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The parasite ecology of the San Miguel Island Fox, *Urocyon littoralis littoralis*

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution, and Marine Biology

by

Jasmine Necole Childress

Committee in charge:

Professor Erika Eliason, Co-Chair

Dr. Kevin Lafferty, Co-Chair

Dr. Christopher L. Jerde

Professor Cherie Briggs

March 2023

The dissertation of Jasmine Necole Childress is approved.

Cherie Briggs

Christopher L. Jerde

Kevin Lafferty, Co-Committee Chair

Erika Eliason, Co-Committee Chair

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VITA OF JASMINE NECOLE CHILDRESS
March 2023

EDUCATION

2015 – 2023 PhD in Ecology, Evolution, and Marine Biology
University of California, Santa Barbara
Advisors: Drs. Erika Eliason and Kevin Lafferty

2011 – 2015 Bsc, Biology
Florida Southern College
Advisor: Dr. Gabriel Langford

PUBLICATIONS

Miller-ter Kuile, A, Apigo, A, Bui, A, Butner, K, **Childress, JN**, Copeland, S, ... & Young, H. S. 2022. Changes in invertebrate food web structure between high-and low-productivity environments are driven by intermediate but not top-predator diet shifts. *Biology Letters*, *18*(10), 20220364.

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PROFESSIONAL EMPLOYMENT

2022-2023	USGS Pathways Internship Program
2021	Teaching assistant. Ecology of Noninfectious Diseases (EEMB 50)
2020	Instructor of Record. Parasitology (EEMB 111)
2019-2021	Wildlife Conservation Consultant. US Geological Survey
2019	Teaching assistant. Ecology of Infectious Diseases (EEMB 40)
2016-2020	Teaching assistant. Parasitology (EEMB 111)
2016	Teaching assistant. Entomology (EEMB 118)
2015, 2020	Teaching assistant. UCSB Invertebrate Zoology (EEMB 112, 116)

AWARDS

2022	NSF Postdoctoral Research Fellowship in Biology (PRFB), Stanford University | UCSB EEMB Graduate Fellowship (Summer)
2021	Fulbright Switzerland | Swiss Government Excellency Award | UCSB EEMB Graduate Fellowship
2020	UCSB EEMB Graduate Student Fellowship | Schmidt Family Foundation Mentorship Grant
2019	NSF Graduate Research Internship Program with US Geological Survey
2018	American Society of Parasitology (ASP) Outstanding Student Presentation Award | ASP Travel Award | UCSB Individualized Professional Skills Graduate Student Award
2017	NSF Graduate Research Internship Program with US Geological Survey
2016	NSF Graduate Research Fellowship (GRFP) | UCSB EEMB Graduate Student Fellowship
2015	Fulbright Mexico Semi-finalist | ASP Outstanding Oral Presentation Award | Florida Southern College Honor Walk Most Distinguished Undergraduate
2014	ASP Willis A. Reid, Jr. Travel Award | Florida Southern College Collaborative Research Grant
2013	NSF Research Opportunity Award Summer Funding
2012	NSF Research Experience for Undergraduates Award
2011	American Fisheries Society Hutton Junior Fisheries Biology Grant

PROFESSIONAL SERVICE

2021	Panelist. UCSB CSEP Summer Programs: Getting in, surviving, and thriving in grad school
2020-2021	Inaugural member of the EEMB Dept. Diversity, Equity, and Inclusion Committee
2020-2021	Lecture Series Director. UCSB Graduate Students for Diversity in Science
2018-present	Member. Ecological Society of America
2017-2023	Founder. UCSB EEMB Diversity, Equity, Inclusion, and Wellness Committee
2017-2019	Mentor. UCSB Black Student Engagement Program
201-2018	Organizer and Keynote Speaker. UCSB I Heart STEM Conference
2015-2023	Member and Outreach Chair (2017). UCSB Black Graduate Student Association
2014-present	Member. American Society of Parasitology

Reviewer

2022-2023	Guest Editor. *Environmental DNA*
2015-present	Environmental DNA, Tropical Ecology, Journal of Parasitology

PRESENTATIONS

(* – invited; ** – award received; **OP** – oral presentation; **PP** – poster presentation)

2022 ***Juneteenth Speaker**: Preventing the parasite-induced decline of the San Miguel Island fox. Ecological Society of America

2021 ***Keynote Talk**: Trajectory of Parasitology: Looking to the future using environmental DNA detection

2020 ***Seminar Speaker**. Amphibian Parasite Ecology in Yosemite National Park (COVID-19 cancellation). California State University Fresno Biology Department

2019 *Science Communication and Interpretation in the National Parks Workshop. 2019. Second Century Stewardship

2019 ***Guest Lecture**. Stanford University Bio2N: Global Change and the Ecology of Infectious Disease

2019 **OP.** "How do invasive bullfrogs affect the amphibian-parasite community of YNP?" Sierra Nevada Aquatic Research Consortium (SNARC) Workshop and Conference

2018 ****OP**. "How does order of infection by a virus, fungus, and parasite affect within-host community dynamics of amphibians?" ESA and ASP

2015 ****OP.** "Reproductive plasticity in the nematode *Gyrinicola batrachiensis*: Is reproductive strategy dependent upon tadpole developmental time?" ASP

2014 **"**Where are the infected snails? Searching for an alternative sentinel organism for Gordian Worms (Phylum Nematomorpha)" **PP** ASP and **OP** Southwestern Society of Parasitology

ABSTRACT

The parasite ecology of the San Miguel Island Fox, *Urocyon littoralis littoralis*

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The Channel Islands foxes are a popular conservation success story after managers, scientists, and zookeepers worked together to remove non-native predators, restore native habitat, and facilitate on-island captive breeding programs. However, although other island fox populations have recovered well, this success story may have been told too soon for the San Miguel Island (SMI) fox. This population experienced a decline of more than 70% between 2015 and 2018 and has been slow to recover since. This decline is attributed, in large part, to parasitism. Notably, a recently identified acanthocephalan parasite, *Pachysentis canicola*, has drawn considerable attention from Channel Islands National Park (CINP) managers. This parasite has not been detected on other California Channel Islands (CCI) or in mainland California canids, and little is known about the ecology of other helminth species that infect the SMI fox. Here, we sought to 1) describe the helminth assemblage of SMI foxes and the biogeographical patterns of parasitism across the CCI; 2) compare traditional methods of detecting infected foxes with new molecular DNA metabarcoding techniques; and 3) determine the spatial and temporal distribution of fox parasites. In addition to recording seven species of fox parasites, we successfully detected parasite DNA as well as DNA from prey items that may facilitate transmission using scat

metabarcoding. Further, we established a baseline for the effects of temperature, rainfall, and habitat type on the prevalence and densities of parasite eggs across the island. The CINP needs a conservation plan to prevent parasite-induced extinction of the San Miguel Island fox, a vulnerable and charismatic species that exists nowhere else in the world. My dissertation provides a foundation for combining traditional and novel tools for managing wildlife threatened by emerging infectious diseases.

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1.0 Abstract

Biogeography theory predicts that island diversity is a nested subset of mainland diversity, and that island biodiversity increases with island size and decreases with island distance from mainland. The same should apply to parasite communities in island hosts. We therefore hypothesized that helminth assemblages would be more diverse in mainland foxes than in island foxes, and in foxes on large/nearby islands than on small/distant islands. We reviewed the literature on gray fox (*Urocyon cinereoargenteus*) and Channel Islands fox (*Urocyon littoralis* spp.) parasites. Then, at the smallest island in our study, San Miguel Island (SMI), we inspected fox feces for parasite diagnostic stages and dissected invertebrates to look for larval parasites. Helminth communities did not follow predictions from island biogeography theory. Helminth richness on islands was not significantly correlated with island size or distance from mainland. Unexpectedly, helminth richness was highest on the smallest island, San Miguel. Observed parasite richness from island foxes do not conform to predictions from island biogeography, as there is no clear relationship between island fox helminth richness and island size or distance from mainland. Surprisingly, SMI has a higher observed helminth richness than other islands and the mainland. The unexpectedly high parasite richness on SMI may be due to under reporting as well as the presence of an island-endemic parasite, *Angiocaulus gubernaculatus,* and two recent parasite introductions: the previously unreported nematode, *Calodium hepaticum*, and the acanthocephalan, *Pachysentis canicola*. Regardless, these patterns of parasite diversity

across islands appear to impact fox health and might explain variation in long-term population persistence from island to island.

1.1 Introduction

Many species endemic to islands have a high risk of extinction. A case in point are the Channel Islands foxes (*Urocyon littoralis* sspp.) that diverged from the gray fox (*Urocyon cinereoargenteus*) after being transported to *Santarosae* island off the coast of southern California, USA, by Native American Chumash peoples around 10,000 calendar years BP (Walker, 1980). With rising sea levels, *Santarosae* split into separate islands, three of which now support genetically distinct subspecies of the Channel Islands fox: San Miguel, Santa Rosa, and Santa Cruz (Rick et al., 2009). Three more islands to the south also support distinct fox subspecies: Santa Catalina, San Nicolas, and San Clemente (Moore & Collins, 1995). Island foxes are smaller, tamer, and more diurnal than the ancestral gray fox (Coonan et al., 2010). They are now an iconic species at the Channel Islands National Park (CINP). Yet, like most species, they are infected with a diverse community of pathogens and parasites. Previous work has focused on the potential impacts of distemper, adenovirus, parvovirus, coronavirus, herpesvirus, *Toxoplasma*, and *Leptospira* (Clifford et al., 2006). Here, we consider how helminth parasites might now threaten fox persistence at San Miguel Island.

In the 1990s, predation by golden eagles reduced island fox populations to the point they were declared endangered (Roemer et al., 2001). To recover the species, wildlife managers and scientists focused on two measures to reverse this decline; i) relocating golden eagles, and ii) captive breeding of the critically low fox population (Coonan et al., 2010). The San

Miguel Island (SMI) fox (*U. littoralis littoralis*) rebounded from a low of just 15 individuals in 1999 to a high of 650 by 2015 (Coonan et al., 2005). Although fox populations on other islands have recovered well, this success story may have been told too soon for SMI foxes. The fox population of SMI fox declined by more than 70% between 2015 and 2018 and has been slow to recover since (Shaskey, 2019; Dillon & Shaskey, 2022).

San Miguel Island has drawn particular interest from managers and researchers because the sharp fox decline coincided with a newly observed acanthocephalan parasite, which has not been found in other Channel Islands foxes or mainland canids in general. After the acanthocephalan was reported in 2012, the number of trapped foxes in poor condition increased, fox reproductive success decreased, and mortality associated with peritonitis due to mass infection by this parasite increased significantly (Coonan et al., 2015; Dillon and Shaskey, 2022). Infection severity by other SMI fox parasites like the Spirurid nematode *Spirocerca* sp. also increased during this period, and park managers have detected more emaciated foxes in recent years (Dillon and Shaskey, 2022). To mitigate further population decline and possible parasite-induced extinction on SMI, the Channel Islands National Park (CINP) has sought further research to understand the extent to which parasitism impacts fox health and population persistence.

The California Channel Islands have been called “California’s Galapagos” and have been subject to many biogeographical studies (Westman, 1983; Smith & Carpenter, 2006; McGlaughlin et al., 2014). Reduced richness on smaller islands and islands farther from the mainland are common predictions from island biogeography (MacArthur & Wilson, 1967). Parasite ecologists have further explored these predictions by incorporating patterns of parasite dispersal to predict variation in parasite communities among host populations

(Dobson & May, 1986; Combes, 2001; Keane & Crawley, 2002; Torchin et al., 2003). For instance, helminth richness in Channel Islands deer mice (*Peromyscus maniculatus* sspp.) decreases with distance from the mainland (Lafferty et al., 2010). Additionally, island fox ectoparasite diversity is lower on smaller islands, with the smallest island, San Miguel, supporting the lowest diversity (Harris et al., 2013).

In this study, we aimed to explore whether patterns of fox helminth diversity in relation to island size and distance from mainland are consistent with findings from previous biogeographical studies of Channel Islands parasite communities. We, therefore, hypothesized that fox helminth richness would increase with island size and decrease with island distance from the mainland. We also hypothesized that the smallest island, San Miguel, would support the lowest fox helminth diversity. To compare parasite richness across fox populations, we reviewed the literature on gray fox and island fox parasites. A previous parasite assessment conducted on captive island foxes between 2002 and 2005 by Sohn and Thomas (2005) provided the bulk of the island fox information. We added unpublished parasite information from island fox necropsies. We also inspected SMI fox feces for parasite diagnostic stages (eggs and larvae), and dissected SMI invertebrates for larval and juvenile parasites to identify possible intermediate hosts.

1.2 Methods

1.2.1 Inspection of fox feces

San Miguel Island (34°02’06” N, 120°21’30” W) is roughly 36 km^2 and is one of five islands that make up the Channel Islands National Park (CINP) (Figure 1). The island lies 42 km from the coast of southern California, USA. The climate is cooler, foggier, wetter and

windier than the mainland, with an annual temperature range of 2 to 33°C and mean annual precipitation of 36 cm (*Western Regional Climate Center*). Habitat diversity on SMI is limited to grassland with shrubs (somewhat degraded by historical sheep ranching), and beaches with dune-lined vegetation. As per park regulations, these habitats are free from permanent human inhabitance and limited in use. Outside of the 1.6-km trail from Cuyler Harbor to the campground and ranger station, hiking is forbidden unless accompanied by a National Park Service (NPS) ranger.

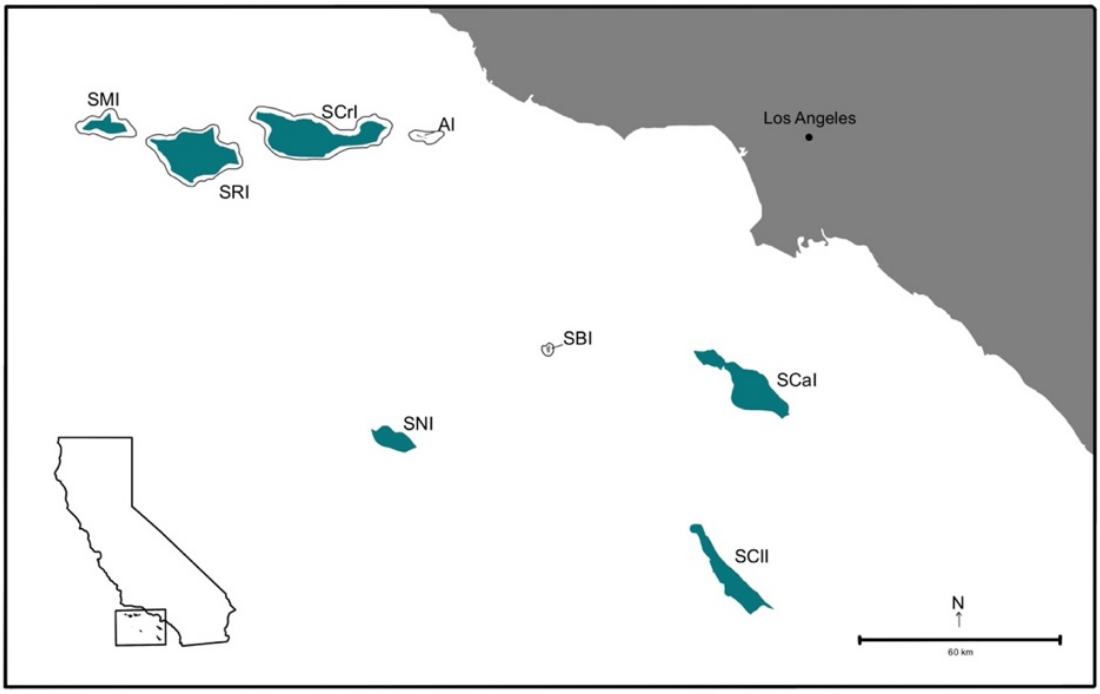

Figure 1. Map of the California Channel Islands with fox-dwelling islands shaded in blue and the CINP boundaries outlined in black. Islands: San Miguel (SMI), Santa Rosa (SRI), Santa Cruz (SCrI), Anacapa (AI), Santa Barbara (SBI), Santa Catalina (SCaI), San Nicolas (SNI), and San Clemente (SClI).

We collected 405 fresh scat samples along trails on SMI. From February 2019 to January 2021, ten scats were collected monthly around the SMI ranger station (NPS staff added considerably to these collections) for the purpose of understanding seasonality. In addition to these 239 samples, 166 scat were collected from 24 September to 24 October 2020 for an

island-wide spatial study. All scats were homogenized upon collection and stored at -20°C until processed.

We used the standing fecal sedimentation and fecal flotation methods to search for helminth diagnostic stages (eggs and larvae) from each homogenized scat (mean and standard deviation (sd) of each sample: 10.4 ± 5.7-g). Sediments and floats were examined under light microscopy (100-400x total magnification). For fecal sedimentation, we used a mean subsample of 2.0 ± 0.3-g (sd) and mixed with DI water to break up large pieces. Then, we sieved the mixture into a 50-mL Falcon tube and filled it with fresh water. After 10 minutes, we poured off the supernatant and refilled the Falcon tube with fresh water. We repeated this process until the supernatant was clear, after which we removed all water. The remaining pellet (between 0.25-0.5mL) was transferred to microscope slides and examined under a compound scope. For fecal flotation, we used a mean subsample of 1.92 ± 0.3-g (sd) and followed the Standard Centrifugation Fecal Examination Technique with a fixed-head centrifuge as described by Dryden et al. (2005). We used Vedco Feca-Med standardized sodium nitrate with specific gravity of 1.25 – 1.30 as the flotation solution. For each sample, we recorded the date collected, date processed, total sample mass, subsample mass, subsample volume, volume searched, and the number and identity of all helminth eggs and larvae. We calculated the mean and standard deviation of helminth richness for individuals SMI fox scats. We discontinued flotation analysis after processing 239 samples due to the better performance of fecal sedimentation (acanthocephalan eggs are too dense to be detected by flotation methods).

1.2.2 Invertebrate dissections

Invertebrates were collected between November 2018 to March 2021 by opportunistic hand capture, inspection under boards set up for herp monitoring, transects baited with oatmeal, cups placed on trails in shallow, existing holes, and hand collection on and around fox scat. Pitfall and other common invertebrate collection traps were not used so as to preserve Native American Chumash heritage sites and reduce the encounter with potential unexploded ordinance as the US military used to conduct explosives test on SMI (Williams et al., 2017). For invertebrate dissections, we targeted both taxa that are known to facilitate parasite transmission (e.g. *A. gubernaculatus* infects gastropods as intermediate hosts) as well as those that are hypothesized intermediate hosts for SMI fox parasites (e.g. orthopterans that may facilitate the life cycle of *P. canicola*). In total, we dissected 945 individuals from the following orders: Coleoptera, Dermaptera, Gastropoda, Hemiptera, Hymenoptera, Isopoda, Orthoptera, and Zygentoma (see Supplemental Material 1 for list of taxa).

1.2.3 Parasite tabulation

We conducted a literature search to tabulate the helminths that are found in San Miguel Island foxes, other Channel Islands foxes, and mainland gray foxes. Mainland gray foxes range from the southern half of North America to northern South America (Venezuela and Colombia), except for the northwest mountain areas of the U.S (Wilson & Reeder, 2005). For this study, our "mainland" classification included gray fox parasites recorded from the pacific west coast of Washington, USA, to Baja California, Mexico. This was to capture a realistic overview of the fox helminths that are present today as well as those that could have been present under the same climatic environment from when island foxes diverged from

mainland foxes around 10,000 years ago. For each species, we searched for trematodes, cestodes, nematodes, and acanthocephalans published in peer-review articles or agency reports. We classified distributions from the individual Channel Islands (San Miguel (SMI), Santa Rosa (SRI), Santa Cruz (SCrI), San Nicolas (SNI), San Clemente (SClI), Santa Catalina (SCaI)) and/or the mainland.

We used a generalized linear model (GLM) with Poisson distribution with log link function to test the effects of island size (Size) and distance from mainland (Distance) on helminth richness (Richness) for the six island fox populations. Model selection was based on Bayesian information criterion (BIC; Schwarz, 1978). We formulated five hypotheses. The first model reflects a null hypothesis that the richness of island fox helminths does not vary among islands and does not depend on measured variables (M_0: Richness ~ constant). We assessed two univariate models (M_1: Richness ~ Size; M_2: Richness ~ Distance), one multivariate model (M_3: Richness ~ Size + Distance), and one model that included an interaction term for island size and distance (M_4: Richness ~ Size*Distance). We determined the best model fit by using the lowest BIC value. We assess the evidence between models using the difference between model BIC values (ΔBIC) of all models from the model with the lowest BIC value. Interpretation of model evidence followed guidance by Jerde et al, (2019), where candidate models with $\Delta BIC < 7$ should be retained as possible explanatory hypotheses for further consideration with more data. All analyses and figures were generated in R version 3.6.3 (R Core Team, 2020) using the *tidyverse*, *stats, broom*, and *ggplot2*, and *ggrepel* packages (Wickham et al., 2019; Robinson et al., 2022; Slowikowski et al., 2022; Wickham et al., 2022).

1.3 Results

1.3.1 Inspection of fox feces

We detected six helminth species using fecal sedimentation, and the mean richness for individual SMI fox scat was 3.3 ± 1.3 (sd) species. Of the 405 scat samples processed, 99.5% (403) contained eggs from at least one helminth (Table 1). We recovered 622,558 (392,691 sedimentation and 229,867 flotation) eggs representing five previously reported SMI fox helminths and the first report of *Calodium hepaticum* eggs. Although *C. hepaticum* prevalence was low (13.3%), the density of eggs was the highest at nearly two orders of magnitude greater than any other species (2677±1589 eggs/gram feces).

The most common helminth was *Uncinaria stenocephala* with a prevalence of 93.8% and 96.2% for sedimentation and flotation, respectively. Other nematodes recovered included eggs of *Spirocerca* sp. and larvae of the lungworm, *Angiocaulus gubernaculatus.* The remaining helminth eggs included the tapeworm, *Mesocestoides corti,* present in 1.7% of samples and the acanthocephalan, *P. canicola*, present in 58.5% of samples. Of note, we found live juvenile nematodes sporadically in sedimentation samples, with the longest period between the freeze and thaw/examination being 11 months (freezing temperatures do not occur on contemporary SMI).

Table 1. Prevalence and mean intensity for helminth eggs recovered from San Miguel Island fox scats. % = percent prevalence (number of samples); Density = mean number of eggs per gram of infected scat examined; CI = lower and upper 95% limits for density.

Helminth	Sedimentation (n = 405)			Flotation (n = 239)		
	% (n)	**Density**	**CI**	**% (n)**	**Density**	**CI**
Angiocaulus gubernaculatus	75.6 (306)	82.9	23.9-141.9	34.7 (83)	99.5	0-216.4
Spirocerca sp.	57.3 (232)	14.1	10.2-18.1	19.7(47)	44.2	0-97.8

Calodium hepaticum	13.3 (54)	2677.0	1088-4265		16.3 (39)	2499.6	0-5044
Uncinaria stenocephala	93.8 (380)	36.5	29.6-43.2		96.2 (230)	30.9	24.6-37.6
Mesocestoides corti	1.7 (7)	21.2	0-48.9		0.0	-	-
P. canicola	58.5 (237)	40.9	32.5-49.2		0.0	-	-

1.3.2 Invertebrate dissections

We recovered a single third stage larva (L3) of the nematode species *A. gubernaculatus* from one San Miguel shoulderband snail (*Helminthoglypta ayresiana*, aka the Ayre's snail). There were no other larvae recovered from the remaining 16 snails dissected. We also observed no other helminths from the remaining 928 invertebrates dissected (see Supplemental Material 1 for list of taxa).

1.3.3 Parasite tabulation

We found a total of 12 papers describing 10 helminth species from mainland gray foxes. One paper exclusively described helminths from mainland gray foxes. Nine papers exclusively surveyed island foxes, and two papers described helminths from both mainland and island foxes. All papers described helminths observed from individual necropsy reports. Fecal sedimentation for this study added two helminth species previously undescribed from SMI. We found no accounts of gray fox helminths from Washington state, Oregon, or Baja California, Mexico. Additionally, there were no reports of trematode infection from mainland or island foxes.

Two parasite species were reported from all fox populations (islands and mainland): the cestode, *M. corti*, and the nematode *Spirocerca* sp. (Figure 2, Table 2). Only island

populations were infected with the hookworm *U. stenocephala*. SMI had four parasites not reported from other locations: *A. gubernaculatus, C. hepaticum*, *Toxascaris leonine*, and *P. canicola* Santa Rosa Island also had a tapeworm species not reported from other locations: *M. manteri* (Voge, 1955). The California mainland had three tapeworms (*M. variabilis*, *Taenia laticollis*, and *T. serialis*) and a heartworm (*Dirofilaria immitis*) not reported from the islands.

Table 2. Helminth species described from mainland gray foxes (M) and Channel Islands foxes (SMI, SRI, SCrI, SNI, SClI, SCaI) with types, range, and reference sources.

Taxa	Type	Range	Ref
Cestoda			
Mesocestoides corti	tapeworm	M, SMI, SRI, SCrI, SNI, SClI, SCaI	32,33,47
M. variabilis	tapeworm	M*	46
M. manteri	tapeworm	SRI*	46
Taenia [Hydatigera] laticollis	tapeworm	M	47
T. serialis (syn: *T. laruei*)	tapeworm	M	47
Nematoda			
Dirofilaria immitis	nematode	M	25
Spirocerca lupi	nematode	M, SMI, SRI, SCrI, SNI, SClI, SCaI	6,25
Uncinaria stenocephala	nematode	SMI, SRI, SCrI	6,7
Angiocaulus gubernaculatus	nematode	SMI	15,44
Toxoascaris leonina	nematode	SMI	
Calodium hepaticum	nematode	SMI	
Acanthocephala			
P. canicola.	acanthocephalan	SMI	4,9

*Reported yet unconfirmed species identification

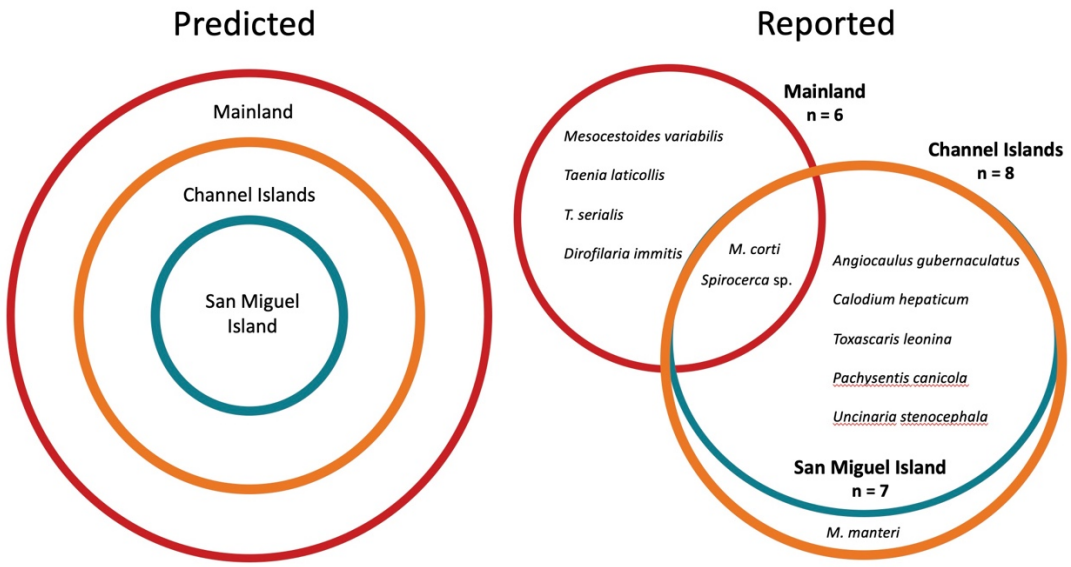

Figure 2. The predicted (left) versus the reported (right) patterns of helminth richness from the San Miguel Island fox (*U. littoralis littoralis*), the Channel Islands fox (*U. littoralis*), and the mainland gray fox (*U. cinereoargenteus*).

Of the five models we generated to test the effects of island size and distance on helminth richness using univariate, multivariate, and multiplicative relationships, the best model was multivariate and included terms for both island size and distance (M_3; BIC = 23.964, Table 3). However, because ΔBIC for the other four models all falls under 7, we cannot exclude any of the models as possible explanatory hypotheses for helminth richness. Thus, there is no clear relationship between island fox helminth richness and island size or distance from mainland. San Miguel Island was an outlier as the smallest island with the highest helminth diversity (Figure 3).

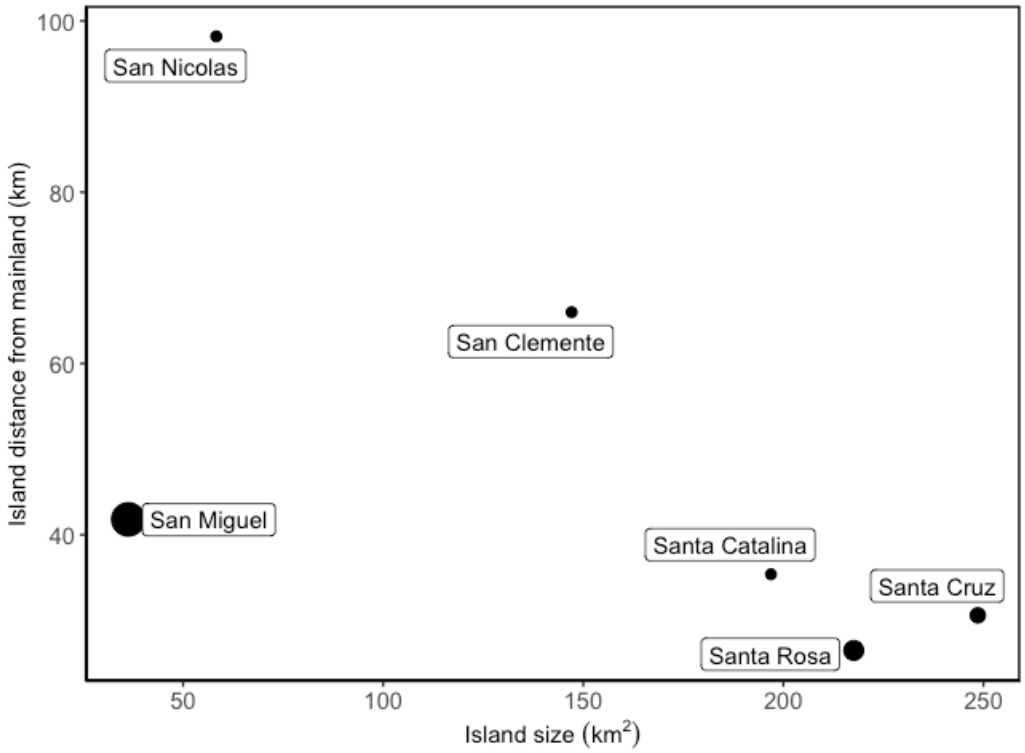

Figure 3. Island size (x-axis) and distance from mainland (y-axis) with helminth richness representing the size of each point. We expected high richness (i.e. larger points) to occur in larger islands closer to the mainland (bottom right quadrant), and low richness (i.e. smaller points) to occur in smaller islands farther from the mainland (upper left quadrant). The highest fox helminth richness occurs on the smallest island of San Miguel.

Table 3. The influence of island size and distance from mainland on fox helminth richness recorded from the California Channel Islands off the coast of southern California (with 6 islands as observations).

Model	k	log-likelihood	Parameter (SE)	BIC	ΔBIC
M_0: Richness ~ constant	1	-11.494	β_0: 1.204 (0.224)	24.781	0.817
M_1: Richness ~ Size	2	-11.041	β_0: 1.581 (0.433) β_1: -0.003 (0.003)	25.666	1.702
M_2: Richness ~ Distance	2	-11.018	β_0: 1.650 (0.506) β_1: -0.009 (0.01)	25.619	1.655

M_3: Richness ~ Size + Distance	3	-9.294	β_0: 3.039 (0.846) β_1: -0.006 (0.003) β_2: -0.022 (0.013)	23.964	0.0
M_4: Richness ~ Size*Distance	4	-9.206	β_0: 2.685 (1.176) β_1: -0.002 (0.001) β_2: -0.001 (0.024) β_3: -0.0002(0.0003)	25.578	1.614

k: number of parameters included in the model; log-likelihood: measure of model fitness; Parameter (SE): parameter values with standard error; BIC: Bayesian information criterion; ΔBIC: Difference of Bayesian information criterion to each model from the most parsimonious model; Size: island size in km^2; Distance: island distance from mainland in kilometers.

1.4 Discussion

Observed parasite richness from island foxes do not conform to predictions from island biogeography. Specifically, there is no clear relationship between island fox helminth richness and island size or distance from mainland. Surprisingly, SMI has a higher observed helminth richness than other islands and the mainland. High richness on SMI creates more concern for pathogenic parasites. Yet, how managers address this problem may depend on where the parasites originated. Some parasites may be endemic, and merit conservation, others may be recent introductions, and merit eradication.

We were surprised that observed fox helminth richness was greater on islands than the mainland and higher on smaller islands. These patterns invite several hypotheses. One hypothesis is that parasite communities are more diverse on islands compared to the mainland because island fox populations are denser, and high host density permits a wider range of parasites to persist (Morand and Poulin, 1998; Arneberg, 2002; Kamiya et al., 2014). One factor inconsistent with this explanation is that most island populations suffered

a bottleneck that should have extirpated parasites dependent on high host density. Yet, contemporary parasite communities remain richest on SMI, of which all foxes today are descended from just 15 individuals.

Another hypothesis is that parasite communities are larger on islands compared to the mainland because wider diet breadth on the islands increases the diversity of trophically transmitted parasites. In other systems, island species are documented to have a broader diet breadth than mainland counterparts due to a reduction of interspecific competition and niche expansion (Lomolino, 2005; Eloy de Amorim et al., 2017). Broader definitive host diets also facilitate the trophic transmission of parasites, benefiting generalist infection strategies (Park, 2019). However, we do not have evidence that island foxes are more generalist than mainland foxes. In fact, all foxes are generalists, and island foxes have relatively few vertebrate prey items due to the low rodent diversity across the islands (Cypher et al., 2014). A formal comparison between the diet breadths of island foxes and mainland foxes could be tested to determine whether the diet breadth hypothesis explains the diversity of helminths on San Miguel Island.

It is also possible that the differences in diversity are due to error. For instance, *M. manteri* was reported from Santa Rosa Island (Voge, 1955), but has never been confirmed. We suspect this species might be subsumed within another *Mesocestoides* species (something which requires additional study to confirm). If *M. manteri* is not a valid species, we can reduce true island parasite richness by one. Furthermore, observed parasite community diversity increases with sampling effort. In other words, all else being equal, the number of species observed increases (to an asymptote) with the number of hosts examined. Error is particularly high for low-prevalence parasites that have low detection probabilities

at low sample sizes. Although we have thorough parasite documentation from island foxes, we only have three reports describing helminths from mainland foxes. Further, those reports do not indicate sample size or prevalence data. Regardless, island foxes have been well-sampled given their conservation need, and mainland foxes have likely been under sampled for parasites. For example, *A. gubernaculatus* has been described from California mustelids, and *U. stenocephala* has been described from the gray fox in the tropical highlands of central Mexico (Hernández-Camacho et al., 2011), suggesting that they are also plausible in California mainland gray foxes. On the one hand, more systematic parasite surveys of mainland foxes might help uncover unreported parasite species. On the other hand, inspection of type material might help assess whether *M. manteri* is a valid island endemic. These two actions could even reverse the unexpected pattern of higher richness on islands compared to the mainland.

1.4.1 Which parasites affect fox health?

Some of the parasites we observed from the literature and recovered through fecal inspections have threatened fox health, whereas others cause no significant pathology. CINP managers have long been concerned with *Spirocerca* sp., as it can be lethal in SMI foxes due to the unusual location of infection. Adult spirocercids from mainland canids tend to aggregate in the esophagus. Yet, *Spirocerca* sp. of SMI foxes tend to aggregate in the colon and form nodules associated with many fox mortalities (Coonan, 2003). Treatment of spirocercosis is difficult due to the location in island foxes, and may pose threats to non-target helminths (Coonan, 2003; Sohn and Thomas, 2005). It remains unclear if the *Spirocerca* species in island foxes belong to the same species as mainland foxes, assumed to

be *S. lupi*. This question could be solved with integrated classical and molecular characterization of specimens.

The most ubiquitous helminth of SMI foxes is the hookworm, *U. stenocephala*. Its life cycle is direct with adult worms shedding eggs into the intestinal tract, which are then passed to the environment through fox feces. Larvae then hatch from eggs and eventually penetrate the skin of a fox and migrate to the intestinal tract. Although the migration of the hookworm includes the blood vessels, heart, lungs, and intestines, this worm has been implicated in few deaths of SMI foxes (Coonan et al., 2005). A common nematode that has contributed to SMI fox deaths occasionally is *A. gubernaculatus*. This lungworm is found in the cardiopulmonary vasculature of foxes and was identified from the heart blood of deceased individuals (Coonan et al., 2005; Faulkner et al., 2001; Sohn and Thomas, 2005). Pathology occurs when the parasite causes extensive lesions in the lung.

With the finding of *C. hepaticum*, we expand host records to include the SMI fox, *U. littoralis littoralis*. This nematode has a cosmopolitan distribution, including from in nature and within zoos, infecting a wide variety of mammals (Fuehrer, 2014b, 2014a). *C. hepaticum* has a direct life cycle, and previous descriptions indicate that foxes may serve as paratenic hosts when eggs pass through the fox's digestive system after consuming an infected prey item. We suspect this occurs on SMI when a fox eats an infected deer mouse, but it remains to be seen whether worms infect fox livers. Liver infection by *C. hepaticum* may cause pathology, including fibrosis, cirrhosis, and hepatitis. Further work on this relationship is needed to determine whether infection with *C. hepaticum* is incidental.

The only tapeworm recovered from fox feces was *M. corti*. This tapeworm was in only 1.7% of sedimentation samples. Although we recovered few eggs, necropsies of SMI foxes

often report finding adults in the intestinal tracts. The proposed first intermediate hosts of *M. corti* on SMI are ants, and the second intermediate host is the SMI deer mouse (Padgett & Boyce, 2004, 2005). There have been only a few reports of tapeworm-induced mortality of SMI foxes (Coonan et al., 2010; LWoods [DVM, California Animal Health & Food Safety Laboratory System in Davis, California], necropsy reports).

The most concerning parasite to fox health seems to be the acanthocephalan tentatively identified to the genus *P. canicola*. This parasite has been implicated in the recent decline of SMI foxes as adult worms can anchor into and perforate the intestinal lining, leading to secondary bacterial infections, and eventually death. Worms reproduce in the intestinal tract, and eggs are shed into the environment though fox defecation. Acanthocephalans require an arthropod intermediate host which we failed to identify in our many invertebrate dissections.

1.4.2 Island endemic parasites?

It is possible that the nematodes, *A. gubernaculatus* and *U. stenocephala*, are cases of relictual endemism due to past evolutionary and geographical change. The type-host for *A. gubernaculatus* is reported as the North American badger, *Taxidea taxus*, with reports from other mustelids in California (Dougherty, 1946). No mustelids occur on San Miguel, though the Channel Island spotted skunk, *Spilogale gracilis amphiala*, is present on Santa Cruz and Santa Rosa Islands (Faulkner et al., 2001). Oddly, this parasite has not been reported from mainland gray foxes. Perhaps this is a case of parasite island syndrome, which predicts frequent host switching and reduced specialization (Nieberding et al., 2006; Pérez-Rodríguez et al., 2013). It also seems possible that the SMI *Angiocaulus* could have diverged over time from *A. gubernaculatus.* Indeed, our discovery that this nematode uses

the endemic San Miguel shoulderband snail, *H. ayresiana*, as an intermediate host adds to the potential that it is endemic to San Miguel Island. The hookworm *U. stenocephala*, has a slightly broader distribution across the three northern island fox populations, with the highest prevalence on SMI (Coonan, 2003; Coonan et al., 2005). This hookworm has been described from domestic dogs and foxes worldwide but not from mainland foxes along the Pacific west coast (Washabau and Day, 2012). Therefore, it could be endemic, or just under studied.

The California Channel Islands include many relictual endemics – species that were present throughout California in the cooler, wetter Pleistocene, that found refuge from past climate, biological, and geographical change on the islands' temperate microclimates. Molecular comparison of *Angiocaulus* and *Uncinaria* from mainland and island hosts would help determine whether the island populations are relictual endemics, or just disjunct from northern mainland populations. Understanding this would be particularly relevant for determining whether broad-spectrum anti-helminthics administered to SMI foxes pose a risk to endemic parasites. If either parasite is endemic, it is at least as threatened with extinction as the island fox.

1.4.3 Introduced parasites?

The last two parasites unique to San Miguel (*C. hepaticum* and *P. canicola*) have only been seen in recent years. *P. canicola* attaches to the intestinal mucosa, which can be easily observed macroscopically, so it is particularly unlikely that it was missed until 2012. *C. hepaticum* is posited as native to South America but are now broadly found to cause health problems in zoo animals (Dunn, 1963; Fuehrer, 2014a,b). Similarly, *P. canicola* infects a wide variety of canid hosts as well as reptile transport hosts (Amin et al., 2022; Bolette,

1997; Buechner, 1944; Mobedi et al., 2007). The Channel Islands fox recovery program, which involved participation from zoos, never moved foxes between the mainland and islands. This indicates that SMI was not invaded by adult parasites, but rather infected insects could have been moved to SMI accidentally. If so, there is a risk that either species could eventually spread from SMI to the other Channel Islands, suggesting a need for specific biosecurity protocols for preventing their spread to other islands. Life-cycle elucidation through more dissections, followed by morphological and molecular characterization of retrieved larval specimens, could help indicate which invertebrate hosts pose a biosecurity risk.

1.5 Conclusion

The San Miguel Island fox, and island foxes in general, had more parasites than we expected from biogeography predictions. On the one hand, this might be because conservation actions have focused attention on San Miguel Island fox health, leading to higher sampling efforts and parasite detections. On the other hand, it could be due to human movement (past ranching, military operations, and conservation actions) inadvertently spreading parasites to the island. SMI might also harbor parasitic refugees from past climate, biological, and geographical change. These explanations are not mutually exclusive and require further investigation. The foxes on San Miguel Island are struggling to maintain a steady population, and parasites are one of many factors that affect fox health. Other factors include drought and resource limitation. There are likely interactions between stress, malnutrition, and the effects from parasites. Contextualizing SMI fox parasite diversity is a key step in mitigating further population decline.

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Chapter 2: DNA metabarcoding of scat reveals the diet and parasites of the San Miguel Island fox, *Urocyon littoralis littoralis*

2.0 Abstract

The San Miguel Island fox, *Urocyon littoralis littoralis*, is a species that was once on the brink of extinction. After successful recovery efforts by the Channel Islands National Park and affiliates, the population rebounded from a low of 15 individuals in 1999 to an estimated high of 650 individuals by 2015. However, in recent years the population has declined by more than 70%, and the foxes are once more facing the threat of extinction. This population decline has coincided with the detection of a new acanthocephalan parasite and increased infections by other known San Miquel Island (SMI) fox parasites. Here, we used DNA metabarcoding of fox scat samples to detect parasites and diet items that may facilitate parasite transmission. We compared our results to diet items and parasites previously reported in the literature. We found that of the approximately 159 potential animal species that foxes might consume, database constraints led to 84 with reference sequences at the species level, yielding a taxonomic ceiling of 52.8% for diet items at the species level. All seven known parasites were uniquely detectable with the COI and 18S barcode regions, yielding a taxonomic ceiling of 100% for parasites. Using metabarcoding, we identified 56 taxa, of which 28 were categorized as diet items and five as parasites. In comparison to previous descriptions using conventional manual scat sorting, we more than tripled the number of known diet items at the genus and species levels. The coverage estimate for diet was 0.93±0.03, leaving a probability of 0.07 for detecting a new (rare) species with one additional sample. For parasites, we detected four of the seven known parasites and one additional parasite, a trematode not known from SMI. These results aligned with the

estimated richness of five. Although we statistically achieved a sampling coverage of 1 for parasites, we failed to detect three known species. This study links diet and parasite ecology using DNA metabarcoding of scat, and the results serve as a baseline to improve the effectiveness of DNA metabarcoding as a tool for parasite ecology. DNA metabarcoding of scat samples enhances our ability to elucidate parasite life cycles and guide the recovery decisions of a vulnerable species.

2.1 Introduction

The Channel Islands National Park (CINP) is home to one of the only island-dwelling carnivores in California, the island fox (*Urocyon littoralis*), which is a species that diverged from the mainland gray fox (*Urocyon cinereoargenteus*) around 10,000 calendar years BP (Moore & Collins, 1995; Rick et al., 2009; Walker, 1980). In 2004, the US Fish and Wildlife Service listed the San Miguel Island (SMI) fox, *Urocyon littoralis littoralis*, and three other island fox subspecies as endangered (US Fish and Wildlife Service, 2004). The listing was primarily due to predation from golden eagles that were attracted to the islands by non-native prey, driving the SMI fox population to just 15 individuals by 1999 (Coonan et al., 2005). Managers, scientists, and zookeepers developed a comprehensive plan to relocate the golden eagles, restore native flora and fauna, and initiate an on-island captive breeding program for island foxes (Coonan et al., 2010). By 2008, the management team released the SMI foxes, after which their numbers grew to a high of 650 (80% CI: 525-875) by 2015 (Coonan et al., 2005). As other island fox populations remained stable, the SMI fox population declined by more than 70% between 2015 and 2018 (Dillon & Shaskey, 2022; Shaskey, 2019). High mortality rates were attributed to a recently identified acanthocephalan

parasite, *Pachysentis canicola*, which has not been detected in other California Channel Islands foxes or mainland California canids (Coonan et al., 2015; Dillon & Shaskey, 2022). Prolonged drought may have also impacted the carrying capacity for foxes on SMI, which is small and has a simpler food web relative to the other Channel Islands (Dillon & Shaskey, 2022).

To better understand the factors contributing to fox success and failure on SMI, we sought information about their helminth parasites and diet. Previous studies that manually sorted scat samples have provided a list of diet items, while necropsy reports and microscopy work have established a list of known parasites (Coonan et al., 2015; Coonan et al., 2005, 2010; Dillon & Shaskey, 2022; Faulkner et al., 2001; Lavoipierre et al., 1986; Padgett & Boyce, 2004, 2005; Sohn & Thomas, 2005; Voge, 1955; Cypher 2014; Chapter 1). Using conventional approaches to study the parasite ecology of the SMI fox (e.g. collect and dissect foxes) is difficult given its remote location and vulnerability to extinction. A further challenge is due to the restriction of setting traps (e.g. pitfalls) to collect invertebrates that may facilitate transmission. This restriction is due to both the preservation of Native American Chumash heritage sites and the minimization of encounters with potential unexploded ordinance. The US military conducted explosives tests on San Miguel Island prior to the transfer of National Park Service management in 1963 (Williams et al., 2017). One major goal of this study was to provide CINP managers with a narrowed list of candidate IH's, which may be subjects of future collection efforts. In this study, we explored how information derived from DNA metabarcoding compares to known SMI fox diet and parasites, as it might be an effective approach for assessing the parasite ecology of island foxes.

All methods, including those described here, have strengths and weaknesses. Past parasitology on island foxes, though extensive, has been limited to opportunistic and labor-intensive necropsies and microscopy work by trained veterinarians and parasitologists. Manual examination of island fox scat has revealed common diet items (Cypher et al., 2014). However, it can miss items that are rare, difficult to identify, degraded, or otherwise misidentified due to observer bias (Granquist et al., 2018; Newmaster et al., 2013; Reynolds & Aebischer, 1991). When identifiable parts are available, such as those associated with arthropods, it can be challenging to identify below order due to difficulty and observer bias (Spaulding et al., 2000). Recent advancements in high-throughput sequencing, such as DNA metabarcoding, offer the potential to alleviate some of the challenges inherent to conventional sampling. This includes identifying an otherwise undetected organism with a higher taxonomic resolution with comparatively less intact contents, time, and expertise (Massey et al., 2021).

Because all organisms shed DNA fragments into their environment via feces, mucus, skin, and other pathways, we can use DNA metabarcoding to indirectly detect target organisms without capture and sacrifice (Beja-Pereira et al., 2009; Valentini et al., 2009; Deiner et al., 2017). In many cases, DNA metabarcoding has enabled faster, non-invasive, and complementary or more comprehensive results than conventional methods, such as camera trapping and visual encounter surveys (Olds et al., 2016; Valentini et al., 2016; Leempoel et al., 2020). Further, DNA metabarcoding can be effective when detecting rare (Thomsen et al., 2012; Thomsen et al., 2016), cryptic (Port et al., 2016), and invasive species (Dejean et al., 2012). Metabarcoding will also allow us to record both diet and parasites simultaneously. Moreover we can potentially use it to enhance the probability of

fox survival by reporting infected individuals for treatment instead of discovering infections through necropsies.

However, DNA metabarcoding is not without constraints that might limit its utility (Barnes & Turner, 2016; Coissac et al., 2012; Taberlet et al., 2012). Particularly, a lack of barcoded taxa deposited in online databases imposes a ceiling on the number of taxa detectable by metabarcoding. This ceiling rises as taxonomic resolution falls (e.g. the ceiling is lower for species than for family). Prior knowledge about the taxa likely to occur at the sampling location can make it possible to infer finer taxonomic detail than is available in databases. For instance, on San Miguel Island, there is only one fox species, and so we are safe to interpret fox sequences to the SMI fox.

For this study, we used DNA metabarcoding to tabulate the parasites and diet items found in scat collected from the SMI fox. Our questions were: 1) what fraction of previously known parasites and diet items can be detected by metabarcoding scat samples (i.e. what is the limit of detectability), 2) what additional diet and parasite taxonomic resolution can metabarcoding provide, and 3) how much sampling effort is needed before we gain sufficient coverage of SMI fox diet and parasite diversity? We used prior knowledge to expand our inference to finer taxonomic scales where appropriate.

2.2 Methods

2.2.1 Study site and sample collection

Off the coast of the Southern California Bight in the USA lies the remote and uninhabited San Miguel Island (34°02’06” N, 120°21’30” W) (Figure 1). This 38.7-km^2 island is part of the Channel Islands National Park (CINP) and, as of 2022, supports an estimated 232

individuals of the endemic island fox subspecies, *U. littoralis littoralis* (Moore & Collins, 1995; Little, 2022). From September through December 2018 and July through August 2019, technicians trapping and releasing foxes as part of the recovery monitoring program saved 40 scat samples tied to GPS coordinates and fox demographic information (individual ID, sex, weight, age, body condition, and reproductive status). Samples were manually homogenized at the time of collection and stored at -20°C until processed.

2.2.2 Database constraints and taxonomic ceilings

Prior to metabarcoding, we generated a list for known or potential diet items and parasites of SMI foxes. We included diet and parasite information from peer-reviewed studies (Cypher et al., 2014b; Faulkner et al., 2001b; Padgett & Boyce, 2004), governmental reports (Dillon & Shaskey, 2022), and records from the Natural History Museums of Santa Barbara and Los Angeles. The species list was harmonized using the Global Biodiversity Information Facility (GBIF) Backbone Taxonomy (GBIF Secretariat, 2021). For each species, we obtained FASTA sequences per nine genetic markers from NCBI (COI, CytB, ITS, ND2, ND4, 12S, 16S, 18S, and RBCL). We included gene regions common for non-animal taxa as negative confirmations. For ease of computing, we capped the number of returned FASTA sequences per taxa at 10 (see Appendix 1, Tables 2 and 3 for full list of taxa). Next, we assessed what fraction of taxa were available for our primers targeting the COI and 18S regions (see Figure A1 for simplified workflow). Doing so set an upper bound, or taxonomic ceiling, for our expected coverage and resolution. Because there may be taxa on San Miguel Island that are not yet reported or identified, we consider this ceiling as a proportion rather than an integer.

2.2.3 DNA extraction, amplification, and sequencing

An overview of the metabarcoding workflow is illustrated in Figure 4. To prepare samples, we used a bleach, ethanol, and flame-sterilized spatula to transfer thawed scat subsamples into a 2mL microcentrifuge tube until 50-75% full (≤ 0.25-g per sample). We extracted DNA from each scat sample following the Qiagen DNeasy PowerSoil Kit (Qiagen, Valencia, California, USA) protocol and included three extraction negatives. All samples were prepared and processed in a dedicated DNA extraction workspace, where surfaces and equipment are routinely sterilized.

DNA was amplified by polymerase chain reaction (PCR) using the following primer sets with an Illumina Nextera adaptor modification: 18S (1391f and EukBr targeting the V9 region; Medlin et al., 1988 Lane, 1991), COI (mlCOIintF and FolDegenRev; Yu et al., 2012; Leray et al., 2013), and a shorter COI (fwhF2 and fwhR2n; Vamos et al., 2017) (Table A1). Amplification was performed in triplicate with the Qiagen Multiplex Mix. We included three PCR negative controls, where dH_2O was used instead of DNA extract. The Kapa Hifi HotStart Ready Mix and Nextera Index Kits were used for the indexing PCR. We confirmed amplification by gel electrophoresis. Triplicates with gel bands were pooled and cleaned with Serapure magnetic beads (Faircloth & Glenn, 2014).

Thermocycler settings for 18S reactions included an initial denaturation step at 95°C for 15 minutes, followed by 13 touchdown cycles of (1) denaturation at 94°C for 30 s; (2) annealing beginning at 69.5°C for 30 s, decreasing by 1.5°C until 50°C was reached; and (3) extension at 72°C for 60 s. Subsequently, we ran 35 additional cycles with a 94°C denaturing temperature for 30 s, 50°C annealing temperature for 30 s, 72°C extension temperature for 60 s, followed by a 10-min final extension at 72°C. Thermocycler settings

for COI reactions included an initial denaturation step at 95°C for 15 minutes, followed by 13 touchdown cycles of (1) denaturation at 94°C for 30 s; (2) annealing beginning at 69.5°C for 30 s, decreasing by 1.5°C until 50°C was reached; and (3) extension at 72°C for 90 s. Subsequently, we ran 40 additional cycles with a 94°C denaturing temperature for 30 s, 50°C annealing temperature for 30 s, 72°C extension temperature for 60 s, followed by a 10-min final extension at 72°C.

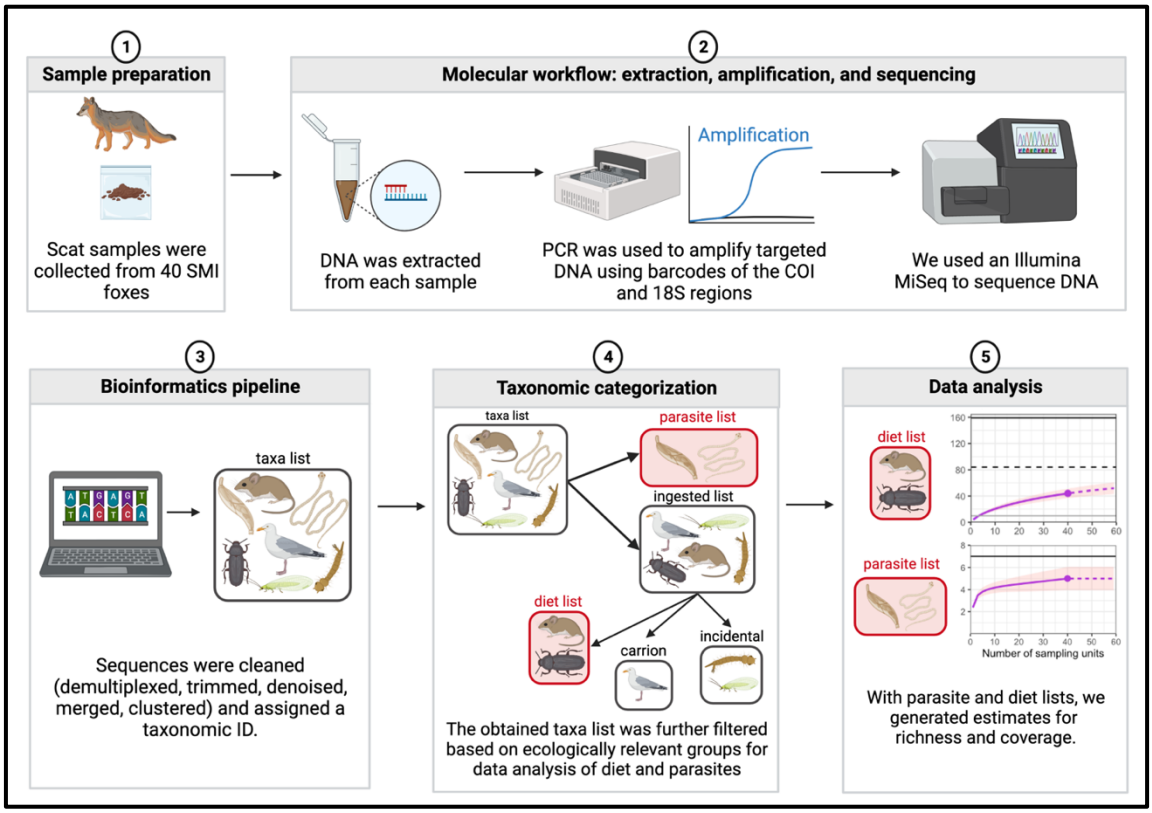


Figure 4. The workflow of this study involved sample collection and preparation, molecular processing, bioinformatic processing, taxonomic categorization, and data analysis. Further details of the taxonomic categorization can be found in Figure 5.

We quantified DNA concentrations of cleaned PCR products with the Qubit dsDNA BR Assay (Thermofisher Scientific, Waltham, MA, USA), then pooled by barcode to equimolar levels. The UC Santa Cruz Paleogenomics Lab sequenced our samples using an

Illumina MiSeq. Reads were recovered by paired-end sequencing using a MiSeq 600 cycle kit and the default Illimunia MiSeq analysis software.

2.2.4 Bioinformatic processing

We trimmed the demultiplexed samples with *cutadapt* (v. 3.3, (Martin, 2011)). Based on the visual inspection of the quality profiles, we used 120 bp for 18S and 180 bp for COI as trimming thresholds for reverse reads. Forward reads were trimmed to 120 bp for 18S and 200 bp for COI, leaving an 80 and 67 bp sequence overlap, respectively. Next, sequences were denoised and merged using the DADA2 Pipeline (v. 1.18.0,Callahan et al., 2016). After accounting for abundances of merged sequence variances, we found that chimeric sequences accounted for about 5% of the merged sequence reads for 18S and 0.5% for COI. Finally, we curated the raw sequence tables with the *LULU* algorithm and clustered reads with *swarm* (v. 3.0, Frøslev et al., 2017; Mahé et al., 2021).

For taxonomic assignment, we used the SINTAX (Edgar, 2016) implementation of the RDP classifier (Wang et al., 2007) in VSEARCH. We used the ANACAPA 18S and COI databases as reference databases for the respective primers (Curd et al., 2019). We removed all sequences with no taxonomic assignment at the phylum level. Subsequently, we set taxonomic assignments to "NA" if they fell below a probability of 60% following Roger et al. (Roger et al., 2022). We excluded taxa that were only present in one sample with 10 or fewer reads, which resulted in one removal: *Chrysops* sp. with four reads in one sample. Before data analysis, taxonomic assignments from all data sources (18S and COI) were harmonized against the GBIF backbone taxonomy.

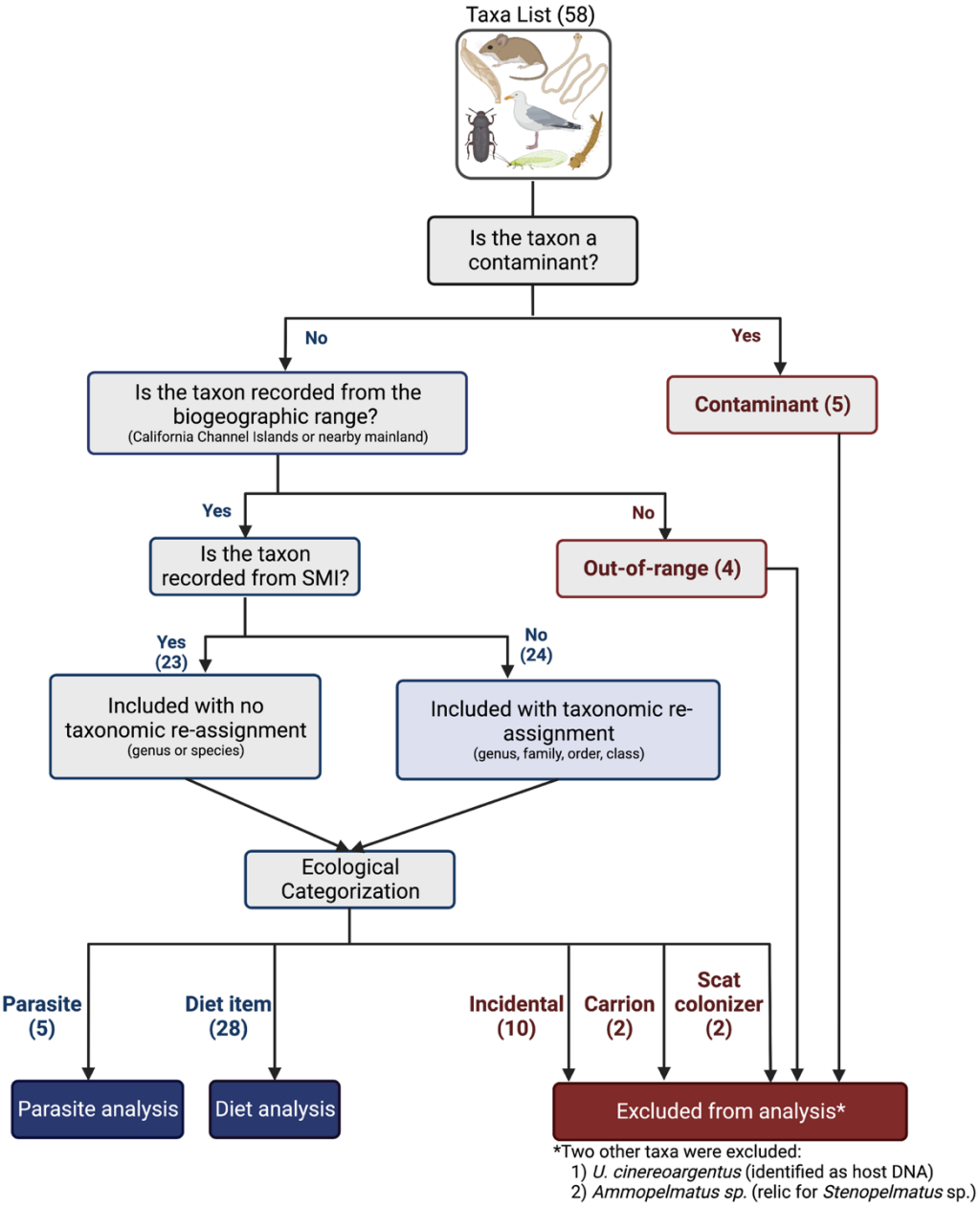
2.2.5 Taxonomic resolution and criteria for inclusion

After identifying OTUs to genus or species level, we applied further exclusion filters to ascertain diet and parasite communities (Figure 5). Firstly, we determined whether a detected taxon was from contamination. If yes, then it was excluded from analysis (five taxa). If no, we considered whether the taxon was within the biogeographic range (California Channel Islands or mainland southern California). If a taxon was not likely to occur on SMI or the biogeographic region, we categorized it as out-of-range (four taxa). If the taxon was recorded from SMI, then it was included with its originally assigned genus or species. If the taxon was reported from the biogeographic range (California Channel Islands or mainland southern California) but not SMI specifically, then we re-assigned identification to the lowest taxonomic level for which a logical basis could be made for presence on SMI. For example the moth *Tricholita fistula* was reassigned to "*Tricholita* sp." because only the genus has been described from SMI. See Appendix 1, Table 4 for the full list of original ID, reassigned ID (if needed), and rationale for reassignment for each detected taxon. In most cases, the re-assigned resolution was coarser than that suggested by the bioinformatics pipeline as we did not want to make conclusions about new species or genera location records in this study. In other cases, the re-assigned ID had a higher resolution (e.g. the slender salamander *Batrachoseps* sp. was re-assigned to *Batrachoseps pacificus* because this is the only species to occur on SMI).

Post biogeographical filtering, there were 47 taxa that were then divided into the following ecological categorizations: parasite, diet item, incidental, carrion, and scat colonizer. 28 organisms were categorized as "diet item" and included in diet analyses. Similarly, five parasites were included in the parasite analyses. Of note, there were two more taxa excluded from analyses. These included the SMI fox (identified as *U. cinereoargentus*)

and *Ammopelmatus* sp. The cricket genus *Ammopelmatus* sp. is now recognized as *Stenopelmatus* sp. Given both genera were detected in the exact same samples, we subsumed all *Ammopelmatus* sp. reads into *Stenopelmatus,* thus including the read data but excluding the extra genus.

Figure 5. The taxonomic workflow for categorizing taxa as excluded from the workflow, included in the parasite analysis, or included in the diet analysis.



2.2.6 Data analysis

Rare species, by definition, tend to account for the low frequency counts in observed data (e.g. singletons) and can heavily influence estimates, such as species richness, Shannon diversity, Simpson diversity (Chao & Jost, 2015). As such, these rare detections hold significant insight about the undetected species in a system (Chao & Jost, 2015). Therefore, accurately measuring or estimating the presence of rare species is vital to making statistical inferences from diversity estimates (Chiu & Chao, 2016). Low frequency counts produced from high throughput sequencing are prone to many types of sequencing errors (Huse et al., 2010; Wen et al., 2017). This may lead to the misidentification of a taxon as unique with subsequent classification as a singleton. Thus, sequencing data are prone to the artificial inflation in a manner unlike macro-community ecological observations. Chiu and Chao (2016) addressed this issue by developing an approach to accurately estimate the number of singletons despite sequencing errors. They used a modified Good-Turing formula (Good, 1953; Good & Toulmin, 1956), which was applied in the ecological framework of estimating the mean relative abundance of a species given the number of times said species appeared. The resulting formula generated more conservative diversity estimates that aligned with the results of data sets with no sequencing errors and was incorporated into the iNEXT package in R (Chiu & Chao, 2016; Hsieh et al., 2016).

To estimate diversity while accounting for potentially spurious sequences, we estimated richness and sampling coverage for diet and parasites using the '*iNEXT*' function in R (v. 4.0.4, R Core Team, 2020). We used the ChaoII estimator, which is a non-parametric approach to account for rare occurrences from observed data and sampling size to extrapolate estimated richness (Chao et al., 2020; Chao & Jost, 2015; Hsieh et al., 2016).

We used sample coverage to assess how well we captured both the diet and parasite communities and to determine the probabilities of missing a new taxon if one additional sample were included. We plotted accumulation and coverage curves separately for each primer as well as the union (COI + 18S) for diet items and parasites. For each accumulation curve, we also plotted a solid black line to represent the known diet or parasites as well as a black dashed line that represents the respective limit of detection for each primer or combination. Unless otherwise specified, we performed all data processing, analyses, and visualization, in R with the following packages: *tidyverse* (v. 1.3.1, Wickham et al., 2019), *phyloseq* (v. 1.34.0, McMurdie & Holmes, 2013), and *eulerr* (v. 6.1.1, Larsson et al., 2022).

2.3 Results

2.3.1 Database constraints and taxonomic ceilings

We identified 159 potential diet items reported from SMI. Of those species, 49% (78) had genetic sequences deposited in GenBank for COI and 27.7% (44) for 18S (Table A2). Combining the COI and 18S barcodes, we reached a taxonomic ceiling at the species level of 52.8% (84) (Figure 6). For parasites, there are seven known species of helminths (worm parasites) of SMI foxes. All but two species had reference sequences at the species or genus level (Table A3). *Angiocaulus gubernaculatus* and *P. canicola* both returned 0 hits for all nine genetic markers at the species and genus level. Thus, at the species level, the taxonomic ceiling with our chosen primers was 71.4% (5/7) of possible SMI fox parasite species (Figure 6). However, a search at the family level for Angiostrongylidae (*A. gubernaculatus*) revealed 20 references for both the COI and 18S regions. Similarly, a search for Oligacanthorhynchidae (*P. canicola*) revealed 71 and 18 references for the COI and 18S

regions, respectively. Given that these two species are the only representatives of their families that infect SMI foxes, we re-assigned detections to species level in our metabarcoding data set. With this adjustment, the taxonomic ceiling for parasites became 100% (7/7) using both barcodes.

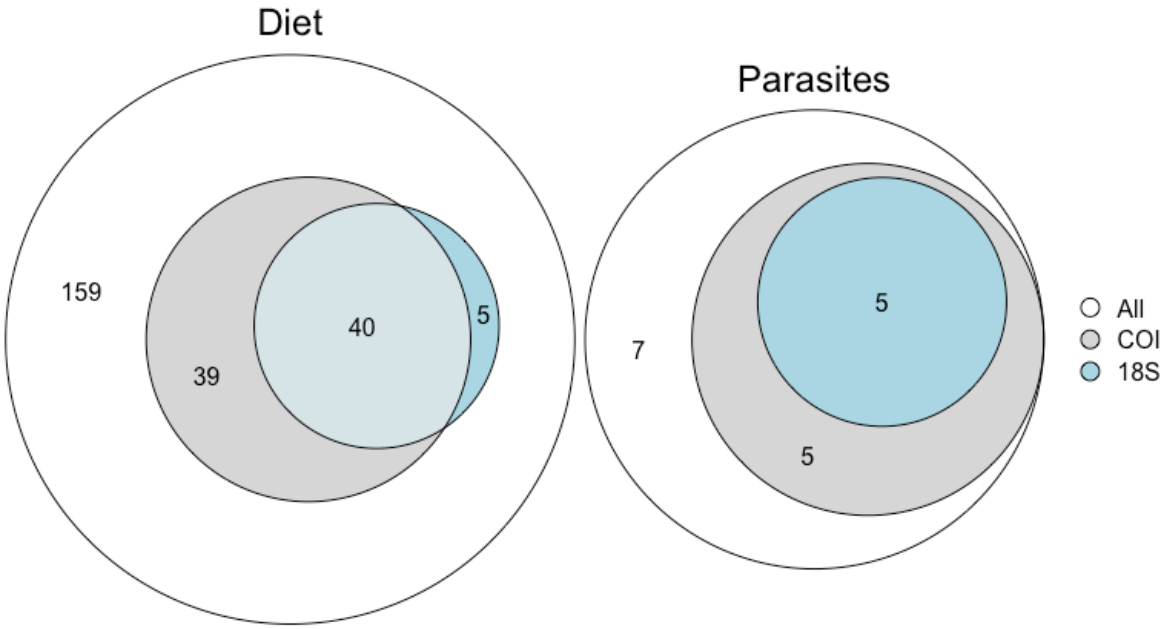


Figure 6. Of the number of potential diet item taxa (n = 159), 84 species have reference sequences for the COI and 18S gene regions (left), yielding a taxonomic ceiling of 52.8% for diet items. Of the number of taxa identified as parasites (n = 7), five species can be detected using a combination of COI and 18S primers (right). Two parasite species, *A. gubernaculatus* and P. canicola were the only representatives of their respective families. As such, they were ultimately identifiable to the species level, bringing the taxonomic ceiling to 100% for parasites.

2.3.2 Metabarcoding results

We obtained too few sequences with the shorter COI primer; therefore, we excluded those data from further analysis. Once we demultiplexed, sorted, trimmed, and merged samples, we obtained 1427 reads for 18S and 2918 for COI. Following primer error filtering, we obtained 1131 reads for 18S and 2524 for COI. After clustering, we retained 896 and 1988 reads for 18S and COI, respectively. Of those sequences, we taxonomically classified 785

OTUs for 18S and 1290 OTUs for COI. We retained all taxa identified to the genus and species level. Because there are many island-endemic arthropods on SMI, we recognize the potential for valid, yet unidentified, taxa due to the lack of database sequences. Therefore, we retained the taxa for which the genus was reported from the island or nearby mainland and re-assigned taxa ID based on the level by which we have confidence (see Appendix 1, Table 4 for taxa list and rationale for inclusion or exclusion).

For COI, one of the three extraction negatives contained five reads identified as island fox DNA. None of the three PCR negatives contained any reads from the COI primer. As for 18S, we recorded a combined 61,378 reads among three OTUs from the extraction negatives. One of those OTUs (36,213 reads) could not be assigned at the Kingdom level. The second OTU (3,163 reads) could not be identified beyond the kingdom Animalia, and the third OTU (22,002 reads) keyed to a fungus, *Apiotrichum montevideense*, of the Basidiomycota kingdom. For the PCR negatives and the 18S primer, there was one sample (17,266 reads) that was not assigned past the kingdom Animalia and one sample that contained (2,967 reads) associated with *Gallus gallus* DNA.

Following our filters for contamination, biogeographic range, and ecological categorization, we excluded a total of 25 taxa for analysis (Table A4). We removed five contaminants (e.g. *Homo sapiens*) and taxa that were likely present in samples of a different system processed concurrently (e.g. *Dasybranchus* sp.). We excluded four taxa that were considered out of range, two flies that are likely scat colonizers, two carrion species (*Larus* sp. and *Zalophus californianus*), and 10 incidentals (e.g. scale insects ingested during plant consumption). Additionally, foxes may opportunistically scavenge other fox carcasses, but we removed all island fox detections as most reads are likely from with host DNA.

We detected 28 diet items (28 for COI; 3 for 18S; Table 4), of which 93% (26) were identified to the genus or species level and confirmed to exist on SMI. The remaining 7% were included with a broader taxonomic classification because they are described from the neighboring Channel Islands or the nearby California coast. For parasites, we identified five unique taxa (three for COI; three for 18S; Table 4). Of those five taxa, four were identified to the genus or species level, while one trematode was classified to the order level.

Table 4. The occurrence, frequency of occurrence (FOO), primer, and type category for the detection of SMI fox diet items and parasites.

Taxa	#Occurrence (n = 40)	FOO %	Primer	Type
Batrachoseps pacificus	6	15	COI	diet
Ceuthophilus hesperus	2	5	COI, 18S	diet
Araneae	2	5	COI	diet
Diachus auratus	1	2.5	COI	diet
Eleodes sp.	3	7.5	COI	diet
Elgaria m. multicarinata	13	32.5	COI	diet
Euxoa sp.	3	7.5	COI	diet
Elkalyce sp.	1	2.5	COI	diet
Forficula auricularia	2	5	COI	diet
Gryllus sp.	1	2.5	COI	diet
Lacinipolia stricta	1	2.5	COI	diet
Lasius sp.	8	20	COI	diet
Leucania sp.	2	5	COI	diet

Melanoplus sp.	2	5	COI	diet
Cancridae sp.	1	2.5	COI	diet
Metepeira sp.	4	10	COI	diet
Scarabaeidae sp.	2	5	COI	diet
Peromyscus maniculatus	33	82.5	COI	diet
Porcellio dilatatus	6	15	COI	diet
Porcellio laevis	3	7.5	COI	diet
Rhynocoris sp.	3	7.5	COI	diet
Solenopsis molesta	1	2.5	COI, 18S	diet
Stenopelmatus sp.	18	45	COI, 18S	diet
Tapinoma sessile	1	2.5	COI	diet
Tibellus sp.	1	2.5	COI	diet
Tinea sp.	1	2.5	COI	diet
Tricholita sp.	1	2.5	COI	diet
Vertigo californica	1	2.5	COI	diet
Angiocaulus gubernaculatus	31	77.5	COI	parasite
Mesocestoides corti	22	55	COI, 18S	parasite
Plagiorchiisa sp. 1	1	2.5	18S	parasite
Pachysentis canicola	8	20	18S	parasite
Uncinaria stenocephala	33	82.5	COI	parasite

2.3.3 Detection of previously reported diet items with metabarcoding

Cypher et al. (2014) reported diet items with a >10% occurrence for SMI foxes from 577 scat samples, resulting in the description of eight animal diet items. With metabarcoding, we confirmed the three animals previously reported to the species level: the island deer mouse (*Peromyscus maniculatus*; 33 metabarcoded samples), the Jerusalem cricket (*Stenopelmatus* spp.; 18 metabarcoded samples), and the earwig (*Forficula auricularia*; two metabarcoded samples). However, we did not detect the sand cricket genus *Cnemotettix*, which was present in over 25% of manually sorted scat samples from Cypher et al. (2014; we detected other orthopterans, see below). We also detected DNA from the following taxa reported by Cypher and colleagues (2014) without genus or species level resolution: beetle, beetle larva, lizard, and grasshopper.

2.3.4 Added diet resolution

Although we cannot distinguish between beetle adults and larvae, we observed one beetle at the species level and one at the genus level. The beetles *Diachus auratus* and *Eleodes* sp. are known to occur on SMI and were present in one and three samples, respectively (Table 4). We also observed DNA of the southern alligator lizard (*Elgaria multicarinata multicarinata*) in 13 samples. One species of grasshopper, *Melanoplus* sp., was recorded from two samples (Table 4).

In addition to adding resolution to previously documented diet items, we report 21 new diet items of the SMI fox. All diet items were identified to the genus or species level apart from two taxa: one spider of the Araneae family and one beetle of the Scarabaeidae family (Table 4). We detected one more vertebrate, the Channel Islands slender salamander, *Batrachoseps pacificus*, in six samples. We also observed two isopods found on SMI:

Porcellio dilatatus in six samples and *P. laevis* in three samples (Table 4). We also observed two spiders, *Tibellus* sp. and *Metepeira* sp., present in one and four samples, respectively. The snail, *Vertigo* sp., was present in one sample (Table 4). The remaining 13 diet items were insects.

There were six unique species of moth observed in the diet (Table 4). Although moths were the most common insect group, the number of detections was low with a range of one to three samples. We observed three species of ant, and *Lasius* sp. was the most prevalent with eight positive samples (Table 4). We observed one true bug, *Rhynocoris* sp., which was present in three samples (Table 4).

2.3.5 Detection of previously reported parasites with metabarcoding

A tabulation of helminth parasites from SMI foxes resulted in seven unique species (Coonan et al., 2015; Coonan et al., 2005, 2010; Dillon & Shaskey, 2022; Faulkner et al., 2001; Lavoipierre et al., 1986; Padgett & Boyce, 2004, 2005; Sohn & Thomas, 2005; Voge, 1955; Chapter 1). There exist five roundworms (Phylum Nematoda), which are transmitted either directly from fox to fox or through the consumption of an infected intermediate host. These include the fox lungworm, *A. gubernaculatus*; the ascarid, *Toxascaris leonina*; the hookworm, *Uncinaria stenocephala*; the spirocercid *Spirocera* sp., and the liver-dwelling *Calodium hepatica*. Additionally, there is one reported tapeworm, *Mesocestoides corti*, and the recently identified acanthocephalan, *P. canicola*.

The full list of parasites detected through metabarcoding are displayed in Table 4. We confirmed the presence of the hookworm, *U. stenocephala.* This parasite was the most common and was present in 82.5% of samples (33/40), aligning with previous reports. The

second most prevalent parasite was the lungworm *A. gubernaculatus*, which we recovered from 77.5% of samples (31/40). The tapeworm, *M. corti*, was present in 55% (22/40) samples, while the acanthocephalan was present in 20% (8/40) samples. While we failed to detect *T. leonina*, *Spirocerca* sp., and *C. hepatica*, we report here the detection of an undescribed trematode species recovered from one sample. We identified the trematode as belonging to the Plagiorchiida order.

2.3.6 Diversity, richness, and coverage

For items recovered in the diet, the COI and 18S primers recovered DNA from 28 and 3 organisms, respectively. The union of the two revealed a total observed richness of 28 unique diet items, while the estimated richness was 37.75 (95% CI: 30.33 - 68.73; Figure 7; Table 5). The union coverage was 0.93 (95% CI: 0.90 – 0.96; Appendix 1, Figures 2 and 3), so the probability of detecting a new diet item with one additional sample was estimated to be 0.07 but may be as high as 0.1.

We observed a total of five parasite species using the combined primer pair, which corresponds to the ChaoII richness estimator (Figure 7; Table 5). Both primers independently detected the tapeworm, *M. corti*. Each primer also amplified two unique species that the other primer failed to detect, highlighting the differences in primer specificity. For the 40 samples used in this study, we reached a sample coverage of 1 for helminth parasites (Appendix1, Figures 2 and 3).

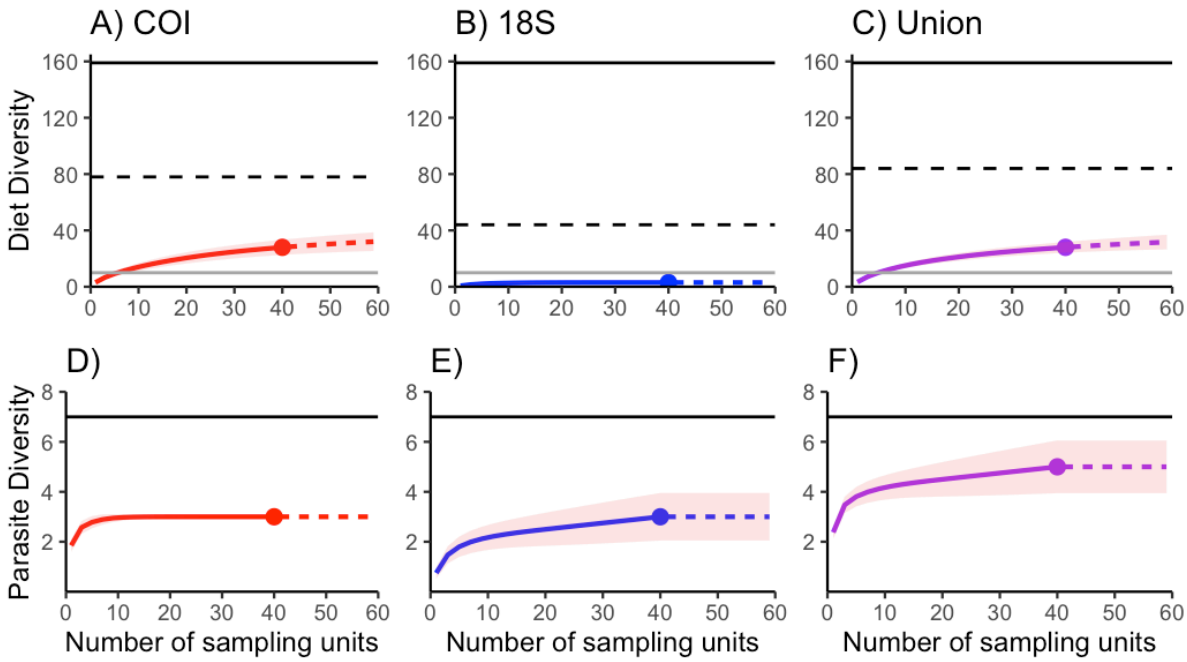


Figure 7. Richness accumulation curves with shaded confidence intervals for diet (top row) and parasites (bottom row) detected by the COI (left column), 18S (middle column), and union of the two markers (right column). The light grey line on the diet figures indicates the previously recorded diet items (10). For parasites, all seven helminths of SMI foxes have been previously recorded. The solid portion of all accumulation represents the interpolated data, while the dashed portion represents the extrapolated data.

Table 5. Species richness inferences for all combinations diet item and parasite by primer combinations. The combination of unique taxa detected by both COI and 18S is referred to as "Union."

Group (Primer)	S(obs)	S(Est) ChaoII	S(est) 95% CI	Coverage	Coverage 95% CI
Diet (COI)	28	37.83	(30.49 - 66.75)	0.91	(0.87 - 0.95)
Diet (18S)	3	3	(3.00 - 3.18)	1	(1.00 - 1.00)
Diet (Union)	28	37.75	(30.33 - 68.73)	0.93	(0.90 - 0.96)
Parasites (COI)	3	3	(3.00 - 3.01)	1	(1.00 - 1.00)
Parasites (18S)	3	3	(3.00 - 4.49)	1	(1.00 - 1.00)
Parasites (Union)	5	5	(5.00 - 6.41)	1	(1.00 - 1.00)

Note: Observed, S(obs), and estimated, S(est), species richness given the sampling effort for this study, by target taxa and primer combination. Coverage indicates how well a sample represents the species that are present and detectable in the system.

2.4 Discussion

This study demonstrates the ability to use DNA metabarcoding to simultaneously detect diet items and parasites from a vulnerable species, of which opportunities to study are rare or difficult to carry out (e.g. necropsies, scat sorting that requires taxonomic expertise, island accessibility, etc.).

We observed all but one of the animal diet items that were previously described from manual scat sorting. Additionally, we used 40 samples compared to the 577 used by expert taxonomists in the manual scat sorting survey. Yet, there remains a large gap in detectability since only half (84/159) of the known potential diet have available reference sequences. Of those 84 with reference sequences, we observed 28 diet items in our samples. While the parasite community is much more limited at seven known species, we still only detected about half (4/7) of the known species. While this is a puzzling find, the detection rates are likely influenced by several factors, such as the possibility of undescribed endemic species on SMI, the lack of reference sequences from described island endemics, or both. Other factors include, but are not limited to, the true absence of organisms from the specific samples used in this study or the inability to extract or amplify DNA due to chosen lab protocols and primer specificities.

2.4.1 Diet, parasites, and fox health

There are several parasites of concern to managers of the SMI fox population. The most relevant is the recently recovered acanthocephalan, *P. canicola*, which was present in 20% of our samples. There were no noticeable differences between the diets of samples that were positive versus negative for the acanthocephalan DNA. This is not surprising as diet

detections represent a snapshot in time, whereas parasite detections likely represent an integration over time. *P. canicola* is a parasite that uses SMI foxes as the definitive host and a terrestrial arthropod as the intermediate host (IH). The invertebrate intermediate host(s) remain unknown on SMI, but the presence of the Jerusalem cricket, camel cricket, and various other invertebrates, may help narrow the search for candidate intermediate hosts.

We also detected two additional parasites that have complex life cycle (i.e. use intermediate hosts) as well as one parasite that has a direct life cycle. We observed DNA of the tapeworm, *M. corti*, which requires two intermediate hosts and one definitive host to facilitate transmission. This tapeworm species uses ants as the first intermediate host, the island deer mouse as the second intermediate host, and the SMI fox as the definitive host (Padgett and Boyce, 2004; Padgett and Boyce, 2005). With metabarcoding, we detected all hosts of this life cycle. The third known parasite with a complex life cycle was *A. gubernaculatus*. This lungworm is found in the cardiopulmonary system of foxes across the Channel Islands and was described from the heart blood of deceased SMI foxes by Faulkner et al. (2001). This parasite uses the snail, *Helminthoglypta ayresiana*, but we did not detect this snail with metabarcoding. The last known parasite we detected was the nematode *U. stenocephala*. This hookworm is the most common parasite recovered from SMI foxes (Coonan, 2003; Coonan et al., 2005). This was also true for the metabarcoding data as this nematode was present in 79% of samples.

One parasite of note that we failed to detect in our samples was *Spirocerca* sp. This parasite is of concern to managers as it can be lethal in SMI foxes due to the location of infection. While adult Spirocercids from other canids tend to aggregate in the esophagus, Spirocercids of SMI foxes tend to aggregate in the colon and form lethal nodules.

Spirocerca sp. has caused many fox mortalities on SMI (Coonan, 2003). Eggs of *Spirocerca* sp. are relatively small at about 35x15 μm (van der Merwe et al., 2008). In comparison, the *U. stenocephala* eggs are double the size, ranging from 65-90x30-55 μm (Saari et al., 2019). Furthermore, eggs of this this parasite have been found at 57% prevalence but low egg densities (14.1 ± 3.9 eggs/g-scat; Chapter 1). Compared to *U. stenocephala* (94% prevalence; 36.5 ± 6.8 eggs/g-scat) and *A. gubernaculatus* (76% prevalence; 82.9 ± 59 X eggs/g-scat), low egg densities decrease the chances of detecting *Spirocerca* sp. egg DNA (Chapter 1). The lack of detection via metabarcoding may be attributed to true absence in the subsamples used for metabarcoding (< 0.25g), or the initial DNA concentrations were too low to be extracted and amplified, as might occur if the DNA is tightly bound within the egg (Amoa et al. 2020). While other studies have examined the results of various molecular approaches for isolating and extracting DNA of tapeworm and trematode eggs, experiments that explore mechanical methods may elucidate the threshold needed to reliably detect this parasite species (Oberhauserová et al., 2010; Øines et al., 2014; Amoa et al. 2020).

2.4.2 DNA metabarcoding as a tool for parasite ecology

A concern for researchers and managers lies in whether scat metabarcoding is a cost-effective, efficient, and accessible tool that may compliment or replace conventional methods used to study parasite ecology. While the data is not included in this study, we performed fecal sedimentation and flotation on the same 40 samples. A comprehensive cost-benefit analysis is underway to compare the performance and resources (time, money, expertise, etc.) needed to determine the cost-effectiveness of metabarcoding versus traditional methods. DNA metabarcoding of diet items, especially those comprised of

mainly invertebrates, may be more efficient that manual methods. Watts et al., (2019) showed that DNA metabarcoding was a more effective approach compared to the manual identification that was laborious and required morphological expertise. Lastly, DNA sequencing methods are becoming faster, cheaper, and more comprehensive (Deiner et al., 2017; Goldberg et al., 2016; McElroy et al., 2020). As costs continue to fall, this will increase the accessibility of metabarcoding by managers and conservationists alike.

One area of concern not addressed in this study, but important nonetheless, regards the effectiveness of DNA metabarcoding as a tool to assess worm burdens from read abundance data. We chose to exclude analyses pertaining to read abundances of diet items and parasites. Like egg counts used in conventional approaches, metabarcoding data represent proxy measurements in place of direct observations, and there is much debate on the usefulness of read abundance data (Schenk et al., 2019; Shelton et al., 2023). Parasites tend to exhibit aggregated distributions and seasonal variation based on life cycle demands (Crofton, 1958; Shaw & Dobson, 1995; Wilson et al., 1996). It is possible that relatively high read abundances are indicative of individual sloughing or increased reproductive outputs (i.e. egg shedding) rather than an increased number of worms and a higher host burden. More research is needed to ascertain the relationship between proxy measurements, such as egg counts and read abundances, and infection burdens on hosts. Barnes and Turner (2016) highlight many studies that have examined the relationship between DNA concentrations estimates of free-living organism abundance from environmental samples (soil, water, air). Yet, there remains a gap in understanding for the same associations with helminth parasites. Until the correlative measures between proxies, such as DNA read

abundances or concentration, and biomass (i.e. number of worms) are explored, we remain hesitant to include more than prevalence data.

2.4.3 Limitations for interpretation

For DNA metabarcoding to be an effective tool, especially for parasite ecologists, we must recognize the potential pitfalls that can arise. As with other sampling methods, DNA metabarcoding is prone to false negative and positives and may lead to erroneous interpretations for diet and parasite detections. Each stage of the molecular, bioinformatic, and ecological interpretation workflow can lead to misinterpretations.

Beginning with molecular workflow, we must 1) approach the study with *a prior* knowledge based on the system and relevant to the study objectives; 2) recognize the tradeoffs between primer specificity and sensitivity; and 3) account for the inherent possibility of data errors due to the DNA extraction, amplification, and sequencing pipelines. For example, we know plants make up a significant part of the SMI fox diet (Cypher et al., 2014). Yet, we deliberately choose not to use plant-sensitive markers as plants do not serve as intermediate hosts for helminth parasites. Using the two COI and 18S primers maximized the chances of detecting invertebrate diet items and parasites. The differential detectability of the two primers were highlighted several times in our data. One example was the tapeworm, *M. corti*, present in 21 of the 18S samples but only 10 of the COI samples.

As with other survey methods, DNA metabarcoding is prone to false negative and positive errors (Darling & Mahon, 2011). Because we have the additional challenge of working on a small, remote island with endemic species, many of those species lack

reference sequences. Here, we outline the possibility of false negatives and positives based on the bioinformatic process – namely the quality of reference databases. On the one hand, we are prone to false negatives as there may exist described species that lack reference DNA sequences as well as species that have yet to be described. The latter would indicate that our ceiling of detection for diet is lower than the 'true ceiling,' and thus decreases our true richness and coverage estimates. On the other hand, we may encounter false positives associated with the bioinformatic process. When there exists limited availability of reference sequences for a given site or system, the taxonomic assignments can lead to erroneous ID's (Darling et al., 2021). An assessment of various bioinformatic pipelines using mock communities has demonstrated major differences at the taxonomic assignment step, which could lead to erroneous ecological interpretations (Mathon et al., 2021). In our study, we attempted to reduce misinterpretations by re-assigning the taxonomic ID of organisms to the taxonomic level by which are aligned with SMI species records. Our data will remain publicly available, so that future analyses may be re-run with more complete reference databases and achieve even greater resolution.

A third area prone to false positives and negatives is based on the ecological interpretations of the data, irrespective of the bioinformatic workflow. Here, we generated a plausible list of 159 species that may be present in fox diet based on SMI species records. The absence of species with reference sequences may be attributed to the absence of the DNA from the 40 samples used in the study, or because they do not exist in the general SMI fox diet. We also recorded several taxa that were categorized as non-diet items (incidental, scat colonizer, etc.). We were careful to distinguish between detections that may have resulted from incidental ingestion rather than intentional consumption. Because parasites can

be facilitated through both intentional consumption and incidental ingestion (Combes, 2001), we reported those species in Table A4. We also scrutinized taxa that could have been detected because they colonize scat post-defection. Metabarcoding alone is unable to distinguish between diet versus fecal colonizer. Instead, we used our assumptions about fox diets (e.g., prey class and size) and knowledge about insect food sources. *A priori* knowledge of the system may be coupled with colonization experiments to disentangle the nuances of diet as measure by scat metabarcoding. An additional area ripe for exploration is the possibility of detecting parasites of diet items and reporting as parasites of the focal host. This may reasonably explain the observation of trematode parasite DNA, but further study is required for certainty.

2.4.4 Sampling effort needed for adequate coverage

Whereas we corroborated the majority of previously described diet items and further added resolution, there is still much to be gleaned from SMI fox diet. Whether missed diet items were absent from the chosen samples or absent from the fox diet in general remains to be seen. For parasites we detected four known and perhaps one additional species. While we achieved a sampling coverage of 1 for parasites, we failed to detect other parasites known to infect SMI foxes. Perhaps this reflects poorly chosen or developed assays, the distribution of parasites in space and time, the methods used to extract DNA, or a combination. It has been shown that using multiple genetic markers increased species richness estimates in systems where detection probabilities were low using conventional survey methods (da Silva et al., 2019; McElroy et al., 2020). Future studies that focus on the development of helminth-specific primers will lead to more robust richness and coverage findings.

2.4.5 Future directions

We see many avenues to further explore diet and parasite ecology through DNA metabarcoding. Currently, we are running cost-benefit and performance analyses of conventional parasite detection methods (fecal flotation and sedimentation) and DNA metabarcoding to determine which methods are more effective. We are also using the same two COI and 18S primers from this study to process samples that were taken from about 50 necropsied fox intestines from San Miguel. From this study, we will correlate proxy measurements (egg counts and DNA read abundances) with actual worm counts. With the necropsy samples, we will compare identifiable diet items to this study with the goal of disentangling scat colonizing species from diet items. Lastly, we have demographic information (age, sex, body condition, etc.) associated with each scat sample. We will analyze which, if any, factors are associated with helminth infections.

2.5 Conclusion

This study is an initial effort to combine parasite and diet ecology of a vulnerable species with emerging non-invasive methods to monitor disease and inform the development of conservation plans. DNA metabarcoding of SMI fox scat achieved detections of most known parasites and suggested another parasite species. To this extent it was partly confirmatory to past work. DNA metabarcoding also detected most of the previously documented diet items and more than tripled the number of known items at the genus or species level. Thus, metabarcoding had far more resolution and sensitivity that traditional diet sampling. Although we achieved adequate sampling coverage for both diet and parasites, there remains

room to explore seasonal patterns of detection and correlations between DNA read abundances and parasite burdens. Further, we hope to combine these data with fox demographic information to enhance the management and conservation plans of the San Miguel Island fox.

2.6 Acknowledgments

This work was supported by the USGS NRPP 78763 awarded to KL and the CINP. JNC was supported by the NSF Graduate Research Fellowship Program under Grant No. 2139319, the NSF Graduate Research Internship Program, and the USGS Pathways program. We thank Laura Shaskey, Juliann Schamel, Stacy Baker, Annie Little of the Channel Islands National Park for their instrumental roles in project coordination and logistical planning as well as the many CINP Biologists and Technicians who helped collect scat samples over the years.

Chapter 3: Spatial and temporal patterns of helminth parasites from the San Miguel Island fox, *Urocyon littoralis littoralis*

3.0 Abstract

Parasite induced decline is of considerable concern for managers of the San Miguel Island (SMI) fox, *Urocyon littoralis littoralis*, and a total of seven helminth parasites are known to infect SMI foxes. While much attention is paid to the effects of parasites on fox hosts, less research has focused on the effects of the ecosystem on parasite distribution. As with all free-living species, the prevalence and abundance of parasites are influenced by biotic and abiotic factors. Here, we recorded the egg prevalence and abundance SMI fox scat samples using fecal sedimentation to identify the following helminth parasites: *Angiocaulus gubernaculatus*, *Calodium hepaticum*, *Mesocestoides corti*, *P. canicola*, *Spirocerca* sp., *Toxascaris leonina*, *Uncinaria stenocephala*. Our objectives were to determine if egg prevalence and density were influenced by 1) temperature and rainfall, and 2) habitat type. We generated a third objective specifically for *P. canicola*: to determine the influence of habitat type and distance from proposed site of introduction, as this species is non-native. For the temporal component of our study, we found varying effects of temperature and rainfall on parasite egg prevalence and densities, with patterns varying from species to species. For the temporal study, we found no effects of habitat type on parasite distribution, but marginal evidence for the probability of P. canicola prevalence decreasing as distance from hypothesized location of introduction increasing. We conclude that more data are needed to disentangle the nuances of spatial and temporal helminth distributions of the SMI fox.

3.1 Introduction

The San Miguel Island (SMI) fox, *Urocyon littoralis littoralis*, is of considerable conservation concern. After the population rebounded from 15 individuals in 1999 to an estimated 650 (80% CI: 525-875) by 2015, it is once more at risk (US Fish and Wildlife Service, 2004; Coonan et al., 2010; Coonan et al., 2005, Shaskey, 2019). The population declined by more than 70% since 2018 (Shaskey, 2019; Dillon & Shaskey, 2022), and increased mortality rates are attributed, in part, to the recently identified acanthocephalan parasite, *Pachysentis canicola*. This parasite has a global distribution and infects a wide variety of carnivores as well as reptiles as paratenic hosts (Amin et al., 2022; Bolette, 1997; Buechner, 1944; Mobedi et al., 2007). However, it is assumed to be introduced on SMI, as it has not been detected on other California Channel Islands nor in mainland California canids (Chapter 1). Not only did the most recent fox decline coincide with the discovery of *P. canicola*, but infection severity by other parasites, such as the nematode *Spirocerca* sp., also increased during the same period (Shaskey, 2019). A total of seven helminth parasites are known to infect SMI foxes, and the Channel Islands National Park (CINP) has sought further research to understand the spatial and temporal patterns of helminth parasites from the SMI fox.

While much attention is paid to the effects of parasites on fox hosts, less research has focused on the effects of the ecosystem on parasite distribution. As with all free-living species, the prevalence and abundance of parasites are influenced by biotic and abiotic factors (Combes, 2001). Abiotic factors, such as temperature, water acidity, and precipitation have been shown to influence the presence and abundance of parasites (Galaktionov, 1996; Marcogliese et al., 2016; Marcogliese & Cone, 1996; Pech, 2010). For

example, Wood and colleagues (2013) showed that rainfall and temperature explained most of the seasonal variation of nematode egg counts from infected horses, with higher egg counts reported during the spring and summer seasons. Helminth infections in African elephants have also been shown to vary across the landscape based on vegetation types (King'ori et al., 2020).

The spatial and temporal patterns of helminth parasites, and their subsequent egg productions, must be incorporated when assessing transmission risks and accounted for when developing conservation plans of their hosts. Currently, there is no effective drug treatment to combat *P. canicola* infections of island foxes. For nematode infections, anti-helminthic resistance is not uncommon in mammal populations that receive sustained interventions (Gillis-Germitsch et al., 2020; Kaplan, 2004; Wood et al., 2013). This remains a long-term concern of managers who have administered anti-helminthic drugs in the past. Management-based strategies to mitigate infections, such sequestering infected individuals in certain locations or at specific times of the year, may provide more reliance than drug treatments. The implementation of management-based controls could depend on abiotic factors, such as temperature, rainfall, or habitat type.

On SMI, there are four general types of habitat that been described (Halvorson, 1992). Grassland communities encompass the largest areas of the island, reflecting the history of sheep ranching from 1850 to 1948 (Halvorson, 1992). Coastal sage scrub, *Haplopappus* scrub, and sand barrens are patchy throughout the island. Daily rainfall and temperature measurements are noted from the SMI Ranger Station when the island is staffed, and data are made accessible through the Western Regional Climate Center (WRCC, 2023). The overarching goal of this study was to assess the influences of spatial (habitat)

and temporal (temperature and rainfall) variables on SMI fox helminths as measured by fecal egg counts.

We inspected SMI fox scat samples using fecal sedimentation to identify the following helminth parasites: *Angiocaulus gubernaculatus*, *Calodium hepaticum*, *Mesocestoides corti*, *P. canicola*, *Spirocerca* sp., *Toxascaris leonina*, *Uncinaria stenocephala*. Our objectives were to determine if egg prevalence and density were influenced by 1) temperature and rainfall, and 2) habitat type. We generated a third objective specifically for *P. canicola*: to determine the influence of habitat type and distance from proposed site of introduction. This parasite is non-native, and it is hypothesized that the site of introduction occurred at the enclosures built to facilitate the breeding program during the initial recovery of the SMI fox. Because the research equipment and personnel used in the breeding program were staged at the ranger station, we also considered the effect of distance from ranger station on *P. canicola* egg prevalence and density.

For the temporal component of this study, we hypothesized that temperature and rainfall would influence the patterns of helminth egg prevalence and density for all helminths. Specifically, we predicted increased egg prevalence and densities as temperature decreased and rainfall increased. We expected these patterns because cooler, wetter conditions slow rates of egg desiccation on the landscape, which benefits parasites that are transmitted directly and indirectly (Haukisalmi & Henttonen, 1990; Kates, 1965; Stromberg, 1997). Further, cooler and wetter conditions tend to increase activity for the invertebrates on SMI that may facilitate helminths with indirect life cycles (Nancy Duncan, 2004; Osborne & Wright, 2018; Weissman & Lightfoot, 2007). For the spatial component, we hypothesized that grassland would exhibit higher egg prevalence and density compared to other habitat

types because grasslands dominate SMI. Lastly, we hypothesized that *P. canicola* egg prevalence and density would be highest at the site of hypothesized introduction (breeding pens or ranger station) and decrease as distance from site increased, reflecting the dispersal of the parasite across the island.

3.2 Methods

3.2.1 Study system and sample collection

All scat samples were collected with GPS coordinates from San Miguel Island, located off the coast of the Southern California Bight (34°02'06" N, 120°21'30" W; Figure 8A). The remote and uninhabited island is 38.7-km^2 in size and is a part of the Channel Islands National Park (CINP). For the temporal component of this study, 10 samples per month were collected around the ranger station for 24 months (February 2019 to January 2021). We analyzed 239 samples for this study as only nine samples were obtained in April 2019. For the spatial component, we intended to systematically collect scat samples from 16 evenly distributed grids across the island (Figure 8B). However, we restricted our efforts to the island trail system as CINP protocols prevent off-trail collections due to potential encounters with unexploded ordinance and culturally preserved Chumash Native American sites. In total, 164 samples were collected across the island trail system from 24 September to 24 October 2020 (Figure 8C).

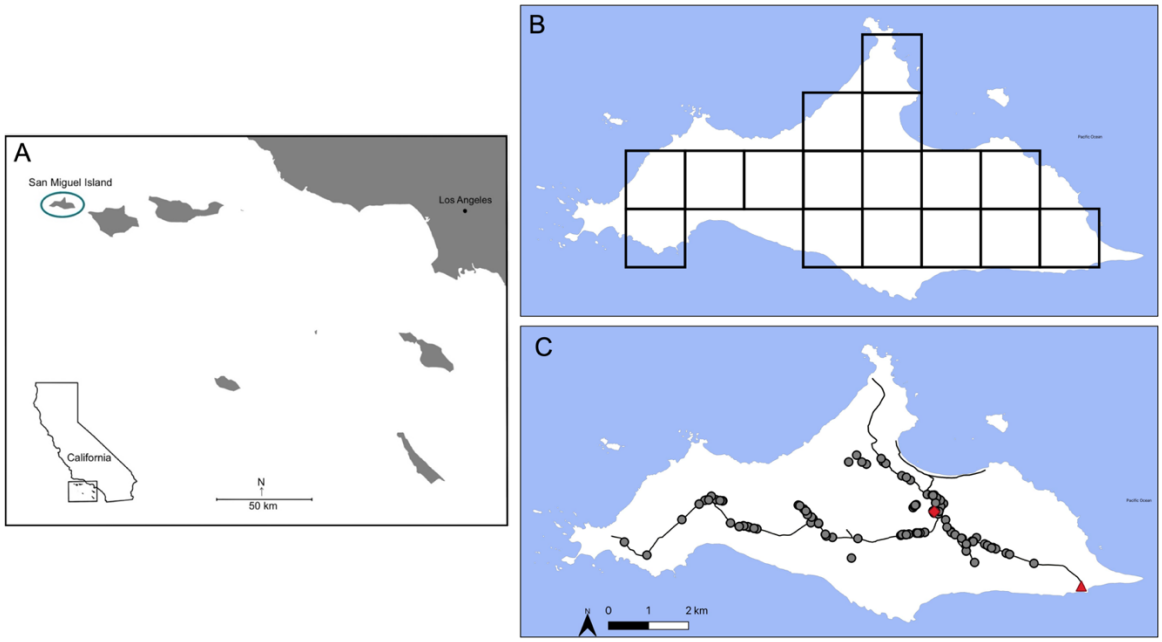


Figure 8. (A) The location of San Miguel Island (SMI; blue circle) in relation to the other California Channel Islands and the southern California coast; (B) The proposed sampling grid for SMI; (C) The sampling scheme of 164 scat samples (grey circles) collected between 24 September to 24 October 2020. The red triangle indicates the location of the historical on-island fox breeding pens and hypothesized introduction location for the acanthocephalan parasite. The trail system is indicated in the solid black line, the breeding pens are indicated with the red triangle, and the ranger station is indicated with a red diamond.

3.2.2 Fecal sedimentation

All scats were homogenized upon collection and stored at -20°C until processed. We used the standing fecal sedimentation method to search for helminth eggs from each homogenized scat as described in Chapter 1 (mean and standard deviation (sd) of each sample: 10.4 ± 5.7-g). We used a mean subsample of 2.0 ± 0.22-g (sd) and mixed with it deionized water to break up large pieces. Then, we sieved the mixture into a 50-mL Falcon tube and filled it with fresh water. After 10 minutes, we poured off the supernatant and refilled the Falcon tube with fresh water. We repeated this process until the supernatant was clear, after which we removed all water. The remaining pellet (between 0.25-0.5mL) was

transferred to microscope slides. Sediments were then examined under light microscopy (100-400x total magnification).

3.2.3 Temporal analysis

For each of the 239 temporal samples, we recorded the prevalence and density (number of eggs per gram scat) counts for eggs of each helminth species. We separated our temporal and spatial analyses for two reasons. Firstly, the temporal data were collected from one location for the duration of the study. Secondly, the rainfall and temperature data are reported for the whole island, so we could not integrate fine scale temporal data into a combined spatiotemporal model. Parasites can have immediate and delayed responses to temperature and rainfall (Shearer and Ezwena 2020). However, no time lags were considered (temperature and rain data were taken from the month the feces were collected) to reduce the potential for overfitting.

Given the time dependency of our count data, we used **g**eneralized **l**inear **a**uto**r**egressive **m**oving **a**verage (GLARMA) models to determine the effects of temperature and rainfall on parasite egg prevalence and density. The AR components were applied based on the estimated and partial autocorrelation functions using the residuals from a generalized linear model. We selected ARMA components of one as monthly egg prevalence and density values were positively correlated with the previous month's observations. Although this model allows the incorporation of multiple cyclical trends, we did not include annual dependence (e.g. AR = 12) as we lacked sufficient data. GLARMA models, which do not require transformation of the data from count into continuous form, allow us to ensure that time dependence is accounted for and maintain the data's inherent form through the use of

discrete distributions of counts (Davis et al., 1999; Dunsmuir & Scott, 2015). GLARMA models have become popularized for modelling the incidence of infectious diseases, such as coronaviruses and rabies, with varying distributions (e.g. Poisson, binomial, and negative binomial; Barría-Sandoval et al., 2021; Lachica et al., 2018). Here, we used a binomial distribution for prevalence data and a Poisson distribution for density data. Although we considered using a negative binomial distribution for the density models, we could not evaluate for overdispersion due to the limits of 10 samples per month. However, inspection of the data indicates that there may indeed exist overdispersion in these data. Future studies might consider incorporating more than 10 samples per month to make evaluation for overdispersion feasible.

For each model, selection was based on Bayesian information criterion (BIC, a.k.a. the Schwarz Information Criterion or SIC because it is not an inherently Bayesian statistics model selection approach; Schwarz, 1978; Dennis et al., 2019). We formulated five hypotheses for each parasite species separately for mean prevalence (Prev) and egg density (Dens). The first model reflects a null hypothesis that the prevalence or density of island fox helminths is constant through time and does not depend on measured variables (M_0: Prev or Dens ~ constant). We assessed two single variable models to explore the effects of average monthly temperature (Temp in °C) and rainfall (Rain in mm; M_1: Prevalence or Density ~ Temp; M_2: Prevalence or Density ~ Rain), one two-variable model (M_3: Prevalence or Density ~ Temp + Rain), and one model that included an interaction term for temperature and rainfall (M_4: Prevalence or Density ~ Temp*Rain). Model selection occurred using the difference between BIC values (ΔBIC), and we chose the model with the lowest BIC value as best fit. Interpretation of models followed guidance by Jerde et al, (2019), where

candidate models with ΔBIC < 7 should be retained as possible explanatory hypotheses for further consideration with more data. Unless otherwise specified, all analyses and figures were generated in R version 3.6.3 (R Core Team, 2020) using the *tidyverse*, *nlme*, and *glarma*, *cowplot*, and *broom* (Dunsmuir et al., 2018; Pinheiro et al., 2023; Robinson et al., 2022; Wickham et al., 2019).

3.2.4 Spatial analysis

We used a generalized linear model (GLM) with binomial distribution with logit-link function and a Poisson distribution with log-link function to test the effects of habitat on parasite prevalence and density, respectively (Wilson et al., 1996). We assigned habitats to each sample location based on Halvorson (Halvorson, 1992). The samples were collected from four distinct sub-habitats on San Miguel Island: grassland (126 samples), sand (or barren; 20 samples), *Haplopappus* sp. scrub (17 samples), coastal sage scrub (1 sample). We formulated two hypotheses for prevalence and density measures for each parasite species. The null model tested the hypothesis that parasite prevalence (Prev) or egg density (Dens) is constant and does not depend on measured variables (M_0: Prevalence or Density ~ constant), whereas model one (M_1) tested whether habitat influenced parasite prevalence or density (M_1: Prevalence or Density ~ Habitat).

For *P. canicola* only, our spatial models also incorporated distances from hypothesized site of introduction. One hypothesized location of introduction for *P. canicola* is the captive fox breeding pens, as foxes were held in this location from 1999 to 2008 as part of the CINP recovery program and is indicated with a red triangle in Figure 8C (Coonan et al., 2010). A second hypothesized location of introduction is the ranger station, as this is

where all personnel and equipment (that might have harbored infective invertebrates) are staged prior to implementing a research project. We recorded the distance in meters (m) from each sample location to both the site of the breeding pens and ranger station using QGIS (QGIS Development Team, 2023). We used a generalized linear model (GLM) with Poisson distribution with log-link function to test the effects of distance from ranger station (RS) or breeding pens (BP) and island sub-habitat (Habitat) on *P. canicola* prevalence (Prev) and egg densities (Dens). For these models, we considered prevalence and density as dependent variables and distance from source (RS or BP), habitat, and their first order interactions as potential effects. For all spatial models, selection was based on BIC values as described for the temporal analysis.

3.3 Results

3.3.1 SMI fox helminth diversity

We detected six helminth species using fecal sedimentation, and the mean richness for individual SMI fox scat was 3.3 ± 1.3 (sd) species. Of the 403 scat samples processed, 99.5% (401) contained eggs from at least one helminth. We recovered a total of 386,184 eggs: 262,160 were recovered in the samples used for the temporal analysis and 124,024 were recovered in the samples used for the spatial analysis. The most common helminth was *U. stenocephala* with a prevalence of 90.4% and 96.3% for temporal and spatial samples, respectively (Table 6). Other eggs recovered included those of the nematodes *Spirocerca* sp. (48.1% temporal; 62.2% spatial), *Angiocaulus gubernaculatus* (65.3% temporal; 84.6% spatial), and *Calodium hepaticum* (17.2% temporal; 6.7% spatial). Eggs of one tapeworm species, *Mesocestoides corti,* was observed in 2.5% and 0.6% of temporal and spatial

samples, respectively. The parasite of most concern, the acanthocephalan *P. canicola*, was present over half of all samples (60.7% temporal and 54.9% spatial). Of note, we found live juvenile nematodes sporadically in sedimentation samples, with the longest period between the freeze and thaw/examination being 11 months.

Table 6. Prevalence and mean intensity for helminth eggs recovered from San Miguel Island fox scats. % = percent prevalence (number of samples); Density = mean number of eggs per gram of infected scat examined; sd = standard deviation for density.

	Temporal (n = 239)		Spatial (n = 164)	
Helminth	**%**	**Density (± sd)**	**%**	**Density (± sd)**
Angiocaulus gubernaculatus	65.3 (156)	28 ± 89.3	84.8 (139)	113.7 ± 687.1
Calodium hepaticum	17.2 (41)	455.9 ± 3279	6.7 (11)	188.6 ± 1339.4
Spirocerca sp.	48.1 (115)	8.5 ± 35.3	62.2 (102)	7.6 ± 20.7
Uncinaria stenocephala	90.4 (216)	29.4 ± 89.3	96.3 (158)	41.4 ± 72.5
Mesocestoides corti	2.5 (6)	0.6 ± 7.6	0.6 (1)	0.01 ± 0.08
Pachysentis canicola	60.7 (145)	25.3 ± 82.7	54.9 (90)	22.2 ± 49.5

3.3.2 Temporal analysis – GLARMA

We detected eggs of the tapeworm, *M. corti*, from only 7 of the 403 samples we processed for this study. These data were insufficient to model the time series influence of temperature and rainfall on tapeworm egg prevalence or density. Figure 9 shows the prevalence and egg density dynamics for each parasite species with monthly rainfall and precipitation data. For *P. canicola*, *U. stenocephala*, and *C. hepaticum*, the best model for prevalence was the null (M_0), where there was no influence of mean monthly temperature or rainfall on prevalence (Table 7). However, ΔBIC values for all other models regarding *P. canicola* and *C. hepaticum* prevalence fell below seven, indicating the need for more data to disentangle the potential influences of temperature and rainfall. We also retained all models for *U.*

stenocephala, except for M_4, which accounted for the interaction between temperature and rainfall (Table 7).

For the canine lungworm, *A. gubernaculatus*, the best model for prevalence was M_4, which considered the interaction of temperature and rainfall. However, the ΔBIC for the model that included temperature and rainfall fell below seven (M_3), so both models were retained. Egg prevalence for *A. gubernaculatus* was negatively correlated with both temperature and rainfall, consistently. Lastly, for *Spirocerca* sp., the top-ranked model accounted for temperature only (M_1). The results of this model indicate that egg prevalence for *Spirocerca* sp. was negatively correlated with temperature. Because the ΔBIC values for all other Spirocerca sp. prevalence models also fell below seven, we cannot exclude any one hypothesis without considering more data. Fit and diagnostic plots of all retained parasite prevalence models can be found in Appendix 2.

The top-ranked model for egg densities of *U. stenocephala*, *Spirocerca* sp., and *C. hepaticum* was M_4, which included the interaction between temperature and rainfall (Table 7). All three parasite species were differentially influenced by temperature and rainfall. Whereas the egg density of *U. stenocephala* was positively correlated with both temperature and rainfall, egg density Spirocerca sp. was negatively correlated with temperature and rainfall. Still egg density for *C. hepaticum* showed yet another pattern, with a negative correlation with temperature yet positive correlation with rainfall. *A. gubernaculatus* egg density was also negatively correlated with temperature, as demonstrated by M_4 (Table 7). However, the ΔBIC values for models one and three fell below seven *A. gubernaculatus* egg density. Thus these three models were all retained. Lastly, the top-ranked model for acanthocephalan egg density accounted for temperature only (M_1). Although the ΔBIC

values for the models that incorporated temperature and rainfall fell below seven (M_3 ΔBIC: 1.25 and M_4 ΔBIC: 4.28), all models indicated a positive correlation between acanthocephalan egg density and temperature (Table 7). The fit and diagnostic plots of all retained models are shown in Appendix 2.

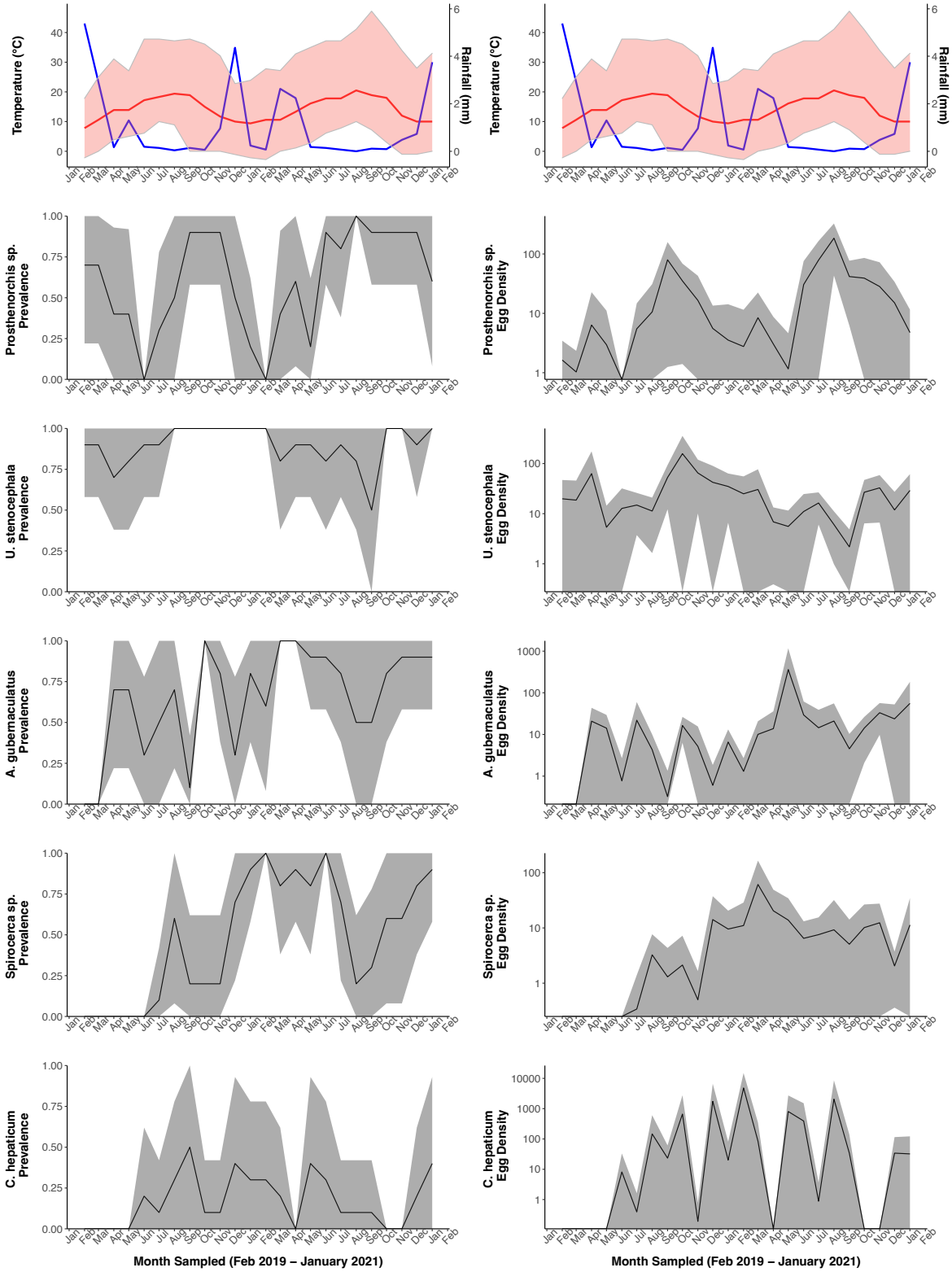


Figure 9. The top row in both columns shows the mean, min, and max temperature in ºC (red line and ribbon) with the maximum rainfall in mm (blue line). The remaining figures in the left column show the prevalence (n = 10) of parasite eggs in scat samples with confidence intervals shaded in grey. The remaining figures in the right column show the log

distribution of parasite egg density (per gram feces) with confidence intervals shaded in grey. All figures show the same 24-month study period on San Miguel Island from February 2019-January 2021. (Second row) *P. canicola* dynamics, (Third row) *U. stenocephala* dynamics, (Fourth row) *A. gubernaculatus* dynamics, (Fifth row) *Spirocerca* sp. dynamics, and (Sixth row) *Calodium hepaticum* dynamics.

3.3.3 Spatial analysis

For *U. stenocephala*, *A. gubernaculatus*, *Spirocerca* sp., and *C. hepaticum* the top-ranked model was M_0, indicating no influence of habitat type on egg prevalence and density. The ΔBIC values for all other models were greater than seven, and therefore, excluded (Table 8). We originally generated seven models to test the effects of distance from possible introduction site (BP or RS) and habitat type on *P. canicola* prevalence and density (Figure 10). However, the models that included interactions terms between BP or RS and habitat type were excluded (two models) because not all parameters were estimable. Of the viable models for *P. canicola* prevalence, M_1 performed best (Table 9). This model supported the hypothesis that scat samples located farther from breeding pens have a decreased probability of infection compared to scat samples located closer. Interestingly, we also retained the model that considered distance from the ranger station because the ΔBIC value fell below seven (M_2; Table 9). The model that incorporated distance from breeding pens generally predicted higher probabilities of prevalence compared with the model that incorporated distance from ranger station (Figure 11). For *P. canicola* egg density, the best model was M_5, and all other models were excluded (Table 9). Whereas there was no effect of *Haplopappus* scrub habitat, there were significant and positive effects of both grassland and sand habitats on *P. canicola* egg density.

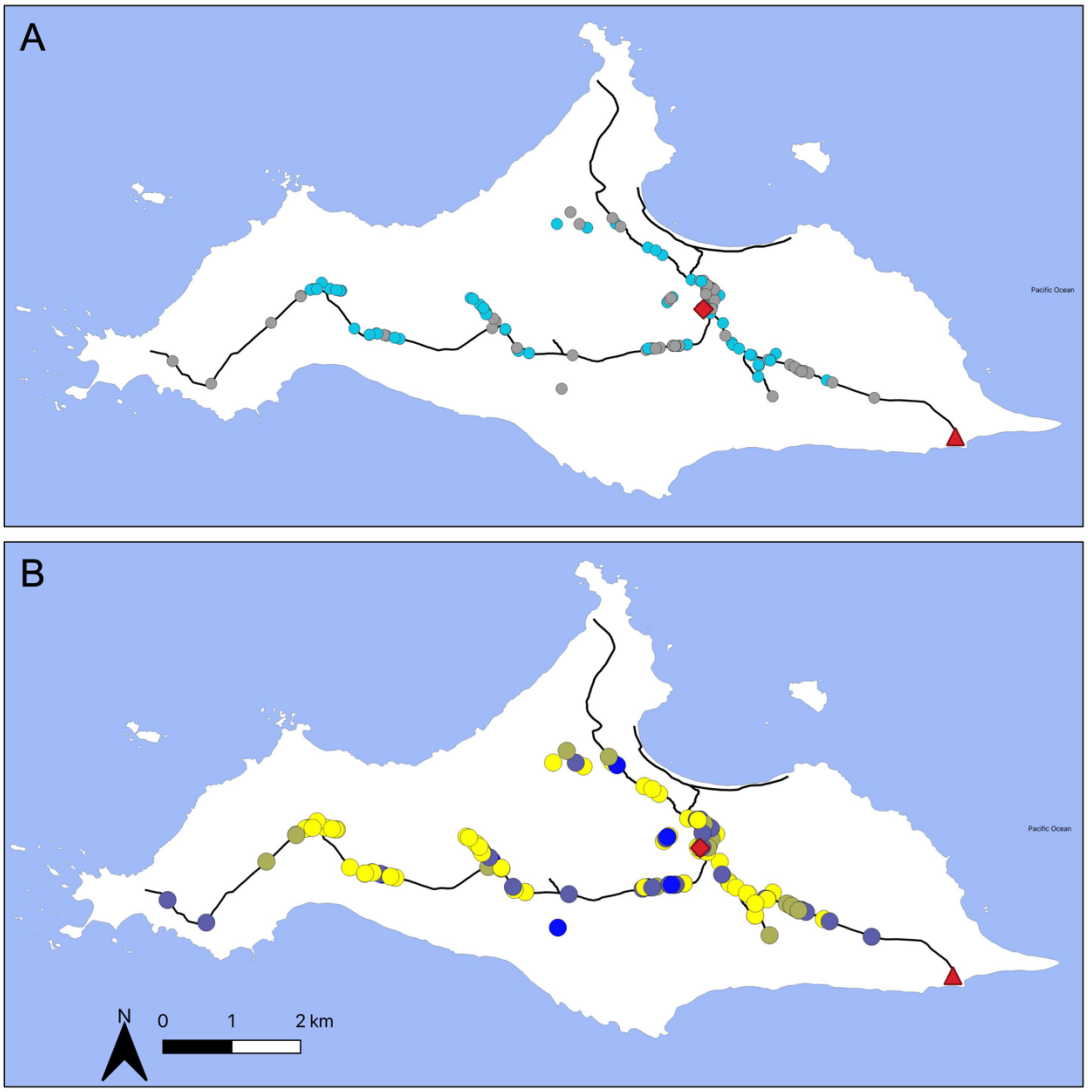


Figure 10. (A) A map of samples taken across SMI between 24 September and 24 October 2020. Samples positive for *P. canicola* eggs are shown in blue while negative samples are shown in grey; (B) the same map of samples with a gradient indicating the number of *P. canicola* eggs per gram scat (values closer to zero are shown in yellow shades, while values closer to the maximum value of 372.3 eggs/gram feces are shown in blue shades). On both maps the trail system is indicated in the solid black line, the breeding pens are indicated with the red triangle, and the ranger station is indicated with a red diamond.

3.4 Discussion

Of the seven helminth species known to infect SMI foxes, we detected the eggs of six species. *Toxascaris leonina* was the only parasite we did not recover through scat sedimentation, although this species has only been documented from two adult worms

recovered from one fox necropsy (see Chapter 1). We failed to find a consistent pattern of temperature, rainfall, or habitat type for all parasite species. However, there are indications that a selection of parasites responds to temperature, rainfall, and habitat type. Specifically, for the acanthocephalan, *P. canicola*, there is evidence that the parasite may be widespread across the island, but more data is needed to disentangle the spatial patterns of this parasite.

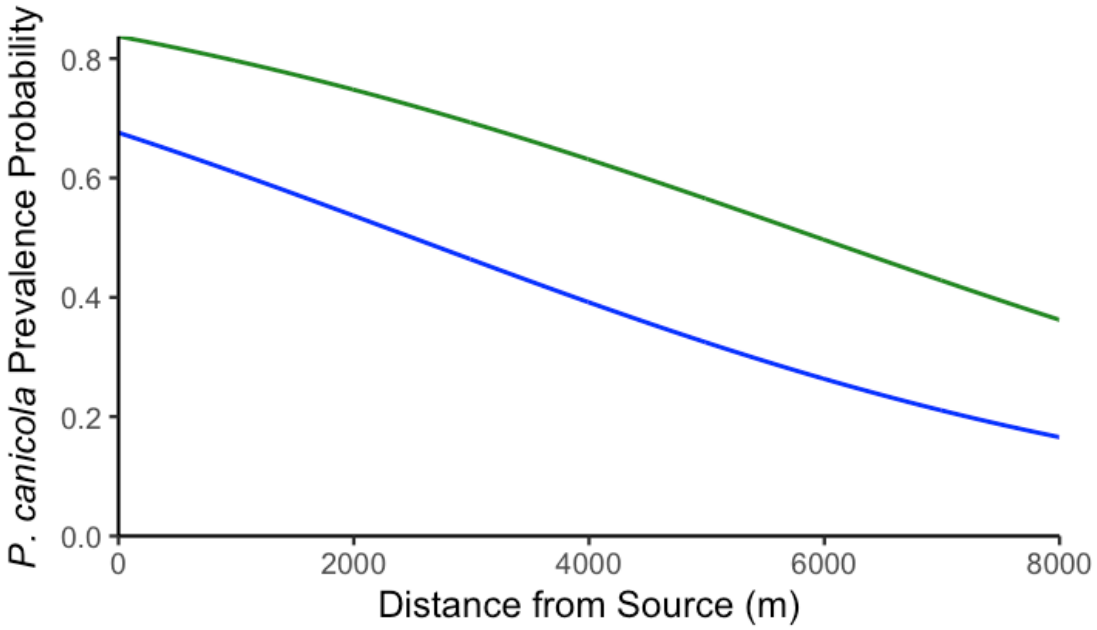


Figure 11. The results of the models retained for acanthocephalan prevalence probability as a function of distance from breeding pens (top ranked M_1, green line) or distance from ranger station (M_3, blue line).

3.4.1 Temporal patterns of SMI fox helminths

Although the tapeworm, *M. corti*, was too rare to include in the temporal or spatial analyses (7 detections from 403 total samples), we know that this species is common from SMI foxes. Yet, manual detection methods, such as fecal sedimentation and flotation (not performed for this study), consistently fail to detect tapeworm eggs. In an unpublished portion of this

dissertation, we found that 55% (22/40) of samples were positive for tapeworm DNA, while only one of those samples returned positive with fecal sedimentation. This finding indicates that while tapeworm infections are common in SMI foxes, eggs are not shed consistently through space and time. As the life cycle for *M. corti* involves three required hosts (Padgett & Boyce, 2004, 2005), the egg production might be tightly timed to facilitate the infection of the subsequent host, and we may have missed the possibly narrow shedding window with only 10 samples per month analyzed.

For all species, there are indications that temperature affects the distribution of egg prevalence. Because we retained many models for each species, including the null, we cannot make definitive conclusions about the magnitude of effects. This indicates that eggs from these species may be produced and shed year-round. Importantly for transmission, there was marginal evidence for the increase of egg densities with varying trends of temperature and rainfall for three species: *U. stenocephala*, *Spirocerca* sp., and *C. hepaticum*. *U. stenocephala* densities were higher with warmer and wetter periods of the year, whereas *Spirocerca* sp. densities were higher with cooler and dryer periods. Of these three species, both *Spirocerca* sp., and *C. hepaticum* require intermediate hosts to facilitate transmission, and their egg density patterns may coincide with the activities of coprophagous beetles, rodents, and reptiles that are known to facilitate these parasites (van der Merwe et al., 2008; Fuehrer 2014a,b). While *C. hepaticum* is a widely distributed parasite, it may not be a true parasite of SMI foxes. Rather, its observation may be dependent on passing through the fox intestinal system upon digestion of an infected prey item. This would align with the combination of low prevalence yet extremely high relative densities when we observed *C. hepaticum* in this study (range: 0 – 26389 eggs/gram; Figure

9). While *Spirocerca* sp. is a confirmed parasite of the SMI fox, the pattern of increased egg density correlated with decreased temperatures and rainfall does not follow previously reported patterns. This may be due, in part, to the fact that we observed egg counts rather than adult worms, as others have shown increased worm burdens associated with elevated temperatures (Bailey, 1972; Martín-Pérez et al., 2020).

Although there were multiple models retained for both *A. gubernaculatus* and *P. canicola*, the reoccurring trend was that egg densities increased with decreasing temperatures. With the minor fluctuations of temperature (-2.8 to 10.0 ºC) and rainfall (0 to 5.38 mm) for this two-year study, the margins for warm to cool and dry to wet are slim. Yet, these relatively small fluctuations, may exert influence on the intermediate hosts that facilitate these two parasite species. Whereas the snail, *Helminthoglypta ayresiana*, is the recorded intermediate host for *A. gubernaculatus*, the intermediate host(s) for *P. canicola* remains unknown. Suspected intermediate hosts include terrestrial arthropods, such as the Jerusalem cricket (*Stenopelmatus* sp.) among other orthopterans and coleopterans (Chapter 1). These intermediate and candidate hosts exhibit increased activities with cooler and wetter environments (Nancy Duncan, 2004; Osborne & Wright, 2018; Weissman & Lightfoot, 2007). We suspect that inferences with more confidence may be obtained with additional data collected continuously for more than two years and combined with patterns of intermediate host activities.

3.4.2 Spatial distribution of SMI fox helminths

For *U. stenocephala*, *A. gubernaculatus*, *Spirocerca* sp., and *C. hepaticum*, there were no indications that habitat type influenced egg prevalence or densities. While patterns emerged

for *P. canicola* prevalence regarding distance from breeding pens, we also retained the model testing the same hypothesis with the ranger station (Figure 11). Of the models that tested the effect of habitat and distance on *P. canicola* egg density, the one that performed best was M_5, which accounted for habitat type and distance from ranger station. Although this parasite was introduced to the island around 10 years ago, these models provide insight into the uneven spread and rate of distribution.

Widespread distribution of foxes, overlapping territories, and intermediate host activity may also explain the lack of measured influence of sub-habitat on acanthocephalan distribution. Due to the sampling restrictions, we were unable to collect proportional samples from the respective sub-habitats. We unevenly collected data from the four habitats, with grassland dominating the habitat type of samples and only one sample from coastal sage scrub. Although grassland and coastal sage scrub are the most and least common habitats across the island, respectively, more data is needed to determine if there exist true discrepancies of acanthocephalan prevalence and density in relation to sub-habitat type (Halvorson, 1992). Additionally, unmeasured variables, such as soil conditions, host density, proximity to fox dens, and other factors may contribute to patterns of egg dispersion.

3.5 Conclusion

Other studies have examined the patterns of island fox parasites at broader scales, such as between islands (Harris et al., 2013, refer to Chapter 1). Yet, this is the first study to determine what patterns exist for island fox parasites within one island. While many have shown that both abiotic and biotic factors influence the parasite dynamics of sensitive host species (Cattadori et al., 2005; Fontanarrosa et al., 2006; Jones et al., 2013; Morton et al.,

2005), here, we presented data that may trend toward temperature and rainfall impacting the parasite presence and egg densities of SMI foxes. Still more data is needed to elucidate these patterns. As this vulnerable fox species struggles to recover from the latest population decline, understanding the spatiotemporal dynamics of their helminth parasites may hold a key to stabilizing the population once more.

3.6 Acknowledgements

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Table 7. The influence of temperature and rain on helminth prevalence (Prev) or egg density (Dens) from five species of helminth recovered from SMI fox scats over a 24 monthly period.

Category	Model	k	log-likelihood	Parameter (SE)	BIC	ΔBIC
P. canicola Prevalence	M_0: Acanth_Prev ~ constant	2	-62.2509	β_0: 0.4963 (0.2748) ϕ: 0.4326 (0.0799)	130.857	0
	M_1: Acanth_Prev ~ Temp	3	-62.12418	β_0: 0.1128 (0.8015) β_1: 0.0280 (0.0552) ϕ: 0.4207 (0.0836)	133.782	2.9246
	M_2: Acanth_Prev ~ Rain	3	-61.79186	β_0: 0.3666 (0.3115) β_1: 0.0872 (0.0907) ϕ: 0.4438 (0.0791)	133.117	2.26
	M_3: Acanth_Prev ~ Temp + Rain	4	-60.95618	β_0: -0.9546 (1.0507) β_1: 0.0880 (0.0671) β_2: 0.1692 (0.1108) ϕ: 0.4172 (0.0825)	134.624	3.7667
	M_4: Acanth_Prev ~ Temp*Rain	5	-59.3032	β_0: -0.3859 (1.1874) β_1: 0.0539 (0.0732) β_2: -0.5838 (0.4243) β_3: 0.0771 (0.0415) ϕ: 0.4924 (0.0902)	134.496	3.6388
P. canicola Density	M_0: Acanth_Dens ~ constant	2	-428.6972	β_0: 3.0223 (0.0615) ϕ: 0.0737 (0.0047)	863.750	454.54
	M_1: Acanth_Dens ~ Temp	3	-199.8345	β_0: -1.8983 (0.2660) β_1: 0.3122 (0.0143) ϕ: 0.1262 (0.0092)	409.203	0
	M_2: Acanth_Dens ~ Rain	3	-366.6367	β_0: 3.4414 (0.0627) β_1: -0.5546 (0.0621) ϕ: 0.0608 (0.0054)	742.807	333.60
	M_3: Acanth_Dens ~ Temp + Rain	4	-198.8696	β_0: -2.1222 (0.3080) β_1: 0.3225 (0.0158) β_2: 0.0906 (0.0634) ϕ: 0.1276 (0.0092)	410.451	1.2482
	M_4: Acanth_Dens ~ Temp*Rain	5	-198.7946	β_0: -2.0992 (0.3113) β_1: 0.3217 (0.0159) β_2: 0.2061 (0.2761) β_3: 0.0771 (0.0415) ϕ: -0.0117 (0.0270)	413.479	4.2763
Uncinaria stenocephala Prevalence	M_0: Hook_Prev ~ constant	2	-31.8383	β_0: 2.3018 (0.3059) ϕ: 0.2811 (0.1845)	70.0327	0
	M_1: Hook_Prev ~ Temp	3	-30.9639	β_0: 3.7264 (1.0908) β_1: -0.0986 (0.0696) ϕ: 0.1586 (0.1954)	71.4619	1.4291
	M_2: Hook_Prev ~ Rain	3	-31.7631	β_0: 2.2259 (0.3525) β_1: 0.0646 (0.1701) ϕ: 0.2673 (0.1886)	73.0604	3.0276

	M_3: Hook_Prev ~ Temp + Rain	4	-30.7154	β_0: 4.6613 (1.7542) β_1: -0.1498 (0.1014) β_2: -0.1735 (0.2467) ϕ: 0.1236 (0.2018)	74.1431	4.1104
	M_4: Hook_Prev ~ Temp*Rain	5	-30.6681	β_0: 4.7383 (1.7931) β_1: -0.1523 (0.1027) β_2: 0.0615 (0.8174) β_3: -0.0255 (0.0836) ϕ: 0.1113 (0.2083)	77.2264	7.1937
Uncinaria stenocephala Density	M_0: Hook_Dens ~ constant	2	-301.9928	β_0: 3.2993 (0.0554) ϕ: 0.0747 (0.0065)	610.341	234.53
	M_1: Hook_Dens ~ Temp	3	-280.0552	β_0: 4.5826 (0.1957) β_1: -0.1022 (0.0142) ϕ: 0.1403 (0.0069)	569.644	193.83
	M_2: Hook_Dens ~ Rain	3	-299.0393	β_0: 3.3789 (0.0628) β_1: -0.0664 (0.0277) ϕ: 0.0741 (0.0066)	607.612	231.80
	M_3: Hook_Dens ~ Temp + Rain	4	-267.3668	β_0: 5.2205 (0.2474) β_1: -0.1385 (0.0172) β_2: -0.1273 (0.0245) ϕ: 0.1426 (0.0066)	547.445	171.64
	M_4: Hook_Dens ~ Temp*Rain	5	-179.9577	β_0: 3.4602 (0.2951) β_1: 0.0006 (0.0193) β_2: 0.8303 (0.0873) β_3: -0.0988 (0.0089) ϕ: 0.1852 (0.0060)	375.805	0
A.gubernaculatus Prevalence	M_0: Angio_Prev ~ constant	2	-77.5244	β_0: 0.6180 (0.1805) ϕ: 0.1752 (0.0699)	161.405	19.124
	M_1: Angio_Prev ~ Temp	3	-77.5041	β_0: 0.74796 (0.6699) β_1: -0.0090 (0.04480) ϕ: 0.1761 (0.0698)	164.542	22.261
	M_2: Angio_Prev ~ Rain	3	-71.8462	β_0: 0.9922 (0.2239) β_1: -0.3145 (0.0946) ϕ: 0.2015 (0.0793)	153.226	10.946
	M_3: Angio_Prev ~ Temp + Rain	4	-67.6166	β_0: 3.8036 (1.0554) β_1: -0.1756 (0.0627) β_2: -0.5583 (0.1360) ϕ: 0.1696 (0.0914)	147.945	5.6650
	M_4: Angio_Prev ~ Temp*Rain	5	-63.1951	β_0: 3.6640 (0.9826) β_1: -0.1815 (0.0578) β_2: -2.5137 (0.8209) β_3: 0.2011 (0.0814) ϕ: 0.0601 (0.0941)	142.280	0.0000
A. gubernaculatus Density	M_0: Angio_Dens ~ constant	2	-49.9581	β_0: 1.0024 (0.1665) ϕ: 0.0124 (0.0932)	105.805	10.451
	M_1: Angio_Dens ~ Temp	3	-46.4568	β_0: 2.7201 (0.7087) β_1: -0.1141 (0.0447) ϕ: -0.0098 (0.1032)	101.747	6.393

	M_2: Angio_Dens ~ Rain	3	-49.9106	β_0: 0.9664 (0.2002) β_1: 0.0427 (0.1395) ϕ: -0.0003 (0.1014)	108.654	13.300
	M_3: Angio_Dens ~ Temp + Rain	4	-44.9503	β_0: 4.0705 (1.1275) β_1: -0.1861 (0.0648) β_2: -0.3066 (0.1758) ϕ: 0.0173 (0.1123)	101.678	6.3244
	M_4: Angio_Dens ~ Temp*Rain	5	-40.3159	β_0: 3.7299 (0.9538) β_1: -0.1915 (0.0543) β_2: -6.1798 (2.6758) β_3: 0.5818 (0.2673) ϕ: 0.2103 (0.1441)	95.3541	0
Spirocera sp. Prevalence	M_0: Spiro_Prev ~ constant	2	-51.0082	β_0: 0.3025 (0.2857) ϕ: 0.3781 (0.0887)	107.905	5.9650
	M_1: Spiro_Prev ~ Temp	3	-46.5536	β_0: 2.9509 (0.8740) β_1: -0.1719 (0.0553) ϕ: 0.2837 (0.0948)	101.940	0.0000
	M_2: Spiro_Prev ~ Rain	3	-49.5394	β_0: 0.1485 (0.2843) β_1: 0.2106 (0.1260) ϕ: 0.3536 (0.0925)	107.912	5.9717
	M_3: Spiro_Prev ~ Temp + Rain	4	-46.4371	β_0: 2.6746 (1.0381) β_1: -0.1575 (0.0626) β_2: 0.0690 (0.1429) ϕ: 0.2887 (0.0955)	104.652	2.7115
	M_4: Spiro_Prev ~ Temp*Rain	5	-46.4346	β_0: 2.6745 (1.0371) β_1: -0.1579 (0.0627) β_2: -0.0040 (1.0447) β_3: 0.0071 (0.1014) ϕ: 0.2872 (0.0977)	107.591	5.6509
Spirocera sp. Density	M_0: Spiro_Dens ~ constant	2	-170.0601	β_0: 2.0491 (0.0879) ϕ: 0.0673 (0.0105)	346.476	30.110
	M_1: Spiro_Dens ~ Temp	3	-160.4401	β_0: 3.3807 (0.3100) β_1: -0.0987 (0.0230) ϕ: 0.0743 (0.0126)	330.414	14.048
	M_2: Spiro_Dens ~ Rain	3	-156.99	β_0: 1.6871 (0.1224) β_1: 0.2332 (0.0417) ϕ: 0.0837 (0.0129)	323.514	7.1486
	M_3: Spiro_Dens ~ Temp + Rain	4	-156.1056	β_0: 2.3509 (0.5001) β_1: -0.0424 (0.0317) β_2: 0.1737 (0.0586) ϕ: 0.0836 (0.0132)	324.923	8.5577
	M_4: Spiro_Dens ~ Temp*Rain	5	-150.2377	β_0: 2.5743 (0.4976) β_1: -0.0655 (0.0323) β_2: -0.5621 (0.2343) β_3: 0.0772 (0.0240) ϕ: 0.0606 (0.0165)	316.365	0
C. hepatica Prevalence	M_0: CaHe_Prev ~ constant	2	-33.9122	β_0: -1.3591 (0.2226) ϕ: 0.1623 (0.1822)	73.7133	0
	M_1: CaHe_Prev ~ Temp	3	-33.6942	β_0: -0.8286 (0.8109) β_1: -0.0365 (0.0545) ϕ: 0.1389 (0.1855)	76.2217	2.5084

	M_2: CaHe_Prev ~ Rain	3	-33.0011	β_0: -1.5384 (0.2749) β_1: 0.1771 (0.1283) ϕ: 0.1886 (0.1887)	74.8354	1.1221
	M_3: CaHe_Prev ~ Temp + Rain	4	-32.9988	β_0: -1.6097 (1.1172) β_1: 0.0045 (0.0687) β_2: 0.1825 (0.1526) ϕ: 0.1900 (0.1896)	77.7755	4.0622
	M_4: CaHe_Prev ~ Temp*Rain	5	-30.1142	β_0: -1.6966 (1.1177) β_1: 0.0300 (0.0698) β_2: 4.0990 (2.0872) β_3: -0.3875 (0.2076) ϕ: 0.2278 (0.1929)	74.9507	1.2374
C. hepatica Density	M_0: CaHe_Dens ~ constant	2	-16010.83	β_0: 6.0116 (0.0088) ϕ: -0.0119 (0.0004)	32028.0	4719.3
	M_1: CaHe_Dens ~ Temp	3	-15536.18	β_0: 6.9752 (0.0311) β_1: -0.0702 (0.0022) ϕ: -0.0138 (0.0004)	31081.9	3773.2
	M_2: CaHe_Dens ~ Rain	3	-15838.91	β_0: 6.1347 (0.0107) β_1: -0.1239 (0.0070) ϕ: -0.0107 (0.0004)	31687.3	4378.6
	M_3: CaHe_Dens ~ Temp + Rain	4	-14055.59	β_0: 8.9279 (0.0452) β_1: -0.1796 (0.0029) β_2: -0.4546 (0.0094) ϕ: -0.0136 (0.0003)	28123.8	815.2
	M_4: CaHe_Dens ~ Temp*Rain	5	-13646.4	β_0: 9.0068 (0.0443) β_1: -0.1727 (0.0028) β_2: 0.7136 (0.0397) β_3: -0.1277 (0.0044) ϕ: -0.0129 (0.0003)	27308.6	0

k: number of parameters included in the model; log-likelihood: measure of model fitness; Parameter (SE): parameter values with standard error; BIC: Bayesian information criterion; ΔBIC: Difference of Bayesian information criterion to each model from the most parsimonious model; Temp: mean monthly temperature (°C); Rain: maximum monthly precipitation (in mm). Parameter definitions: β_0 = Intercept, β_1 = Temp (or Rain in each M_2), β_2= Rain, β_3 = Interaction between Temp and Rain, ϕ: accounts for the serial dependence in the response process.

Table 8. The influence of habitat [Habitat] on helminth egg prevalence (Prev) or density (Dens) from SMI fox scats sampled across the island from 24 September to 24 October 2020.

Category	Model	k	log-likelihood	Parameter (SE)	BIC	ΔBIC
Uncinaria stenocephala Prevalence	M_0: Hook_Prev ~ constant	1	41.61	β_0: 0.963 (0.1471)	-73.03	0
	M_1: Hook_Prev ~ Habitat	2	41.84	β_0: 1.0 (0.189) β_1: -0.032 (0.191) β2: -0.059 (0.195) β3: -0.05 (0.194)	-58.19	14.8
Uncinaria stenocephala Density	M_0: Hook_Dens ~ constant	1	-934.621	β_0: 41.449 (5.658)	1879.44	0
	M_1: Hook_Dens ~ Habitat	2	-932.965	β_0: 6.50 (72.4) β_1: 32.93 (72.69) β2: 63.19 (74.50) β3: 25.43 (74.19)	1891.43	12.0
Angiocaulus gubernaculatus Prevalence	M_0: Angio_Prev ~ constant	1	-64.9000	β_0: 0.847 (0.028)	140.00	0
	M_1: Angio_Prev ~ Habitat	2	-64.5540	β_0: 1.00 (0.363) β_1: -0.143 (0.365) β2: -0.177 (0.373) β3: -0.200 (0.372)	154.60	14.6
Angiocaulus gubernaculatus Density	M_0: Angio_Dens ~ constant	1	-1303.535	β_0: 113.72 (53.65)	2617.27	0
	M_1: Angio_Dens ~ Habitat	2	-1303.141	β_0: 14.8 (691.87) β_1: 124.91 (694.61) β2: 7.45 (711.92) β3: 17.89 (708.95)	2631.78	14.5
Spirocera sp. Prevalence	M_0: Spiro_Prev ~ constant	1	-114.0000	β_0: 0.622 (0.038)	238.20	0
	M_1: Spiro_Prev ~ Habitat	2	-111.8000	β_0: 1.033e-14 (4.845e-01) β_1: 0.657 (0.486) β2: 0.529 (0.498) β3: 0.500 (0.497)	249.10	10.9
Spirocera sp. Density	M_0: Spiro_Dens ~ constant	1	-728.804	β_0: 7.610 (1.613)	1467.81	0
	M_1: Spiro_Dens ~ Habitat	2	-727.57	β_0: 9.233e-13 (0.21) β_1: 8.952 (20.78) β2: 4.382 (21.29) β3: 2.275 (21.21)	1480.66	12.8
Calodium hepatica Prevalence	M_0: CaHe_Prev ~ constant	1	-5.4510	β_0: 0.067 (0.019)	21.10	0
	M_1: CaHe_Prev ~ Habitat	2	-3.6530	β_0: 8.756e-16 (0.251) β_1: 0.087 (0.252) β2: -1.025e-15 (2.578e-01) β3: -9.528e-16 (2.567e-01)	32.81	11.7

Calodium hepatica Density	M_0: CaHe_Dens ~ constant	1	-1413.006	β_0: 188.6 (104.6)	2836.21	0
	M_1: CaHe_Dens ~ Habitat	2	-1412.512	β_0: 1.243e-12 (1.348e+03) β_1: 2.454e+02 (1.353e+03) β2: -1.740e-12 (1.387e+03) β3: -1.078e-12 (1.381e+03)	2850.52	14.3

k: number of parameters included in the model; log-likelihood: measure of model fitness; Parameter (SE): parameter values with standard error; BIC: Bayesian information criterion; ΔBIC: Difference of Bayesian information criterion to each model from the most parsimonious model; Habitat: Island sub-habitat (grassland, coastal sage scrub, *Haplopappus* scrub, or sand); Parameter definitions: β_0 = Intercept, β_1 = Grass habitat, β_2= *Haplpappus* habitat, β_3 = Sand habitat

Table 9. The influence of sample distance from hypothesized source of introduction (either Breeding Pens [BP] or Ranger Station [RS]) and habitat [Habitat] on *P. canicola* egg prevalence [Prev] or density [Dens] from SMI fox scats sampled across the island from 24 September to 24 October 2020.

Metric	Model	k	log-likelihood	Parameter (SE)	BIC	ΔBIC
P. canicola Prevalence	M_0: Acanth_Prev ~ constant	1	-112.9	**β**0: 0.1957 (0.1569)	230.9	8.0
	M_1: Acanth_Prev ~ BP_Dist	2	-106.4	**β**0: 1.638 (0.447) **β**1: -2.755e-04 (8.007e-05)	222.9	0
	M_2: Acanth_Prev ~ RS_Dist	2	-107.6	**β**0: 0.7347 (0.2346) **β**1: -0.000294 (0.0000956)	225.4	2.5
	M_3: Acanth_Prev ~ Habitat	4	-111.6	**β**0: 14.57 (882.74) **β**1: -14.31 (882.74) **β**2: -14.92 (882.74) **β**3: -14.37 (882.74)	243.6	20.7
	M_4: Acanth_Prev ~ BP_Dist + Habitat	5	-105.3	**β**0: 15.38 (0.083) **β**1: -2.952e-04 (8.649e-05) **β**2: -13.70 (0.083) **β**3: -13.85 (0.083) **β**4: -13.10 (0.083)	236.1	13.2
	M_5: Acanth_Prev ~ RS_Dist + Habitat	5	-106.5	**β**0: 15.07 (882.7) **β**1: -0.00031 (0.0001) **β**2: -14.35 (882.7) **β**3: -14.57 (882.7) **β**4: -13.39 (882.7)	238.5	15.6
P. canicola Density	M_0: Acanth_Dens ~ constant	1	-5301.8	**β**0: 3.100 (0.016)	10608.7	323.2
	M_1: Acanth_Dens ~ BP_Dist	2	-5250.0	**β**0: 3.517 (0.0438) **β**1: -8.253e-05 (8.335e-06)	10510.2	224.7
	M_2: Acanth_Dens ~ RS_Dist	2	-5226.1	**β**0: 3.305 (0.023) **β**1: -1.237e-04 (1.056e-05)	10462.4	176.9
	M_3: Acanth_Dens ~ Habitat	4	-5186.8	**β**0: 1.792 (0.408) **β**1: 1.379 (0.409) **β**2: 0.331 (0.417) **β**3: 1.417 (0.411)	10393.9	108.4
	M_4: Acanth_Dens ~ BP_Dist + Habitat	5	-5147.7	**β**0: 2.009 (0.409) **β**1: -7.903e-05 (9.102e-06) **β**2: 1.530 (0.409) **β**3: 0.613 (0.418) **β**4: 1.733 (0.412)	10321.0	35.5
	M_5: Acanth_Dens ~ RS_Dist + Habitat	5	-5130.0	**β**0: 1.979 (0.409) **β**1: -0.000115 (0.00001) **β**2: 1.349 (0.409) **β**3: 0.448 (0.417) **β**4: 1.558 (0.411)	10285.5	0.0

k: number of parameters included in the model; log-likelihood: measure of model fitness; Parameter (SE): parameter values with standard error; BIC: Bayesian information criterion; ΔBIC: Difference of Bayesian information criterion to each model from the most parsimonious model; Distance: distance from hypothesized source (in meters); Habitat: Island sub-habitat (grassland, coastal sage scrub, *Haplopappus* scrub, or sand); Parameter definitions: $\boldsymbol{\beta}_0$ = Intercept, $\boldsymbol{\beta}_1$ = Distance (from BP or RS), $\boldsymbol{\beta}_2$ = Grass habitat, $\boldsymbol{\beta}_3$= *Haplpappus* habitat, $\boldsymbol{\beta}_4$ = Sand habitat.

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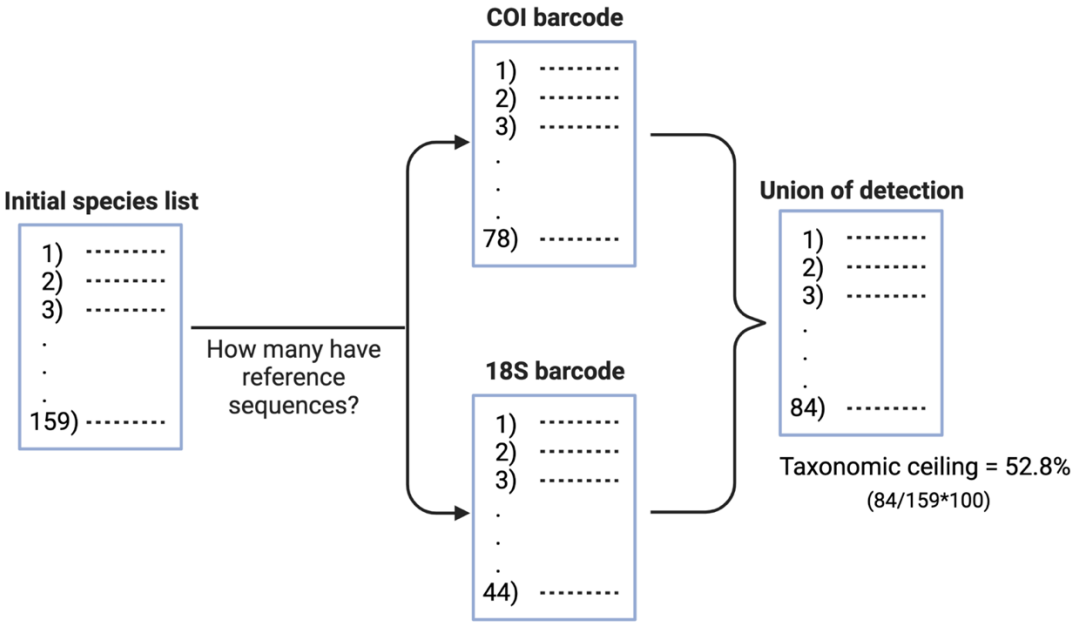
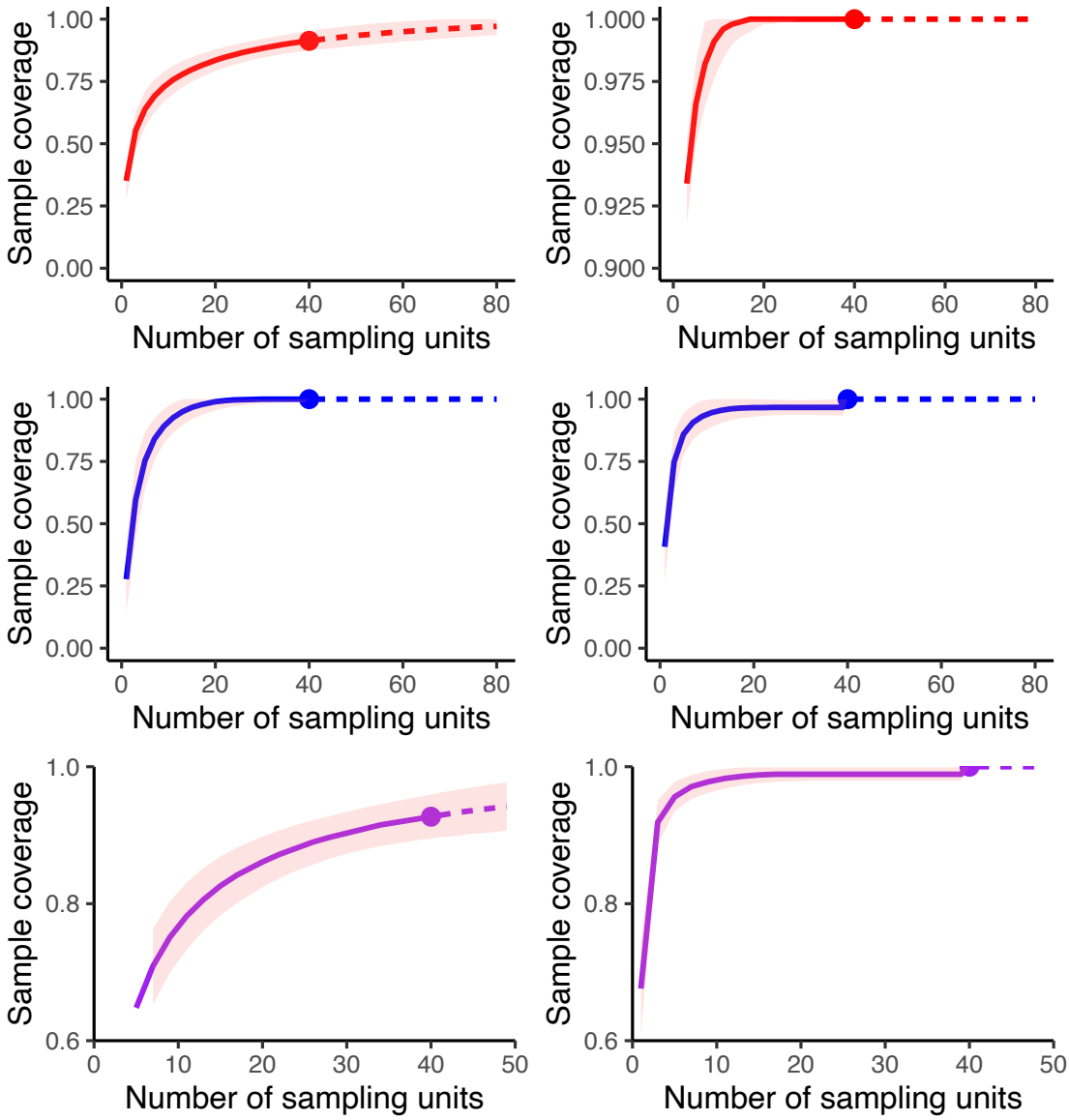
Appendices

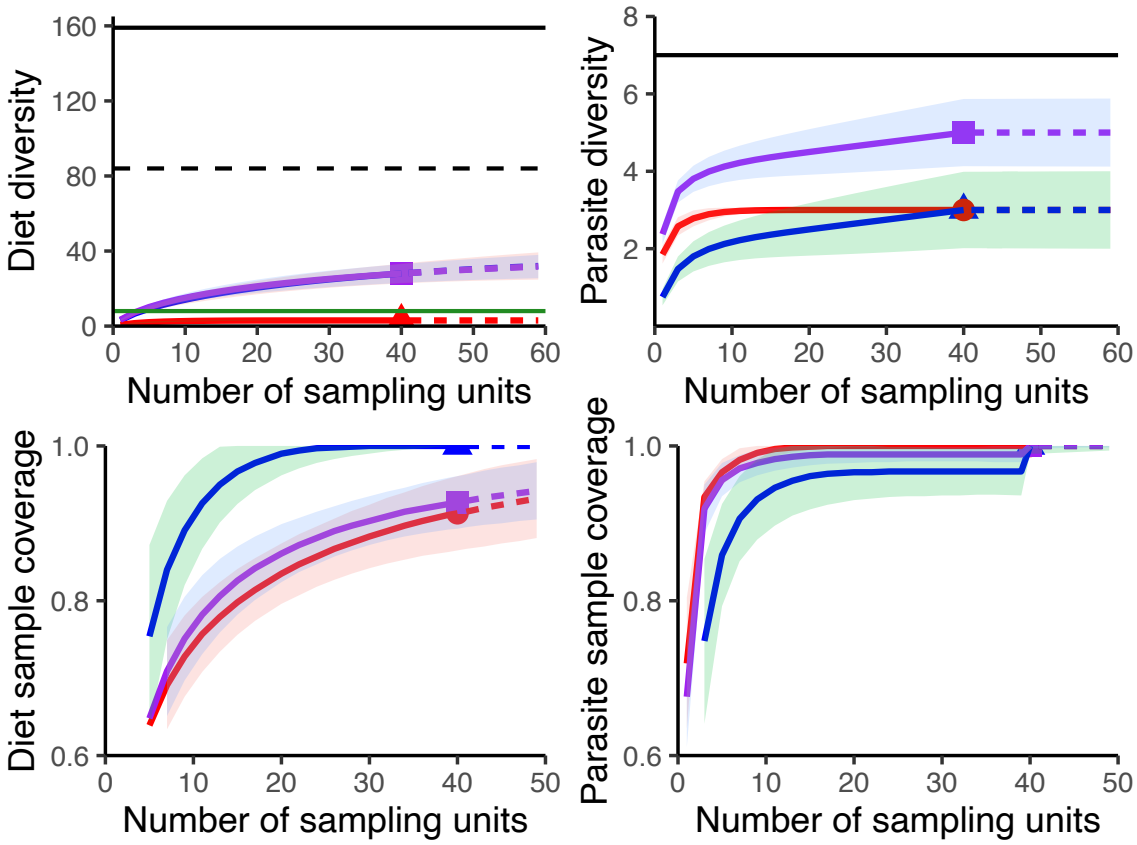
Appendix 1, Chapter 2



Appendix 1, Figure 1. The workflow for calculating the taxonomic ceiling for diet items of San Miguel Island foxes using two barcodes from the COI and 18S gene regions and GenBank. The taxonomic ceiling (52.8%) is the proportion of unique taxa that were detected (84) divided by the number on the initial species list (159). The same workflow was used to determine the taxonomic ceiling for parasite species detection as well.



Appendix 1, Figure 2. Coverage curves for diet items (left column) and parasites (right column). COI coverages is shown on the top row in red; 18S in the middle row in blue; the union of COI+18S on the bottom row in purple. The solid portion of all coverage curves represents the interpolated data, while the dashed portion represents the extrapolated data.



Appendix 1, Figure 3. The combined accumulation curves (top) and coverage curves (bottom) for diet items (left) and parasites (right). The red lines represent detections with the COI primer, the blue lines represent detections with the 18S primer, and the purple lines represent the union of detection between the two. In the top left panel, the solid black line indicates number of potential diet items on SMI (159), the dashed black lined indicates the number of items with reference sequences, and the solid green line represents the number of taxa confirmed from manual scat sorting (10).

Appendix 1, Table 1. The chosen barcodes and respective oligonucleotide sequences, target taxa, and references for the 18S and COI gene regions.

Gene region	Barcode	Oligonucleotide sequence (5′ – 3′)	Target taxa	Reference
18S	1391f EukBr	GTACACACCGCCCGTC TGATCCTTCTGCAGGTTCACCTAC	vertebrates; parasites	Medlin et al., 1988; Lane, 1991
COI	mlCOIintF FolDegenRev	GGWACWGGWTGAACWGTWTAYCCYCC TANACYTCNGGRTGNCCRAARAAYCA	arthropods; parasites	Yu et al., 2012; Leray et al., 2013
COI	fwhF2 fwhR2n	GGDACWGGWTGAACWGTWTAYCCHCC GTRATWGCHCCDGCTARWACWGG	arthropods; parasites	Vamos et al., 2017

Appendix 1, Table 2. The generated list of SMI fox diet items and how many times they have been sequenced for the following genes: COI, CytB, 12S, 16S, 18S, ITS, RCBL, ND2, ND4.

species	COI	CytB	ITS	ND2	ND4	r12S	r16S	r18s	rbcl
Acanthoscelides napensis	0	0	0	0	0	0	0	0	0
Acanthoscelides pauperculus	0	0	0	0	0	0	0	0	0
Acanthoscelides pullus	0	0	0	0	0	0	0	0	0
Aenigmaticum californicum	1	0	0	0	0	1	1	1	0
Agabus	10	10	0	1	1	9	10	10	0
Akephorus marinus	0	0	0	0	0	0	0	0	0
Aleochara sulcicollis	7	0	0	0	0	0	1	0	0
Aleochara valida	1	0	0	0	0	1	0	0	0
Amara sp.	0	0	0	0	0	0	0	0	0
Amartus tinctus	0	0	0	0	0	0	0	0	0
Amblyderus obesus	0	0	0	0	0	0	0	0	0
Amblysellus punctatus	0	0	0	0	0	0	0	0	0
Anasa tristis	10	0	0	0	0	0	10	2	0
Anisodactylus californicus	0	0	0	0	0	0	0	0	0

Anthidium	10	0	0	0	0	0	1	4	0
Anthonomus subvittatus	0	0	0	0	0	0	0	0	0
Aphodius lividus	0	0	0	0	0	0	0	0	0
Apleurus jacobinus	0	0	0	0	0	0	0	0	0
Apsena grossa	0	0	0	0	0	0	0	0	0
Bactericera lavaterae	0	0	0	0	0	0	0	0	0
Batrachoseps pacificus	1	6	0	0	0	0	0	0	0
Bembidion transversale	9	0	0	0	0	0	0	2	0
Bembix americana	0	0	0	0	0	0	0	1	0
Bembix amoena	0	0	0	0	0	0	0	0	0
Bisnius albionicus	0	0	0	0	0	0	0	0	0
Blapstinus discolor	0	0	0	0	0	0	0	0	0
Bledius albonotatus	0	0	0	0	0	0	0	0	0
Bledius fenyesi	0	0	0	0	0	0	0	0	0
Bombus californicus	10	0	0	0	0	10	10	0	0
Cafius canescens	8	0	0	0	0	1	1	1	0
Cafius lithocharinus	1	0	0	0	0	1	1	1	0
Cafius seminitens	2	0	0	0	0	1	1	1	0
Calathus ruficollis	10	0	0	0	0	0	0	2	0
Cardiophorus tenebrosus	0	0	0	0	0	0	0	0	0
Carpophilus ligneus	0	0	0	0	0	0	0	0	0
Cercyon fimbriatus	10	0	0	0	0	0	0	0	0
Cercyon luniger	1	0	0	0	0	0	0	0	0
Chlorochroa uhleri	10	0	0	0	0	0	1	0	0
Cicindela oregona	10	0	0	0	0	0	0	0	0
Cnemotettix caudulus	0	0	0	0	0	0	0	0	0
Cnemotettix pulvillifer	0	0	0	0	0	0	0	0	0
Cnemotettix spinulus	0	0	0	0	0	0	0	0	0
Coccinella californica	2	0	0	0	0	0	0	0	0
Coccinella novemnotata	4	0	0	0	0	0	0	0	0
Coccinella septempunctata	10	1	0	2	1	4	7	10	0
Coelus globosus	4	0	0	0	0	0	0	0	0
Coelus pacificus	10	0	0	0	0	0	0	0	0
Collops cribrosus	1	1	0	0	0	0	0	1	0
Collops crusoe	0	0	0	0	0	0	0	0	0
Coniontis lata	0	0	0	0	0	0	0	0	0
Coniontis santarosae	0	0	0	0	0	0	0	0	0

Copidita quadrimaculata	0	0	0	0	0	0	0	0	0
Corticarina fuscula	10	0	0	0	0	0	0	1	0
Corticarina milleri	0	0	0	0	0	0	0	0	0
Cupido amyntula	10	0	0	0	0	0	0	0	0
Cyclocephala melanocephala	10	0	0	0	0	0	1	0	0
Cyclocephala pasadenae	0	0	0	0	0	0	0	2	0
Cymbiodyta dorsalis	1	0	0	0	0	0	0	1	0
Dermestes frischii	10	2	0	1	1	2	4	1	0
Diachus auratus	4	0	0	0	0	1	0	0	0
Dichelonyx pusilla	0	0	0	0	0	0	0	0	0
Elassoptes marinus	0	0	0	0	0	0	0	0	0
Eleodes acuticauda	2	0	0	0	0	1	0	0	0
Eleodes gigantea	0	0	0	0	0	0	0	0	0
Eleodes inculta	0	0	0	0	0	0	0	0	0
Eleodes littoralis	2	0	0	0	0	1	0	0	0
Eleodes osculans	1	0	0	0	0	0	0	0	0
Elgaria multicarinata	1	10	0	9	10	1	1	0	0
Emerita analoga	10	0	0	0	0	1	10	2	0
Endeodes basalis	1	1	0	0	0	0	0	1	0
Endeodes insularis	0	0	0	0	0	0	0	0	0
Enochrus carinatus	0	0	0	0	0	0	0	0	0
Enochrus hamiltoni	10	0	0	0	0	0	0	0	0
Epantius obscurus	0	0	0	0	0	0	0	0	0
Epeolus	10	0	0	0	0	0	0	3	0
Eschatocrepis constrictus	0	0	0	0	0	0	0	0	0
Eurygaster amerinda	10	0	0	0	0	0	0	0	0
Eusattus politus	0	0	0	0	0	0	0	0	0
Euspilotus scissus	2	2	0	2	2	1	1	1	0
Forficula auricularia	10	0	0	0	0	3	10	7	0
Habropoda miserabilis	0	0	0	0	0	0	0	0	0
Hadrotes crassus	10	0	0	0	0	1	1	1	0
Haplotrema sp.	0	0	0	0	0	0	0	0	0
Heliococcus clemente	0	0	0	0	0	0	1	1	0
Helminthoglypta ayresiana	0	0	0	0	0	0	0	0	0
Helops bachei	0	0	0	0	0	0	0	0	0

Hemicrepidius californicus	0	0	0	0	0	0	0	0	0
Heterosilpha ramosa	0	0	0	0	0	0	0	0	0
Hippodamia convergens	10	3	0	2	2	4	4	2	0
Hippodamia quinquesignata	0	0	0	0	0	0	0	0	0
Hyperaspidius comparatus	0	0	0	0	0	0	0	0	0
Hyperaspidius vittigera	0	0	0	0	0	0	0	0	0
Hypocaccus bigemmeus	2	0	0	0	0	0	0	0	0
Hypocaccus gaudens	0	0	0	0	0	0	0	0	0
Hypocaccus lucidulus	10	0	0	0	0	0	0	0	0
Iliotona cacti	0	0	0	0	0	0	2	0	0
Ipochus fasciatus	0	0	0	0	0	0	0	0	0
Lasius niger	10	2	0	1	1	0	10	1	0
Leconectes striatellus	2	1	0	1	1	2	2	0	0
Lepisma saccharina	5	2	0	2	2	2	3	6	0
Liodessus	10	0	0	0	0	2	10	4	0
Listroderes costirostris	0	0	0	0	0	0	0	0	0
Lopidea nigridia	10	0	0	0	0	0	0	0	0
Megalorchestia californiana	7	0	0	0	0	0	0	0	0
MeIanopleurus fuscus	0	0	0	0	0	0	0	0	0
Meloe barbarus	0	0	0	0	0	0	0	0	0
Meloe strigulosus	10	0	0	0	0	0	0	0	0
Microtes nicola	0	0	0	0	0	0	0	0	0
Morsea californica	0	0	0	0	0	0	0	1	0
Necrobia rufipes	10	0	0	0	0	1	2	3	0
Neduba morsel	0	0	0	0	0	0	0	0	0
Neduba propstl	0	0	0	0	0	0	0	0	0
Neopachylopus sulcifrons	1	0	0	0	0	0	0	3	0
Nicrophorus nigrita	2	0	0	0	0	0	0	1	0
Nyctoporis carinata	10	0	0	0	0	0	0	0	0
Ochthebius	10	10	0	10	10	10	10	10	0
Okanagana hirsuta	0	0	0	0	0	0	0	0	0
Okanaoana catalina	0	0	0	0	0	0	0	0	0
Oniscus asellus	10	1	0	0	0	1	7	4	0
Peromyscus maniculatus	10	10	0	4	10	10	8	10	0

Phenacoccus madeirensis	10	0	0	0	0	0	4	4	0
Phyconomus marinus	0	0	0	0	0	0	0	0	0
Phyllobaenus funebris	0	0	0	0	0	0	0	0	0
Platynus brunneomarginatus	0	0	0	0	0	0	0	1	0
Polites sabuleti	10	0	0	0	0	0	0	0	0
Pontomalota opaca	6	0	0	0	0	0	4	3	0
Prionosoma podopioides	1	0	0	0	0	0	0	0	0
Pseudosermyle catalinae	0	0	0	0	0	0	0	0	0
Pterostichus menetriesii	1	0	0	0	0	0	0	1	0
Rhyncolus	10	0	0	0	0	0	0	1	0
Sanfilippodytes	10	0	0	0	0	3	5	10	0
Saprinus lugens	0	0	0	0	0	0	0	1	0
Scantius aegyptius	7	0	0	0	0	1	1	1	0
Scaphinotus punctatus	0	0	0	0	0	0	0	0	0
Sceloporus occidentalis becki	0	0	0	0	0	0	0	0	0
Scirtetica clementina	0	0	0	0	0	0	0	0	0
Scymnus nebulosus	3	0	0	0	0	0	0	0	0
Serica mixta	0	0	0	0	0	0	0	0	0
Sibinia maculata	0	0	0	0	0	0	0	0	0
Sitona californicus	0	0	0	0	0	0	0	0	0
Sitona cockerelli	0	0	0	0	0	0	0	0	0
Stenolophus anceps	1	0	0	0	0	0	0	0	0
Stenopelmatus mahogani	10	0	0	0	0	0	0	0	0
Tachysphex tarsatus	9	0	0	0	0	0	0	0	0
Tanystoma maculicolle	2	0	0	0	0	0	0	0	0
Tapinoma sessile	10	10	0	0	0	10	1	1	0
Tarphiota fucicola	6	0	0	0	0	0	3	2	0
Tarphiota geniculata	4	0	0	0	0	0	2	0	0
Tasgius ater	2	0	0	0	0	0	0	0	0
Thinopinus pictus	10	0	0	0	0	0	0	0	0
Tiaja cruzensis	0	0	0	0	0	0	0	0	0
Tiaja insula	0	0	0	0	0	0	0	0	0
Tomarus gibbosus	0	0	0	0	0	0	0	0	0
Trichochrous calcaratus	0	0	0	0	0	0	0	0	0
Trigonoscuta miguelensis	0	0	0	0	0	0	0	0	0

Trimerotrotis santabarbara	0	0	0	0	0	0	0	0	0
Vertigo californica	1	10	0	0	0	0	10	10	0
Xanthochilus saturnius	0	0	0	0	0	0	0	0	0
Xerasia grisescens	1	1	0	0	0	0	0	1	0

Appendix 1, Table 3. The generated list of SMI parasites and how many times they have been sequenced for the following genes: COI, CytB, 12S, 16S, 18S, ITS, RCBL, ND2, ND4.

Species	COI	r18s	CytB	ITS	ND2	ND4	r12S	r16S	rbcl
Helminth									
Angiocaulus gubernaculatus	0	0	0	0	0	0	0	0	0
Mesocestoides corti	2	8	1	0	2	2	4	2	0
Pachysentis canicola	0	0	0	0	0	0	0	0	0
Spirocerca sp.	10	6	3	0	1	1	1	1	0
Calodium hepaticum	10	10	10	0	10	10	10	10	0
Uncinaria stenocephala	10	8	0	0	0	0	4	0	0
Toxascaris leonina	6	10	1	0	1	0	7	1	0

Appendix, Table 4. List of unique taxa with genus assignments with status of whether or genus presence is confirmed on San Miguel Island or a possibility; which primer was used to detect the taxa; its reassigned ID for analysis; decision to include or exclude from the study and the rationale for such decision.

Taxonomic Assignment	Status	Primer	Re-assigned ID	Decision	Type	Rationale
Batrachoseps sp.	yes	COI	Batrachoseps pacificus	include	diet item	Previously recorded on SMI
Ceuthophilus hesperus	yes	COI, 18S	Ceuthophilus hesperus	include	diet item	Previously recorded on SMI
Cyclosa turbinata	yes	COI	Araneae sp. 1	include	diet item	Genus recorded from nearby mainland CA; cosmopolitan orb weaver spiders. Could be incidental ingestion or collection
Diachus auratus	yes	COI	Diachus auratus	include	diet item	Previously recorded on SMI
Eleodes sp.	yes	COI	Eleodes sp.	include	diet item	Previously recorded on SMI
Elgaria panamintina	yes	COI	Elgaria m. multicarinata	include	diet item	Previously recorded on SMI
Euxoa sp.	yes	COI	Euxoa sp.	include	diet item	Previously recorded on SMI
Everes amyntula	maybe	COI	Elkalyce sp.	include	diet item	Genus is a synonym. Corrected genus has been recorded from SMI
Forficula sp.	yes	COI	Forficula auricularia	include	diet item	Previously recorded on SMI
Gryllus	maybe	COI	Gryllus sp.	include	diet item	Gryllus sp. have been recorded from neighboring Santa Rosa and Santa Cruz islands
Lacinipolia stricta	yes	COI	Lacinipolia stricta	include	diet item	Previously recorded on SMI
Lasius sp.	yes	COI	Lasius sp.	include	diet item	Previously recorded on SMI
Leucania farcta	yes	COI	Leucania sp.	include	diet item	noctuid moths on SMI
Melanoplus sp.	yes	COI	Melanoplus sp.	include	diet item	Previously recorded on SMI
Metacarcinus gracilis	yes	COI	Cancridae sp. 1	include	diet item	crancidae on SMI
Metepeira sp.	yes	COI	Metepeira sp.	include	diet item	Previously recorded on SMI
Mimeoma maculata	maybe	COI	Scarabaeidae sp. 1	include	diet item	Family cosmopoliton. Genus Ligyrus recorded from SMI
Peromyscus maniculatus	yes	COI	Peromyscus maniculatus	include	diet item	Previously recorded on SMI
Porcellio dilatatus	yes	COI	Porcellio dilatatus	include	diet item	Previously recorded on SMI

Porcellio laevis	yes	COI	Porcellio laevis	include	diet item	Previously recorded on SMI
Rhynocoris kumarii	yes	COI	Rhynocoris sp.	include	diet item	Previously recorded on SMI
Solenopsis molesta	yes	COI, 18S	Solenopsis molesta	include	diet item	Previously recorded on SMI
Stenopelmatus	yes	COI, 18S	Stenopelmatus sp.	include	diet item	Previously recorded on SMI
Tapinoma sessile	yes	COI	Tapinoma sessile	include	diet item	Previously recorded on SMI
Tibellus chamberlini	yes	COI	Tibellus sp.	include	diet item	SMI spider
Tinea occidentella	maybe	COI	Tinea sp.	include	diet item	Genus recorded from nearby mainland CA; Could be incidental
Tricholita fistula	yes	COI	Tricholita sp.	include	diet item	noctuid moths on SMI
Vertigo californica	yes	COI	Vertigo californica	include	diet item	Previously recorded on SMI
Excluded						
Amblyseius eharai	maybe	18S	Amblyseius sp.	exclude	incidental	Genus recorded from nearby mainland CA; predatory mite
Ammopelmatus fuscus	yes	COI	Ammopelmatus fuscus	exclude	relic	Likely a reflection of genetic deposits prior to the genus change to Stenopelmatus sp.; was collasped to Stenopelmatus
Anolis sp.	maybe	18S	Anolis sp.	exclude	contamination	contamination
Australothrips bicolor	maybe	18S	Australothrips bicolor	exclude	out of range	Genus only recorded from Austalia
Blaesoxipha cessator	maybe	COI	Blaesoxipha sp.	exclude	scat colonizer	Genus is cosmopolitan and recorded from nearby mainland CA; cosmopolitan fly. Could be incidental ingestion or colonizer
Chrysis leptomandibularis	maybe	18S	Chrysis sp.	exclude	incidental	Genus recorded from Santa Cruz island
Culicoides sp.	maybe	18S	Culicoides sp.	exclude	incidental	Genus recorded from mainland CA
Cynoglossus lineolatus	implausible	18S	Cynoglossus lineolatus	exclude	out of range	Indo-pacific; could be lab contamination
Dasybranchus	no	COI	Dasybranchus	exclude	contamination	contamination
Drosophila	maybe	18S	Drosophila	exclude	contamination	contamination

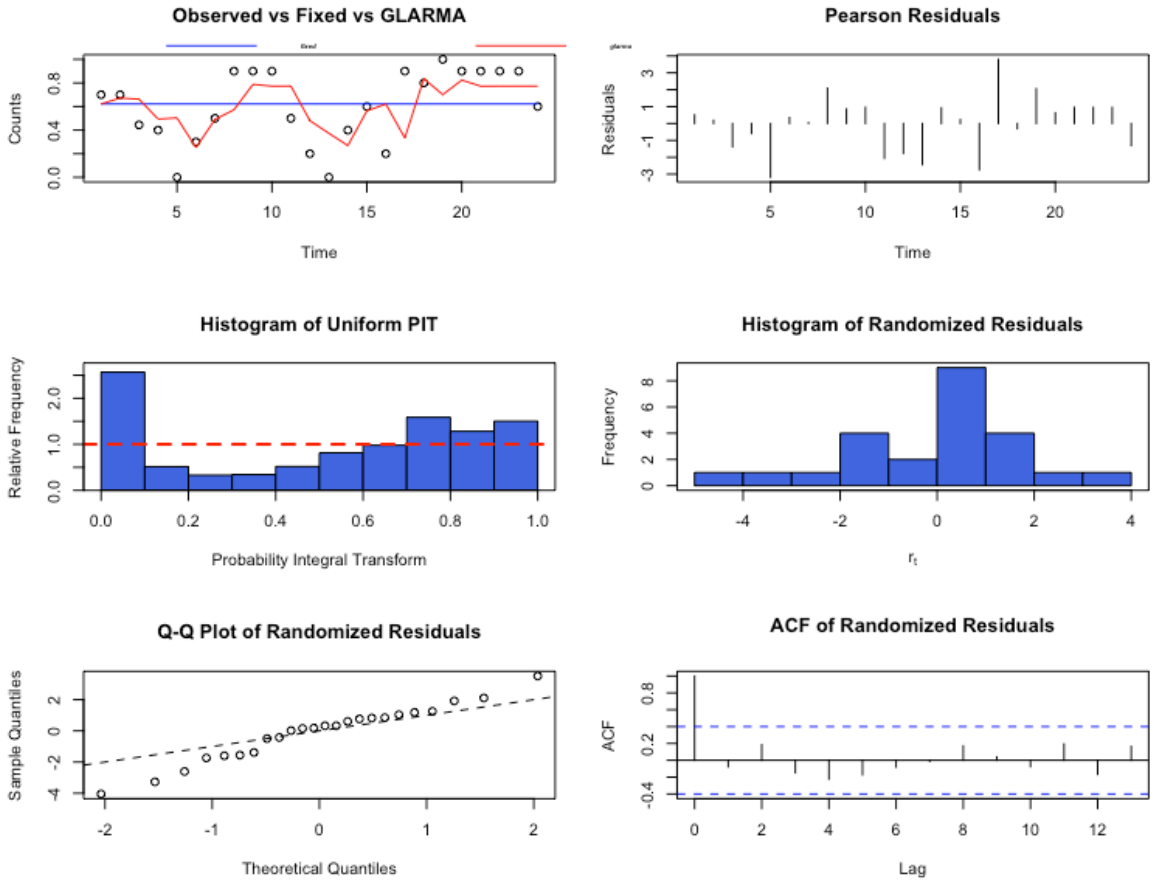
Entomobrya atrocincta	maybe	COI	Entomobrya sp.	exclude	incidental	Genus recorded from nearby Santa Cruz Island; springtail
Eucalliphora sp.	maybe	COI	Calliphoridae	exclude	scat colonizer	Family recorded from Anacapa Island
Fahrenholzia reducta	maybe	18S	Fahrenholzia sp.	exclude	incidental	Genus recorded from nearby mainland CA; rodent louse
Felicola subrostratus	maybe	18S	Trichodectidae	exclude	incidental	Cosmopolitan, recorded from North America; mammal chewing lice
Gallus gallus	no	COI, 18S	Gallus gallus	exclude	contamination	contamination
Glyptapanteles sp.	maybe	COI	Braconidae	exclude	incidental	Genus recorded from nearby mainland CA; parasitoid wasp likely ingested from plant consumption
Homo sapiens	yes	COI	Homo sapiens	exclude	contamination	contamination
Larus sp.	yes	COI	Larus sp.	exclude	carrion	Previously recorded on SMI
Micropsectra nigripila	maybe	COI	Microspectra sp.	exclude	incidental	Genus recorded from nearby mainland CA; midge
Munnopsurus	implausible	18S	Munnopsurus	exclude	out of range	deep sea crab
Paronellides praefectus	maybe	18S	Paronellides praefectus	exclude	out of range	Genus recorded from Tasmania and South America only
Pulvinaria sp.	maybe	COI	Hemiptera sp. 1	exclude	incidental	Genus recorded from nearby mainland CA; scale insect
Trioza eugeniae	maybe	18S	Trioza sp.	exclude	incidental	Genus recorded from nearby mainland CA; sap-sucking bug
Urocyon cinereoargenteus	yes	COI	U. l. littoralis	exclude	host	Removed for conflation with host DNA
Zalophus californianus	yes	COI	Zalophus californianus	exclude	carrion	Previously recorded on SMI

Appendix 2, Chapter 3

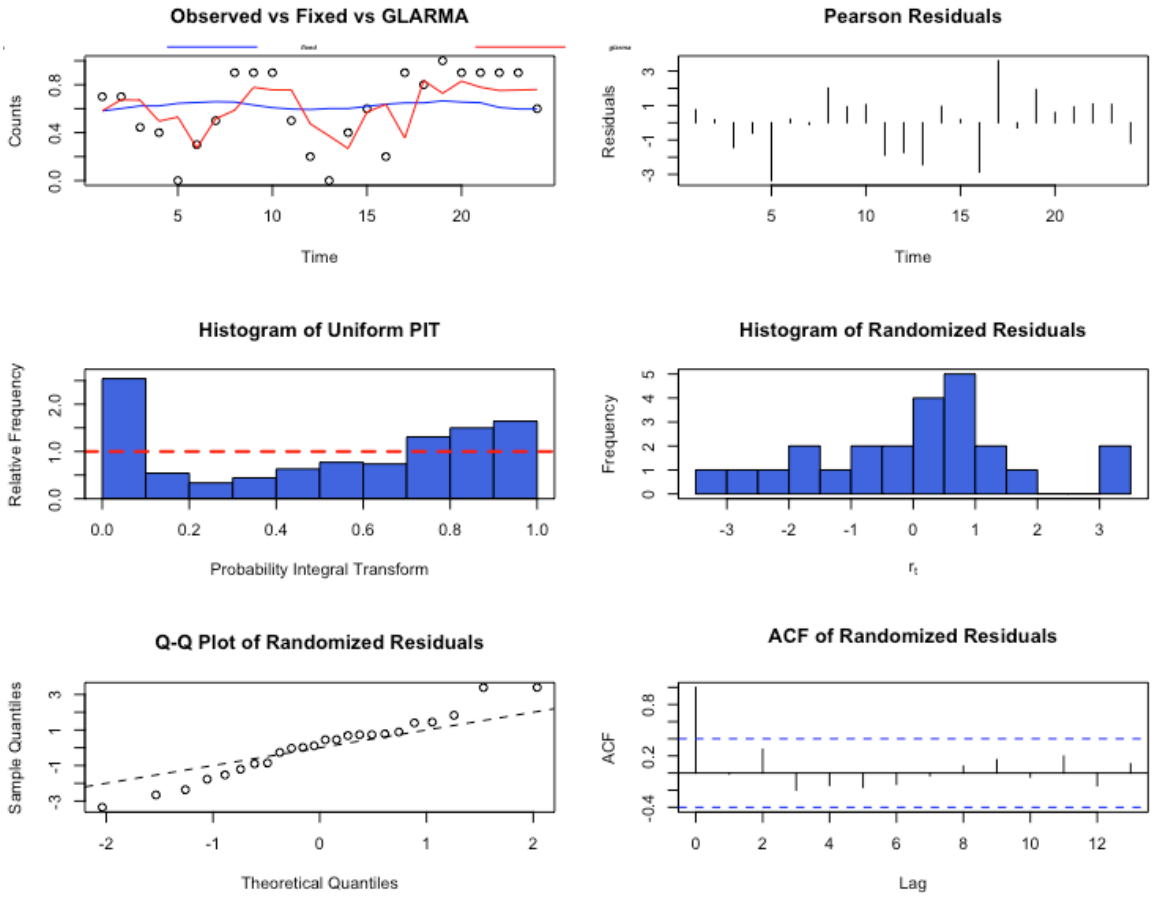
Fit and Diagnostic plots for time series GLARMA models
For all top left panels, the open circles or black dashes represent the observed data, while the red line indicates the GLARMA model fit.

1 | Acanthocephalan (*P. canicola*)
Acanthocephalan Prevalence: All five models retained

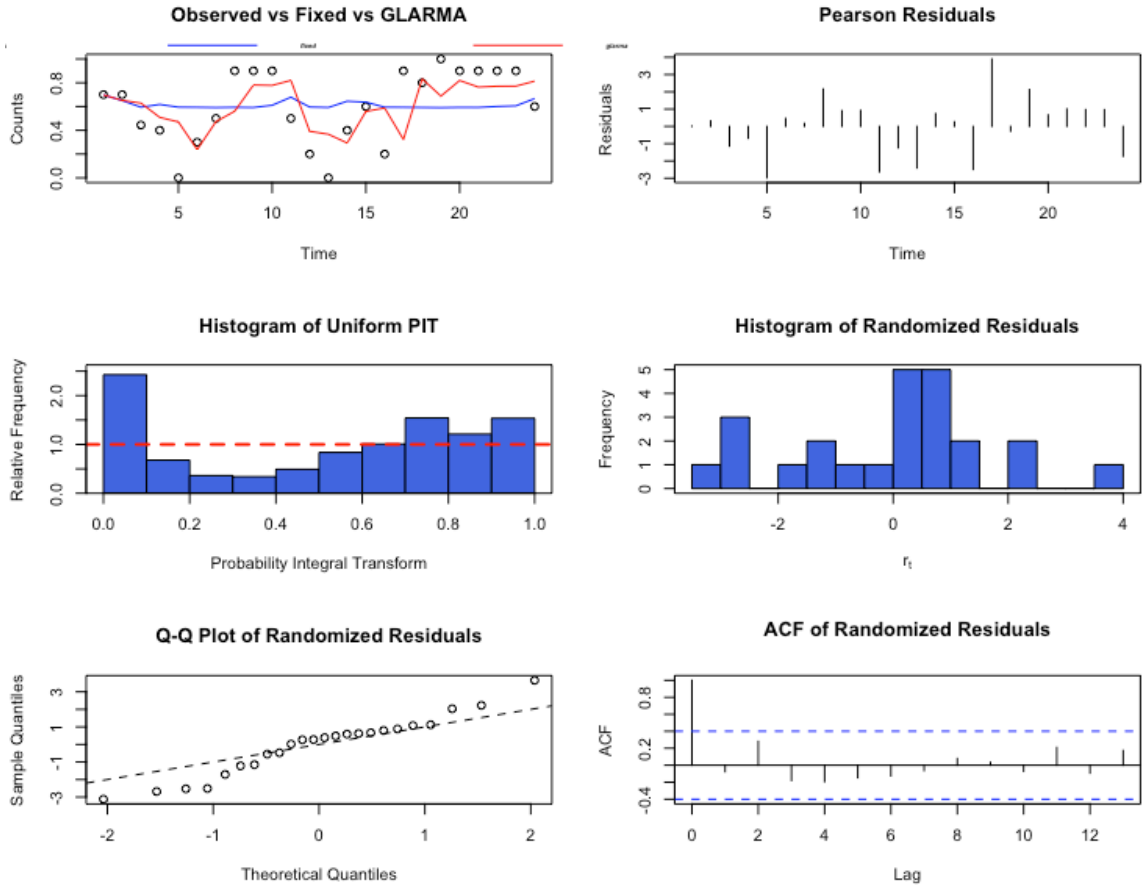
1. M_0: Acanth_Prev ~ constant



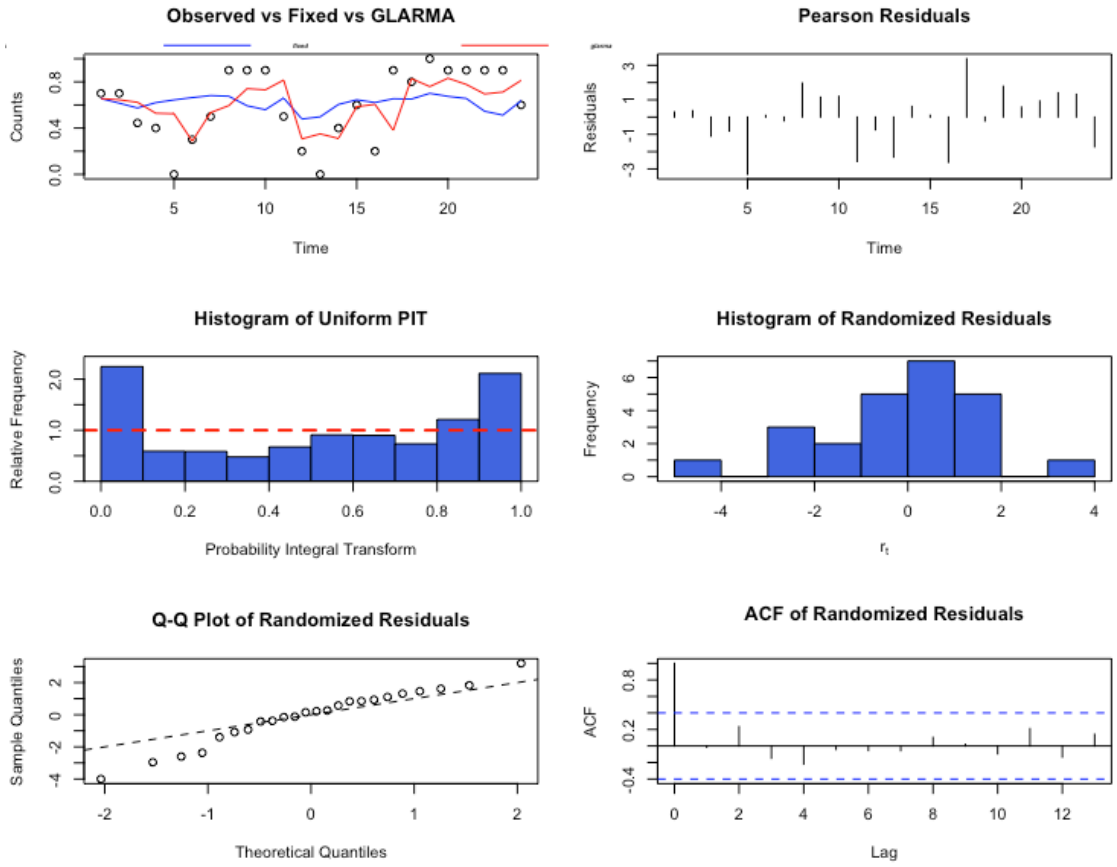
2. M_1: Acanth_Prev ~ Temp



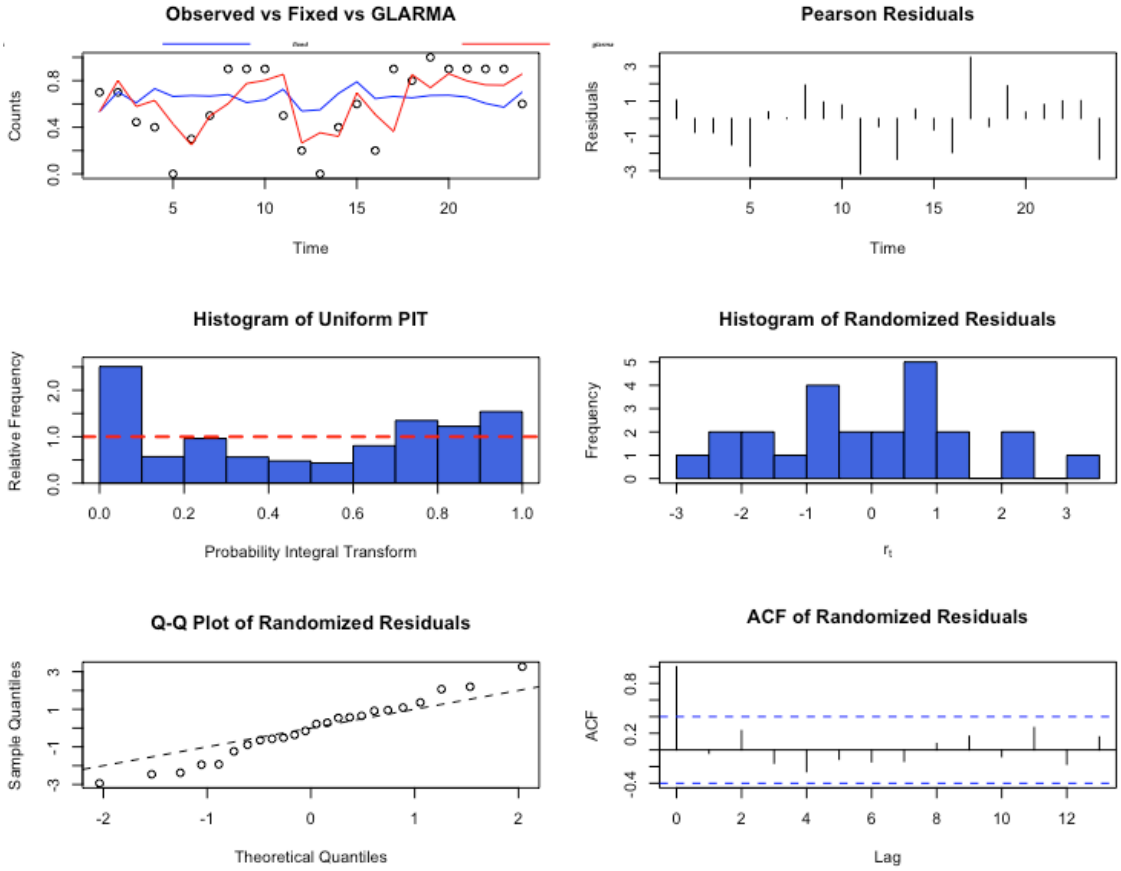
3. M_2: Acanth_Prev ~ Rain



4. M_3: Acanth_Prev ~ Temp + Rain

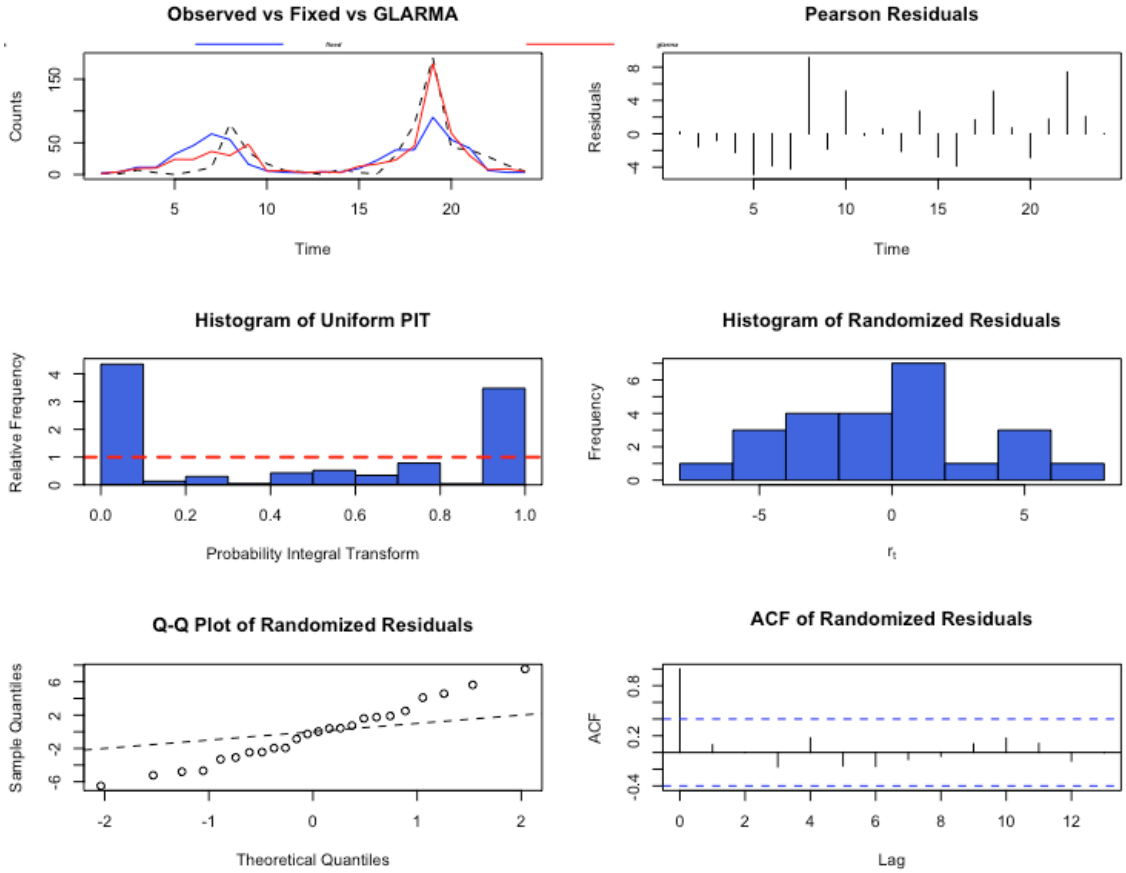


5. M_4: Acanth_Prev ~ Temp*Rain

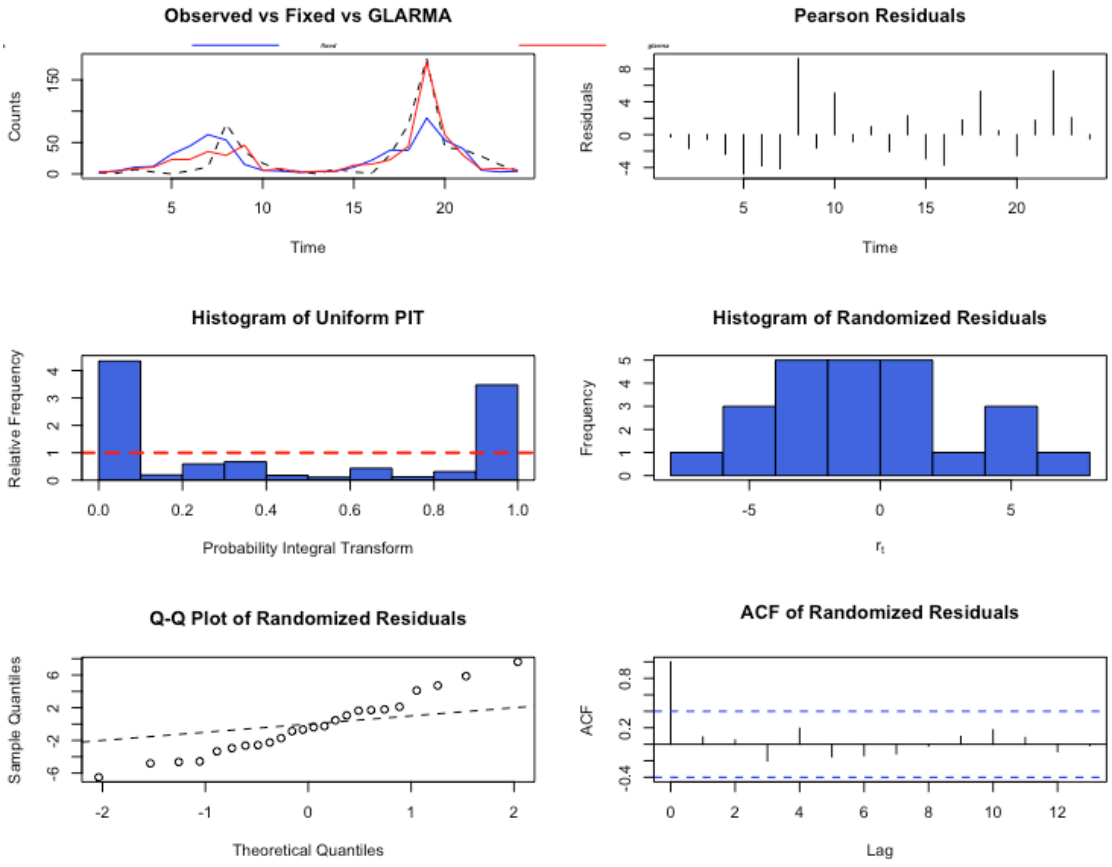


Acanthocephalan Density: M_1, M_3, and M_4 retained

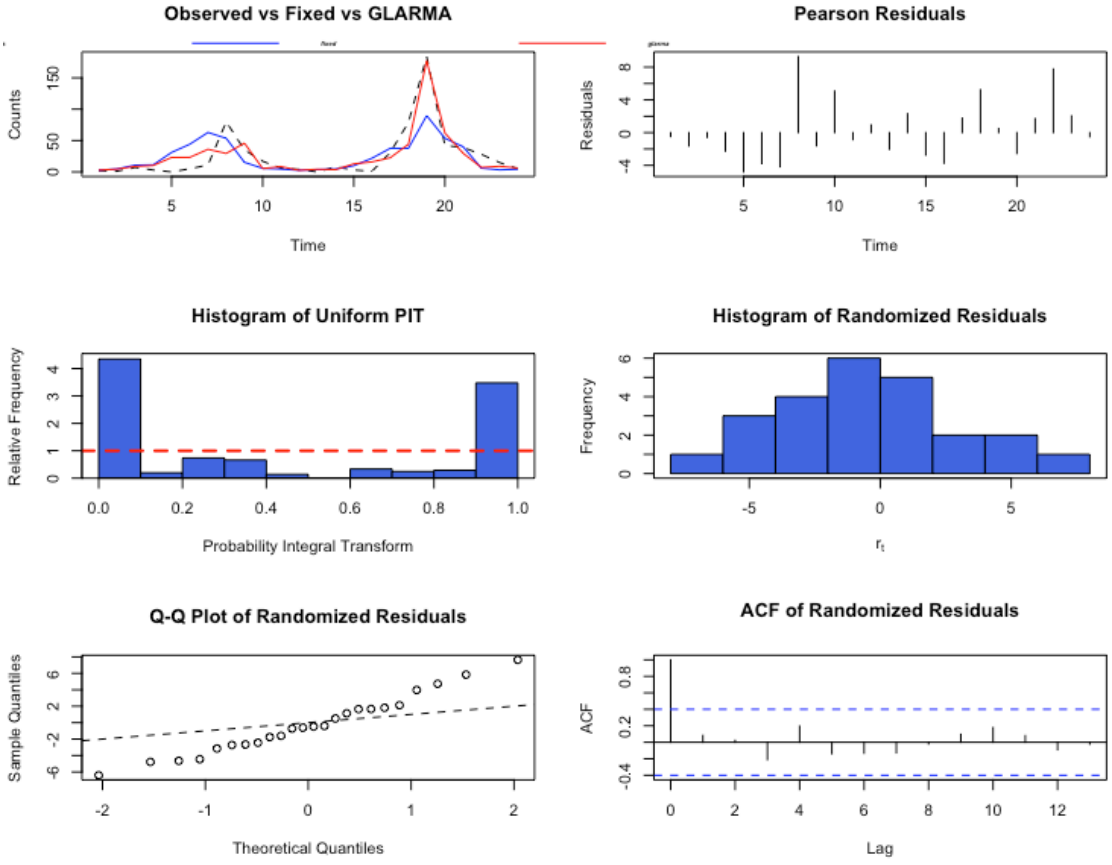
M_1: Acanth_ Dens ~ Temp



M_3: Acanth_Dens ~ Temp + Rain



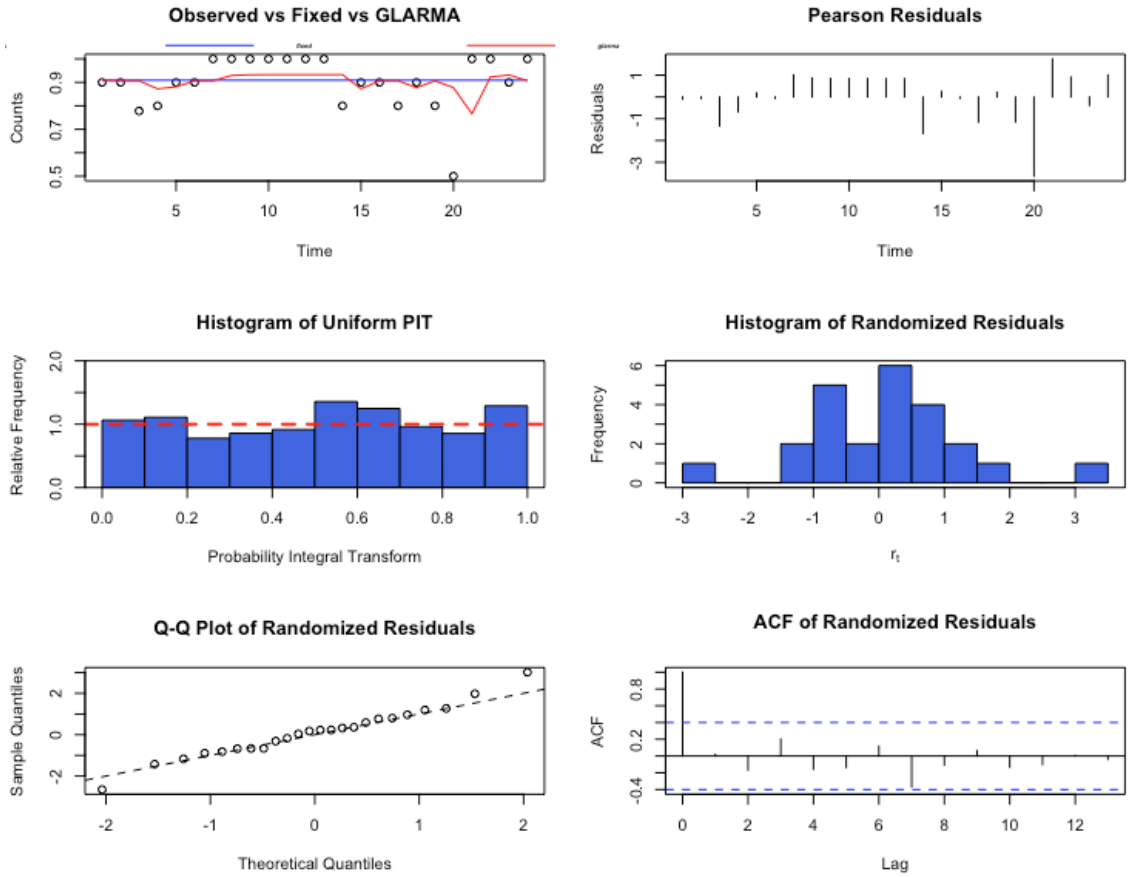
M_4: Acanth_Dens~ Temp*Rain



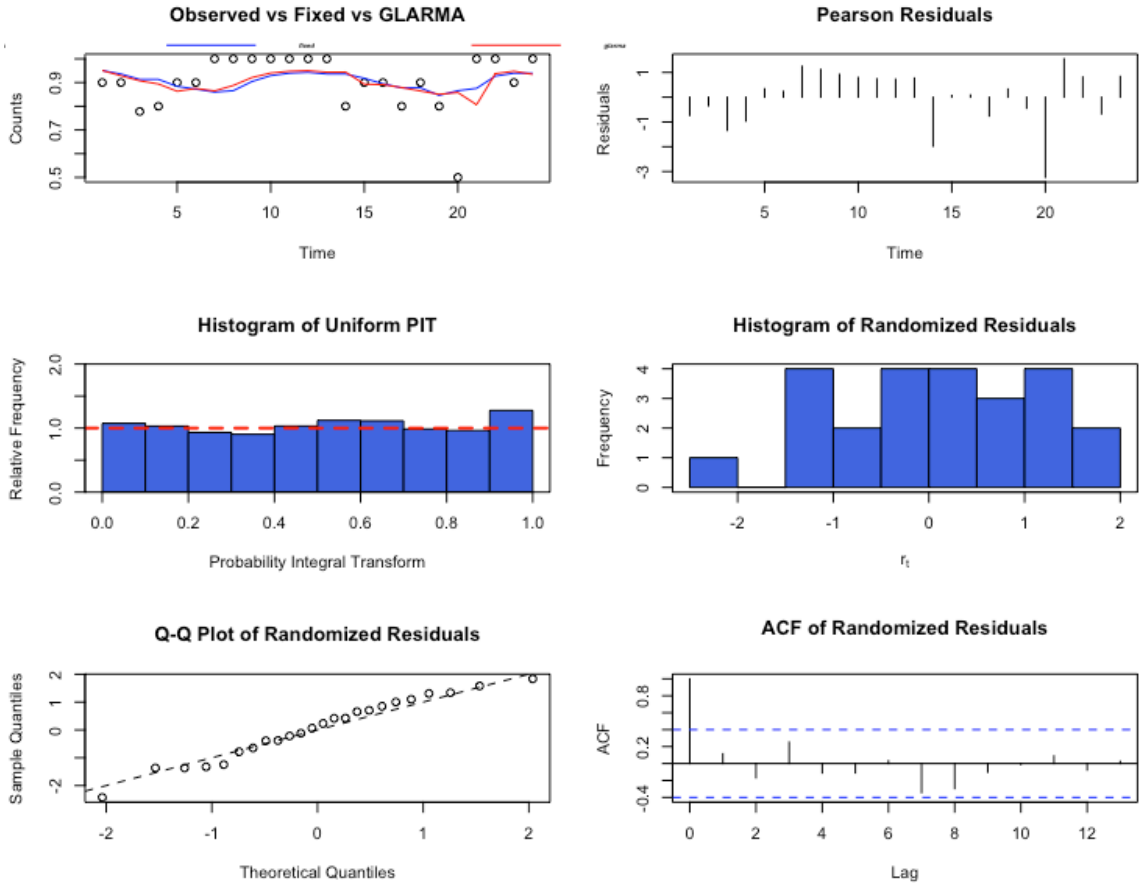
2 | Hookworm (*Uncinaria stenocephala*)

Hookworm Prevalence: M_0, M_1, M_2, M_3 retained

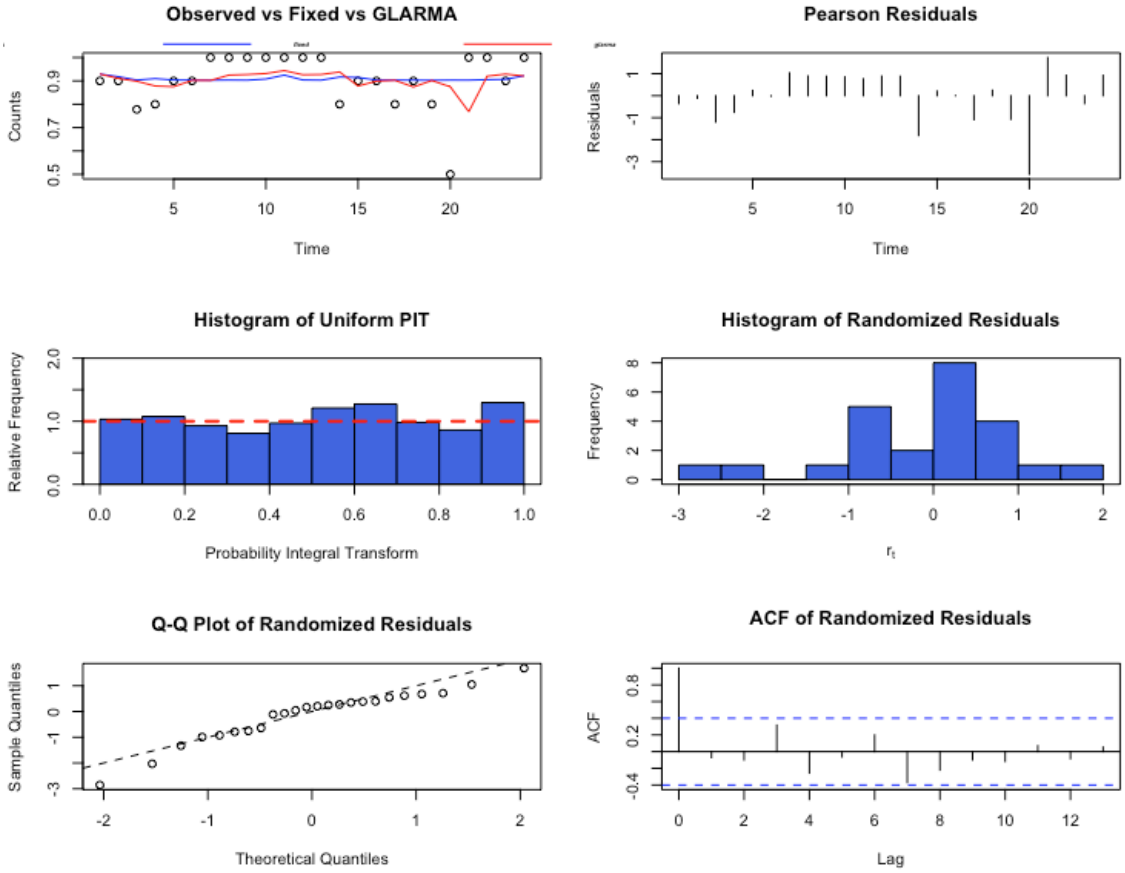
M_0: Hook_Prev ~ constant



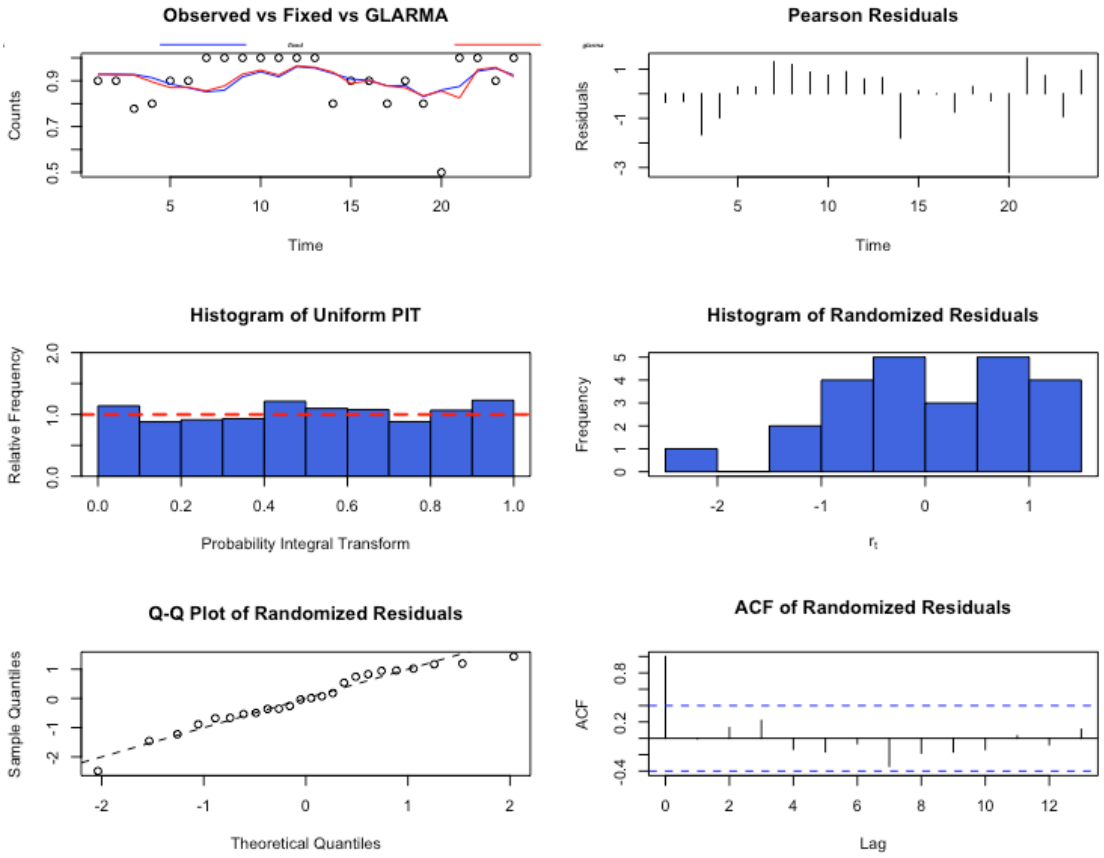
M_1: Hook_Prev ~ Temp



M_2: Hook_Prev ~ Rain

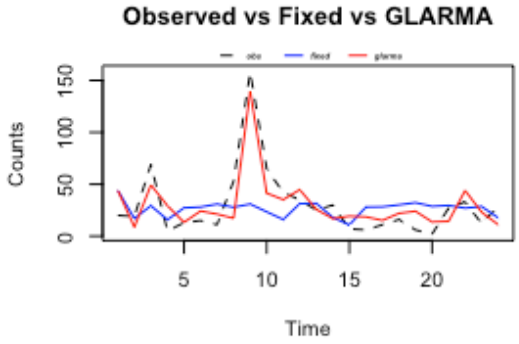


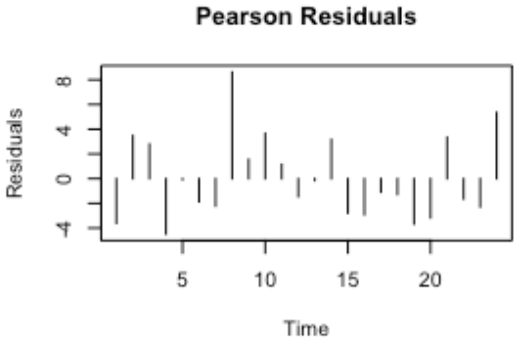
M_3: Hook_Prev ~ Temp + Rain

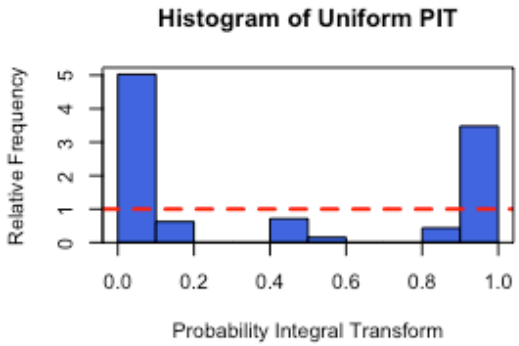


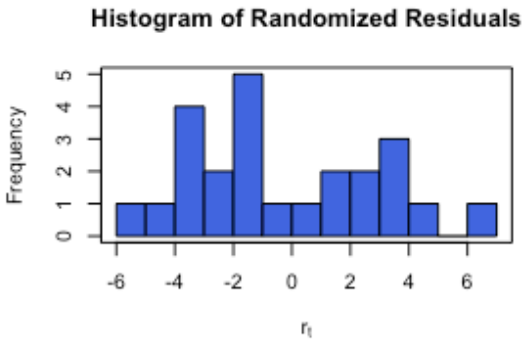
Hookworm Density: M_4 retained

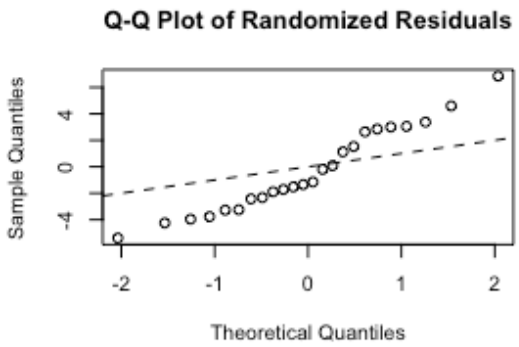
M_4: Hook_Dens~ Temp*Rain

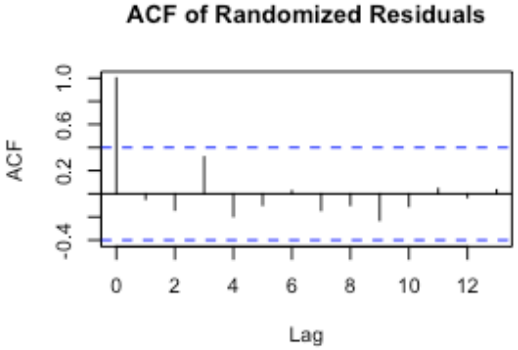








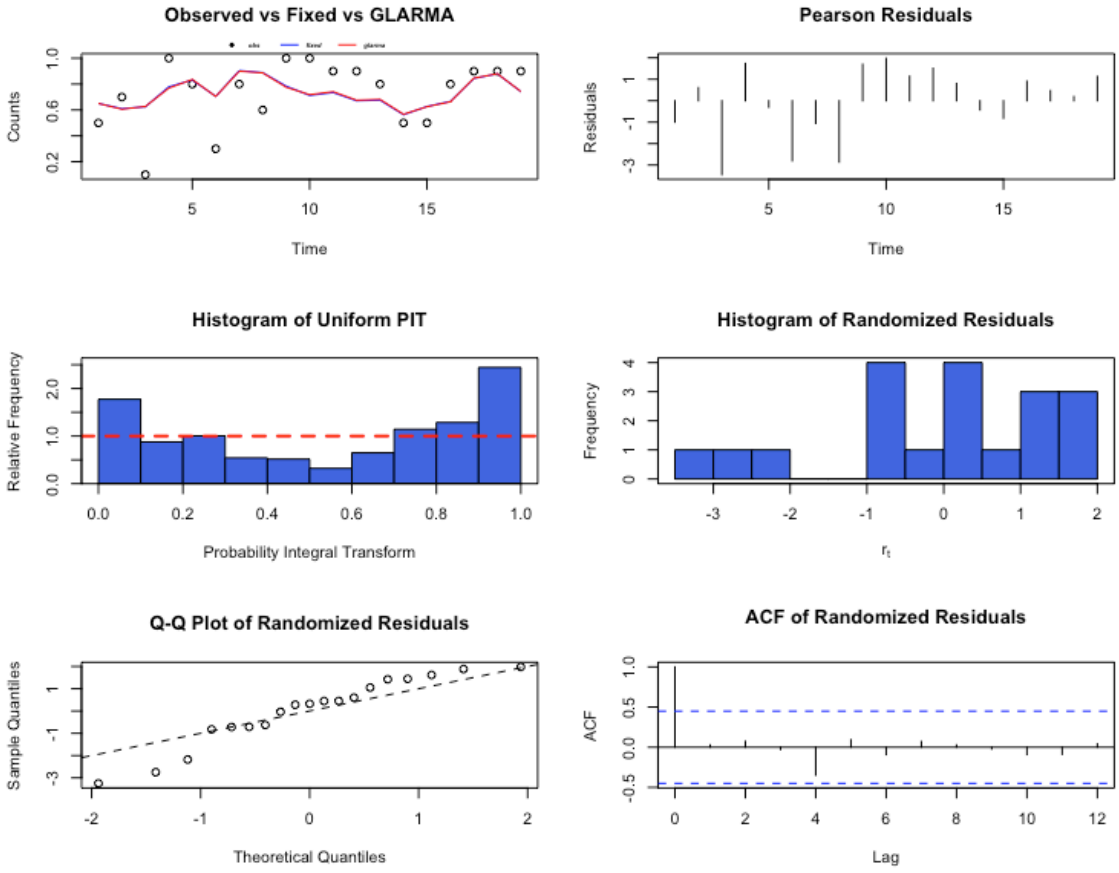




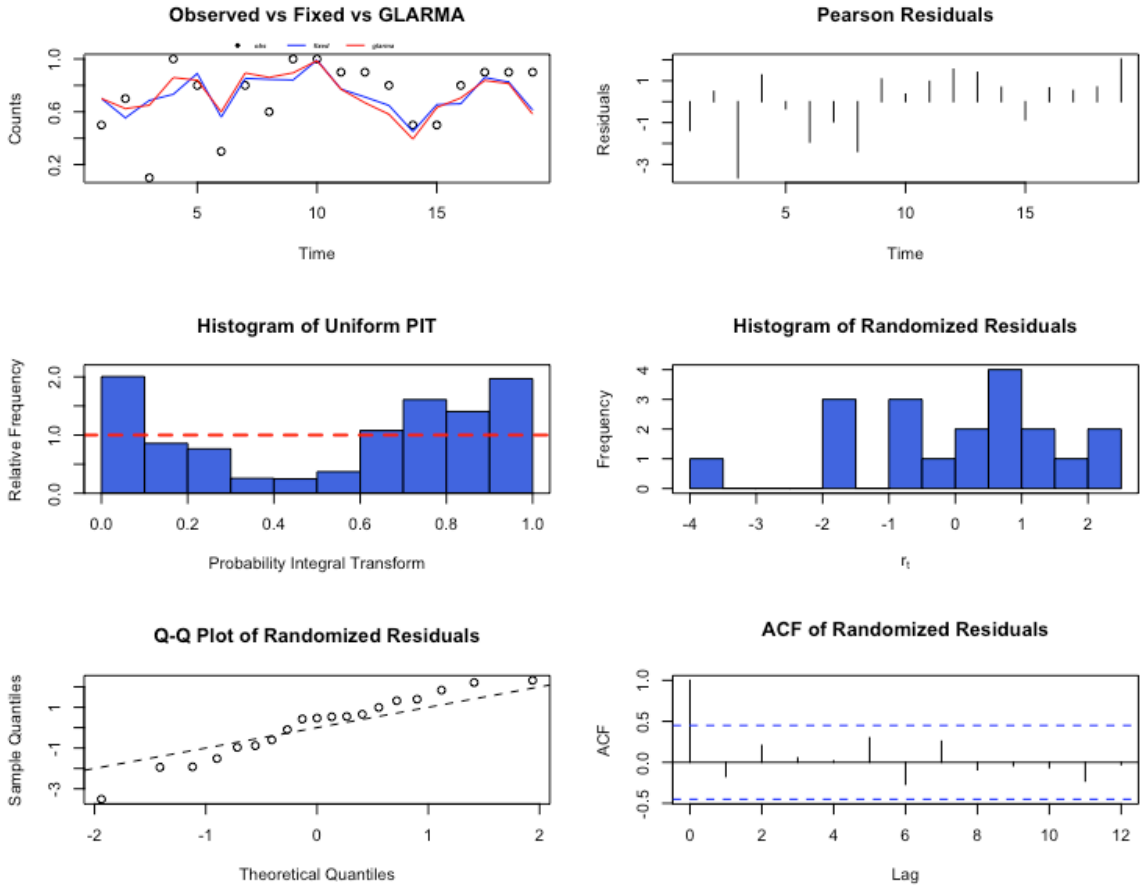
3 | Canine lungworm (*Angiocaulus gubernaculatus*)

A. gubernaculatus Prevalence: Retain M_3 and M_4

M_3: Angio_Prev ~ Temp + Rain

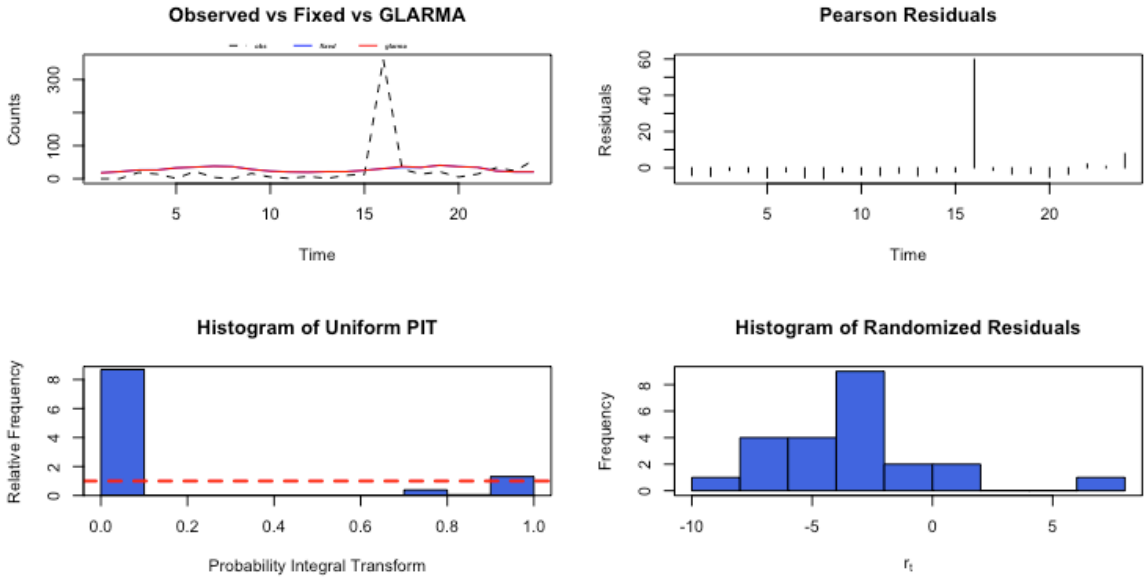


M_4: Angio_Prev ~ Temp*Rain

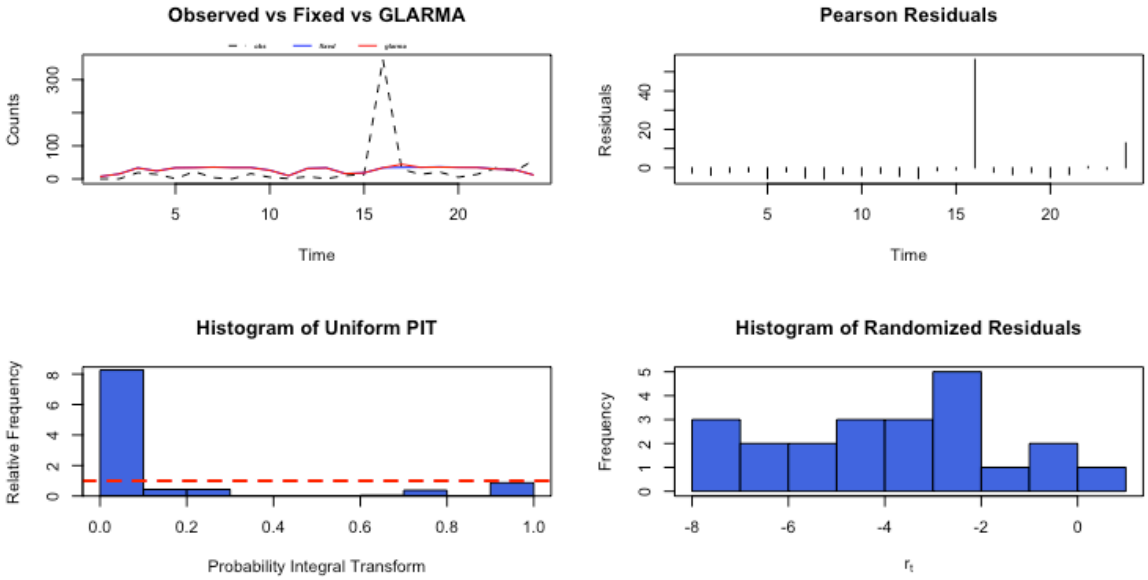


Angio Density: Three models retained all include temperature

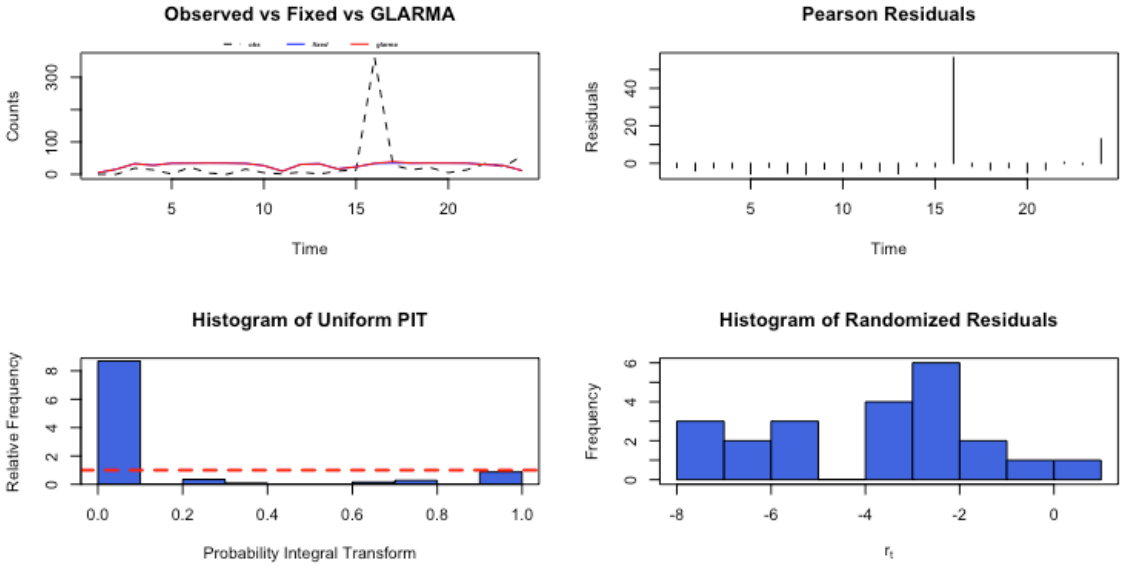
M_1: Angio_ Dens ~ Temp



M_3: Angio_Dens ~ Temp + Rain



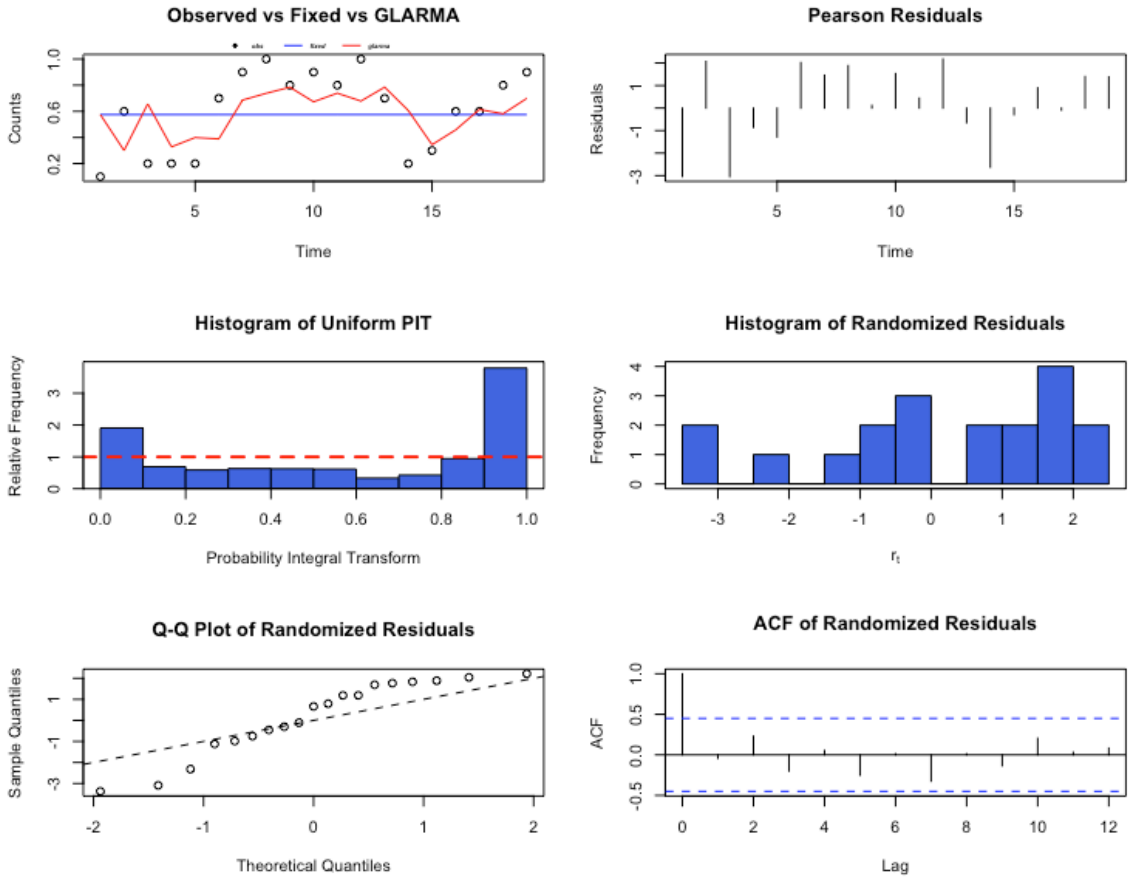
Angio_Dens~ Temp*Rain



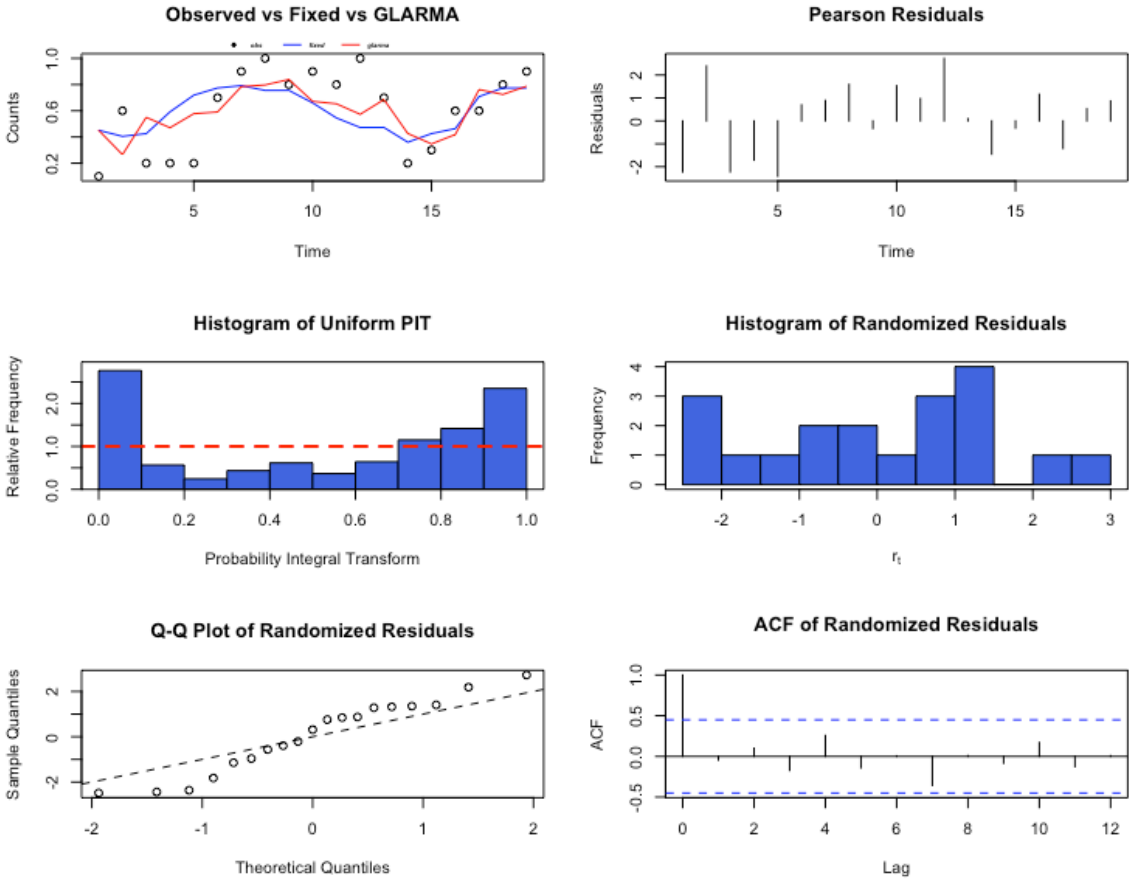
4 | *Spirocerca* sp.

Spirocerca Prevalence:All five models

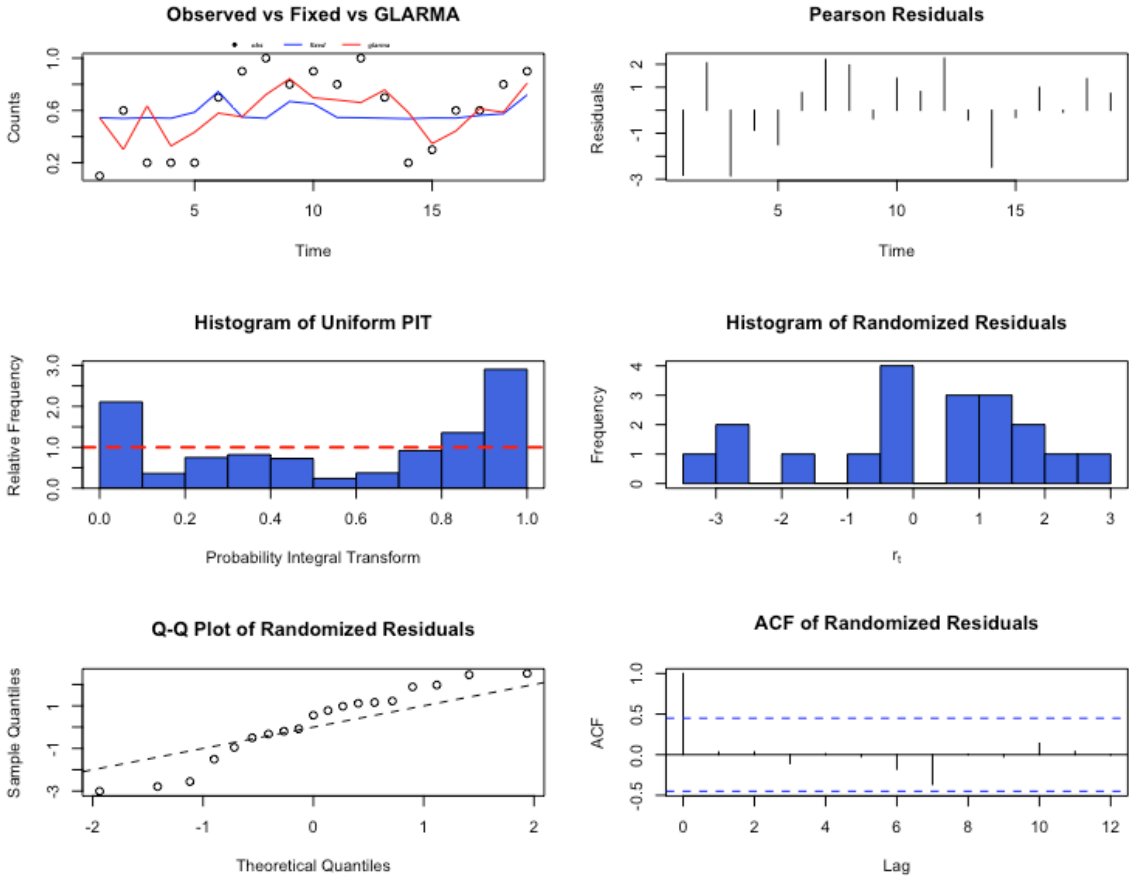
M_0: Spiro_Prev ~ constant



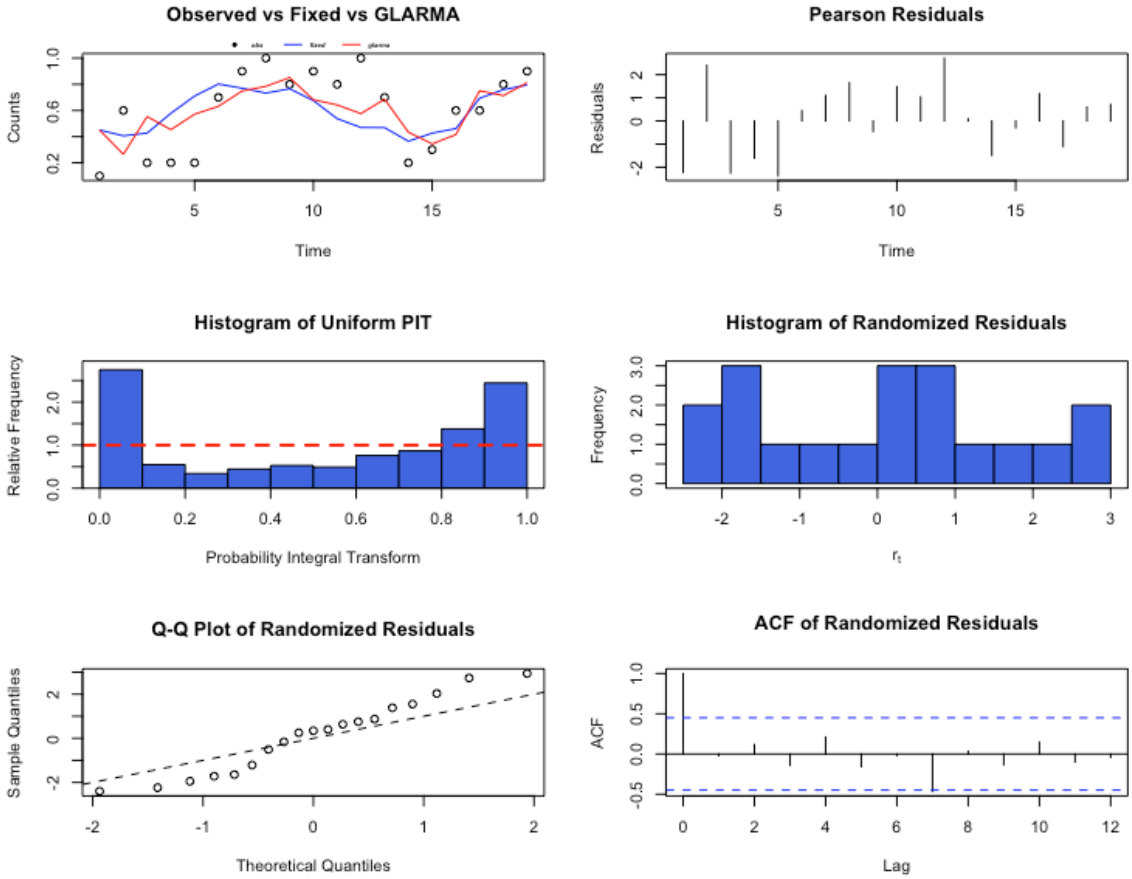
M_1: Spiro_Prev ~ Temp



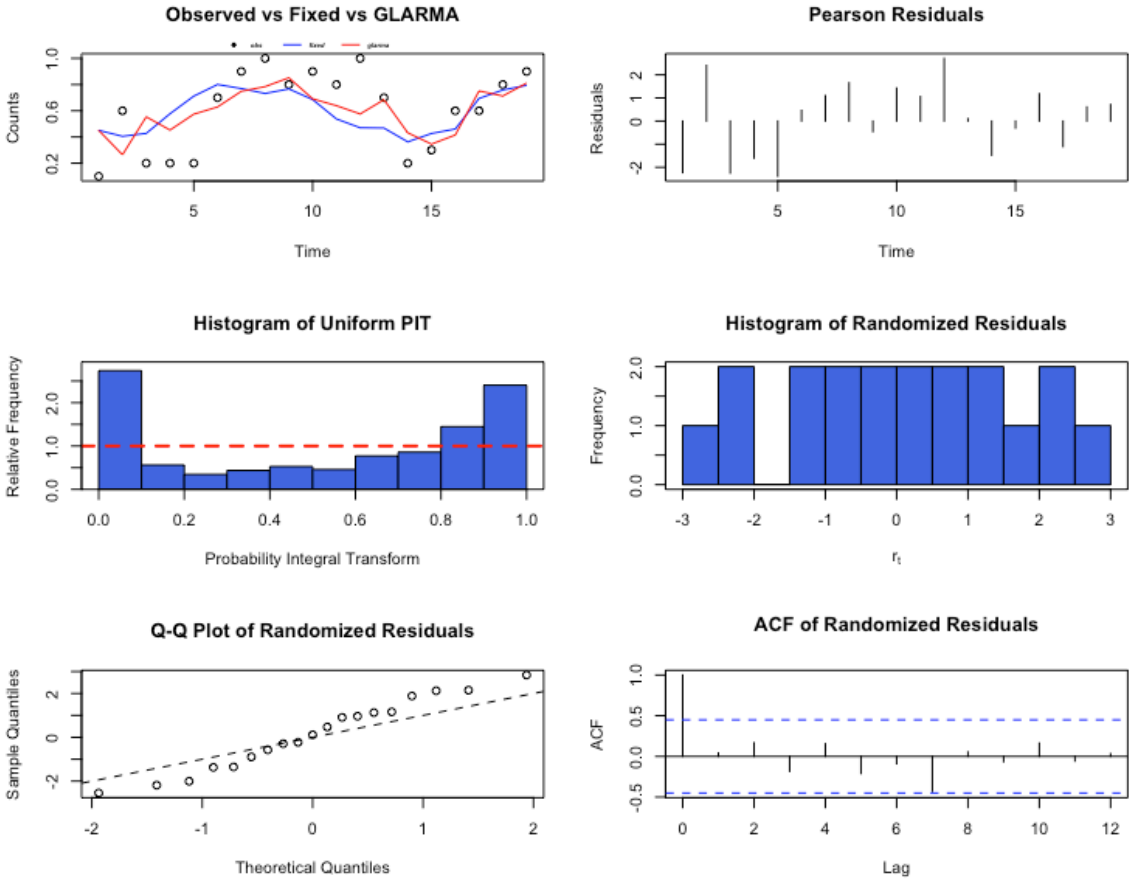
M_2: Spiro_Prev ~ Rain



M_3: Spiro_Prev ~ Temp + Rain

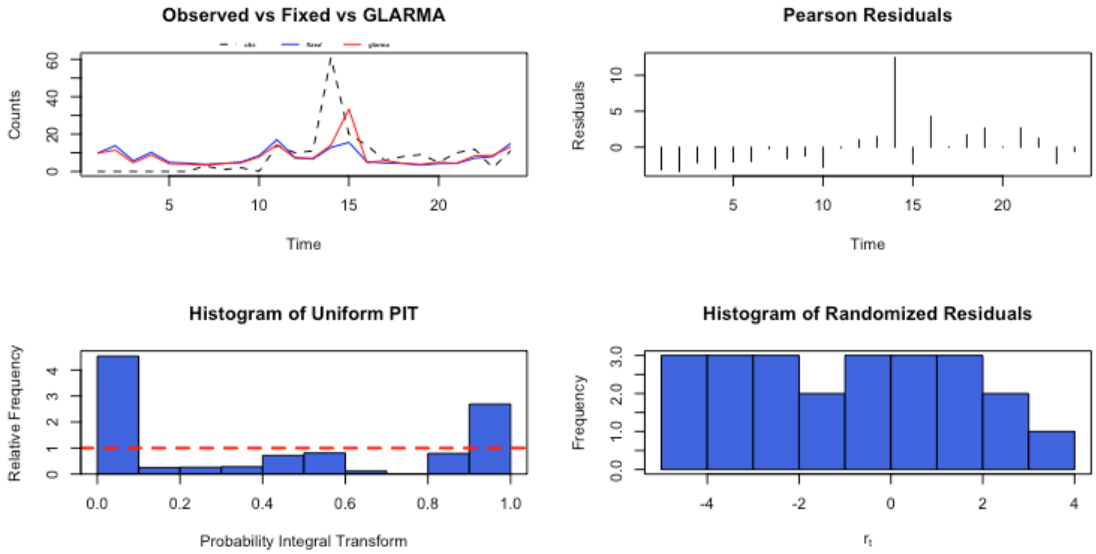


M_4: Spiro_Prev ~ Temp*Rain



Spirocerca Density: only M_4 retained

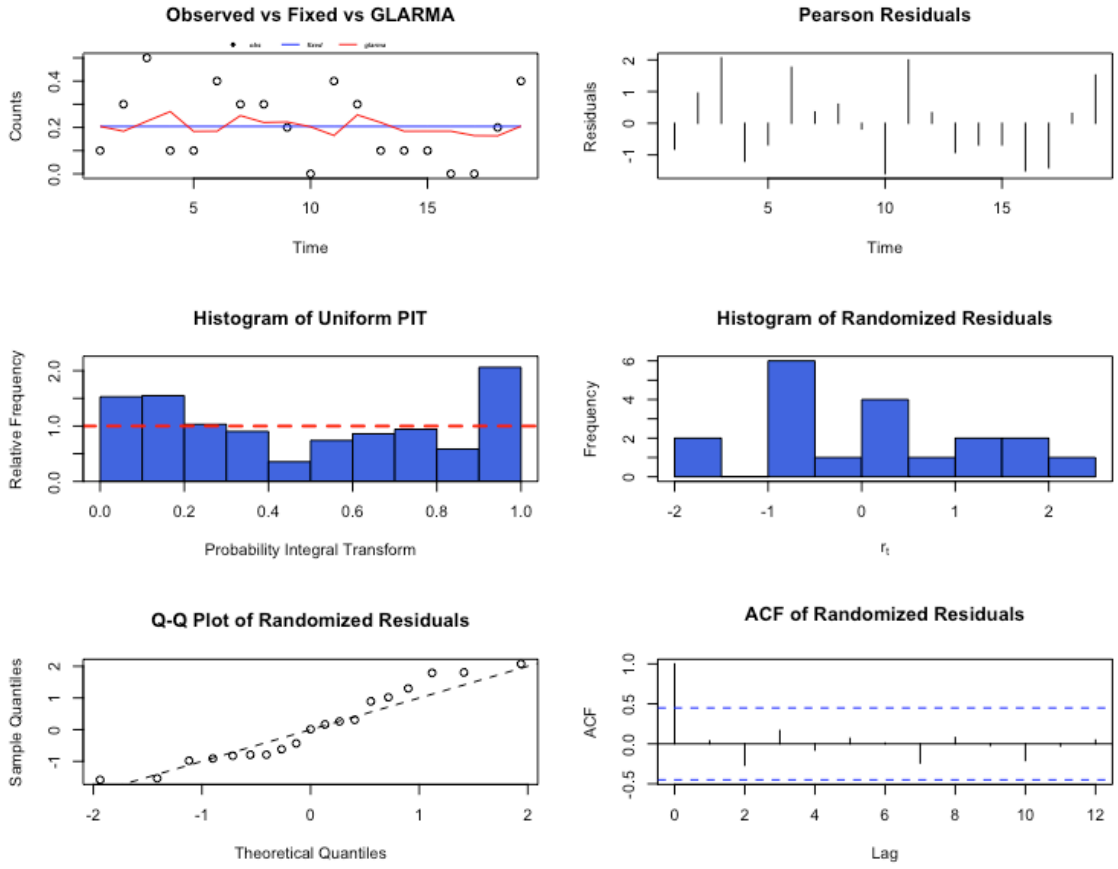
M_4: Spiro_Dens~ Temp*Rain



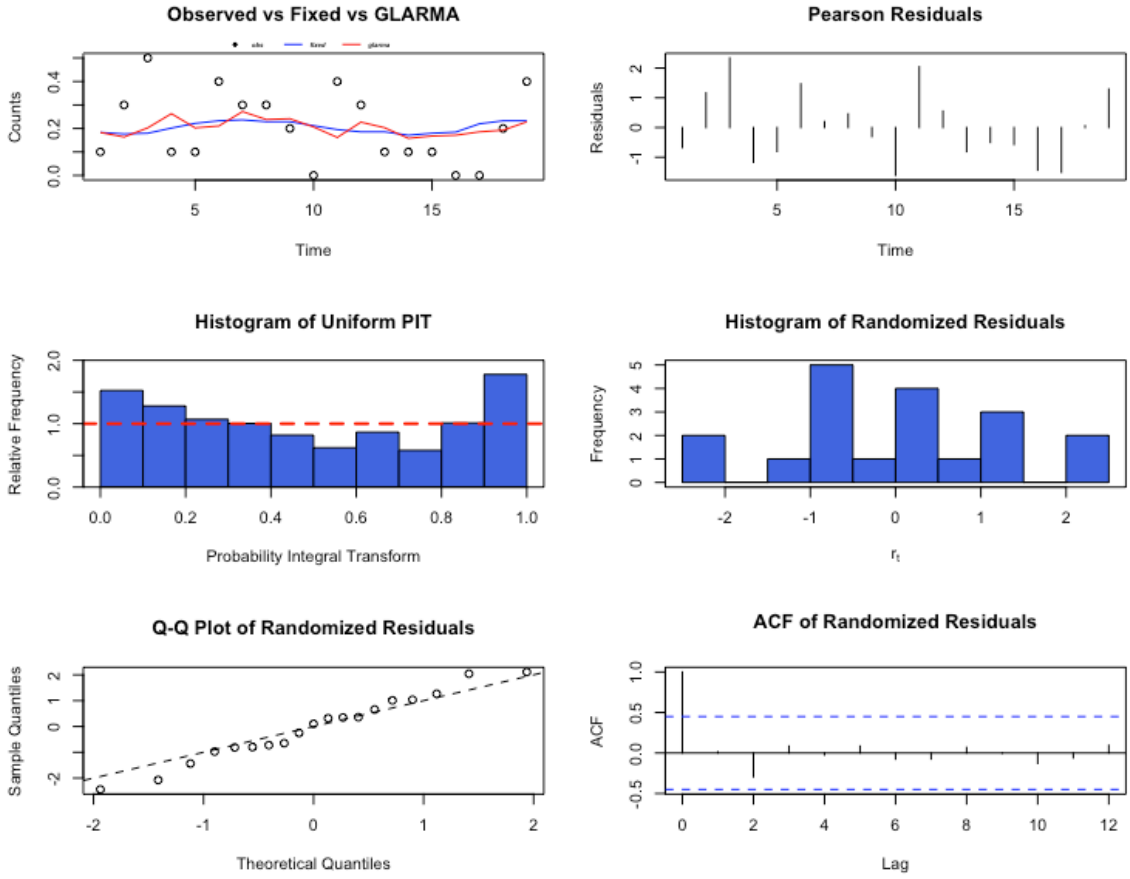
5 | *Calodium hepatium*

Calodium hepaticum Prevalence: All five models retained

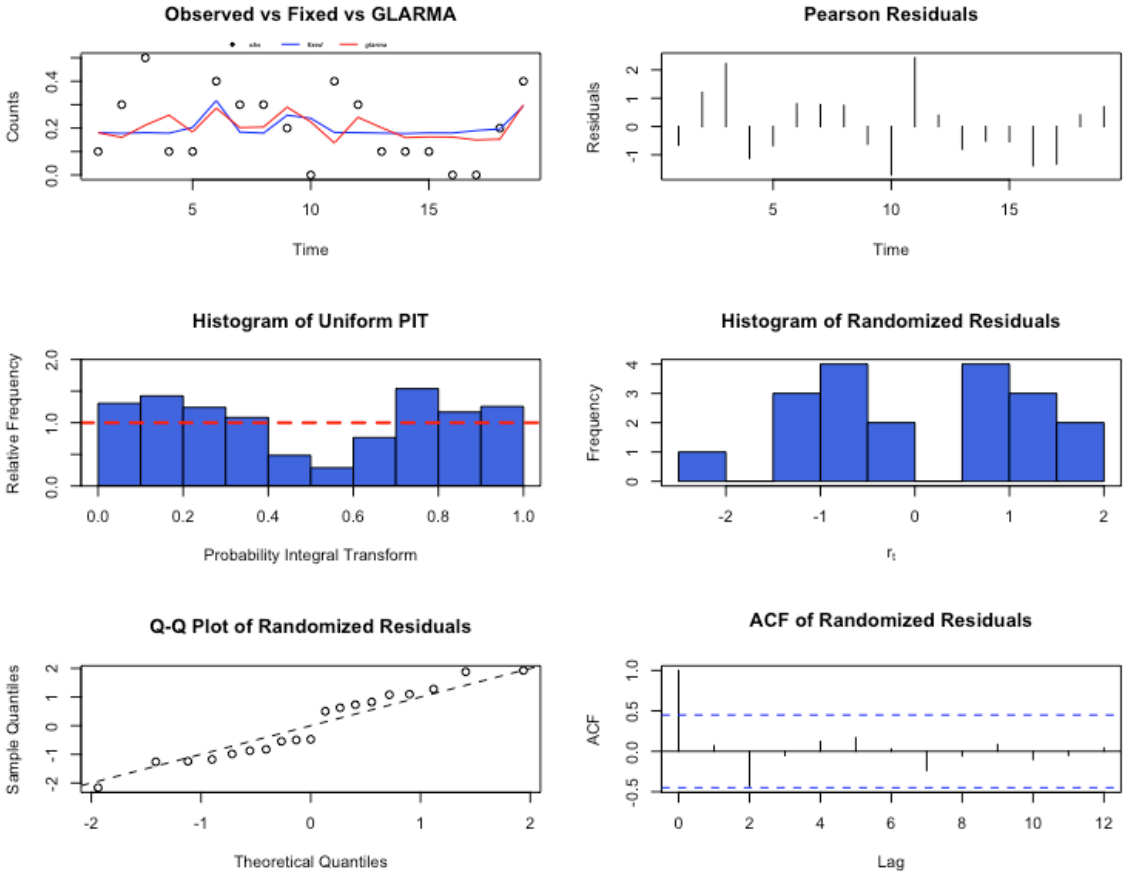
1. M_0: CaHe_Prev ~ constant



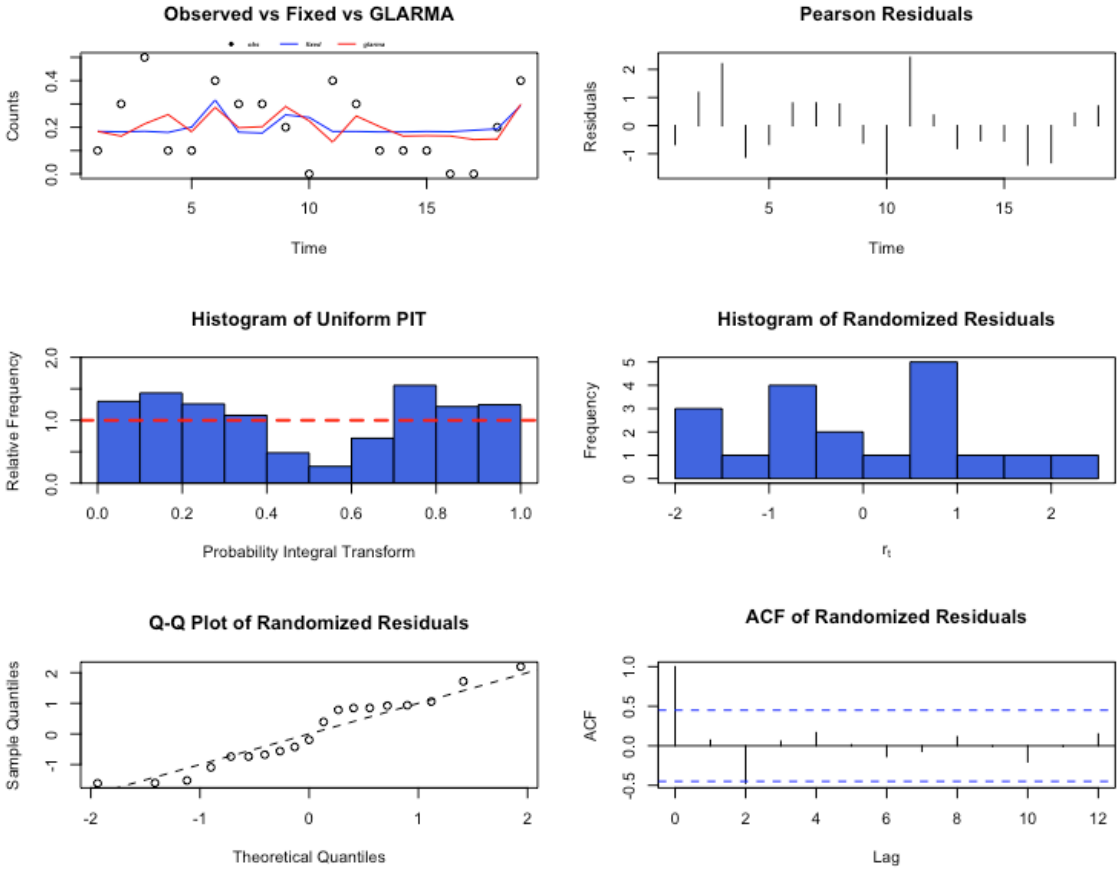
2. M_1: CaHe_Prev ~ Temp



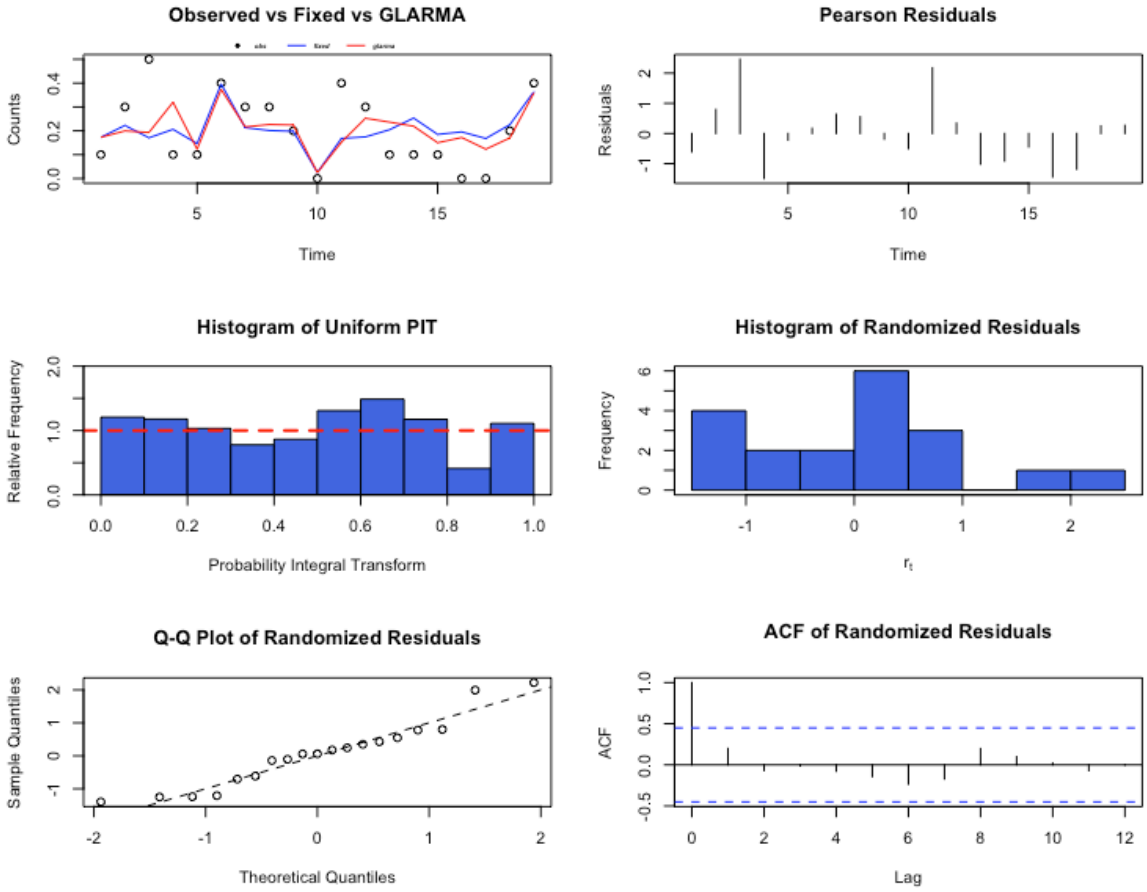
3. M_2: CaHe_Prev ~ Rain



4. M_3: CaHe_Prev ~ Temp + Rain



5. M_4: CaHe_Prev ~ Temp*Rain



Calodium hepaticum Density: M_4 retained

M_4: CaHe_Dens~ Temp*Rain