasite systems (e.g., grouse, arctic ungulates, raccoon roundworm). These well-studied systems reveal patterns and suggest predictions about wildlife nematodes in general (Rose *et al.* 2014). For example, the raccoon roundworm, *Baylisascaris procyonis*, is a model organism for comparing mechanisms that alter nematode transmission in a wildlife host that interacts with humans. Similarly, the domestic dog, *Canis familiaris*, and its relatively well studied parasite fauna provide a familiar example of spillover and spillback from an introduced host. Throughout this review we will highlight how these and other well-studied examples help us predict how human actions affect wildlife nematodes.

Creating new host-parasite interactions

Introduced species introduce parasites

Although invasive species lose many parasites compared to populations in their native ranges, an estimated 12% of parasite species do invade along with their hosts (Torchin *et al.* 2003). A case in point is the domestic dog which diverged from wolves over 20,000 years ago and then dispersed around the globe with humans (Thalmann *et al.* 2013). Of the 51 nematode species documented from domestic dogs, about 17 originated in dogs (Figure 1.1). Two thirds of those 17 nematodes occur in native wildlife and likely represent spillover from

dogs. Repeated host introductions, as has occurred for species such as dogs and rats, increase the probability that parasites will invade. For example, the exotic rat lungworm, *Angiostrongylus cantonensis*, now infects native wildlife, like the tawny frogmouth (*Podargus strigoides*) from once rat-free regions such as Australia (Ma *et al.* 2013; Qvarnstrom *et al.* 2013). With continued international trade and human movement, introductions like these will only accumulate with time.

An even greater source of introduced nematodes than accidental introductions is the commerce of pets and livestock. For example, the aquarium trade and mosquito control programs released guppies (*Poecilia reticulata*) and mosquito fish (*Gambusia affinis*) infected with the Asian nematode, *Camallanus cotti*, which is now common in endemic Hawaiian stream fishes (Font 1998). Similarly, Japanese eels (*Anguilla japonica*) imported to Europe for food introduced the swim-bladder worm, *Anguillicoloides crassus* to the endangered European eel (*Anguilla anguilla*). *Anguillicoloides crassus* is a good example of an introduced parasite that can impact wildlife health. Although infection in Japanese eels is asymptomatic, severe pathology may be contributing to spawning failure in European eels (Kirk 2003; Kennedy 2007). This increased pathology in the acquired host could stem from naïve hosts having little resistance or tolerance. We can't help but notice harmful introduced parasites (Strauss *et al.* 2012; Lymbery *et al.* 2014), but many others, like the gut parasite, *Trichuris muris*, which jumped from introduced black rats to native deer mice in the California Channel Islands (Smith & Carpenter 2006), cause little damage. Such host switching appears to be common in nematodes, and is likely to happen when hosts are related and use similar resources (Hoberg & Brooks 2008). Overall, although some introduced parasites impact native host populations; most introduced nematodes probably have minor impacts and go unnoticed.

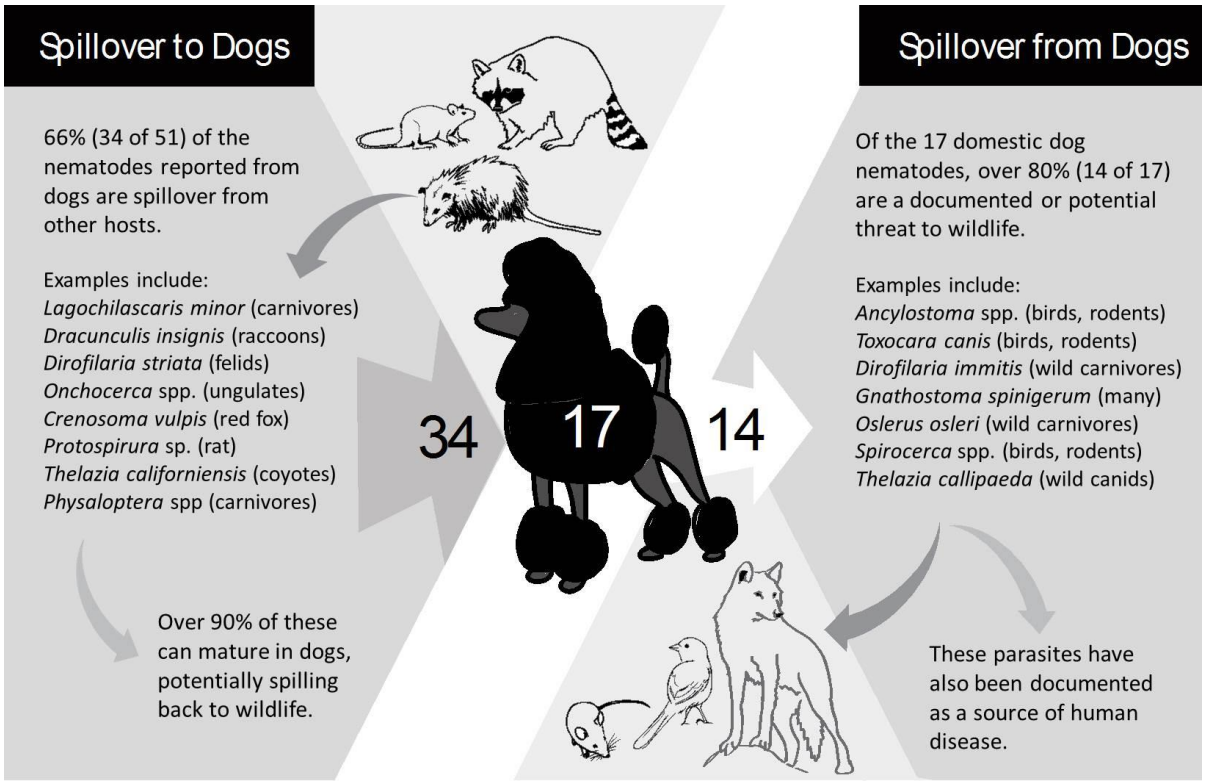

Figure 1.1: Domestic dog (*Canis familiaris*) nematode parasites: how common is parasite spillover?
The domestic dog is host to at least 51 parasitic nematodes. Two thirds of these infections are spillover from wildlife hosts and over 90% of these can mature in dogs and can spillback into wildlife. Although only one third of the nematodes recorded from dogs are dog parasites, 80% represent a disease risk for other wildlife. (Vincent et al. 1976; Hendrix et al. 1987; Panciera & Stockham 1988; Lanfredi et al. 1998; Anderson 2000; Bimi et al. 2005; Toparlak et al. 2005; Otranto et al. 2007; Sréter & Széll 2008; Shalaby et al. 2010; Simón et al. 2012; Eleni et al. 2014)

Introduced species alter disease dynamics of native nematodes

Introduced species can act as alternative hosts or reservoirs for endemic parasites, increasing transmission to native species via spillback (Kelly *et al.* 2009). Dogs have picked up most (~34 of 51) of their recorded parasite diversity from native hosts in their introduced range (Figure 1.1). For these native parasites, dogs increase total host density, which amplifies transmission back to native hosts. For example, the dog heartworm, *Dirofilaria*

immitis, was likely endemic in red wolf (*Canis rufus*) populations (Phillips & Scheck 1991; Simón *et al.* 2012). The parasite persisted even after the red wolf was declared extinct in the wild, suggesting that current infections in the recovering wolf population probably represent parasite spillback from domestic dogs (and the expanding coyote population) (1989; Phillips *et al.* 2003; Pedersen & Antonovics 2013). The spillover and spillback seen in dog and wildlife nematodes suggests that for every host introduction there is likely host switching as introduced parasites invade native hosts and native parasites colonize the introduced host. When introduced species amplify native parasite transmission, it reduces the threshold host density set by the native host, making it possible for sustained parasite transmission even if the native host declines. If an introduced host is more tolerant to infection than the native host, a parasite can theoretically drive its original host extinct (De Castro & Bolker 2005).

Fortunately, introduced species are often not suitable hosts for native parasite fauna (Torchin *et al.* 2003), and their presence, under certain conditions, might reduce transmission through a dilution effect. A dilution effect could occur if introduced hosts become a sink for infective stages or vectors. Knowing the extent to which infective stages limit nematode transmission is critical to understanding the potential for the dilution effect via introduced species. Transmission dilution through introduced species seems most likely to happen for nematodes with frequency-dependent transmission such as vector-transmitted filarial worms (Dobson 2004). For instance, because filarial worms are host specific in African rainforest birds (Sehgal *et al.* 2005), introduced bird species might (theoretically) divert blood-sucking flies, reducing vector transmission to competent native hosts. The dilution effect is often posited as a biodiversity benefit (Keesing *et al.* 2006). Ironically, it should be strongest when introduced species dominate communities (Telfer & Bown 2012).

Range expansion and human-mediated parasite spillover

Like species introductions, range expansion and increases in anthropophilic wildlife populations can lead to novel host-parasite interactions and conservation concerns for co-occurring species due to parasite spillover. Some parasite populations have benefitted from having human associated hosts, such as white-tailed deer (*Odocoileus virginianus*) and raccoons (*Procyon lotor*) (Gehrt 2003; Côté *et al.* 2004). White-tailed deer have expanded due to a decrease in hunting and reforestation, following a switch from firewood to fossil fuels. Deer now overlap with moose (*Alces alces*), and the spillover of the deer meningeal worm, *Parelaphostrongylus tenuis*, causes serious pathology and is predicted to cause moose declines where moose and deer now overlap (Schmitz & Nudds 1994; Lankester 2010). Similarly, raccoons and raccoon parasites have spread into eastern United States forests, resulting in Allegheny woodrat (*Neotoma magister*) extirpation (LoGiudice 2003). Woodrats contaminate their food caches with raccoon roundworm eggs as they feed on seeds in raccoon latrines; and then the migrating larval roundworms often kill them (LoGiudice 2001). Raccoon roundworm spillover into woodrats is a novel host-parasite interaction, and mammals and birds in Japan, Europe, and Russia may also be at risk (Figure 1.2). For both raccoon roundworm and the deer meningeal worm, host use is flexible for at least one life stage: the adult meningeal worm infects several ungulates, and larval raccoon roundworms have been recovered from over 100 bird and mammal species (Anderson 2000; Page 2013b). This broad host use increases spillover risk to both wildlife and humans in the parasite's native range and suggests that such nematodes might be the most successful and devastating invaders.

Parasite spillover from humans also threatens wildlife. Reverse zoonotic disease risk (aka "anthropozoonosis") increases as humans move into wildlife habitats (Messenger *et al.*

2014). Several human nematodes, including *Ascaris lumbricoides*, *Strongyloides stercoralis*, and *Trichuris trichiura,* infect non-human primates, especially where habitat alteration brings humans and wildlife into contact (Eley *et al.* 1989; Gillespie *et al.* 2010; Howells *et al.* 2011; Sá *et al.* 2013). For example, forest fragmentation leads to higher parasitism in red colobus monkeys (*Piliocolobus tephrosceles*) due to spillover of strongyle nematodes from villagers (Gillespie & Chapman 2006). However, parasites that use human and non-human primates might not always engage in cross-species transmission. For example, the strongyle *Oesophagostomum bifurcum* infects patas monkeys (*Erythrocebus patas*), mona monkeys (*Cercopithecus mona*), olive baboons (*Papio anubis*), and humans in Ghana. However, each parasite population is structured according to host species (de Gruijter *et al.* 2005). Although proximity to humans should increase human parasite spillover, concurrent changes in wildlife behavior might reduce parasitism. The reduced worm burden in baboons that raid human crops is attributed to reduced exposure to trophically-transmitted infective stages in wild prey and increased resistance due to better body condition (Weyher *et al.* 2006). Given the frequency that human nematodes are detected in wild primates, campaigns to reduce nematode infections in humans could also reduce nematode spillover into wildlife, leading to a win-win for biodiversity and human health.

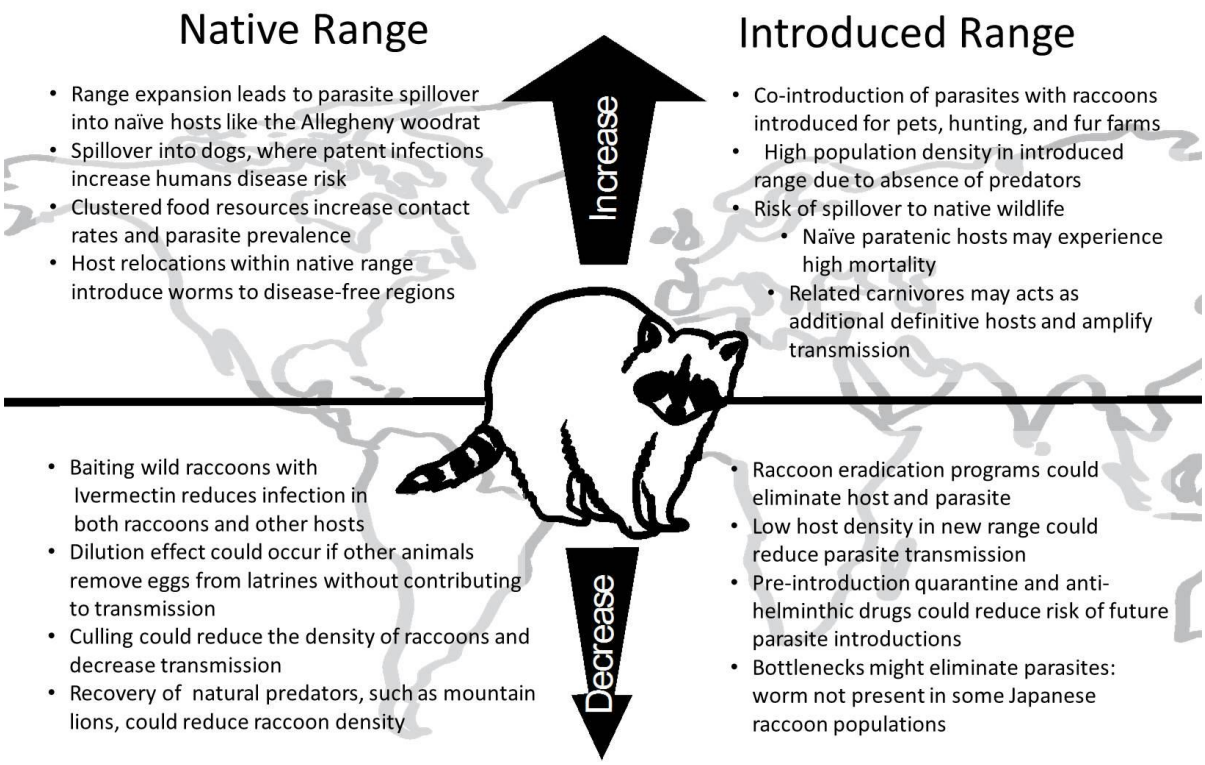

Figure 1.2: Raccoon roundworm (*Baylisascaris procyonis*): A threat to wildlife in both the native and introduced raccoon range.
Human impacts can alter invasion and infection dynamics of the raccoon roundworm, in both its native and introduced ranges, resulting in either amplified or reduced transmission risk to wildlife and humans (Bowman 2000; Kazacos 2001; LoGiudice 2003; Wright & Gompper 2005; Matoba *et al.* 2006; Blizzard *et al.* 2010b; Page *et al.* 2011b)

Changing dynamics in established host-parasite interactions

Parasites thrive in intact ecosystems (Hudson *et al.* 2006). However, humans often alter natural host-parasite dynamics, which are a complex function of host-parasite contact rates, host density, and parasite survival. Human impacts that increase any of these parameters might also increase wildlife parasitism (Arneberg *et al.* 1998).

Contact rates

Human actions can increase disease transmission through increasing host species' contact rates. Contact rates increase when wildlife, like raccoons, aggregate around supplemental food resources, leading to increased parasite transmission (Wright & Gompper 2005). Similarly, Bahamian rock iguanas (*Cyclura cychlura*) aggregate at sites where tourists feed them, and this correlates with higher hookworm and pinworm prevalence and intensity (Knapp *et al.* 2013). The impact of supplemental feeding on wildlife is likely a tradeoff for wildlife health; additional calories might support a larger population, however, when food resources are clumped, increased contact rates are likely to increase parasite transmission. The net effect will depend on the host's tolerance to infection.

Habitat fragmentation also alters transmission dynamics. For example, mice are more exposed to raccoon roundworm in a fragmented agricultural landscape due to changes in the feeding habits in both raccoons and mice. Raccoons forage on crops, and then, when mice forage in raccoon latrines for undigested corn, the increased contact with infective stages results in higher raccoon roundworm prevalence in mice (Page *et al.* 2001a). Even depleted species can have high local densities in a patchy landscape. For instance, habitat fragmentation has concentrated endangered colobus and mangabey monkeys, and this leads to a higher gastrointestinal nematode prevalence (Mbora & McPeek 2009). Although any increase in contact rates should increase parasitism, this is most worrisome when conditions concentrate threatened species and the nematode is pathogenic. When habitat fragmentation increases aggregation, threatened species do not gain the health benefits associated with rarity.

Host density

Wildlife declines due to hunting, fishing, and habitat degradation, should reduce, or even eliminate host-specific nematodes (Dobson & May 1987). A striking example is when the swim bladder nematode, *Cystidicola stigmatura*, disappeared from lake trout (*Salvelinus namaycush*) following overfishing (Black 1983, 1985). In general, parasites with complex life cycles decline under fishing pressure (Wood & Lafferty 2014). Moreover, in primates, threatened hosts harbor fewer parasite species, suggesting that parasites are less likely to persist in small, isolated host populations (Altizer *et al.* 2007). Host-specific nematodes should be highly sensitive to host population changes, and these relationships become more complex when additional hosts are present in the lifecycle. The more complex the life cycle, the more chances there are for transmission to fail (Rudolf & Lafferty 2011).

Even though complex life cycles might be more difficult to complete, increased intermediate host and vector density can increase disease even when wildlife host populations are stable or in decline. Arthropod vectors often proliferate in response to dams and changing agricultural practices. Such human modifications can increase filarial nematode infection in humans (Patz *et al.* 2000) and presumably could for wildlife. Furthermore, at high latitudes, mosquito populations increase with recent increases in temperature and humidity. A warmer climate seems to increase exposure of moose and reindeer to the filarial nematode, *Setaria tundra*, by fueling mosquito abundance and driving ungulates into swampier microhabitats where transmission occurs (Laaksonen *et al.* 2010). Yet another example is that increasing temperatures result in higher muskox (*Ovibos moschatus*) parasitism by the nematode *Umingmakstrongylus pallikuukensis*, because rising temperatures both increase larval parasite development rate and reduce winter mortality of the intermediate-host slug (Kutz *et al.* 2005; Jenkins *et al.* 2006). Eutrophication is another environmental factor that can favor

intermediate hosts such as oligochaetes. Oligochaetes infected with *Eustrongyloides* nematodes are eaten by fish, which, in turn, are eaten by birds, and the resulting trophic transmission sequence leads to high infection intensity and increased nestling mortality for birds at eutrophic sites (Spalding & Forrester 2009). For these reasons, humans often affect wildlife diseases indirectly through their effects on intermediate hosts and vectors (Dobson & May 1986).

Additional alternative definitive host species can also increase disease impacts, because parasite population dynamics are become less linked to the density of a single host species. For example, the cosmopolitan bird parasite, *Dispharynx spiralis*, is present in endemic Galapagos finches, and although the nematode's origin is unclear, disease risk in native birds increases due to spillover from the high worm burdens in island chickens (*Gallus gallus domesticus*). Parasitism is high in the chickens due to host biology, feeding habits, and captive-rearing methods, and these domestic fowl act as a reservoir for the nematode (Gottdenker *et al.* 2005). Because there are now around 23 billion domestic fowl and five billion domestic ungulates on earth (FAO 2014) and most domestic animal nematodes are generalists that can spillover to wildlife (Walker & Morgan 2014) animal husbandry could be the main way that human actions put wildlife at risk to parasitic nematodes.

Larval parasite survival

Disease transmission should decrease when abiotic conditions reduce larval parasite survival (Hoberg *et al.* 2008; Marcogliese 2008; Lafferty 2009). However, such consequences are less likely when crowding or acquired immunity plays a strong regulating role in the parasite population, as seen by comparing the rabbit stomach worm, *Graphidum strigosum* and the rabbit intestinal worm, *Trichostrongylus retortaeformis* (Hudson *et al.*

2006). In other words, parasite intensity is less sensitive to variation in exposure rates when other factors limit infrapopulation abundance.

Because infective stages like nematode eggs and larvae are exposed to environmental conditions, each species should evolve an optimal physiological tolerance for development and survival. Climate change shifts an organism's optimal temperature to higher latitudes, and might allow parasites and their intermediate hosts or vectors to invade from lower latitudes (Kutz *et al.* 2005; Hernandez *et al.* 2013a). At the same time, warming should hasten the mortality rate of eggs and larvae of locally adapted parasites, perhaps excluding them over time. Similarly, unfavorable abiotic conditions associated with environmental degradation should reduce juvenile parasite survival and decrease parasite transmission. For example, petrochemical pollution is correlated with decreased helminth infection in the cotton rat, *Sigmodon hispidus,* via both reducing arthropod intermediate host and free-living nematode juvenile survival (Faulkner & Lochmiller 2000). Furthermore, annual burning and herbicide treatments reduce *Syphacia peromysci* and *Nippostrongylus muris* infections in the white-footed mouse, *Peromyscus leucopus,* by altering mouse behavior and parasite free-living stage survival (Boren *et al.* 1993). However, environmental stressors that increase infective-stage mortality might also stress hosts and increase susceptibility to infection. In addition, stress could reduce the survivorship of infected hosts. Consequently, the idiosyncratic impact on reduced parasite and host survival, versus increased host susceptibility, will determine whether environmental stress reduces or facilitates parasites, but most stressors probably result in parasite declines (Lafferty & Holt 2003).

Implications for managing wildlife nematodes

For those cases where human actions increase parasitic nematodes in wildlife, effective mitigation at the host population level will require understanding the most important transmission pathways. If an introduced parasite spills over to native wildlife, culling or eradicating the introduced host might reduce the parasite in wildlife hosts. For parasites that spillover from pets or livestock, giving more antihelminthics to domestic animals might be merited. However, once the parasite establishes transmission within the native fauna, control strategies are much more limited. Baits with antihelminthics are one possible way to control nematodes. Raccoon antihelminthic baiting can reduce roundworm prevalence in both raccoons and rodents and fenbendazole laced salt licks can reduce lungworm prevalence in bighorn sheep (*Ovis canadensis*) (Goldstein *et al.* 2005; Page *et al.* 2011a; Page *et al.* 2014). Such baiting might be easier when wildlife aggregate into reserves or habitat fragments. Although humans introduce many nematodes and alter wildlife-nematode dynamics, impacts to wildlife health are not often noticeable. In part, this is because parasitic nematodes are natural parts of intact ecosystems, and their effects are mostly minor compared with other challenges in wildlife conservation. In those cases where human impacts do increase nematode parasites in wildlife, a better understanding of parasite ecology can point wildlife managers to mitigating solutions.

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2. *Baylisascaris procyonis* demography and egg production in a California raccoon population

Abstract

California has more reported human raccoon roundworm cases than any other state, due to large and overlapping human and raccoon populations. Infection by this parasite, *Baylisascaris procyonis*, is relatively benign in raccoons, but can cause severe pathology in other species. Disease risk is driven by environmental egg contamination, which increases with raccoon density, worm intensity, and worm prevalence. To improve knowledge about risk to humans and other species, 189 raccoons from southern California were examined to investigate how host age and season affect worm abundance, demography, and fecundity. Adult worms were present in animals as young as 10 weeks and 100% of 4-month old raccoons were infected. Although 80% of sampled raccoons hosted adult *B. procyonis*, prevalence and abundance were lower in older animals. There were more worms in juvenile than in adult raccoons, resulting in a convex age-intensity profile. Coupled with raccoon demography, this drove fall peaks in parasite abundance and egg production. Eggs per-gram feces averaged 4,606 ± 661 (SE), and this output increased with worm intensity with no evidence that crowding reduced parasite size or fecundity. High parasite egg outputs from hosts in this California raccoon population increase human exposure risk, and this risk could be reduced by management strategies that target heavily infected juvenile raccoons.

Introduction

There are 5 million raccoons in North America, and most host the raccoon roundworm, *Baylisascaris procyonis.* This parasite can cause severe, even fatal, disease in other animals (Riley *et al.* 1998; Gehrt 2003). Infection has been linked to declines in the threatened Allegheny woodrat and is an increasingly recognized human zoonosis (Kazacos 2001; Sorvillo *et al.* 2002; Gavin *et al.* 2005; Page 2013b). As raccoon populations expand, information on host and parasite demography may illuminate better management of this zoonotic parasite.

The zoonotic potential of *Baylisascaris procyonis* derives from its complex lifecycle, in which raccoons are the definitive host. Living in the raccoon small intestine, female worms release eggs into the environment with host feces (Snyder & Fitzgerald 1987). These eggs concentrate at communal raccoon defecation sites, termed latrines, and become infective after approximately 2 weeks of development. If ingested by a young raccoon, juvenile worms hatch from these eggs, remain in the gut and develop into adults. If ingested by another species, such as a rodent or bird, worms leave the gut and migrate through host tissue until impeded by a host response (Sprent 1952). If an infected host is eaten by a raccoon, juvenile worms will then develop into egg-producing adults (Kazacos 2001) and contact with these eggs exposes both humans and other animals to baylisascariasis.

Baylisascariasis risk varies by season and region, and is greatest in areas with high raccoon density, parasite prevalence and intensity. Prevalence and intensity vary with land use, raccoon abundance and geographic region (Page et al., 2008, 2009). In the United States, prevalence is lowest in the south and highest in the midwest and along the west coast (Kazacos 2001). In northern regions, harsh winter conditions are thought to reduce parasite survival and contribute to annual cycles in parasite abundance (Kidder *et al.* 1989; Sarkissian

et al. 2015). However, a fall peak in parasitism also occurs in raccoons from milder climates (Evans 2002b), suggesting that infection seasonality could also be driven by other factors such as host age structure.

Raccoons are born in the spring (Gehrt 2003), and by fall, this juvenile host cohort is heavily infected with roundworms. Raccoons under 4 months of age ("pre-weaned") are susceptible to infection via eggs and acquire infections prior to leaving their natal den. Due to either age-based resistance or acquired immunity, raccoons older than 4 months ("weaned") are no longer susceptible to eggs but can be infected by eating paratenic hosts (Kazacos 1983b, 2001; Reed *et al.* 2012). Although parasite intensity and prevalence are consistently lower in adult raccoons (Kazacos 2001), the processes that generate these patterns remain unclear as ascarids are long lived (Olsen *et al.* 1958) and all raccoons over a year are typically categorized as adults (Snyder & Fitzgerald 1985, 1987; Blizzard *et al.* 2010a; Hernandez *et al.* 2013b). Infection in adult raccoons could result from high residual burdens in only 1 to 2 year old animals with no further transmission to older individuals, or by new infections acquired at lower rates across all adult age classes. Distinguishing between a continuous low reinfection rate and a lack of transmission to adults requires well-resolved raccoon age intensity profiles, coupled with adult and immature parasite intensity data. J4 and small immature worms are often not included in raccoon roundworm surveys; however, analyses that include these more ephemeral stages could provide insight into the timing of transmission, intensity-dependent parasite establishment, and seasonal patterns in disease risk.

Disease risk in humans, raccoons, and other wildlife increases with environmental egg contamination. Infected raccoons shed 20,000-26,000 eggs per gram feces, with single worms producing 115,000-179,000 eggs per day (Kazacos 1982; Snyder & Fitzgerald 1987).

Although egg production is expected to increase with worm burden, parasite fecundity could be limited by crowding effects as in other nematodes (Sinniah & Subramaniam 1991; Tompkins & Hudson 1999; Irvine *et al.* 2001; Walker *et al.* 2009; Romeo *et al.* 2014). Mature female *B. procyonis* range in length from 7 to 20 cm (Kazacos 2001); however, the relationship between parasite size, fecundity and crowding is unknown. If crowding occurs, then parasite size, fecundity and longevity could decline with intensity.

Raccoons from coastal southern California were examined to explore relationships between host age, season and parasite demography. Roundworm prevalence and abundance peaked in juvenile raccoons, contributing to seasonal infection patterns. Infection intensity increased egg production with no evidence of a crowding effect, even in high-intensity infections. These results reveal a heavily infected area in coastal California, and suggest that young raccoons should be targeted to reduce risk of parasite spillover to humans.

Materials and Methods

To document host and parasite demography, I counted and measured *B. procyonis* from wild raccoons and then aged these hosts using skull morphology and dentition. I received 189 raccoons from trappers (n = 176) or as road kill (n = 13) in Santa Barbara County, California from 2012 to 2015. To age hosts, skulls were cleaned by dermestid beetles and age was determined using suture closure (Junge & Hoffmeister 1980), tooth eruption (Montgomery 1964), and tooth wear (Grau *et al.* 1970). As both male and female raccoons reached adult size when about 8 months old (Figure 2.1), skull morphology, not host size or sexual maturity, was used to determine age. Age was binned into 8 similar sized classes (based on host development and aging accuracy) to calculate age-dependent prevalence.

To determine parasite presence and abundance, animals were either dissected immediately or frozen before processing. Each host was measured and sexed and then all *B. procyonis* were recovered from the intestines. After all visible worms were removed from the gut, for 183 hosts the remaining intestinal contents were collected by dividing the gut into sections and then scraping and washing the material from each section into a beaker of saline. This solution was decanted several times to remove fine particulate matter, rinsed with near-boiling water in the final decanting step to kill eggs, and then preserved in 70% ethanol. These preserved gut contents were then sorted under 12-30x magnification and all immature worms were removed, counted and measured.

Worms were classified as immature or adult based on gonad presence and then adult and immature worms were analyzed separately. Parasite prevalence, intensity, and abundance were calculated following Bush et al. (1997) and the relationships between adult parasite abundance and season, host age and sex for pre-weaned (≤4 months) and weaned (>4 months) animals were analyzed using generalized linear models with a log link function and a negative binomial error distribution to account for over-dispersion. Immature parasite abundance patterns were examined with the same models used for adult worms, including adult parasite abundance as a covariate to test for concomitant immunity. All statistics were done in R 3.3.0 (R Core Team 2016) and mean abundance was reported with non-parametric bootstrapped 95% confidence intervals calculated in the R package "boot" (Canty & Ripley 2016) and prevalence was reported with 95% Bayesian confidence intervals estimated with the "prevalence" package (Devleesschauwer *et al.* 2014). Model selection was done with backward stepwise regression using the F test. These models were then used to examine the relationship between season, sex and host age and adult and immature parasite abundance.

Immature worm counts included both J4 and developing adults, however intensity dependent expulsion is most likely to affect late J4 stages (Roepstorff *et al.* 1997; Morimoto *et al.* 2003). To differentiate fourth and fifth stage *B. procyonis*, 7 worms ranging in size from 2 mm to 50 mm were mounted on graphite and examined with environmental scanning electron microscopy. Using identified differences in cuticle structure (Figure 2.2), an additional 95 worms were then staged and measured to determine average size at 4th molt. As late J4 and early fifth stage worms overlapped in size, to test for evidence of intensity dependent parasite establishment, I examined the relationship between the presence and number of worms less than 10mm (J4), and the presence and number of established worms greater than 30 mm. I used generalized linear models with negative binomial error distributions for abundance data and logistic regressions for presence/absence data. I included host age and season in weaned raccoon models, but used only host age in pre-weaned models because age and season were highly correlated (adjusted $R^2 = 0.36$, $p < 0.001$) in these very young animals. As developing worms reach patency in 1-2 months (Kazacos 2001), these immature stages provide more insight into transmission timing than would be gained from analyzing only long lived adult parasites.

After examining intensity dependent effects on parasite establishment, I used adult parasite measurements to test for crowding effects in established infections. I weighed all adult female worms (n = 1,916) to the nearest 0.001g and measured all adult worms (with gonads present, n = 2,783) to the nearest millimeter. I established that worm size was a proxy for fecundity by examining the relationship between worm size, uterus weight, and in-utero egg counts. For 471 female worms from 48 hosts, I preserved worms in 5% formalin and then removed and weighed the uterus. For 27 worms from 11 hosts, the whole uterus was homogenized using a diluted (5%) bleach solution in a Tenbroek tissue grinder (Wheaton,

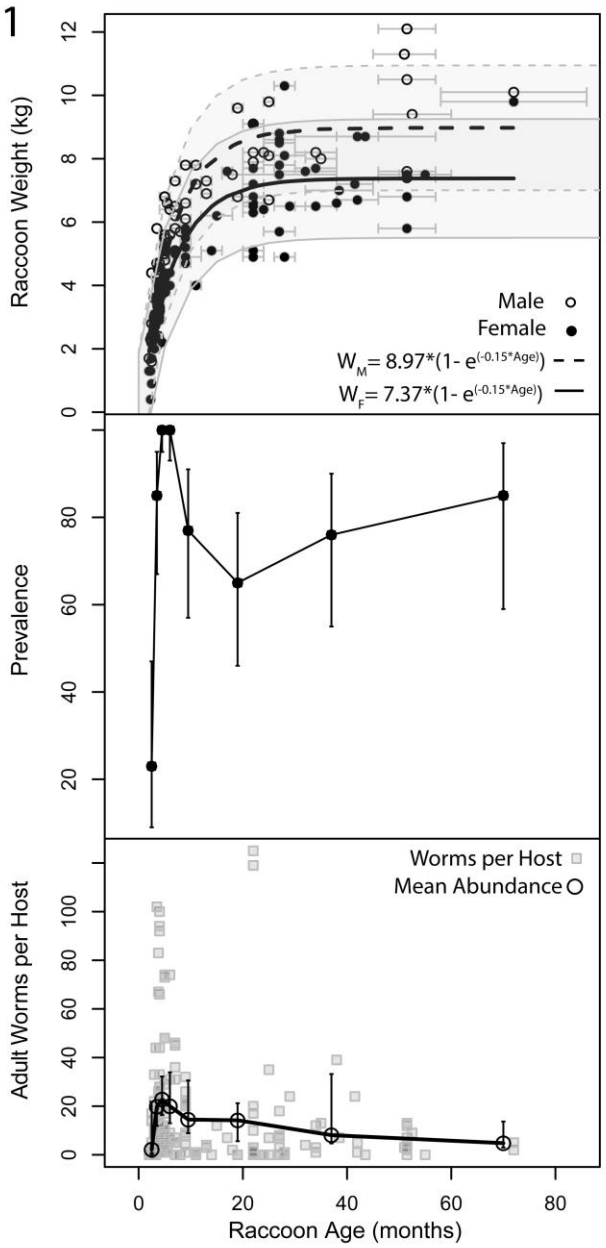
Millville, New Jersey) and filtered through 625 micron mesh. Eggs were counted using 50 to 60 grid cells on a Sedgwick counter slide (Wildco, Yulee, Florida) and mean eggs per grid cell then used to calculate total uterine eggs. I used linear models to first establish the relationships between worm size, uterus weight, and uterine egg counts, log transforming data to normalize residuals when needed. I then tested for crowding effects on reproductive allocation by examining how parasite intensity and female worm biomass affected uterus weight, including host age and season as covariates in linear models. Then, to test for crowding effects on worm size, I examined how parasite intensity and biomass affected mean worm length, again including host age and season as covariates.

To measure egg production and test for crowding effects on parasite fecundity, a fecal sample was collected from 170 hosts and then eggs per-gram (EPG) feces were counted using the McMaster technique (Page *et al.* 2005; Zajac & Conboy 2006). Feces (4 g) were mixed with Sheather's sugar solution (26 milliliters, specific gravity 1.27), aliquoted into 1.5 ml tubes and stored at 4 C before counting. For each host, 2 to 4 samples were counted by filling a 2-chambered McMaster slide (Chalex Corporation, Portland, Oregon) with 30 ml of well-mixed solution, letting eggs rise for 30 min, and then searching for eggs under 40-100x magnification. Eggs per-gram feces was calculated by multiplying the averaged egg counts of each host by the dilution factor (fecal sugar solution volume/subsample volume) and dividing this by fecal sample weight. Fecal egg counts were square-root transformed to meet linear regression assumptions, and then linear models were used to establish a relationship between female parasite intensity (and biomass), host age class and eggs per-gram feces. Crowding effects on parasite fecundity were then tested for by examining how EPG per female worm varied with parasite intensity (Paterson & Viney 2003; Romeo *et al.* 2014).

Results

In this California raccoon population, host age and seasonal factors drove annual patterns in *B. procyonis* abundance (Figure 2.1). Infected raccoons hosted 1 to 125 adult worms (mean intensity: 18.8, standard deviation (SD): 23.6) with adult roundworm present in 152 (80.4%) of 189 examined raccoons (Table 2.1). Sampled raccoons ranged in age from 2 months to over 8 years old however 68% of animals were under a year old. Parasite prevalence and abundance (Figure 2.1) varied with host age with lower mean intensity in adult raccoons (13.7 ± (SD) 25.2) than in juveniles (age <12 months, 21.1 ± (SD) 23.1). Animals as young as 10 weeks harbored adult worms and released eggs, and for animals 4 months and younger, the best parasite abundance model included only host age (GLM, $\beta = 1.32$, $p < 0.0001$). In these pre-weaned hosts, abundance increased with age ($\beta = -0.024$, $p = 0.0035$) and all animals 4 to 7 months old hosted at least 1 adult *B. procyonis*. Although seasonal infection patterns in pre-weaned raccoons disappeared once age was included in statistical models, among weaned animals, adult parasite abundance was significantly lower in spring compared to other seasons ($\beta = -1.02$, $p = 0.01$).

Figure 2.1: *Baylisascaris procyonis* in raccoons.
Raccoon weight increased with host age, with males generally larger than females. Animal age is plotted with error bars representing the estimated age range (top). Mean *Baylisascaris procyonis* prevalence (middle) and abundance (bottom) increased with age for juvenile raccoons and then declined in adults.



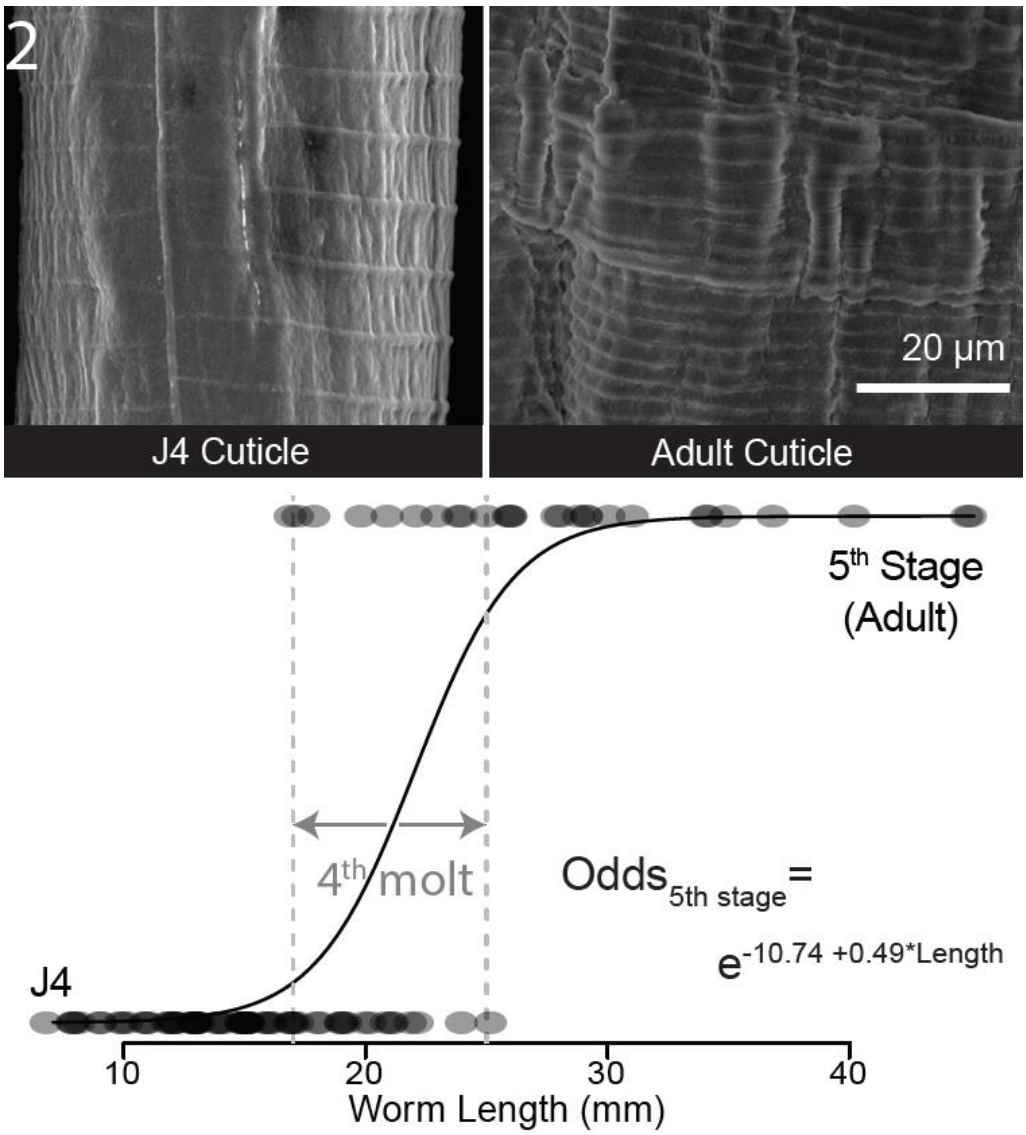


Figure 2.2: Comparison of J4 and 5th stage *Baylisascaris procyonis.*
Environmental electron scanning microscopy of *Baylisascaris procyonis* cuticle from J4 (top left) and immature adult (top right) worms, showing wider annules in juvenile worms. Both images use the same 20 µm scale bar. Late J4 and early fifth stage immatures overlapped in size; with the 4th molt occurring between 17 and 25 mm (logistic regression of molt stage and worm length, bottom).

Including immature worms, prevalence of *Baylisascaris procyonis* rose to 90.7% (n = 183). Although most raccoons hosted between 1 and 200 immature worms, one adult raccoon had over 2,000 J4 stage worms. Because this outlier significantly influenced analyses, this host was excluded from immature worm abundance regressions and mean abundances were

reported both with and without this value (Table 2.1). Immature worm prevalence was higher in juvenile than in adult raccoons (Table 2.1), however mean intensity did not significantly differ among these age classes (t-test, $p = 0.50$). There was no significant relationship between immature parasite abundance, host age and adult worm abundance in either pre-weaned or weaned raccoons, and season had no effect on immature worm abundance in weaned hosts.

Immature worms measured from 1 to 136 mm in length (mean = 9 mm, SD = 15mm), however 80% were less than 10 mm long. Although late J4 and early 5th stage immature worms overlapped in length, these stages could be distinguished based on annuli widths (Figure 2.2). The molt from J4 to adult occurred when worms were between 15 to 20 mm. Across all sampled raccoons, J4s (< 20 mm) outnumbered immature fifth stage worms (20 - 40 mm) 8.5 to 1, even excluding the raccoon with over 2,500 J4s (Figure 2.3). Early J4s (< 10mm) infected 65% of raccoons and although prevalence was higher in younger animals (72% pre-weaned versus 47% weaned), intensity did not significantly differ between these age classes (pre-weaned: 9.8 ± (SD) 13.8, weaned: 13.8 ± 30.6). Early J4 abundance declined with host age for both pre-weaned and weaned raccoons (GLM [pre-weaned], $\beta = -0.66$, $p = 0.0124$; [weaned] $\beta = -0.03$, $p = 0.036$). In pre-weaned raccoons, there was no relationship between established worms and J4 abundance, however the probability of detecting J4s decreased with increasing numbers of established worms (logistic regression, $\beta = -0.028$, $p = 0.011$). In weaned raccoons, established worm abundance did not significantly affect the presence or abundance of J4 stages, however hosts with at least 1 established worm also had significantly more J4s (GLM, $\beta = 2.5$, $p = 0.0002$). J4s infected raccoons in all age classes and these immature stages were substantially more abundant than mature parasites.

Most, but not all raccoons infected with adult worms released *B. procyonis* eggs in their feces. Fecal assays detected 80.5% of adult parasite infections, with missed infections due to sporadic female egg production and infection with only male worms. Although the parasite sex ratio was 54% female, 10 infected raccoons hosted no mature female worms and thus released no eggs. Even when female worms were present, eggs were not always detected. Only 60% of hosts with 1 or 2 female worms released eggs; however eggs were recovered in 96% of infections with at least 3 female worms. Fecal assay sensitivity increased with increasing intensity because, on average, more heavily infected raccoons released more *B. procyonis* eggs.

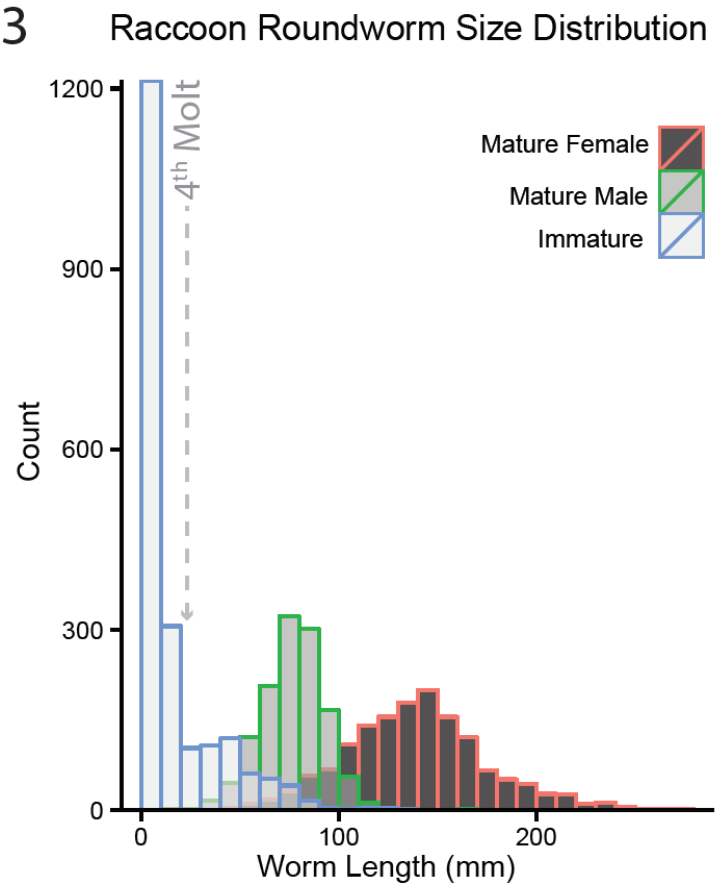


Figure 2.3: *Baylisascaris procyonis* size distribution in raccoons.
Average size at the 4th molt is marked with a dashed line and J4 outnumber fifth stage immatures 8.5 to 1.

Egg production increased with worm burden, with no evidence that crowding reduced egg output at high intensities (Figure 2.4). Infected raccoons with positive fecal assays released 8 to 50,979 eggs per-gram feces (mean 4,606 ± 661(Standard Error)) and EPG increased with female worm intensity (Figure 2.4., $R^2 = 0.41$, $F_{1,131} = 91.03$, $p < 0.0001$) and biomass ($R^2 = 0.53$, $F_{1,123} = 132.8$, $p < 0.0001$). As expected given their higher infection intensities, juvenile raccoons released more eggs per gram feces (EPG 3,894 ± 660 (mean ± SE)) than did adult raccoons (1,779 ± 560), however age effects disappeared when worm intensity was included in egg production models. Per-worm fecundity did not decline with increasing intensity (Figure 2.4, $F_{1,131} = 0.93$, $p = 0.34$) with female worms producing, on average, 385 ± 46 eggs per-gram feces. If raccoons produced 40 to 200 g feces per day (Kazacos, 1982; Reed et al., 2012), then hosts released 15,000 to 77,000 eggs per day for every female worm present. In general, egg counts increased with intensity, but even controlling for intensity, egg production varied over 3 orders of magnitude among hosts.

Female worm biomass captured more variance in egg production than did female worm intensity due to size-based differences in parasite fecundity. Mature female worms ranged in length from 47 to 278 mm (mean 139 ± (SE) 1 mm) and larger worms produced more eggs. In-utero egg counts correlated with uterus weight ($F_{1,25} = 92.29$, $p < 0.0001$), which increased with worm length ($F_{1,507} = 762.7$, $p < 0.0001$). Crowding did not affect mean worm length (Figure 2.4, $F_{1,136} = 1.09$, $p = 0.30$) or uterus weight ($F_{1,46} = 0.0028$, $p = 0.96$); however adult female worms were smaller in pre-weaned hosts (t-test, $p < 0.0001$). As worm size increased with host age, and worm size correlated with fecundity, EPG per worm should have also increased with host age. However, this pattern only occurred if fecal volume was set proportional to host weight.

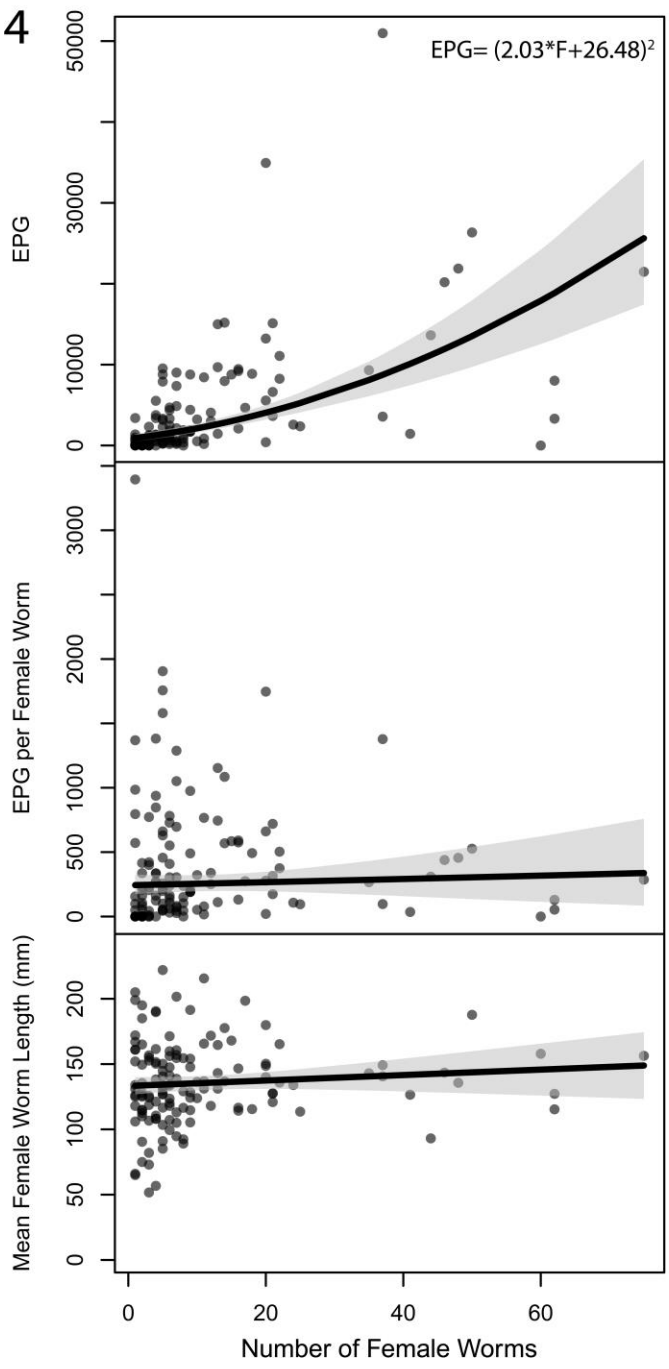


Figure 2.4: *Baylisascaris procyonis* egg production.
Eggs per gram feces (EPG) increased with intensity (top). No crowding effects were detected as neither EPG per female worm (middle) nor mean female worm length decreased with increasing female worm intensity (bottom).

Table 2.1: Prevalence and abundance of *Baylisascaris procyonis* in adult and juvenile raccoon age classes.

Raccoon Age (Month)	Host sample size (imm)*	Adult parasites			Immature parasites		
		% Prevalence (95% CI)	Range	Mean abundance (95% CI)	% Prevalence (95% CI)	Range	Mean abundance (95% CI)
Juvenile	129 (124)	84 (77-89)	0-102	17.5 (14-21.8)	79.0 (71-85)	0-181	13.0 (9.8-18.1)
<3	17 (14)	23 (9-47)	0-17	2.2 (0.4-5.9)	79 (53-94)	0-55	15.9 (7.2-28.2)
[3,4)	26 (24)	85 (67-95)	0-102	19.8 (11.9-32.2)	96 (82-100)	0-62	13.6 (9.0-22.3)
[4,5)	37	100 (95-100)	1-100	22.8 (16.4-33.9)	86 (70-93)	0-50	8.8 (5.7-14.5)
[5,7)	27	100 (93-100)	1-74	20 (13.0-30.6)	78 (60-90)	0-181	19.1 (10.2-40.1)
[7,12)	22	77 (57-91)	0-46	14.5 (8.9-21.2)	55 (34-74)	0-109	10.0 (3.0-28.6)
Adult	60 (59)	73 (61-83)	0-125	10.1 (6.3-20.1)	54 (42-66)	0-2649 0-72†	52.1, 7.3† (4.3-12.7)†
[12,26)	26	65 (46-81)	0-125	14.2 (5.6-33.2)	50 (32-68)	0-2649 0-72 †	107.5, 5.8† (1.8-18.4)
[26,48)	21 (20)	76 (55-90)	0-39	8.1 (4.7-13.7)	57 (36-76)	0-57	9.2 (4.3-19.4)
>48	13	85 (59-97)	0-13	4.8 (3-7.7)	54 (28-78)	0-36	7.4 (2.6-16.6)
All	189 (183)	80 (74-86)	0-125	15.1 (12.3-18.9)	71 (64-77)	0-2649 0-181†	25.6, 11.2† (8.8-15.5)†

*Immature worm data available for 183 of 189 hosts. †Excluding outlier with 2,649 immature worms

Discussion

In this California raccoon population, raccoon demography influenced parasite abundance. Prevalence and intensity were higher in juvenile raccoons than in adults. Every 4 to 7 month old raccoon carried at least 1 adult roundworm whereas over 40% of older animals were uninfected. Although raccoons can live nearly 2 decades (Gehrt 2003), in this study, the majority were under a year old. As a result, most sampled raccoons were in the most infected age classes, leading to high mean worm intensity. High worm intensity did not lead to crowding, suggesting that worm abundance predicts egg output.

The fall-winter peak in parasite abundance in this southern California site was driven by host age and differed from the fall peak and winter decline reported from regions with colder winter temperatures (Smith *et al.* 1985; Kidder *et al.* 1989; Evans 2001; Page *et al.* 2005). This difference could be due to the hypothesized self-cure and parasite-induced host death associated with cold winters (Kidder *et al.* 1989; Kazacos 2001; Page *et al.* 2009; Sarkissian *et al.* 2015). Regardless, the demographic pattern in California shows that harsh winters are not needed to drive seasonality. To disentangle demographic effects from weather effects, future parasite surveys in colder regions might consider incorporating both host and parasite age classes into parasite prevalence and abundance analyses.

In this raccoon population, much of the seasonality in infection patterns was driven by the abundance of heavily infected juvenile raccoons. In helminths, higher intensities in younger hosts are often due to higher exposure to infective stages, parasite-induced host death, or acquired immunity (Gregory & Woolhouse 1993; Hudson & Dobson 1995; Duerr *et al.* 2003). Although these mechanisms are not mutually exclusive, for raccoon roundworm there is little evidence of parasite-induced host mortality, nor differential exposure of juvenile and adult raccoons. Healthy raccoons can carry over 200 worms (Kazacos 2001), and

parasite-induced host death has been implicated in reports of only 3 raccoons with intensities of 141, 636 and 1,321 adult worms (Stone 1983; Carlson & Nielsen 1984). Both adult and juvenile raccoons are also exposed to parasite eggs. Before weaning, raccoon kits are exposed to eggs in the den and on their mother's fur (Kazacos 2001) and exposure continues for older raccoons through daily latrine visits and grooming. Poor sampling in older host cohorts can also generate age-intensity peaks (Gregory & Woolhouse 1993; Wilson *et al.* 2001). However, under sampling of adult hosts cannot fully explain the observed patterns as an age intensity peak was also seen for prevalence, which is not affected by sample size (Gregory & Woolhouse 1993). This leaves susceptibility as the most plausible mechanism driving the differences in adult and juvenile raccoon infection patterns.

Changing susceptibility can generate age-intensity peaks and the observed relationships between early J4s, host age, and established parasites suggest that acquired immunity and age-based resistance influenced parasite establishment in this raccoon population. Eggs are thought to infect only the youngest raccoons (Kazacos 2001) and the observed decline in J4 abundance with host age is consistent with the development of age-based resistance in pre-weaned raccoons. Further, in these young hosts, J4s were significantly less likely to be found in animals with larger established worm infrapopulations, suggesting that egg susceptibility declined in the presence of conspecifics. Acquired or concomitant immunity has been documented in other vertebrate host-nematode systems (Eriksen *et al.* 1992; Jungersen *et al.* 1999; Else & deSchoolmeester 2003; Grencis *et al.* 2014) and the patterns observed here suggest that age-based resistance and intensity-dependent reductions in parasite establishment combine to limit transmission of *B. procyonis* as raccoons mature.

Although immature parasite prevalence declined with raccoon age, over 50% of adult raccoons hosted immature worms. As weaned raccoons are thought to be resistant to eggs

(Kazacos 2001), new infections in such hosts likely resulted from trophic transmission. Although vertebrate prey are generally less than 10% of raccoon gut contents (Hamilton 1936; Giles 1939, 1940; Hamilton 1951), J4 presence in these adult raccoons suggests that 50% of adult raccoons consumed an infected paratenic host in the previous month. At this study site, mice host 1 to 40 worms thus 90% of J4 intensities could be attributed to eating a single infected mouse. In this same region, rats can carry over 5000 juvenile worms, suggesting that the one raccoon with over 2600 J4s likely consumed a rat (Chapter 4). Two thousand adult worms would be fatal to a raccoon; however, consistent with experimental studies of *Ascaris suum* establishment in pigs (Roepstorff *et al.* 1997; Morimoto *et al.* 2003), it appears that less than 15% of juvenile *B. procyonis* found in raccoons mature into egg producing adults.

Among hosts with established parasite populations, egg production increased with infection intensity. Some variance in the relationship between intensity and egg production could be due to differences in individual parasite fecundity (Marcogliese 1997; Irvine *et al.* 2001), sporadic egg production (Yeitz *et al.* 2009; Reed *et al.* 2012), or reduced fecundity in senescing worms (Sinniah 1982; Sinniah & Subramaniam 1991). Further, differences in fecal output associated with raccoon health, diet, and size could also add substantial variance to fecal egg counts. Adult raccoons egest more feces than do juveniles, thus measuring eggs per-gram feces underestimates egg production from larger older hosts, compared to smaller juvenile hosts. Adult raccoons do harbor fewer worms (Kazacos 2001), but studies based only on fecal assays will exaggerate this difference because parasite infrapopulations with equal fecundity will produce lower EPG counts in larger raccoons. This has implications for estimating disease risk because EPG is one of the most commonly used measures of parasite

prevalence and environmental egg contamination (Evans 2001; Kazacos 2001; Page *et al.* 2005).

The limited ability to predict EPG from worm intensity was, in part, due to variation in parasite size. Parasite biomass more accurately predicted egg production because larger worms produced more eggs. Had *B. procyonis* growth been resource limited, crowding effects would have been detected as stunting and reduced fecundity in high intensity infections. Although variance in EPG per female worm decreased with increasing intensity; this could be due to averaging egg production across increasingly large parasite infrapopulations, and does not alone provide evidence for crowding. Crowding effects can regulate parasite populations (Keymer 1982; Quinnell *et al.* 1990), but are not observed in all nematode systems (Quinnell *et al.* 1990; Coyne & Smith 1992; Hudson & Dobson 1997; Marcogliese 1997). Density dependent growth and fecundity were not detected in this parasite population, however stunted parasites have been documented in a raccoon with 636 worms (Stone 1983). Here, although *B. procyonis* infected 90% of sampled raccoons, only 6% carried more than 50 adult worms. High intensity infections were rare, suggesting that even if density dependence reduces parasite growth and fecundity, it is unlikely to regulate this raccoon roundworm population. Without crowding effects, parasite responses to management strategies are more predictable as any action reducing *B. procyonis* intensity should also reduce egg production and disease risk.

High worm fecundity, and the potential to cause severe pathology in humans and other hosts, makes raccoon roundworm a threat to human and wildlife health. The greatest disease risk appears to emanate from juvenile raccoons, because they host more worms and release more eggs than older raccoons. Heavily infected juvenile age classes dominate raccoon populations because, due to trapping, vehicle collisions and disease, raccoons rarely

survive beyond 3 years (Gehrt 2003; Fisher 2007). Reducing this raccoon population turnover, in addition to anthelminthic baiting, offers a promising non-lethal alternative to manage raccoon populations.

This chapter is published as "*Baylisascaris procyonis* Demography and Egg production in a California Raccoon Population", by S.B. Weinstein, appearing in *The Journal of Parasitology* 102.6 (2016) and is reprinted with permission from *The Journal of Parasitology* Allen Press Publishing Services. DOI: 10.1645/15-747

3. Fear of Feces? Trade-offs between disease risk and foraging influence animal activity at raccoon latrines

Abstract

Fear of predation alters prey behavior, which can indirectly alter entire landscapes. A parasite-induced landscape of fear might also exist if animals avoid parasite-contaminated resources when infection costs outweigh foraging benefits. To investigate whether animals avoid parasite contaminated sites, and if such avoidance balances disease costs and foraging gains, we monitored animal behavior at raccoon latrines — sites that concentrate both seeds and pathogenic parasite eggs. Using wildlife cameras, we documented over 40 potentially susceptible vertebrate species in latrines and adjacent habitat. Latrine contact rates reflected background activity, diet preferences and disease risk. Disease-tolerant raccoons and rats displayed significant site attraction, while susceptible birds and small mammals avoided these high-risk sites. This suggests that parasites, like predators, might create a landscape of fear for vulnerable hosts. Such non-consumptive parasite effects could alter disease transmission, population dynamics, and even ecosystem structure.

Introduction

Elk fear wolves, hares fear lynx, and tadpoles fear dragonfly larvae; this fear changes where and how much each herbivore forages (Peacor & Werner 2000; White *et al.* 2003; Peckarsky *et al.* 2008). Fear-altered herbivory can have cascading effects on plant communities that alter entire landscapes, inspiring ecologists to coin the term, landscape of fear (Brown *et al.* 1999; Preisser *et al.* 2005). Parasitism bears many ecological similarities to predation (Raffel *et al.* 2008; Kortet *et al.* 2010), landscapes contain parasite biomass that

rivals predator biomass (Kuris *et al.* 2008), and many of these parasites impart substantial fitness costs to their hosts (Schall 2002). Hosts should fear virulent parasites and avoid their associated cues, suggesting that the idea of a predator-induced landscape of fear should also apply to some parasites. Infectious diseases can impact their hosts and infective stages are often aggregated across landscapes, but the extent to which parasite avoidance shapes animal distributions is little understood. Here we describe how a vertebrate community responds to a patchy and pathogenic parasite, the raccoon roundworm.

Avoiding predators seems wise, but not all parasites are worth avoiding. In particular, it can be hard to avoid directly transmitted parasites if doing so forgoes social interactions. For indirectly transmitted parasites, there might be tradeoffs between infection risk and resources. Animals often accept increased predation risk when it permits access to a high value resource (Sih 1980; Pitcher *et al.* 1988; Anholt & Werner 1995; Kotler *et al.* 2004), suggesting that animals might also accept parasite exposure when food has high value or infection costs are low (Lozano 1991; Lafferty 1992; Hutchings *et al.* 2006). For example, fish-eating birds and coral-feeding fish prefer infected food because it is easy to obtain, and the fitness cost from resulting infection is low (Lafferty 1992; Lafferty & Morris 1996; Aeby 2002). As parasite avoidance can reduce foraging opportunities, avoidance behavior should be modulated by trade-offs between infection costs and contaminated resource value.

When the risks outweigh the benefits, animals should avoid places or resources with concentrated infective stages. For instance, oyster-catchers avoid consuming larger more heavily infected cockles (Norris 1999), mammals eat less where tick density is high (Fritzsche & Allan 2012), and many herbivores avoid foraging in vegetation contaminated with feces (Cooper *et al.* 2000; Ezenwa 2004; Fleurance *et al.* 2007; Garnick *et al.* 2010; Sharp *et al.* 2015). This avoidance behavior should be influence by the host's ability to resist

or tolerate infection. Just as prey should "fear" their fiercest predators, hosts should avoid more pathogenic parasites (Brown 1999). A weasel might frighten a mouse but not a moose, and a raccoon roundworm should be more "feared" by the mouse that will develop fatal neurological disease than the raccoon that will develop a relatively benign intestinal infection (Kazacos 2016). This suggests that costly parasites should elicit the greatest avoidance in the most vulnerable hosts.

Although hosts should avoid pathogenic parasites, to be avoidable, infective stages must also be detectable (Lima & Dill 1990; Hart 1994). Ambush predators have larger non-consumptive effects than wider-ranging active predators because prey can identify ambush sites (Schmitz & Suttle 2001; Preisser *et al.* 2007; Thaker *et al.* 2011). Similarly, parasite avoidance should be most pronounced when infection risk is aggregated and detectable. Hosts can flee from biting vectors (Helle *et al.* 1992; Hart 1994) and some skin-penetrating infective stages (Karvonen *et al.* 2004; Taylor *et al.* 2004), but for passively ingested (or otherwise undetectable) infectious stages, avoidance depends on associated cues, such as odor (Kavaliers & Colwell 1995), a carcass (Capinera *et al.* 1976; Turner *et al.* 2014) or feces (Cooper *et al.* 2000). Human disgust towards feces is nearly universal and likely protects against disease (Rozin & Fallon 1987); however, we know relatively little about the extent to which wild animals detect and react to such indirect cues about parasitism risk, in part because it can be difficult to measure how transmission risk varies across a landscape.

A tractable system for quantifying host responses to patchy transmission risk is the North American wildlife community exposed to raccoon roundworm (*Baylisascaris procyonis*). Raccoon roundworm eggs accumulate at communal raccoon defecation sites, and these latrines are an infection source for raccoons and other species. Adult raccoon roundworms cause little pathology to raccoons; but their migrating larvae cause disease

(baylisascariasis) in over 150 bird and small mammal species (Kazacos 2016), such as the Allegheny woodrat, whose decline has been attributed to increased raccoon density (LoGiudice 2003; Page 2013a). Disease severity varies across host species (Kazacos 2016), suggesting that animals suffering the greatest pathology from *B. procyonis* should exhibit the greatest latrine avoidance. Birds and mammals will, however, forage in latrines, which often contain many seeds (Page *et al.* 1999; LoGiudice 2001; Page *et al.* 2001c). If animals balance foraging opportunities with disease risk, the highest foraging rates should occur among disease tolerant (or resistant) granivores. To determine how these high-risk foraging sites alter animal activity, we monitored animal behavior at latrines and control sites, finding that avoidance and attraction varied among species in ways that matched disease risk and diet preferences.

Materials and Methods

Study System

To test how diet preference and avoidance behavior influenced animal activity and parasite exposure, we mapped raccoon latrines and monitored animal activity in an ecological reserve in Santa Barbara County, California (Coal Oil Point Reserve, 34.4105° N, -119.8779° W). Raccoon roundworm infects 80-90% of raccoons in the county (Moore *et al.* 2004; Weinstein 2016), infected raccoons can release over a million *B. procyonis* eggs per day (Kazacos 1982), and the dense network of raccoon latrines at Coal Oil Point could expose all wildlife species to baylisascariasis.

Table 3.1: Predicted diet and baylisascariasis risk for common terrestrial vertebrates at Coal Oil Point Reserve. Granivory is classified as "frequent" for primarily granivorous species and "occasional" for species that primarily eat other foods but are known to eat grains. As communal defecation sites and territorial markers, we expect latrines to attract raccoons, but not alter the behavior of other non-granivorous species with no disease risk. Regardless of diet preference, animals with high (typically lethal) pathology should avoid these sites. In contrast, for animals with no, low or moderate pathology, latrine responses should be mixed, reflecting trade-offs between diet preferences and disease risk. See Appendix for additional information and references on diet, susceptibility and pathology.

Taxon	Granivory	Susceptible	Infection site	Pathology	Predicted response
Rabbits (*Sylvilagus* spp.)	No	Yes	Brain, eyes, viscera	High	Avoid
Squirrels (*Otospermophilus beecheyi*)	Frequent	Yes	Brain, viscera	High	Avoid
Mice (*P. maniculatus, R. megalotis*)	Frequent	Yes	Brain, viscera, eyes	High	Avoid
Birds (ground foraging passerines)	Frequent	Yes	Primarily brain, also viscera	High	Avoid
Rattus spp.	Frequent	Yes	Viscera (primarily gut), brain	Moderate	Mixed
Skunk (*Mephitis mephitis*)	Occasional	Probable	Gut lumen	Low	Mixed
Opossum (*Didelphis virginiana*)	Occasional	No	NA	None	Mixed
Raccoon (*Procyon lotor*)	Occasional	Yes	Gut lumen	Low	Attract
Bobcat (*Felis rufus*)	No	No	NA	None	None
Reptiles	No	No	NA	None	None

To predict latrine avoidance and attraction we classified common vertebrates in the reserve based on disease risk from *B. procyonis* and potential foraging benefits from latrines (Table 3.1). Disease risk is non-existent for opossums, felids, and reptiles and low for raccoons and skunks (Miyashita 1993; Kazacos 2016). In contrast, exposure puts most rodents, passerine birds and lagomorphs at high risk for developing fatal neurological disease (Evans 2002a; Kazacos 2016). These species also vary in their diet preferences. Opossums, rodents and some birds are known to forage in latrines (Page 1998; LoGiudice 2001), suggesting that the partially digested seeds in raccoon feces might attract omnivorous and granivorous species in the reserve (Jameson & Peters 2004). In contrast, latrines should be less attractive to carnivores, like bobcats and lizards, and herbivores such as rabbits. Accounting for disease risk and foraging benefits suggests that most mesopredators should ignore latrines, herbivorous rabbits should avoid them, and granivorous birds and mammals should face a tradeoff between foraging and disease risk.

Latrine Mapping

Latrines are most abundant in wooded areas (Pedlar *et al.* 1997; Beasley *et al.* 2007), and are often on logs or against tree trunks (Page *et al.* 1999; Smyser *et al.* 2010). To map latrines, we first searched all wooded and willow thicket habitat except the 10% inaccessible due to impenetrable poison oak. To survey non-wooded habitats, we searched all landscape features and game trails where latrines frequently occurred and then searched an additional 18 randomly selected 100m^2 plots, ultimately searching 82% of this non-wooded habitat.

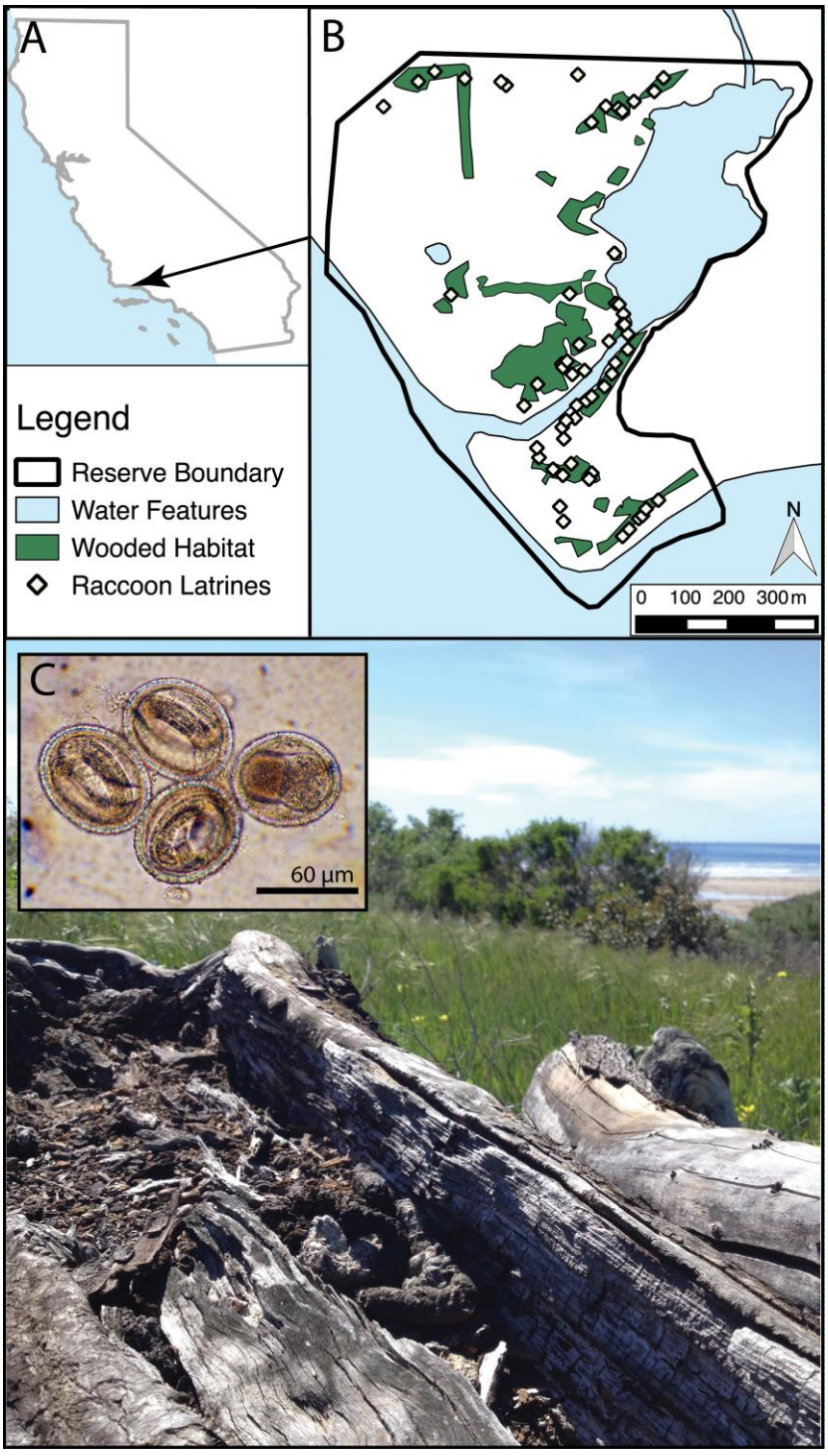


Figure 3.1: Raccoon latrines at Coal Oil Point Reserve.
Raccoon latrines are common, but patchy, at Coal Oil Point Reserve (34.407197°, -119.878388°) in Santa Barbara County, California (A, B). Latrines are easily identified by the presence of raccoon feces, and these communal defecation sites can contain millions of embryonated *Baylisascaris procyonis* eggs (C).

Camera set up and data analysis

We set camera traps to examine animal activity at latrines and in latrine-adjacent habitat. To monitor animal behavior at latrines, from December 2012 through April 2015, we deployed 225 cameras sets at 50 different latrines (Figure 3.1). To control for animal habitat use, for 116 latrine sets, we placed a second matched "latrine-adjacent" camera 5-15 meters from the first camera to compare activity at latrines to background activity patterns (See Figure S1 in Supporting Information). Latrines were monitored with one camera set 1-2 meters from the latrine and, in the paired sets, the second latrine-adjacent camera was set in similar habitat, aimed at microhabitat (e.g., an open patch, or fallen log) resembling the latrine site. Cameras (M-880 series MCG-12631 and 12594 and MFH-DGS-M80XT, Moultrie, Birmingham, Alabama, USA) used a passive infrared sensor that triggered within a second after detecting either heat or motion and were set to take three to four photos in a rapid sequence ("4 Shot Fast" in M80XT and M-880 12594 and "3 Burst" in M-880 12631) with a 5 - 30 second delay between each series. Diurnal photos were in color and low light photos were in black and white, illuminated with an infrared (850 nm) "Low-Glow" flash. We used the same camera model and settings within pairs and both cameras were set on the same day, and at a similar distance, height and angle to standardize sampling effort.

We identified animals in camera trap photos and recorded when animals were in contact with or foraging in latrines. Repeated species observations could represent multiple visits from the same animal, or sequential visits from multiple animals. However, we counted animal observations as unique events if size and markings distinguished the individual or 15 minutes had elapsed since that species was photographed (Meek *et al.* 2014). Parasite exposure should increase with time spent at latrines (Page *et al.* 2001b), thus we counted camera triggers and used this as a proxy for time (Figure S2). We defined contact as animals

touching the latrine and recorded which contacts included foraging, defined as eating, scratching and sniffing, or holding material in the mouth or paws. As house, harvest and deer mice were difficult to differentiate in nocturnal photographs, we grouped these rodents together as "mice". We identified other animals to species whenever possible and unknown species accounted for less than 0.5% of observations.

We tested for latrine attraction and avoidance behavior using our paired latrine and latrine-adjacent cameras, including all species observed at least 10 times in latrine and latrine adjacent cameras. For these 11 taxa, we compared time spent in latrines and latrine-adjacent sites using a paired t-test that included all sets in which the animal was detected in at least one camera. For all comparisons, we adjusted p-values to control family-wise error rate using the p.adjust function with Hochberg's method in R (R Core Team 2016).

Results

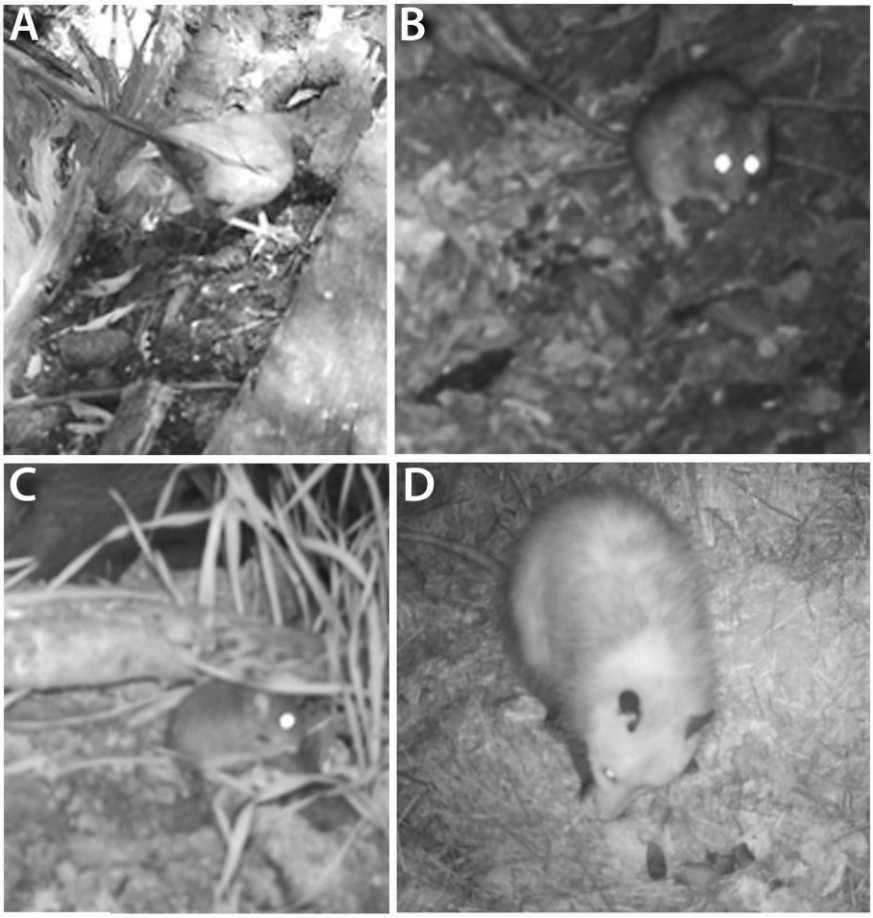
The cameras recorded 61 bird, mammal, and reptile species in and around raccoon latrines. From 2065 latrine observation days, we recorded 2482 unique animal visits, of which 65% included physical contact with raccoon feces. Raccoons were the most common latrine visitors, with 3.2 ± 4.6 (SD) raccoon observations per week. Rats, western fence lizards, mice, California towhees, striped skunks, Virginia opossums, and brush rabbits were also common at latrines and, in total, at least five reptile, nine mammal, and 20 bird species were found at latrines (Table 3.2).

Table 3.2: Counts of observed animal latrine contacts and contacts that included foraging, by species.

Animal in latrine	Contacts	Foraging
Mesopredators		
Raccoon (*Procyon lotor*)	813	0
Striped skunk (*Mephitis mephitis*)	70	4
Opossum (*Didelphis virginiana*)	64	6
Bobcat (*Lynx rufus*)	19	0
Small mammals		
Black rat (*Rattus rattus*)	217	118
Mice (*P.maniculatus, R.megalotis, M. musculus*)	66	16
Brush rabbit (*Sylvilagus bachmani*)	36	8
California Ground Squirrel (*Otospermophilus beecheyi*)	13	1
Merriam's Chipmunk (*Tamias merriami*)	2	0
California Vole (*Microtus californicus*)	1	1
Reptiles		
Western fence lizard (*Scleroporus occidentalis*)	154	-
Western skink (*Plestiodon skiltonianus*)	7	-
Striped racer (*Coluber lateralis*)	2	-
Gilbert's skink (*Plestiodon gilberti*)	1	-
Southern alligator lizard (*Elgaria multicarinata*)	1	-
Birds		
California towhee (*Pipilo crissalis*)	63	17
Song sparrow (*Melospiza melodia*)	23	4
Golden-crowned sparrow (*Zonotrichia atricapilia*)	12	2
White-crowned sparrow (*Zonotrichia leucophrys*)	10	2
California thrasher (*Tozostoma redivivum*)	8	0
Northern flicker (*Colaptes auratus*)	7	4
Bewick's wren (*Thryomanes bewickii*)	6	2
Hermit thrush (*Catharus guttatus*)	6	1
Mourning dove (*Zenaida macroura*)	5	3
California quail (*Lophortyx californicus*)	4	1
Spotted towhee (*Pipilo maculatus*)	4	2
Nutmeg mannikin (*Lonchura punctulata*)	3	0
American crow (*Corvus brachyrhynchos*)	1	0
Anna's hummingbird (*Calypte anna*)	1	1
House finch (*Carpodacus mexicanus*)	1	1
Killdeer (*Charadrius vociferus*)	1	1
Western bluebird (*Sialia mexicana*)	1	0
Western scrub-jay (*Aphelocoma californica*)	1	0
Yellow-rumped warbler (*Dendroica coronata*)	1	0

Most latrine visits did not involve foraging; however, for the 56 bird and mammal species observed at latrines and in adjacent habitat, 36% were observed foraging in feces at least once (Table 3.2). Brush rabbits foraged on vegetation sprouting within feces, opossums picked up and ate feces, and both rats and mice picked through fecal material (Figure 3.2). Besides raccoons, rats were the most common latrine visitor and 54% of rat contacts included foraging.

Figure 3.2: Animals observed foraging in latrines.
(A) California towhee, (B) black rat, (C) deer mouse, and (D) Virginia opossum.



Testing for attraction and avoidance

Controlling for background habitat use, we found evidence of both latrine avoidance and attraction (Figure 3.3). Rats and raccoons showed significant attraction to latrines, both

spending over three times as much time at latrines compared to matched adjacent sites (Rat: Mean[latrine contacts per camera set] = 6.6 ± 10.3 (SD), adjacent = 2.0 ± 2.9, $p = 0.04$; Raccoon: latrine = 5.0 ± 4.9, adjacent = 1.9 ± 3.5, $p < 0.001$). Although western fence lizards spent more time at latrines than any other animal, these reptiles were equally common at adjacent basking sites (latrine = 4.5 ± 10.3, adjacent = 3.4 ± 5.8, $p = 0.88$). Like lizards, mesopredators such as bobcats, opossums, and skunks showed neither avoidance nor attraction (Bobcat: latrine = 0.6 ± 0.6, adjacent = 0.7 ± 0.7; Opossum: latrine = 1.5 ± 2.2, adjacent = 1.2 ± 1.5; Skunk: latrine = 2.1 ± 4.2, adjacent = 2.0 ± 3.4; all $p > 0.60$). In contrast, sparrows, California towhee, mice, squirrel and brush rabbit all spent less time at latrines than at matched adjacent sites. Although this avoidance behavior was statistically significant only for rabbits at the taxon level (latrine = 1.1 ± 2.4, adjacent = 3.6 ± 4.2, $p=0.015$), when combined, rodents (excluding *R. rattus*) and birds spent significantly less time at latrines than at matched adjacent sites (latrine = 3.24 ± 6.07, adjacent = 6.25 ± 10.54, $p = 0.01$).

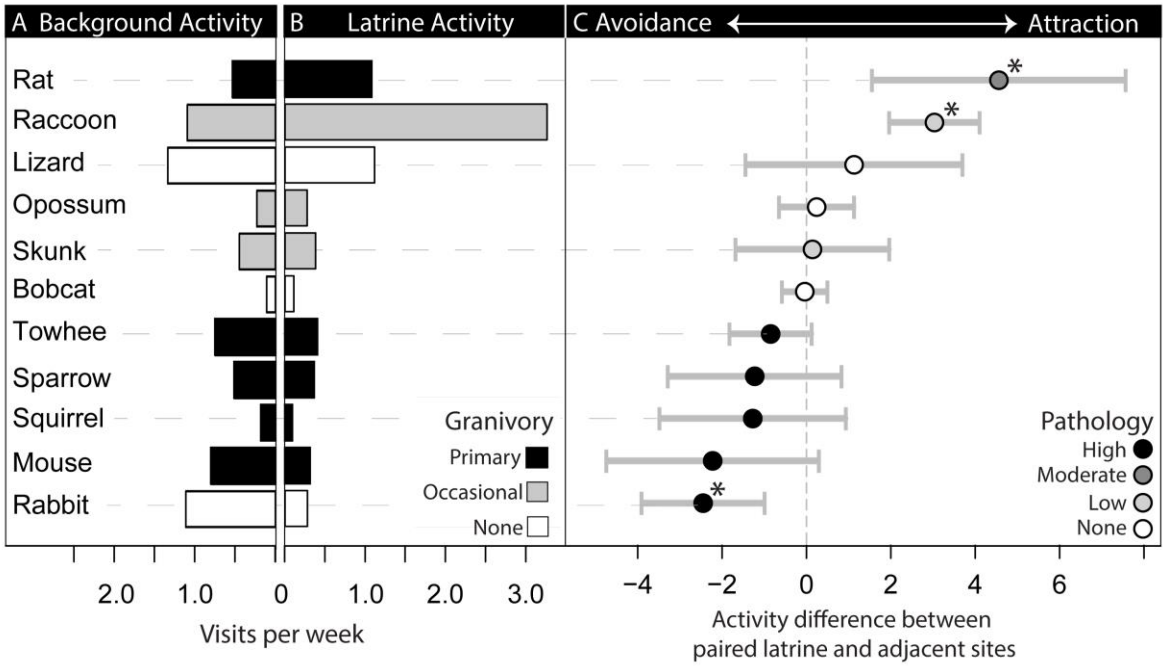


Figure 3.3: Latrine contact reflected background activity, diet preferences and disease risk. Besides raccoons, rats and western fences lizards were the most common latrine visitors (B). Paired comparison of time at latrines and matched adjacent sites revealed significant attraction for raccoons and rats, and avoidance behavior for native birds and small mammals (C). Mean activity difference between paired sites is represented as a point, shown with 95% confidence intervals. Bars are colored according to diet preference and points according to pathology, as detailed in Tables 3.1 and 3.1S.

Discussion

The Coal Oil Point terrestrial habitat averages one parasite-infested and food-laden raccoon latrine per hectare. Species' activity around latrines was determined by their habitat use, whether they fed on resources within latrines, and the extent to which they were sensitive to disease caused by raccoon roundworm. Parasite contaminated latrines altered animal activity suggesting that parasites, like predators, might also induce a landscape of fear.

Although animals altered their behavior around latrines, habitat use also influenced latrine contact. For example, most perching birds and raptors were rarely observed at latrines or in latrine adjacent habitat; however these species were abundant in other habitats in the

reserve (Holmgren 2001). In contrast, western fence lizards were often in both latrine and in adjacent habitat. For lizards, latrines were neither risky nor valuable, and were thus neither avoided nor sought out. Similarly, mesopredators (other than raccoons) showed no significant latrine attraction or avoidance, they simply encountered these sites as they moved through the landscape.

Rats showed significant latrine attraction. Although *Rattus* species are susceptible to baylisascariasis, these rodents appear to be more tolerant to raccoon roundworm than are other small mammals and birds (Wirtz 1982; Russell 2006; Sapp *et al.* 2016c). Wild caught *R. rattus* exhibit normal behavior despite intensities of over 10,000 larval worms (Table S3.1), suggesting that these rodents tolerate high natural exposure. For rats, access to food in latrines might outweigh the perceived risk, leading to high foraging rates on a contaminated resource.

In contrast to rats, rabbits had little to gain from latrines and much to lose. Consistent with expected trade-offs between diet preference and disease risk, these herbivores avoided latrines the most. Native granivorous foragers also avoided latrines, despite sacrificing foraging opportunities. Overall, species known to suffer from baylisascariasis avoided latrines. Although we hypothesized that this avoidance was due to parasitism, some prey species are thought to avoid predator feces to reduce predation risk (Apfelbach *et al.* 2005; Roth *et al.* 2008). It is hard to untangle what causes avoidance, but, in our system, predator fear might be less likely than parasite aversion because raccoons rarely catch healthy terrestrial vertebrates (Hamilton 1936; Giles 1940; Baker *et al.* 1945; Hamilton 1951; Harman & Stains 1979), whereas *B. procyonis* infects up to 70% of rodents in some populations (Beasley *et al.* 2013) and is estimated to kill 5% of mice (Tiner 1954). Avoiding predators and avoiding parasites are not mutually exclusive incentives. Because predator

feces often contain parasites, there could be synergies between predator and parasite-induced fear.

Altered animal behavior at contaminated foraging sites has potential ecological and evolutionary consequences. When avoidance relies on ephemeral cues such as feces or a carcass, parasites that can outlast these cues can circumvent host avoidance behavior (Hutchings *et al.* 1998; Turner *et al.* 2014). Thus perhaps alongside the widely recognized predator-prey arms race of speed and stealth, there is a subtle race between parasite persistence and host avoidance. This parasite avoidance might alter energy flow through food webs because it reduces foraging for hosts and opportunities for parasites, just as a fear of predation reduces foraging by prey and opportunities for predators (Brown *et al.* 1999). In addition to potential non-consumptive effects on hosts, an aversion to parasites, like fear of predators, might have cascading effects on lower trophic levels (Werner & Peacor 2003). Fecal avoidance can alter vegetation structure (Ödberg & Francis-Smith 1977; Edwards & Hollis 1982) and at Coal Oil Point, latrine avoidance likely reduces vertebrate seed predation, potentially increasing plant recruitment. Such pre-infection trait-mediated parasite cascades are rarely documented (Buck & Ripple 2017), but might be widespread for virulent parasites, like raccoon roundworms, that occur at detectable focal sites. Animals likely face far more exposure to parasites than to predators, yet the extent to which other parasites elicit avoidance and how this behavior impacts disease transmission, population dynamics, and ecosystem structure remains hidden in most ecosystems.

4. Introduced rats and an endemic roundworm: Does *Rattus rattus* contribute to *Baylisascaris procyonis* transmission in California?

Abstract

The introduced black rat, *Rattus rattus*, occurs throughout the native range of the raccoon roundworm, *Baylisascaris procyonis*, and might incorporate into its life cycle if rats consume parasite eggs, acquire viable infections, and are eaten by raccoons. Although rats forage at raccoon latrines, their role in *B. procyonis* transmission remains unknown. Here I tested the potential for rats to amplify *B. procyonis* transmission in California by surveying wild rodents for *B. procyonis* and conducting scavenger trials using motion activated cameras. *Rattus rattus* were infected with *B. procyonis* at intensities more than 100 times greater than that of co-occurring native *Reithrodotomys megalotis* and *Peromyscus maniculatus*. Rodent carcasses were scavenged by opossums, skunks and raccoons, suggesting that these rodents, particularly *R. rattus*, contribute to *B. procyonis* transmission in this coastal California ecosystem.

Introduction

Invasive species create opportunities and challenges for native parasites. Although introduced species often host a depauperate parasite community (Torchin *et al.* 2003; Torchin & Mitchell 2004), some parasites reach high prevalence and intensity in these novel hosts (Pasternak *et al.* 2007; Marr *et al.* 2008; Dunn 2009). Introduced hosts often acquire new parasites (Kelly *et al.* 2009), and when introduced hosts amplify endemic disease transmission, the resulting parasite spillback can impact native species (Hershberger *et al.* 2010; Mastitsky & Veres 2010; Hartigan *et al.* 2011). As introduced species host mostly

newly acquired parasites, this parasite spillback is likely a common but underreported phenomena (Kelly *et al.* 2009). Here I explore how an invasive Old World species, the black rat (*Rattus rattus*) might alter transmission of a pathogenic New World parasite, the raccoon roundworm.

Native to North America, raccoon roundworm (*Baylisascaris procyonis*) is a large ascarid nematode that matures in the raccoon gut. Infected raccoons shed millions of parasite eggs per day and these eggs accumulate at communal defecation sites, termed latrines (Kazacos 1982; Snyder & Fitzgerald 1987; Page 1998). Eggs infect juvenile raccoons; however adult raccoons become resistant to eggs and instead acquire worms from infected birds and small mammals (Kazacos 2001, 2016). These birds and small mammals serve as paratenic (or transport) hosts and are infected when they ingest eggs from foraging or contact with latrines (Page *et al.* 1998; Page *et al.* 1999; Page *et al.* 2001b). Unlike in raccoons, in paratenic hosts, eggs hatch and larval *B. procyonis* migrate through tissues, often causing severe neurological damage. In the brain of a small mammal or bird, a single worm can be fatal, and doses as low as 50 eggs rapidly kill most mice (Tiner 1953b; Sheppard & Kazacos 1997; Sapp *et al.* 2016b). High pathology in these hosts likely increases transmission to raccoons as incapacitated animals become easy prey and larval worms survive up to 6 days in animal carcasses (Sprent 1953). Raccoon scavenging rates are unknown, however once eaten by a raccoon, worms mature into egg producing adults.

Native small mammals and birds are paratenic hosts for *B. procyonis* and, in California, high prevalence of juvenile worms in adult raccoons suggests that raccoons regularly eat these infected animals (Kazacos 2001; Weinstein 2016). Although baylisascariasis is documented from over 150 vertebrate species, most studies on wild paratenic hosts have examined only the white footed deer mouse, *Peromyscus leucopus*

(Kazacos 2016). In these mice, *B. procyonis* prevalence typically varies from 5 to 70%, and mean intensities range from 1 to over 20 worms per infected mouse (Tiner 1954; Page *et al.* 2001a; Page *et al.* 2011b; Beasley *et al.* 2013). Although *P. leucopus* is common throughout the raccoon range (Gehrt 2003; Cassola 2016), raccoons overlap with at least 200 other rodent species (Hafner *et al.* 1998; Reid 2006) that could also serve as paratenic hosts for *B. procyonis*. In particular, in urban and suburban landscapes, introduced rodents such as *R. rattus* often outnumber native species (McKinney 2002) and might also integrate into the *B. procyonis* life cycle.

As a novel host, *R. rattus* might alter *B. procyonis* transmission if its exposure and competence differ from native species. *Rattus rattus* forage in raccoon latrines more often than do native species, suggesting that their parasite exposure might also be higher (Chapter 3). Although *R. rattus* has never been surveyed for *B. procyonis*, high exposure and presumed susceptibility suggest that raccoon roundworm might frequently infect these introduced rodents. To investigate how introduced rats and native mice contribute to *B. procyonis* transmission, I surveyed rodents for *B. procyonis* at a coastal site in southern California. I examined how host species, weight, sex and proximity to latrines influenced parasite abundance and then used host density to estimate *B. procyonis* populations in native and introduced rodents. Finally, to evaluate potential *B. procyonis* transmission from rodents to raccoons, I quantified raccoon scavenging rates on rodent carcasses.

Materials and Methods

I surveyed rodent populations and conducted scavenging trials in an ecological reserve in Santa Barbara County, California (Coal Oil Point Reserve, 34.4105° N, 119.8779°

W). This 63 ha University of California Natural Reserve includes dune, grassland and coastal scrub habitat and is contiguous with a 260 ha open space bordered by the Pacific Ocean and neighborhoods. Over 80% of raccoons in Santa Barbara County host adult *B. procyonis* (Moore *et al.* 2004; Weinstein 2016) and raccoon latrines are common in the reserve. Approximately 35 potential paratenic host species have been seen in contact with these latrines; however, among birds and mammals, rodents visit most frequently (Chapter 3). Over 50% of rat and 20% of mouse latrine contacts include foraging, suggesting that these rodents could be important paratenic hosts in this coastal ecosystem.

To assess *B. procyonis* loads in rodents, I set lines of approximately 30 Sherman traps (XLK Folding Traps, Sherman Inc., Tallahassee, FL) haphazardly throughout the reserve monthly from March 2013 through April 2015. Using 1-3 sherman trap lines run for one night each, each month I collected three to six rodents. In 2015, additional larger wire-mesh traps (Collapsible Squirrel Trap Model 202, Tomahawk Live Trap, Hazelhurst, WI) were also set throughout the study area to target *R. rattus*. All traps were set in the late afternoon, baited with oats and peanut butter, and checked within two hours of sunrise the next morning. This trapping yielded five rodent species and 67 *P. maniculatus*, 55 *R. megalotis*, 20 *R. rattus*, 5 *Mus musculus*, and 1 *Microtus californicus* were collected for parasitological analyses.

Rodents were processed for larval *B. procyonis* following standard raccoon roundworm-specific protocols (Kazacos 2001). The brain and viscera were squashed between glass plates and tissues were examined under a stereomicroscope. The gut was opened lengthwise and contents were examined separately from tissues. To count larvae in musculature, the remaining carcass was skinned and homogenized in a blender with a 1% pepsin acid solution. The homogenate was incubated on a shaker plate at 37°C for 2.5 hours and then filtered through cheesecloth into pint glasses. After this solution settled, it was

decanted two to four times until the supernatant was clear, and then larvae were counted. The status of *B. procyonis* and *B. columnaris* as separate species remains unresolved (L. Camp personal communication), and, based on morphology, all larvae were identified as *Baylisascaris procyonis*.

For each host species, I calculated infection prevalence (± 95% Bayesian confidence intervals), worms per host (abundance ± SD), and worms per infected host (intensity ± SD). Bayesian confidence intervals were estimated using the "prevalence" package, and all analyses were done in R unless otherwise noted (Devleesschauwer *et al.* 2014; R Core Team 2016). I calculated the distance from each trap site to the nearest latrine using mapped latrines from Chapter 3 and the "distance to nearest hub" function in QGIS (QGIS Development Team 2016). I then tested whether species differed in their proximity to latrines using a one-way ANOVA and Tukey HSD test. Controlling for differences in host size and latrine proximity, I compared parasite abundance between *P. maniculatus*, *R. megalotis*, and *R. rattus* with a negative binomial generalized linear model using the "MASS" package (Venables & Ripley 2002; R Core Team 2016). I compared parasite distributions in rat and mice tissues (brain, muscle, gut and other) using a Fisher's exact test. Then, I tested for a difference in worms per gram tissue between *R. rattus* and *P. maniculatus* with a Welch's two sample t-test. For *R. rattus* and *P. maniculatus* I used negative binomial generalized linear models to examine how host sex, weight and latrine proximity influenced within species parasite abundance. Model selection was done by backwards stepwise selection, comparing nested models using p-values with significance set at $p < 0.05$ (Zuur *et al.* 2009). Together, these analyses examined the inter- and intra-specific factors influencing *B. procyonis* abundance in a California rodent community.

To examine how larval *B. procyonis* moved from paratenic hosts to higher trophic levels, I monitored scavenger behavior in the reserve. To simulate incapacitated paratenic hosts, frozen feeder mice (*M. musculus*) with the same color pattern as wild *Peromyscus* spp. were purchased from East Bay Vivarium, Berkeley, California. I put thawed mice at 85 random points in the reserve and monitored them with camera traps (MFH-DGS-M80XT, Moultrie, Birmingham, AL), set to take either a rapid photo series or a 15-30 second video when triggered. After 6 days, I recorded whether the mouse remained and then analyzed photos and videos to determine scavenger and time until scavenging. To examine scavenger efficiency, I compared relative scavenging frequency to scavenger relative abundance in the reserve (C. Moura, unpublished data) with exact binomial tests.

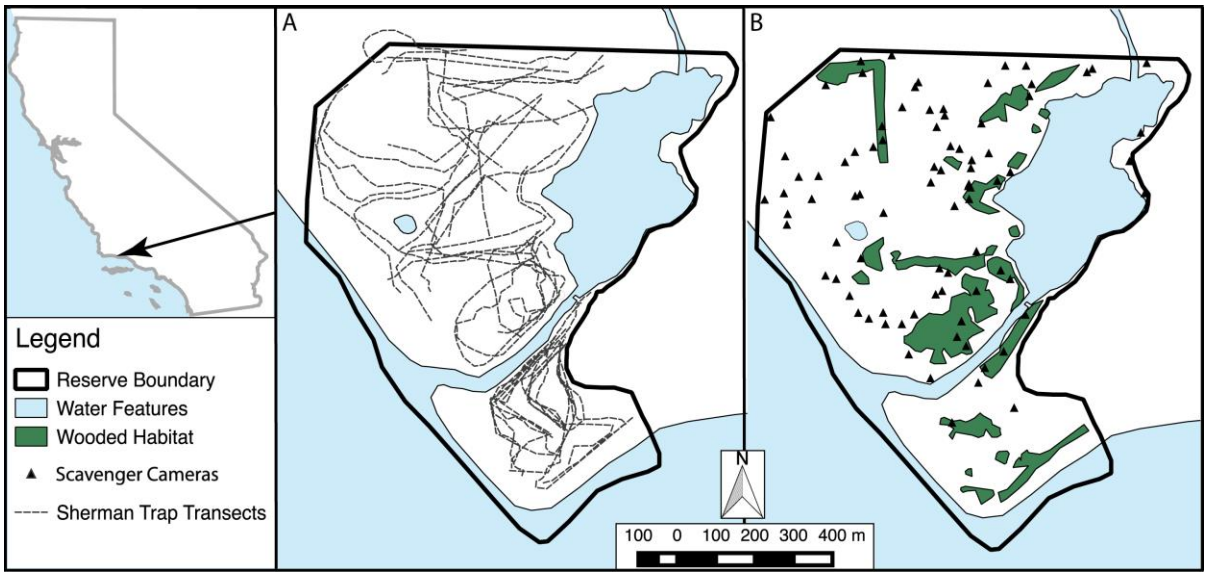


Figure 4.1: Maps of Sherman trap lines and scavenger cameras at Coal Oil Point Reserve. Sherman trap lines (A) were haphazardly set throughout the reserve and scavenger monitoring points (B) were randomly selected using a random point generator.

Results

Introduced rats and native rodents differed in their parasites loads (Figure 4.1). *Mus musculus* and *Microtus californicus* were rarely trapped and I found no *B. procyonis* in the

few individuals examined. Raccoon roundworm infected mice (*R. megalotis* and *P. maniculatus*) and rats (*Rattus rattus*) and no trapped animal exhibited signs of neurological damage such as head tilt, circling or ataxia. For *P. maniculatus* and *R. megalotis*, 45% (95% CI: 33-57%) and 4% (0.7-11%) were infected, with 3.5 ± 6.7 and 0.13 ± 0.13 (abundance ± SD) worms per mouse, respectively. Rats hosted more worms than mice (Table 4.1); 75% of rats (CI: 54-90%) were infected, with 715.1±1437 worms per rat. Raccoon roundworm was common in rodents from this coastal California ecosystem and the average rat hosted almost 1000 times more worms than did the average mouse.

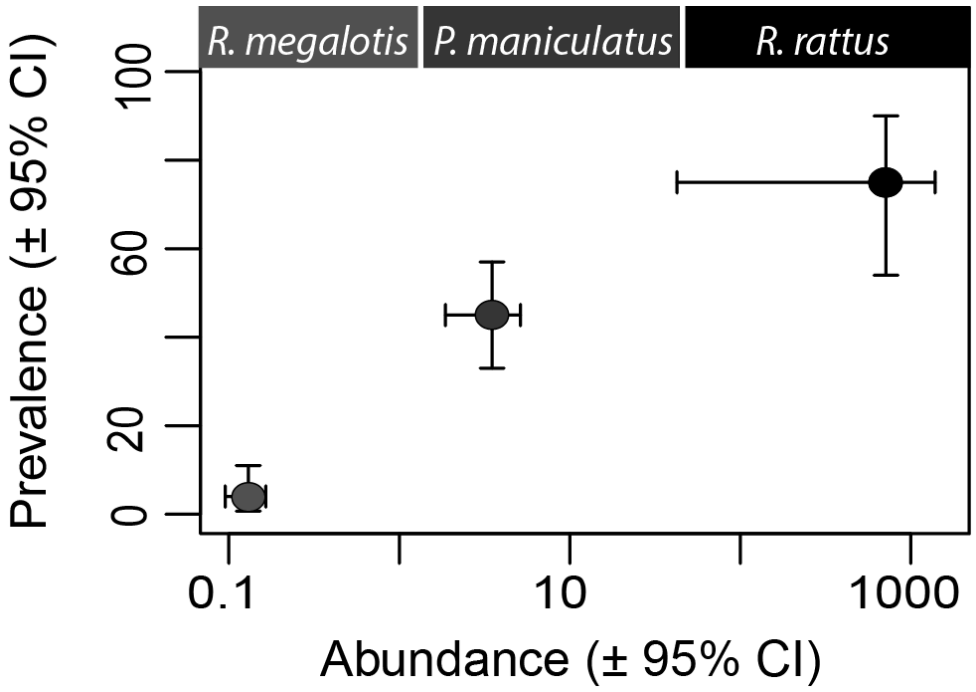


Figure 4.2: Larval *Baylisascaris procyonis* in rodents.
Prevalence versus abundance (worms per rodent) in *Reithrodontomys megalotis*, *Peromyscus maniculatus* and *Rattus rattus* from coastal Southern California.

Factor	β	SE	Z value	p-value
Rattus	**4.25**	**1.53**	**2.78**	**0.005**
Reithrodomys	**-3.49**	**0.62**	**-5.60**	**<0.0001**
Weight	0.007	0.01	0.62	0.54
Distance	-0.004	0.004	-0.96	0.34

Table 4.1: Results from a GLM regression with negative binomial error structure, comparing parasite abundance in *Peromyscus maniculatus* to that in *Rattus rattus* and *Reithrodontomys megalotis* while controlling for host weight and trap proximity to a latrine. The table includes the coefficient estimate (β), standard error (SE), Wald's Z score (Z value) and p-value. Significant factors are in bold.

Rodent species differed in size and proximity to latrines. Trapped *R. rattus* were seven times heavier than were *P. maniculatus*, and although infected *R. rattus* had more worms per gram tissue than *P. maniculatus* (5.5±11.4 versus 0.16±0.3), this difference was not significant ($t(14.0) = 2.1$, $p = 0.05$). Rodent species differed in their average distance from latrines (*R.r:* 36.8±25.0m, *R.m.*: 59.6±54.4m, *P.m.*: 108.6±72.9m; ANOVA, $F(2, 137) = 14.9$, $p < 0.001$). Although the infrequently infected *R. megalotis* did not differ in latrine proximity compared to the more heavily infected *R. rattus* (Tukey HSD, $p = 0.35$), both *R. rattus* and *R. megalotis* were trapped closer to latrines than were *P. maniculatus* (Tukey HSD, both $p<0.001$). Host size and latrine proximity explained some, but not all interspecific differences in parasite load.

Factor	β	SE	Z value	p-value
Rattus				
Sex (M)	**-2.89**	**1.27**	**-2.28**	**0.022**
Weight	**0.03**	**0.01**	**1.99**	**0.047**
Distance	**-0.06**	**0.02**	**-2.36**	**0.019**
Peromyscus				
Weight	**0.25**	**0.08**	**3.19**	**0.0014**

Table 4.2: GLM regression results for the best model for *Baylisascaris procyonis* abundance in *Rattus rattus* and *Peromyscus maniculatus*, including coefficient estimate (β), standard error (SE), Wald's Z score (Z value) and p-value. Significant factors are in bold.

For *P. maniculatus* and *R. rattus*, trap location, size, and sex also influenced within species infection patterns (Table 4.2). For *P. maniculatus*, host sex and latrine proximity had no effect on parasite abundance; however both factors influenced infection in *R. rattus* (Table 4.2). More heavily infected rats were caught closer to latrines and female rats hosted more worms than did males (Male mean: 258.4 ± 406.6, Female: 961 ± 1781.5, Table 4.2). However, if the two female rats with 5064 and 4759 larval worms were excluded, female rats no longer hosted more worms than did males (Female mean: 242.7 ± 339.3). Although only rats showed significant sex and location effects, for both rats and mice, larger individuals hosted more worms.

Larval *B. procyonis* infected rodent gut, liver, lung, muscle, and brain tissues. Parasite distribution in tissues significantly differed between native mice and introduced rats (Fisher's exact test $p < 0.0001$, Table 4.3). Although no mouse hosted more than 40 worms (*P.m.* mean intensity: 7.8 ± 8.2; *R.m.*: 3.5± 3.5), 55% of infected mice had worms in their brains. In contrast, the average infected rat had nearly 1,000 worms (mean intensity: 953.5 ± 1589.9). Over 90% of these larvae were encapsulated in the gut wall and mesenteries (Figure 4.2) and only one worm (out of over 14,300) was found in rat brain tissue. In rats, worms were rarely

recovered from the muscle or brain, and in mice these tissues were the most commonly infected.

To estimate the larval worm population in mice and rats, I multiplied mean parasite abundance by host density estimates. Assuming mouse densities of 5-20 per hectare at Coal Oil Point (A. J. MacDonald, unpublished; Blaustein 1978; Tietje *et al.* 2008), *Peromyscus maniculatus* and *R. megalotis* populations together might host between 700 and 3,000 worms within the reserve. Assuming 5 rats per wooded hectare (Dutson 1974; Clark 1980), the rat population likely supports over 30,000 worms in the reserve, which is an order of magnitude more than in the mice, and 50 times more than the adult worm population in raccoons in the same area (Weinstein 2016, Appendix Ch. 3).

Table 4.3: Distribution of larval *Baylisascaris procyonis* in infected *Rattus rattus* (n=15) and *Peromyscus maniculatus* (n=30). For each tissue, the mean ± SD and percent of total worms recovered in that tissue are reported. "Gut" includes stomach, intestines, cecum, and mesenteries; "Other" includes heart, kidney and any tissues not included in another category. Infected *Reithrodontomys megalotis* are not tabulated; however, one mouse had six worms in the brain and the other had one in the musculature.

	Organ						Intensity
Species	Brain	Muscle	Gut	Liver	Lung	Other	(Total)
R. rattus	0.07 ±0.2 (0.006%)	5.1 ±10.6 (0.5%)	877 ±1457 (92%)	18.7 ±56.7 (2%)	52.7 ±134 (5.5%)	0.07 ±0.2 (0.006%)	954 ±1590
P. maniculatus	2.9 ±4.1 (37%)	3.2 ±3.1 (41%)	1.4 ±3.5 (18%)	0 (0%)	0.2 ±0.7 (3%)	0.2 ±0.4 (2%)	7.8 ±8.1

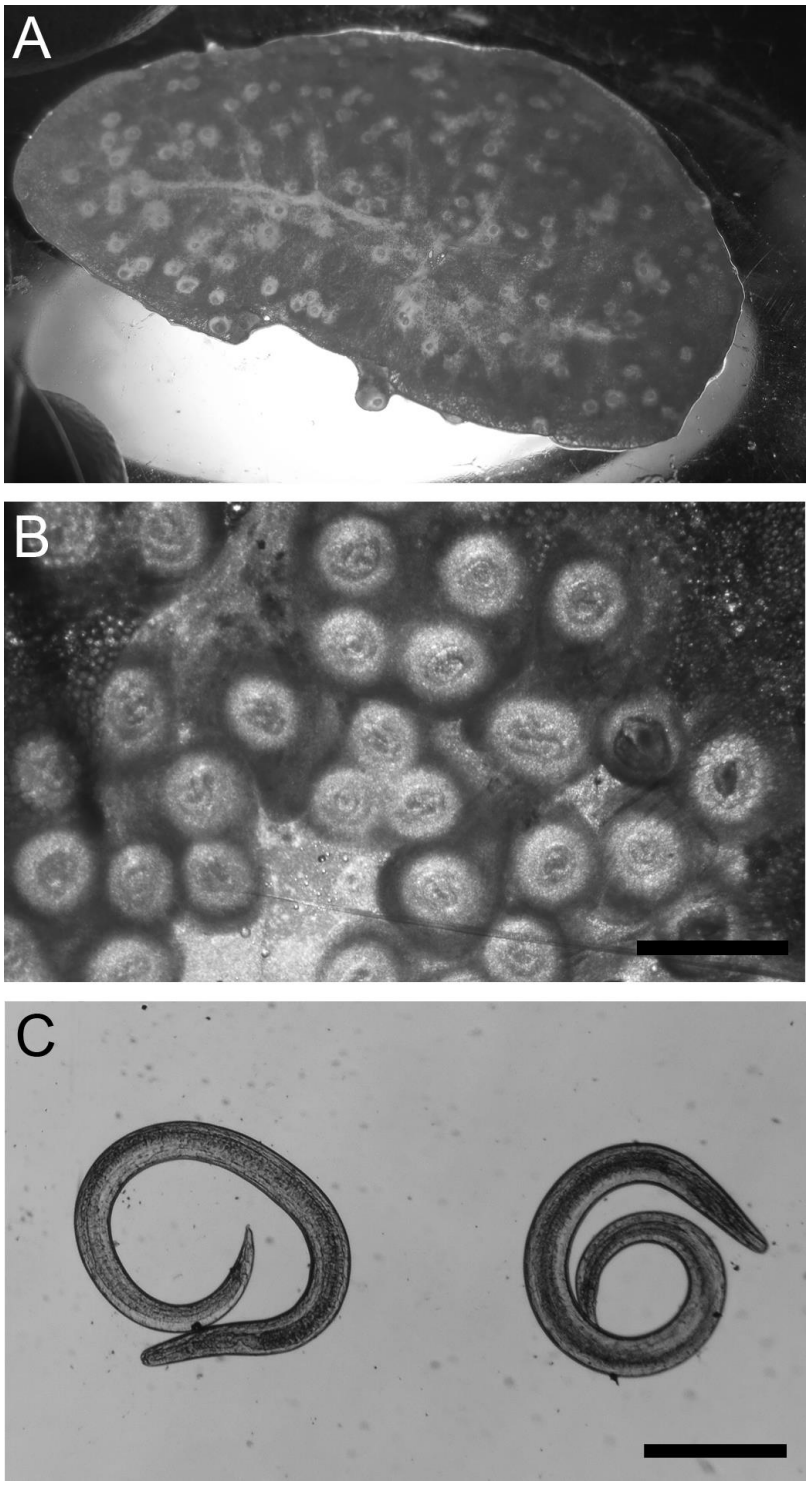
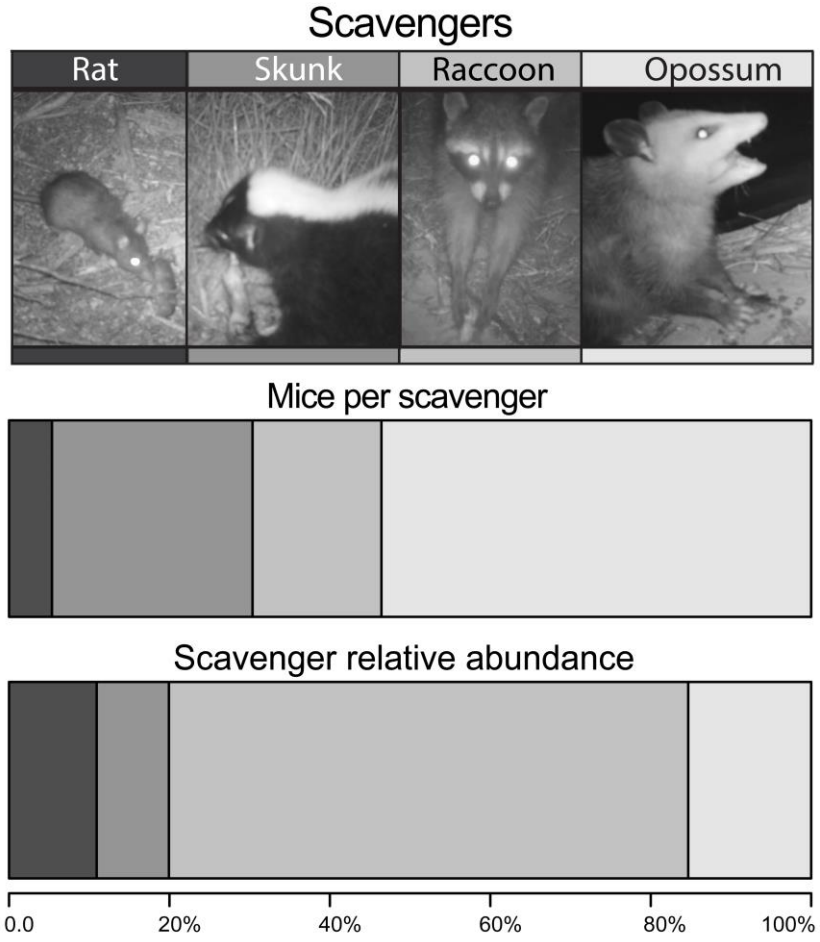


Figure 4.3: *Baylisascaris procyonis* in tissues from a *Rattus rattus* with 5064 larvae. (A) Granulomas were visible in the lungs, shown squashed between glass plates. (B) Larvae in the small intestine and surrounding mesenteries, scale bar = 1500 μm. (C) Two live *B. procyonis* digested from lung tissue, scale bar = 250 μm.

Figure 4.4: Carcass scavenging.
Rats, skunks, raccoons and opossums scavenged rodent carcasses (top). Although raccoons ate 16% of dead mice, skunks and opossums were more efficient scavengers, consuming more carcasses (middle) than expected based on their relative abundance (bottom).



In scavenging trials, raccoons, skunks, opossums or rats picked up 87% of mice within six days. Most mice were scavenged within the first two days (mean: 1.85 ±1.81) and raccoons ate 16% of mice in the 56 trials with identifiable scavengers. Opossums and skunks took more mice than expected given their relative abundance and raccoons took fewer mice than expected (exact binomial test, p-values for raccoon, skunk and opossum < 0.001, Figure 4.3). Although raccoons were over four times more abundant in the reserve than were opossums, opossums scavenged more than three times as many mice.

Discussion

Black rats at Coal Oil Point had the highest *B. procyonis* loads reported in a wild animal population. Parasite loads in these introduced rats exceeded those in native rodents, but not because native mice had limited access to latrines or unusually low infection levels. Most mice in the study area had access to at least one latrine as *Peromyscus maniculatus* home ranges are typically 100 ± 25 m in diameter (Diffendorfer *et al.* 1995; Abramson *et al.* 2006) and most mice were caught within 150 meters of a latrine. Although mice hosted fewer worms than did rats, *P. maniculatus* from this California site hosted parasite loads similar to those reported from *P. leucopus* in the midwestern United States (Tiner 1954; Page *et al.* 2001a; Page *et al.* 2011b; Beasley *et al.* 2013). Although most larval *B. procyonis* surveys have focused on *P. leucopus,* the more widely distributed *P. maniculatus* and *R. rattus* might be important paratenic hosts throughout much of the raccoon range.

Parasite intensity differences between introduced rats and native mice might reflect differences in parasite accumulation or host survival. In both *R. rattus* and *P. maniculatus,* larger individuals hosted more worms, suggesting that parasites accumulated with age. Due to more frequent latrine foraging, rats likely have higher per capita exposure to *B. procyonis* than mice (Chapter 3). Higher exposure combined with a longer life span would lead to higher parasite loads. Life span differences might be due to differences in host life history or to parasite-induced mortality. Baylisascariasis induced mortality has not been measured in *R. rattus*; however, from experimental work with *R. norvegicus* and observed *R. rattus* intensities, *Rattus* spp. appear to be more tolerant of *B. procyonis* compared to smaller mice (Wirtz 1982). In rats, most worms remain in the viscera, and might impact gut, liver and lung functions. In mice about 5% of the larvae migrate to the brain, typically killing their host (Sprent 1952; Tiner 1953b). *Baylisascaris procyonis* is estimated to kill 5% of *P. leucopus* in

Illinois (Tiner 1954) and *P. maniculatus* from California appear to be more susceptible to larva migrans than are *P. leucopus* (Sapp *et al.* 2016b). Baylisascariasis might cause substantial mortality in *P. maniculatus*, and these incapacitated rodents likely transmit infection to raccoons.

Although the extent to which rats and other rodents contribute to *B. procyonis* transmission is difficult to measure, trophic transmission is frequent enough to maintain *B. procyonis* infection in over 50% of adult raccoons in this region (Weinstein 2016). Raccoons scavenged less than 15% of rodent carcasses, and other predators or scavengers ate most rodents. Opossums and cats eat rodents, and both are apparently resistant to infection (Miyashita 1993, but see Kazacos 2016 for opossums; Alden 1995). While worms are lost to these resistant predators, infected rodents put susceptible predators, such as owls, at risk of baylisascariasis (Evans 2002a). High *B. procyonis* prevalence and intensity in rodents, particularly rats, might substantially increase disease risk for native predators and scavengers.

Although introduced *R. rattus* typically host a low diversity parasite community (Torchin *et al.* 2003), in southern California this Old World rodent is heavily infected with endemic *B. procyonis*. Despite a limited evolutionary history with raccoons, *R. rattus* now serve as a reservoir for raccoon roundworm. *Rattus* species likely incorporate into the *B. procyonis* lifecycle wherever they overlap with infected raccoons, potentially increasing baylisascariasis risk for humans, domestic animals, and wildlife.

5. Bird and mammal habitat use and foraging behavior predict community-wide exposure to the pathogenic parasite, raccoon roundworm

Abstract

Raccoon roundworm (*Baylisascaris procyonis*) threatens bird and small mammal populations, but infection risk is patchy. Here we propose and evaluate a model, based on animal habitat preferences and behavior, to predict community-wide exposure to this widespread parasite. We measured animal abundance and foraging behavior in southern California using camera traps, bird surveys and live trapping and used these data to parameterize a function for predicting parasite exposure. We then validated this model by comparing predicted exposures to measured parasite loads in rodents. Although less than 30% of susceptible species came into contact with contaminated sites, high contact rates among ground foraging birds and mammals suggest that infection is likely widespread in these species. Using non-invasive activity-based metrics we identified species warranting more invasive monitoring and this approach could be used to estimate community-wide exposure to any heterogeneously distributed parasite or contaminant.

Introduction

Each day, infected raccoons release enough *Baylisascaris procyonis* eggs to kill every bird and small mammal with which they co-occur (Kazacos 1982; Snyder & Fitzgerald 1987; Weinstein 2016). Although abundant, these pathogenic nematode eggs are also highly aggregated. Most eggs occur in raccoon communal defecations sites ("latrines") suggesting that, for susceptible wildlife, risk is a function of per capita contact with these disease hot spots. Estimation of contact rates is thus critical for parasite management and wildlife

conservation, yet these values are difficult to measure in wildlife populations. Here, using raccoon roundworm as a model infectious agent, we propose a non-invasive way to predict parasite exposure in a heterogeneous transmission landscape and then evaluate the method by comparing predicted exposure to measured parasite loads in rodents.

Raccoon roundworm provides an ideal model parasite to develop these techniques because high infection risk is associated with easily identified sites—raccoon latrines. Maintained by multiple raccoons, these communal defecation sites can persist for years in the same location (Weinstein, unpublished data). In regions where roundworms infect most raccoons, most latrines also contain roundworm eggs (Page *et al.* 2005). These eggs survive for years (Ogdee *et al.* 2016), and most remain within a meter of their original deposition site (Ogdee *et al.* 2017). Eggs infect juvenile raccoons, but worms remain in the gut and cause little pathology (Kazacos 2016). In contrast, when eggs infect most other mammals and birds, larval worms migrate from the gut and cause extensive tissue damage (Sheppard & Kazacos 1997). Larval *B. procyonis* infection ("baylisascariasis") is often fatal and has now been documented in over 150 species, including wildlife, domestic animals, and humans (Kazacos 2016). Baylisascariasis reaches 70% prevalence in some rodent populations (Beasley *et al.* 2013), kills 5% of mice in some regions (Tiner 1954), and is implicated in the decline of at least one endemic rodent (LoGiudice 2003; Page 2013a). Any animal that contacts a latrine risks infection, and because pathology is dose dependent, disease risk increases with contact.

Although any bird or mammal in a region with infected raccoons risks *B. procyonis* exposure, this risk will be modulated by species-specific habitat preferences and foraging behavior. Risk should be higher for animals that prefer habitats with more latrines and, within these high risk habitats, exposure should be amplified for animals that seek out and forage in high risk sites (Page *et al.* 1999; Page *et al.* 2001b). Overall, habitat use puts animals in

possible contact with egg-contaminated latrines, site attraction increases contact frequency, and foraging amplifies exposure at each contact (Figure 5.1). We combined these spatial and behavioral elements into a model for predicting relative per capita *B. procyonis* risk in a patchy transmission landscape. Parameterized with animal activity from a coastal California ecosystem, this model predicts taxon-specific relative baylisascariasis exposure for a bird and mammal community with over 100 species.

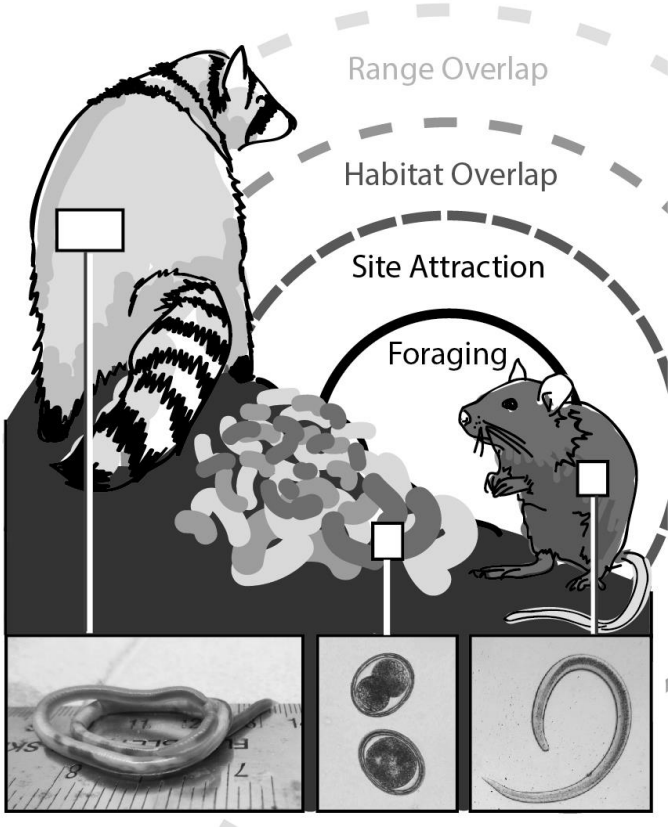


Figure 5.1: Adult *B. procyonis* in raccoons release eggs that accumulate at raccoon latrines. Eggs infect other species and larval worms cause severe pathology. Any susceptible species that co-occurs with infected raccoons risks baylisascariasis, however this risk likely increases with increasing habitat overlap, site attraction and foraging behavior.

Modeling Exposure

Like anthrax, hydatid disease, and toxoplasmosis, *B. procyonis* transmission typically occurs when hosts ingest an infectious stage from the environment (Kazacos 2016). We model this parasite exposure as a function of animal habitat use, latrine attraction and foraging behavior. We assume that any animal sharing a range with infected raccoons has some exposure risk. This relative base exposure, standardized to 1 egg per undefined unit time, is then modified by animal habitat preferences and foraging behavior. Animals that spend more time in latrine-adjacent habitats will be at greater contact risk, and we estimate this habitat overlap by dividing relative abundance near latrines (A_r) by habitat-wide relative abundance (H_r). For a given species, this ratio can be interpreted as the relative fraction of time spent near latrines. Species for which $A_r/H_r > 1$, spend more time in latrine habitats than predicted by their relative abundance in all available habitat. Once in a latrine-associated habitat, animals might seek out or avoid latrines, which can be expressed as time spent at latrines (L_p) divided by time at a paired latrine-adjacent site (A_p). When $L_p/A_p > 1$, species seek out latrines within latrine-associated habitat. Relative exposure due to physical contact, $(A_r/H_r)*(L_p/A_p)*(1$ eggs/time), will be further amplified for species that also forage in latrines. To account for this foraging, we assume that egg exposure per unit time increases when contact includes foraging. To account for this foraging, we add Y*F to the base egg exposure, where F is the proportion of contacts involving foraging and Y is a hypothetical amount by which foraging increases exposure per unit time. We then combine these habitat overlap, site attraction and foraging metrics into Equation 1, representing instantaneous relative parasite exposure in units of eggs per time.

$$\text{Instantaneous Exposure} = (A_r/H_r)*(L_p/A_p)*(1+Y*F) \quad \text{(Eqn 1)}$$

Although relative instantaneous exposure does not predict per capita infection, it can predict relative disease risk for susceptible species. If multiplied by animal lifespan, Equation 1 becomes relative cumulative exposure, measured in eggs. This cumulative exposure, combined with species-specific susceptibility and parasite-induced host mortality can then be used to predict relative parasite loads. Furthermore, absolute contact rates and loads could be calculated given additional information on animal density, time allocation per habitat, and eggs consumed per foraging event.

Study System

To parameterize this exposure model for raccoon roundworm, we collected data on animal habitat use, site attraction, and foraging behavior in an ecological reserve in Santa Barbara County, California (Coal Oil Point Reserve; 34.4105° N, 119.8779° W). Raccoon roundworm infects 80-90% of raccoons in the county (Moore *et al.* 2004; Weinstein 2016), infected raccoons deposit eggs by the millions at their latrines (Kazacos 1982; Evans 2002b; Page *et al.* 2005), and wooded areas in the reserve have up to three latrines per hectare (Chapter 3). Although about 350 terrestrial vertebrates are known from Coal Oil Point Reserve (Holmgren 2001, S. Weinstein, personal observation), *B. procyonis* risk should be greatest for susceptible species that are common in latrine containing habitats, attracted to latrines, and forage at these sites.

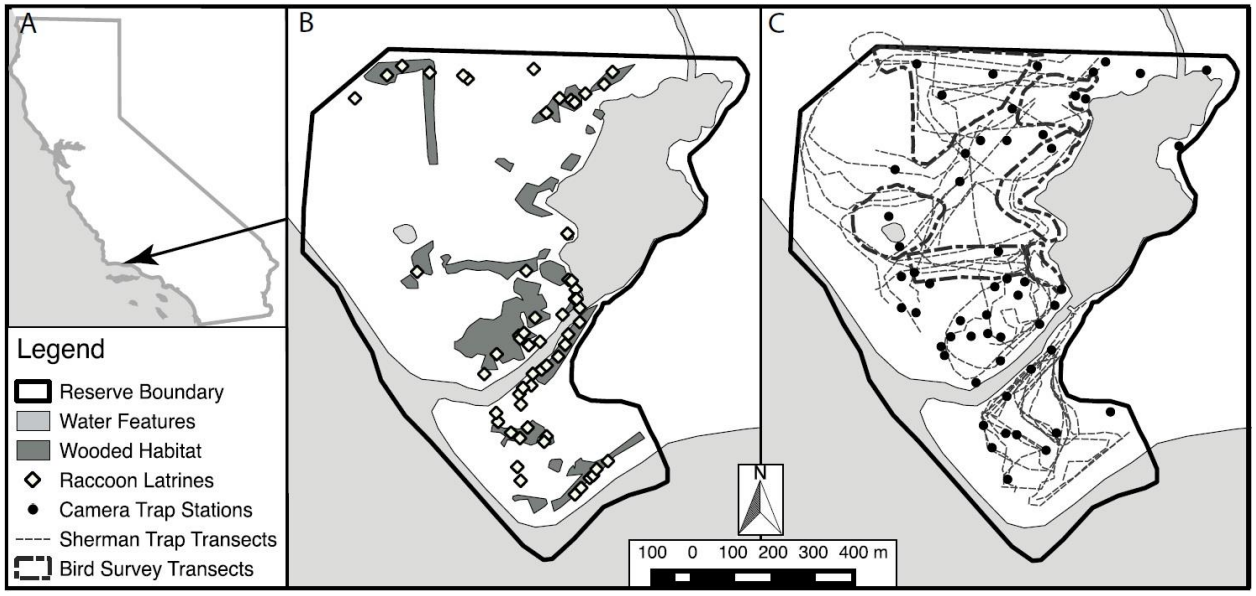


Figure 5.2: Map of latrines, camera trap stations, bird transects, and rodent trap lines at Coal Oil Point Reserve in Santa Barbara County, California

Animal Abundance and Activity

We surveyed birds and mammals throughout the reserve using taxon-specific methods. To calculate reserve-wide relative bird abundance we summed bird counts from monthly bird surveys conducted on a 3.8 km transect from February 2015 and March 2016 (data courtesy of the Coal Oil Point Reserve Bird Monitoring Program, Figure 5.1). To estimate reserve-wide relative mammal abundance, we set camera trap stations (n = 55) throughout the reserve from January through May 2016 (Figures 5.2, 5.1S). We randomly selected eleven stations within wooded, willow thicket, coastal scrub, dune, and grassland habitat and ran two cameras back to back for approximately one week at each station. Cameras (Moultrie M-880 series MCG-12631 and 12594 and MFH-DGS-M80XT, Moultrie, Birmingham, Alabama, USA) used a passive infrared sensor that triggered within a second after detecting either heat or motion. We set cameras to take three to four photos in a rapid sequence ("4 Shot Fast" in M80XT and M-880 12594 and "3 Burst" in M-880 12631) with a

5 - 30 second delay between each series. Each camera had distance markers at one, two and three meters, but as rat and rabbit detection decreased beyond 2 meters, we calculated relative abundance using only observations within 2 meters of the camera (Figure 5.1S). Mammal activity varied by habitat, thus we first calculated relative abundance in each habitat and then extrapolated this to reserve-wide relative abundance based on habitat area. As these camera stations could not always detect mice, we surveyed small (<100g) rodents using Sherman traps (47 trap lines each set for 1 night with ~30 traps, totaling 1376 trap nights) set throughout the reserve monthly from March 2013 through April 2015 (Figure 5.2). Although relative mammal and bird abundances are not comparable, these metrics can be used to compare reserve-wide (H_r) and latrine-adjacent abundance (A_r) within each taxon.

We used data from Chapter 3 to calculate relative abundance in latrine-adjacent habitat (A_r), latrine attraction (L_p/A_p), and foraging (F). Briefly, this study deployed cameras at latrines and matched latrine adjacent sites at Coal Oil Point from December 2012 through April 2015. Using these camera trap data, we calculated the proportion of latrine contacts that included foraging (F) and modeled latrine attraction (L_p/A_p) as time spent in latrines divided by time spent in matched adjacent sites.

To model habitat overlap with latrines (A_r/H_r), for mammals (excluding mice) and birds we calculated relative abundance from reserve-wide mammal cameras, bird surveys, and latrine-adjacent cameras. We consider latrine adjacent habitat to be habitat within 30 m of a latrine and calculate habitat overlap for mice by comparing trap success within 30 meters of a latrine to trap success elsewhere in the reserve. To determine if species preferred or avoided latrine-adjacent habitat, we used an exact binomial test to assess whether relative abundance observed in latrine-adjacent habitat differed from relative abundance reserve-

wide, grouping rare bird species (< 3% of observations) together as either “passerines, excluding corvids (P)” or “non-passerines and corvids (NPC)” (Table 5.1).

Estimated exposure and model validation

We applied Equation 1 to the habitat use, attraction, and foraging data to estimate relative instantaneous exposure for each bird and mammal taxon in the reserve. As the degree to which foraging increases exposure is suspected to be high, but in fact is unknown, we tested prediction sensitivity for Y values ranging from one to 999, corresponding to foraging increasing egg ingestion two to 1,000 times beyond that which would occur through non-foraging contact.

We then compared predicted exposure to measured parasite loads in rodents collected from the same study site. At a community level, exposure is unlikely to correlate with per capita infection due to substantial differences in susceptibility, lifespan, and parasite-induced mortality across unrelated species. However, similar lifespan and susceptibility in mice and rats, and high tolerance in rats (Wirtz 1982; Feng & Himsworth 2014), permits comparisons between predicted relative instantaneous exposure and measured parasite loads for these rodents.

Results

Predicted exposure varied four to eight orders of magnitude across the sampled animal community. Exposure risk was low for most taxa simply due to their habitat preferences. For example, 53 of 85 bird species in the reserve were never seen at latrines or in latrine-adjacent habitat. Although abundant in the region, birds such as hummingbirds and house finches, along with mammals such as rabbits and bobcats, were less abundant around latrines than expected by chance (Figure 5.3A, Table 5.1). In contrast, birds such as

California towhees, California quails, and hermit thrushes, along with skunks (*Mephitis mephitis*) and rats (*R. rattus*), were more abundant in latrine-adjacent habitat than in the surrounding reserve, thereby increasing their per capita exposure (Figure 5.3A, Table 5.1).

Table 5.1: Animal relative abundance and activity. Relative abundance (A_r:H_r) compares latrine-adjacent to habitat-wide relative abundance. Habitat-wide relative bird abundance is based on relative abundance in reserve-wide walking surveys compared to latrine-adjacent cameras, mammals (excluding mice) is based on relative abundance from reserve-wide cameras compared to latrine-adjacent cameras, and mice from trap success data. Activity per month (L_p:A_p) compares activity at latrines and latrine-adjacent sites using data from Chapter 3. P-values from binomial and paired t-tests are adjusted for multiple comparisons with Hochberg's method and coded as: *ns* > 0.05, + <0.05, ++ < .001, +++ <0 .0001, with direction of significant comparisons indicated by a "+" or "-".

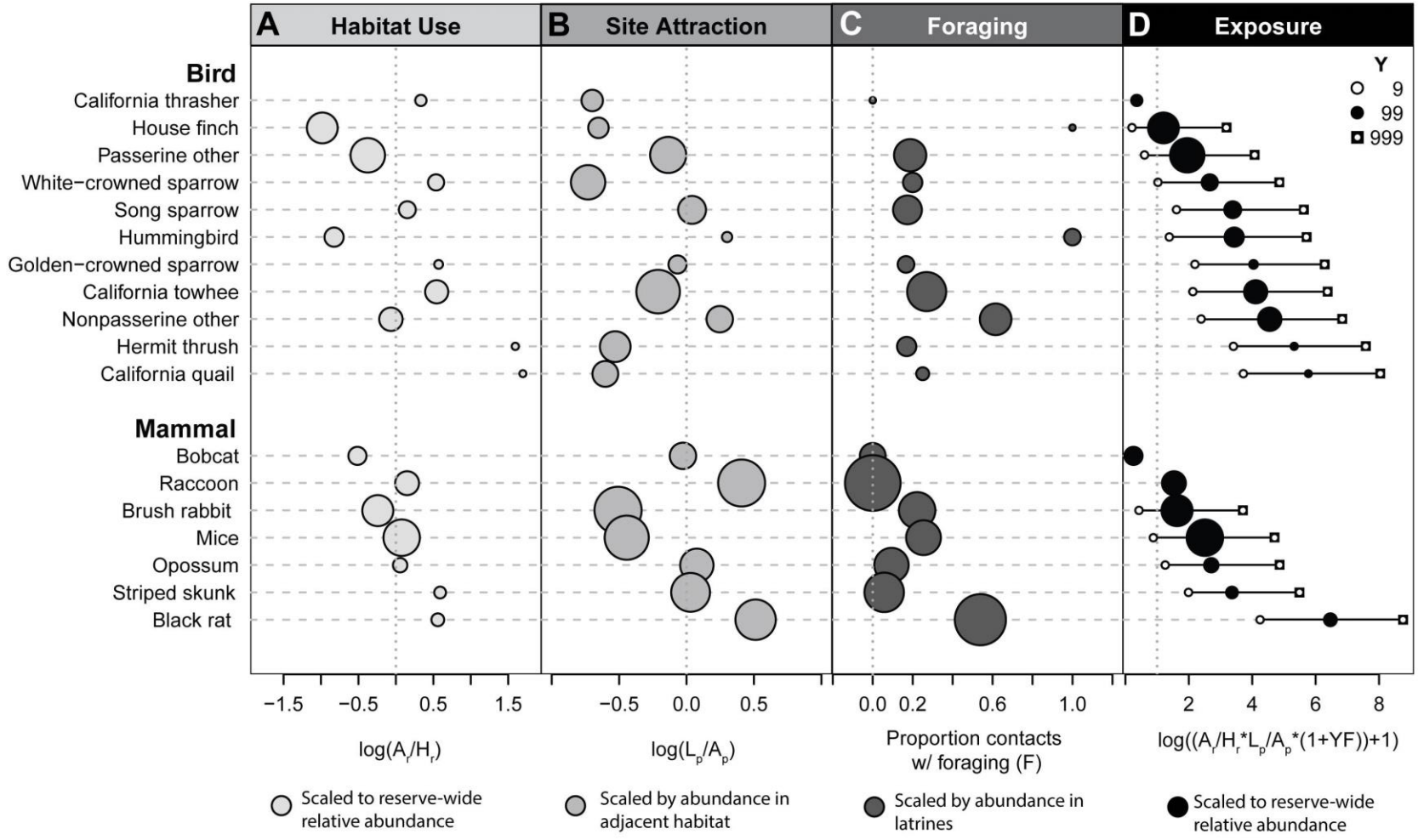
Taxa	Relative abundance A_r:H_r	Binomial test p-value	Activity per month L_p:A_p	Paired t-test p-value	% contacts with foraging
Birds					
California thrasher	2.94 : 1.36	+	0.1 : 0.4		0
House finch	2.6 : 25.4	- - -	0.1 : 0.5		100
Other (P) [1]	16.1 : 38.4	- - -	1.8 : 2.3	*ns*	26
W.c. sparrow[2]	12.6 : 3.7	+ + +	0.2 : 2.3	*ns*	20
Hummingbird	0.9 : 5.9	- - -	0.2 : 0.1		100
G.c. sparrow[3]	2.1 : 0.6	+ +	0.2 : 0.2		17
California towhee	35.0 : 10.0	+ + +	2.3 : 3.6	*ns*	27
Other (NPC)[4]	8.8 : 10.3	- -	2.1 : 0.8	*ns*	62
Hermit thrush	7.9 : 0.2	+ + +	0.2 : 0.8	*ns*	17
California quail	5.0 : 0.1	+ + +	0.1 : 0.7	*ns*	25
Mammal					
Bobcat	3.2 : 10.4	- - -	0.6 : 0.6	*ns*	0
Raccoon	32.4 : 22.9	*ns*	21.2: 6.8	+ + +	0
Rabbit	31.0 : 53.9	- - -	2.5 : 7.3	-	22
Opossum	6.3 : 5.6	*ns*	1.6 : 1.3	*ns*	9
Skunk	12.5 : 3.2	+ + +	2.4 : 2.3	*ns*	6
Rat	14.6 : 4.0	+ + +	11.9 : 3.3	+	54
Mice	32.7 : 27.4	*ns*	1.4 : 3.8	*ns*	24

[1] Passerines, excluding corvids: American robin, Bewick's wren, black phoebe, black-headed grosbeak, blue-gray gnatcatcher, bushtit, common yellowthroat, brown headed cowbird, house wren, nutmeg mannikin, oak titmouse, orange-crowned warbler, song sparrow, spotted towhee, western bluebird, western meadowlark, and yellow-rumped warbler. [2]white-crowned sparrow. [3]golden-crowned sparrow. [4]Non-passerines and corvids: acorn woodpecker, American crow, black-crowned night heron, downy woodpecker, great egret, hairy woodpecker, killdeer, mallard, mourning dove, northern flicker, Nutall's woodpecker, and western scrub-jay.

We combined habitat overlap with foraging behavior and site attraction to estimate taxon-specific relative egg exposure using Equation 1. Rankings were not sensitive to assumptions about how much foraging increased exposure when foraging was assumed to increase exposure more than 100-fold over non-foraging contact (Figure 5.3D). Among birds, the highest predicted exposure risk was for California quail, hermit thrush, golden-crowned sparrow, and California towhee. The abundant sparrows and towhees were also common at latrines (68% of bird contacts), while thrushes and quails represented just 3 and 4% of contacts, respectively. For these relatively rare birds, frequent high risk behavior spread across a small population lead to high predicted exposure.

Among mammals, rats had the highest predicted exposure, with a risk estimated to be around 1000 times greater than that for mice. These differences in predicted exposure matched measured differences in parasite loads in rodents (Figure 5.4). The average rat at Coal Oil Point hosts over 700 B. *procyonis* whereas mice, on average, host less than two (Chapter 4). This match between predicted relative exposure and relative parasite loads in rodents suggests that exposure predictions could be applied community-wide.

Figure 5.3: Predicted egg exposure from habitat use using reserve-wide and latrine-adjacent abundance, site attraction comparing time spent at latrines and latrine-adjacent sites, and foraging as the proportion of latrine contacts with foraging.
Plot A presents the natural log transformed latrine-adjacent relative abundance divided by habitat-wide relative abundance such that species with $A_r/H_r > 0$, are more common in latrine habitats than predicted by their relative abundance in all available habitat. Similarly, Plot B presents the natural log transformed latrine activity divided by latrine-adjacent activity such that species with $L_p/A_p > 0$, are attracted to latrines. A, B, and C were combined using Equation 1 to estimate relative exposure for each taxa (D) using three values for Y, the egg exposure per foraging time.


A Habitat Use
B Site Attraction
C Foraging
D Exposure
Bird
California thrasher
House finch
Passerine other
White−crowned sparrow
Song sparrow
Hummingbird
Golden−crowned sparrow
California towhee
Nonpasserine other
Hermit thrush
California quail
Mammal
Bobcat
Raccoon
Brush rabbit
Mice
Opossum
Striped skunk
Black rat
Y
9
99
999
−1.5 −0.5 0.5 1.5
log(A_r/H_r)
−0.5 0.0 0.5
log(L_p/A_p)
0.0 0.2 0.6 1.0
Proportion contacts w/ foraging (F)
2 4 6 8
log((A_r/H_r*L_p/A_p*(1+YF))+1)
Scaled to reserve-wide relative abundance
Scaled by abundance in adjacent habitat
Scaled by abundance in latrines
Scaled to reserve-wide relative abundance

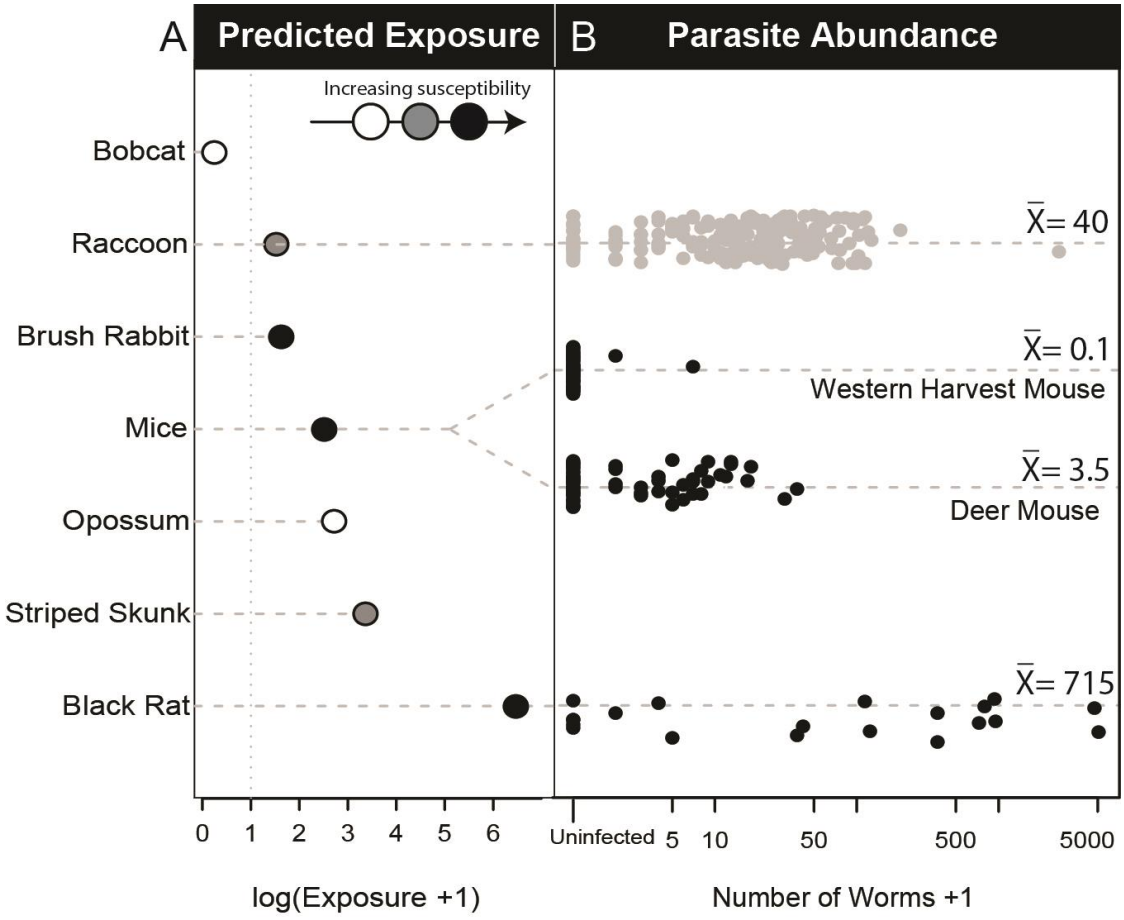


Figure 5.4: Predicted exposure compared to parasite loads from Coal Oil Point Reserve. (A) Predicted egg exposure for each common mammal assuming that foraging increases exposure approximately 100 times that of non-foraging contact (Y=99). Points are shaded by host susceptibility to eggs with black for susceptible, grey for susceptible under limited conditions, and white for resistant (Kazacos 2016). As predicted by egg exposure, parasites were more abundant in rats than in mice (B), as shown by jittered larval parasite counts from rats (*Rattus rattus*) and mice (*Peromyscus maniculatus* and *Reithrodontomys megalotis*).

Discussion

Dozens of latrines, each containing millions of *B. procyonis* eggs, contaminate the terrestrial habitat at Coal Oil Point Reserve. Despite high egg production from raccoons (Weinstein 2016), most potential hosts in this area likely remain uninfected because their activity patterns do not put them in contact with latrines. In particular, although most bird species are susceptible to infection (Wolf *et al.* 2007; Thompson *et al.* 2008), most were not

active near latrines. However, some animals are frequently exposed to *B. procyonis* eggs. We can identify these potentially at-risk species from their habitat preferences, latrine avoidance behavior, and foraging activity. High habitat overlap, latrine attraction, and frequent latrine foraging all increase exposure, and when combined, can result in high parasite loads, as seen in rats.

Even low exposure rates can add up to substantial infection prevalence. For mice, even relatively low estimated instantaneous exposure lead to over 20% infection prevalence (Chapter 4), suggesting that infection could also be common in other species with similar exposure. As most surveys for *B. procyonis* in non-raccoon hosts have focused on mice (e.g. Page *et al.* 2001a; Kellner *et al.* 2012; Beasley *et al.* 2013), parasitism is likely underestimated in other species, particularly ground foraging birds. Raccoon roundworm has been isolated from dead and moribund wild birds (e.g. Evans & Tangredi 1985; Evans 2002a; Kazacos 2016). However, these limited records likely underestimate parasite impacts on bird populations. Using animal time allocation and behavior, we can now identify these at-risk species.

Although a non-invasive method cannot replace dissections as definitive proof of infection, predicting which species are at highest risk can guide more targeted infection surveys. With more targeted surveys, fewer animals will need to be processed with traditional labor intensive and lethal sampling techniques. Here, in a California ecosystem with nearly 100 bird species, predicted exposure suggests that only quail, towhee, thrush, and some sparrows warrant further sampling. Infected raccoons also overlap with susceptible species throughout North America, Europe and Asia and this same non-invasive approach could be used to identify at-risk species anywhere raccoons occur.

Habitat overlap, site attraction and foraging behavior influence *B. procyonis* exposure because infection risk is highly aggregated; however, *B. procyonis* is not the only parasite with heterogeneous transmission risk. Landscape (or spatial) epidemiology incorporates this heterogeneity at large spatial scales (Ostfeld *et al.* 2005; Peterson 2006), but risk can also vary at smaller scales. Schistosome cercaria aggregate 20-30 centimeters below the water surface (Haas *et al.* 2008), anthrax spores concentrate around carcasses (Turner *et al.* 2014), and larval nematodes are found around ungulate fecal clusters (Ezenwa 2004). If high risk sites can be identified, animal behavior can provide insight into exposure risk. Equation 1 predicts *B. procyonis* exposure and could be applied to other aggregated and ingested environmental infectious stages. For infectious agents with alternative transmission routes, this function could be easily modified to predict system-specific contact rates across the relevant animal community. Species with high contact rates might disproportionately contribute to transmission (Lloyd-Smith *et al.* 2005), with ecological consequences determined by host biology. For instance, susceptible species might decline, tolerant hosts might act as reservoirs, and non-competent species might dilute transmission.

Although transmission rates can be estimated using sentinel animals or age intensity patterns (McCallum 2000), these methods are challenging to implement in wild animal populations, might underestimate disease impacts if infected animals die before they are counted, and combine contact and parasite establishment into a single value. Predicting contact rates from space use and risky behavior, as we have done here, offers an alternative non-invasive method for estimating exposure to any infectious agent or contaminant with a heterogeneous transmission risk. Such non-invasive methods may prove critical to conservation of endangered species threatened by such agents, or to predicting pathogen transmission in large and complex ecological communities.

6. Seroprevalence of *Baylisascaris procyonis* infection in Santa Barbara County, California

Abstract

Raccoon roundworm, *Baylisascaris procyonis*, is common in raccoons and can cause devastating pathology in other animals, including humans. There is limited information on the frequency of asymptomatic human infection. We tested 150 adults from California for *B. procyonis* antibodies; 11 were seropositive suggesting that subclinical infection does occur.

Introduction

The raccoon roundworm, *Baylisascaris procyonis*, is a potential health risk to humans. Infected raccoons release eggs in their feces and these eggs accumulate at communal defecation sites ("latrines"). When non-raccoon hosts consume eggs, larva migrans can cause blindness and fatal neurological sequelae (Graeff-Teixeira *et al.* 2016; Kazacos 2016). Less than 5% of migrating larvae reach the brain and experimental studies suggest that host size, infection site, and inoculating dose drive pathology (Kazacos 2016).

Reported human disease cases are rare; however, there is growing evidence for more frequent asymptomatic infections. For example, a recent study showed that 7% of wildlife rehabilitators had *Baylisascaris* specific antibodies (Sapp *et al.* 2016a). Large and heavily infected raccoon populations likely contaminate many regions with *B. procyonis* eggs (Kazacos 2016). As these microscopic eggs can survive for years (Shafir *et al.* 2011), anyone living in regions with infected raccoons likely risks exposure.

A potential high-risk area is Santa Barbara, California. In Santa Barbara, a baylisascariasis case was reported in a toddler in 2002 (Schultz 2002; Kazacos 2016), raccoon roundworm consistently infects over 80% of raccoons (Moore *et al.* 2004; Weinstein 2016) and latrines are abundant in residential areas (J.F. Mendez, unpub. data), potentially exposing residents to infection. Here we describe how we detected subclinical *B. procyonis* infections in adult Santa Barbara residents using a parasite-specific antibody assay.

Materials and Methods

From 2014-2016 we provided public education about raccoon roundworm and offered free testing to healthy adults (ages 18-75 years) who had lived in Santa Barbara County for at least 3 years. Participants were recruited via word of mouth and flyers (distributed primarily at presentations) and through presentations at public outreach events and classes at the university, natural history museum, zoo, and other venues. We also provided information about testing to local wildlife rehabilitators and researchers working with raccoons and *B. procyonis*. We collected serum from a convenience sample of 150 volunteers. This included wildlife rehabilitators (n=5) and researchers (n=7); however we considered results from these 12 individuals separately because their exposure was expected to be higher than for the general population.

Around 5ml of blood was collected from each volunteer, allowed to clot, centrifuged at 1,500 x g for 15 minutes, separated and then stored at -80C. Participants (149/150) filled out a questionnaire on demographics and potential risk factors such as pet ownership, pet and wildlife feeding practices, past raccoon or raccoon feces contact, and frequency of raccoon observations around their neighborhood and residence. No participant reported baylisascariasis symptoms and no clinical examinations were performed. We de-identified

samples and tested for *B. procyonis* IgG using the recombinant *B. procyonis* repeat antigen 1 protein Western blot assay (88% sensitivity, 98% specificity, Rascoe *et al.* 2013; Sapp *et al.* 2016a). Prevalence estimates were generated using Epitools (Sergeant 2016), calculating 95% confidence limits for an imperfect diagnostic assay (Reiczigel *et al.* 2010). We then compared questionnaire responses between seropositive and seronegative participants using exact binomial tests in R (R Core Team 2016).

Results and Conclusions

The 12 researchers and wildlife rehabilitators tested negative for *B. procyonis* antibodies. Among the remaining 138 volunteers, 11 tested positive (apparent prevalence (n=138): 8.0%, Wilson CL: 4.5-13.7%); with an adjusted prevalence of 6.9% accounting for test sensitivity and specificity (Blaker's Exact CL: 2.5-13.4%). All positive individuals had seen raccoons in their neighborhood in the last year and most (7 of 11) had seen one in their yard in the last month. Most positive individuals (9 of 11) reported no contact with raccoons or their feces; in two that reported contact, this potential exposure occurred two and 12 months prior to testing. Individuals with positive serology ranged in age from 20 to 72 years with occupations including engineer, student, administrator, researcher, social worker, zoo volunteer and retired. Some owned dogs, fed animals outside, gardened, and had sand boxes. However, individuals with negative serology gave similar responses (p-value for all comparisons > 0.15) and we found too few infected individuals to identify risk factors (Table 6.1).

Table 6.1: Questionnaire responses from study participants, n=149.*

Variable	Seropositive respondents No. (% of 11)	Seronegative respondents No. (% of 138)
Sex		
Male	3 (27)	54 (39)
Female	8 (73)	84 (61)
Garden regularly	4 (36)	70 (51)
Sandbox at residence	1 (9)	11 (8)
Own a dog	4 (36)	37 (27)
Feed pets outside	1 (9)	11 (8)
Feed wildlife	1 (9)	18 (13)
Contact with raccoon or their feces	2 (18)	20 (14)
Raccoon last seen in neighborhood		
Week	3 (27)	23 (17)
Month	4 (36)	45 (33)
Year	4 (36)	48 (35)
>1 years (or never)	0	22 (16)
Raccoon last seen in yard		
Week	2 (18)	15 (11)
Month	5 (45)	33 (24)
Year	2 (18)	48 (35)
>1 years (or never)	2 (18)	42 (31)

* One seronegative participant did not fill out the questionnaire.

We estimate that approximately 7% of our sample had antibodies to raccoon roundworm; however, this convenience sample does not represent all county residents. Our recruiting strategy likely introduced income, age and education biases, and, by presenting to groups interested in wildlife and outdoor activities we might have selected a sample population with greater exposure risk. Furthermore, because participants could receive their test results, we expect that individuals concerned about past exposure were more likely to participate. Although most baylisascariasis is attributed to *B. procyonis*, cross-reactivity between the *B. procyonis* recombinant antigen assay and other less common *Baylisascaris* species is not well characterized and warrants further study. This survey suggests that subclinical *Baylisascaris* infection occurs in the general population; however, additional studies would improve prevalence estimates. These surveys could also include children because most clinical *B. procyonis* infections occur in individuals less than two years old

(Graeff-Teixeira *et al.* 2016; Kazacos 2016) and it is unclear how long antibodies remain after exposure.

Despite frequent contact with raccoons and their feces, no sampled wildlife rehabilitators or researchers tested positive for *B. procyonis* antibodies. Most wildlife rehabilitators and all researchers examined were aware of *B. procyonis* and took precautions when handling raccoons, feces, or parasites. Although no infection in these high-risk groups could reflect a small sample size, it does suggest that preventive measures are effective.

Subclinical human *Baylisascaris* infections might occur wherever humans and infected raccoons overlap. These infections are likely more widespread than previously assumed and their health risk remains an open question. Subclinical infection might result from lower intensity infection or depend on which tissues are infected (Kazacos 2016). Low intensity infection in organs such as the brain could result in subtle clinical manifestations and understanding the full public health impacts of *Baylisascaris* would require clinical as well as serologic evidence.

This chapter is published Open Access as "Weinstein SB, Lake CM, Chastain HM, Fisk D, Handali S, Kahn PL, Montgomery SP, Wilkins PP, Kuris AM, Lafferty KD (2017) Seroprevalence of *Baylisascaris procyonis* infection among humans, Santa Barbara County, California, USA, 2014–2016. Emerging Infectious Diseases. 23(8). DOI:10.3201/eid2308.170222

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Appendix

Chapter 2 Supplemental Material

Raccoon helminth communities

Table 2.1S: Observed parasites from 182 raccoon small and large intestines. Note that counts include immature parasites such as juvenile (J4) stage *Baylisascaris procyonis*.

Parasite	Number (% of 182)	Intensity range
Nematodes		
Baylisascaris procyonis	166 (91.2)	1-2666
Physaloptera sp.	45 (24.7)*	1-45
Trematodes		
Ascocotyle	5 (2.7)	1-100+
Brachylaima	6 (3.3)	1-7
Fibricola	27 (14.8)	1-100+
Maritrema	20 (11.0)	1-100+
Microphallus	1 (0.5)	1
Pygidiopsis plana	13 (7.1)	1-100+
Cestodes		
Atriotaenia procyonis	35 (19.2)	1-100+
Mesocestoides variabilis	5 (2.7)	1-100+
Acanthocephala		
Profilicollis altmani	38 (20.9)	1-48
Southwellina hispida	22 (12.1)	1-60
Plagiorhynchus cylindraceus	5 (2.7)	1

Chapter 3 Supplemental Material

Table 3.1S. Disease risk and diet preferences for common vertebrates at Coal Oil Point Reserve in Santa Barbara County, California

Taxon	***B. procyonis* exposure outcome**	**Diet**
Rabbits (*Sylvilagus* spp.)	Experimental exposure to 100 eggs generates clinical signs and brain lesions; recorded epizootic baylisascariasis in wild populations (Nettles *et al.* 1975; Jacobson *et al.* 1976; Evans 2002a; Kazacos 2016).	Primarily grasses, will also incorporate stems, leaves, roots and berries from forbs such as thistle, rose, hemlock, clover (Chapman 1974)
Squirrel	Dead and moribund squirrels	Primarily seeds,

(*Otospermophilus beecheyi*)	with neural larva migrans frequently collected around raccoon latrines; in experimental infections, other squirrel species develop fatal neurological symptoms (Evans 2002a; Kazacos 2016).	incorporates herbaceous vegetation (forbs, grasses) in growing season, trace amounts of arthropods (Schitoskey & Woodmansee 1978; Smith *et al.* 2016)
Mice (*P. maniculatus, R. megalotis*)	In *P. maniculatus*, exposures to 50 and 500 eggs are 83% and 100% fatal, respectively. *Reithrodontomys megalotis* develop fatal neurological symptoms from exposure to 250 eggs (Tiner 1953a; Sheppard 1996; Evans 2002a; Kazacos 2016; Sapp *et al.* 2016b).	Primarily seeds, but omnivorous and diet varies with available resources and can also include arthropods, fruits, and green vegetation (Jameson 1952; Whitaker 1966; Webster & Jones 1982).
Birds (common ground foraging passerines including sparrows (white crowned, golden crowned, song), California towhee, California thrasher)	Birds (including passerines) found dead and moribund with neural larva migrans around raccoon latrines (Evans 2002a); numerous (fatal) baylisascariasis outbreaks in captive birds (Richardson *et al.* 1980; Reed *et al.* 1981; Loretti *et al.* 2008; Diab *et al.* 2012) and notably poor ability to encapsulate larvae suggests that most birds are highly susceptible to baylisascariasis (Russell 2006; Kazacos 2016).	Primarily seeds and insects, occasional plant shoots, berries and small fruits. New world sparrows (including towhees) are more granivorous, while the California thrasher consumes relatively more arthropods (Kaufman 2001)
Rattus spp.	In *R. norvegicus*, less than 0.04% of larvae migrate to the brain and most remain in the gut wall; 36% of rats asymptomatic despite experimental acute dosage of 3000 (Wirtz 1982); We have trapped *R. rattus* with no signs of infection despite 12603 larval worms, at Carpinteria Salt Marsh, California	Preferentially consume fruits, nuts and seeds but highly opportunistic (Feng & Himsworth 2014) and also eat green vegetation, roots, arthropods, and small vertebrates (Clark 1981; Grant-Hoffman & Barboza 2010; Ruffino *et al.* 2011)
Raccoon	Usually no clinical sign of infection, however fatal intestinal obstruction is possible for juvenile animals with exceptionally high loads. Egg susceptibility decreases with	Omnivorous, varies with resource availability. Plant foods include berries, nuts, seeds; arthropods, particularly crustaceans when available; vertebrate

	age, with resistance past 4 months (Kazacos 1983a; Stone 1983; Carlson & Nielsen 1984; Kazacos & Boyce 1989; Kazacos 2001, 2016).	predation infrequent but includes eggs, injured animals, nestlings (Lotze & Anderson 1979; Gehrt 2003)
Skunk	Definitive host for related *B. columnaris*, but can also host adult *B. procyonis* suggesting that susceptibility and pathogenicity are similar to raccoons (Tiner 1949; Sapp *et al.* 2017).	Primarily insectivorous, but opportunistically omnivorous, eats vertebrates (rodents, eggs, nestling birds, carrion), vertebrate and invertebrate prey represent 80-90% of diet but will eat fruit, grains and garbage (Wade-Smith & Verts 1982; Rosatte & Lariviere 2003)
Bobcat	No infection in experimentally exposed cats, no documented natural infections despite high exposure through predation on infected rodents; felids presumed resistant to both eggs and larval stages (Miyashita 1993; Kazacos 2016)	Almost exclusively carnivorous, prey varies across season and includes lagomorphs, ungulates, rodents, birds and less frequently herpetofauna, fish, insects and eggs. Grass commonly found in gut likely used as a purgative as with domestic cats (Anderson & Lovallo 2003)
Opossum	No infection in opossums experimentally exposed to eggs, and despite frequent latrine contact, no *B. procyonis* documented from wild opossums (Alden 1995; Page 1998; Page *et al.* 1999; Kazacos 2016)	Omnivorous, but primarily insects, vertebrates and carrion, will also eat garbage and plant material including green vegetation, fruits, nuts and grains (McManus 1974; Gardner & Sunquist 2003)
Reptiles (western fence lizard)	*Baylisascaris* spp. infections are confirmed only from birds and mammals. Although Davis *et al.* (2016) report a *Baylisascaris* larva in a snake, ascarid larvae are difficult to differentiate and this identification is suspect without histological sections or sequencing (L. Camp pers com).	Insects and spiders (Stebbins 2003)

Figure 3.1S: Camera set-up

(A) Latrines were identified based on the presence of feces from at least two raccoon defecation events and most sites contained feces from 10-40 defecations. (B) For latrine monitoring, a single camera (n=225) was positioned to monitor animal activity at the latrine. For a subset of latrine deployments, a second camera (n=116) was added to monitor animal activity at a matched adjacent site.

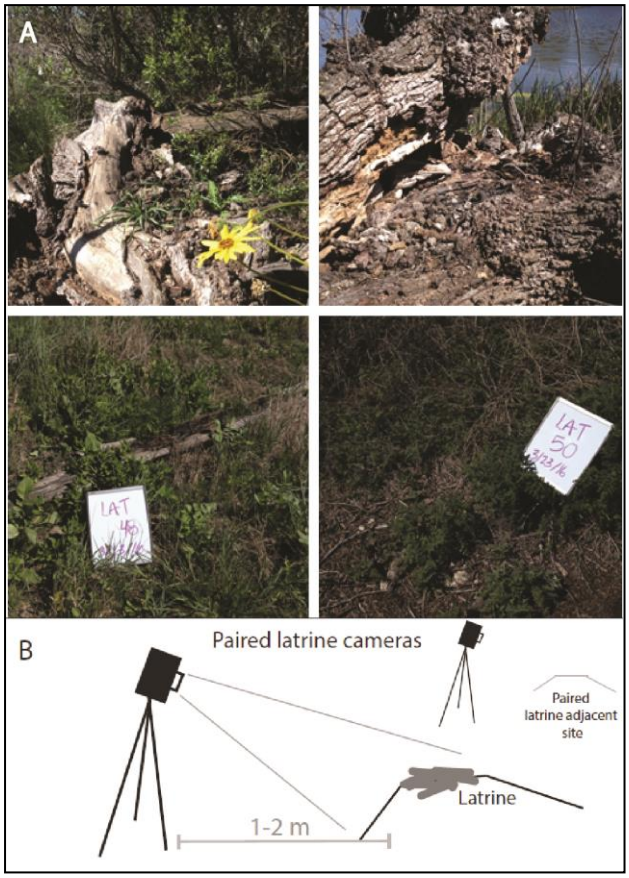
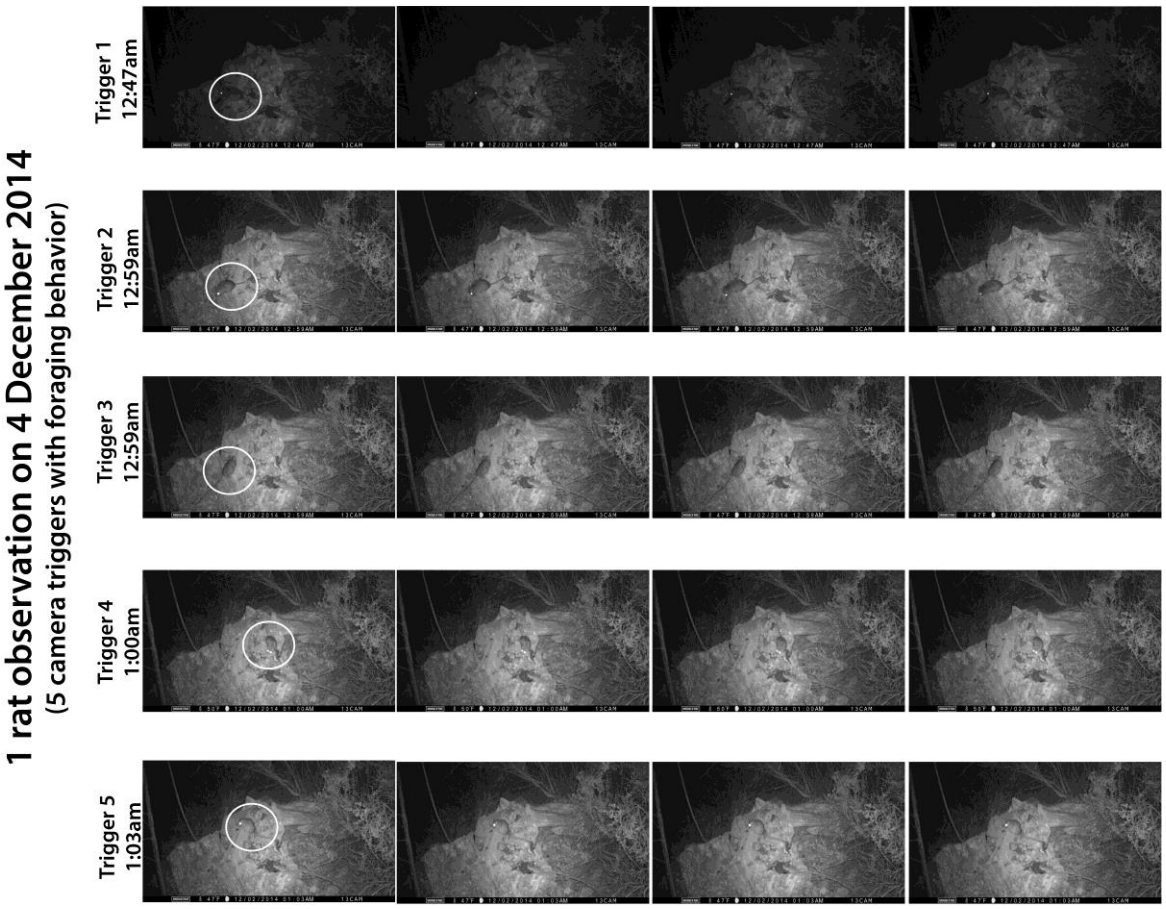


Figure 3.2S: Image analysis

All camera trap images were examined by trained personnel, with each person working together with a more experienced individual until able to identify all species in photographs. All birds were initially coded as "bird" and then identified to species by C.W. Moura. Any animal captured in the camera frame was considered an observation, however, only animals seen in physical contact with a latrine were classified as "latrine contacts." Animals were counted as unique observations if multiple individuals were present, at least 15 minutes had elapsed since the last observation of that species, or animal features clearly distinguished it from previously observed individuals. Latrine and latrine-adjacent comparisons were based

on time spent in each location, using the number of camera triggers as a proxy for time in contact with latrines and matched latrine adjacent sites. For example, in this photo sequence from 12:47 to 1:03 am, all images are counted as the same animal observation because time stamps are within 15 minutes of each other and no features indicate that rats (circled) observed in the 5 triggers are different individuals. Based on the photo sequences, particularly trigger 4 and 5, the animal is also coded as foraging and in contact with the latrine (note that this is the latrine pictured in the top left of Figure S1.A)



Latrine activity by habitat, time, and season

We measured habitat features at each latrine to determine whether local vegetative structure influenced species observed at each site. To control for annual and seasonal variation in plant growth, we surveyed all vegetation over one week in March, 2016. At each latrine site (n=47) we measured canopy cover (openness) using a densiometer and then measured distance to nearest cover that would be sufficient to hide a bird or small mammal (dist.cover). Then, using a one meter square quadrat placed 1.5 meters from the latrine in each of the four cardinal directions we measured percent bare ground (p.bare), percent grass (p.grass), forb (p.forb), shrub cover (p.shrub), and litter depth (litter).

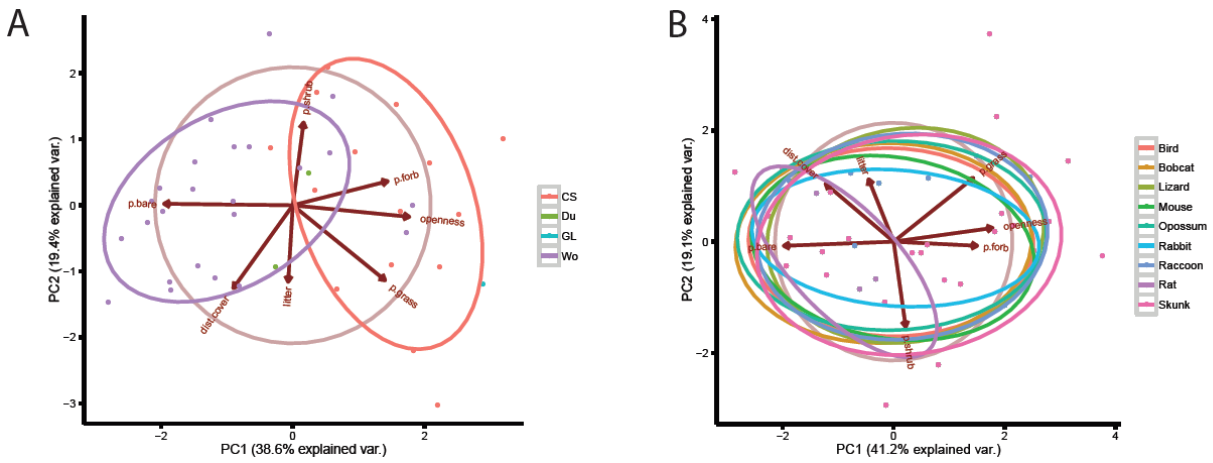


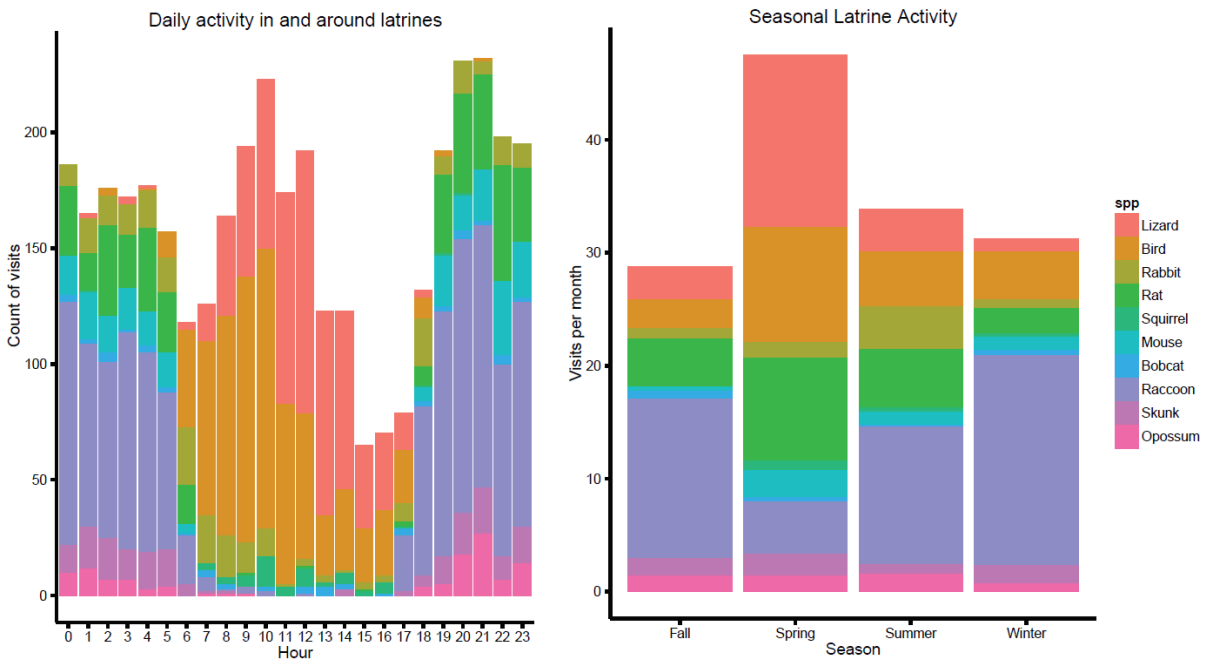
Figure 3.3S (A) Characterization of latrine sites based on habitat characteristics. (B) Animal presence at latrines based on habitat characteristics

Latrines occurred in wooded and willow (Wo), grassland (GL), dune (Du), and coastal scrub (CS) habitat. Wooded habitats were characterized by more canopy cover (less openness) and more bare ground. Latrines were rare in grassland and dune habitat and those sites generally clustered with coastal scrub, and were similarly characterized by less canopy cover and higher percent grass and forb cover than in wooded habitats. Most species visited latrines in all habitat types. Chipmunks were previously not known to occur within the

reserve and their sightings were restricted to a single location. Rat (*Rattus rattus*) populations were distributed throughout the reserve but restricted to wooded and willow habitats with higher canopy cover.

Although most species occurred at latrines in all habitat types, animal activity in and around latrines varied by time of day and season. Birds, lizards and squirrels were most active during the day. Mice, opossums, raccoons, rats, and skunks were active after dark. Bobcats and rabbits were active both day and night. Most animals were seen year round (note that all birds are considered together here), with increased activity, particularly in birds and lizards, in the spring.

Figure 3.4S: Daily and Seasonal animal activity in and around latrines



Estimating raccoon population size at Coal Oil Point Reserve

Population size estimates typically require mark recapture surveys; however, because raccoons consistently use latrines, the combination of latrine use and latrine density can be used to estimate raccoon population size. There were approximately 45 active latrines in the reserve at any given time and the average latrine was visited by 0.46 ± 0.65 (SD) raccoons per day, for an estimated 20.7 latrine visits per day in the reserve. If raccoons are assumed to defecate one to two times per day (Kazacos 1982), and most of these defecation events occurred at latrines, then this network of latrines was likely maintained by a local population of approximately 10-20 animals. This translates to a density of approximately 16-32 raccoons per square kilometer, which is similar to estimates from Coyne *et al.* (1989) and Broadfoot *et al.* (2001) and within densities ranges summarized in Gehrt (2003).

Chapter 5 Supplemental Material

Figure 5.1S: Diagram of camera set up for reserve-wide mammal monitoring