PARASITE-MEDIATED COMPETITION ACROSS AN APPALACHIAN SALAMANDER HYBRID ZONE

by

TIMOTHY L. ODOM

(Under the Direction of Andrew W. Park and Sonia M. Altizer)

ABSTRACT

Understanding the mechanisms behind the maintenance of hybrid zones and the means by which parasitism can either favor or penalize introgression is likely to be crucial for predicting wildlife disease dynamics under both contemporary and future anthropogenic disturbances. In this dissertation, I leverage preserved specimens of two hybridizing *Plethodon* salamanders to determine if infection with shared parasites is a factor influencing their relative fitness and identify host and environmental factors associated with parasite occurrence. In Chapter 2, I explore how parasite community composition and assembly transform across the hybrid zone between *P. teyahalee* and *P. shermani* at the Coweeta Hydrological Station. Chapter 3 investigates how host factors such as body size, estimated degree of introgression, and environmental conditions like rainfall influence the distribution, prevalence, and intensity of individual parasite species among *P. teyahalee,* hybrids, and *P. shermani* in the Coweeta Basin. In Chapter 4, I incorporate preserved specimens from field surveys of gravid female *Plethodon* to estimate the degree to which parasite infections influence host fitness. Lastly, in Chapter 5 I combine insights from trends in parasite community composition and assembly, patterns in host and environmental associations with parasite pressure, and costs of fitness associated with parasite infections to provide commentary on the likely mechanisms influencing the contemporary extent and long-term stability of the hybrid zone between *P. teyahalee* and *P. shermani* at Coweeta Hydrological Station.

INDEX WORDS: Hybrid zone, parasite community, parasite-mediated competition, *Plethodon shermani, Plethodon teyahalee,* EMS framework, historical ecology, museum collections

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DEDICATION

For Travis, Scott, Aunt Charity, Circe and Atlas. Thank you for believing in me. It takes a village, and I’m eternally grateful for mine. I love you.

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CHAPTER 1

INTRODUCTION AND OVERVIEW

Anthropogenic climate change and increased instances of globalization-associated disease invasions have been causally tied to species extinctions and catastrophic losses of biodiversity across broad taxonomic and geographic scales (McCallum et al., 2024). Further, global change is precipitating increased incidence of animal redistributions, increasing the likelihood of novel species assemblages (Pecl et al., 2017) as well as disease spillover (Lafferty 2009). Among such phenomena, climate tracking species can create new species contact zones (Urban 2020), with hybridization between closely related species being a growing consequence (Chunco 2014). Parasite sharing can occur between newly introgressed species following range shifts (Krichbaum et al., 2010), one of many ways that climate change is exacerbating disease threats in wildlife (Kutz et al., 2005; Morales-Castilla et al., 2021). The impacts of parasite sharing on the extent and stability of hybrid zones are poorly understood, with no hard and fast rules emerging (Theodosopoulos et al., 2019; Baird and de Bellocq, 2019). Understanding the mechanisms behind the maintenance of hybrid zones and the means by which parasitism can either favor or penalize introgression is likely to be crucial for predicting wildlife disease dynamics under climate change.

A hybrid zone can be loosely defined as a region of interbreeding of individuals from genetically distinct populations that maintain some degree of reproductive isolation (Barton and Hewett, 1985; Wielstra 2019). The term “hybrid zone” is used interchangeably in the literature with “zone of introgression”. The mechanism responsible for the creation of hybrid zones is introgression, or gene flow between lineages that result in populations of individuals that contain proportions of genetic material attributable to each parent lineage (Rhymer and Simberloff, 1996). These regions of mixed ancestry were originally described as tension zones where hybrids largely possess lower fitness than parent taxa (Barton 1979). It was also initially believed that these introgression events are rare and ephemeral, occurring largely in range boundaries of lineages undergoing speciation, prior to the idea that hybrid zones may themselves be stable, evolutionary outcomes (Dobzhansky 1940). Some estimates now suggest that the evolutionary trajectory of approximately 25% of plant species and 10% of animal species have been influenced by introgression (Arnold 1997; Adavouldi 2021).

Several causal mechanisms have been proposed to explain hybrid zones and the heterogeneity observed in hybrid zones across taxa and ecoregions. The most fundamental mechanism is historical introgression, which refers to historical gene flow or migration of few individuals from localities containing populations of distinct taxa, where gene flow may or may not be ongoing (Pardo-Diaz 2012). The genetic identity of hybrids consists of proportions and combinations of alleles belonging to both parent taxa. However, the genetic contribution of each parent taxa might not be equally represented in hybrids. A number of studies have investigated how parental species can contribute asymmetrical proportions of genetic material to the genomes of their descendants. In a study of hybrid populations of swordtail fish by Schumer et al., (2018) researchers found that the genetic material sequenced from parent taxa was less well represented in hybrid genomes and occurred in regions undergoing relatively higher rates of recombination in hybrids but contain fewer deleterious alleles. These results have been compared to similar patterns observed in *Homo neanderthalensis* genetic material found in human genomes (Sankararaman et al., 2014; Schumer et al., 2018).

Classical arguments for the inherent ephemerality of hybrid zones are easily refuted thanks to rationale provided by gradual developments of ecological theory like Levins migration and metapopulation models which provided foundational work in understanding outcomes of migration of individuals, and consequently genes, between patches or populations (Levins 1970). We now know that several forces can act to maintain hybrid zones long term in natural systems. One mechanism is gene flow. Constant introgression of individuals or genes from populations of parent taxa into regions of range overlap can result in sustained hybrid zones, even in situations where hybrids are less fit than parent taxa. This of course assumes that the population growth rate in patches of hybrids isn't so low that they experience repeated extinction events. As long as migration rates are sufficient to counteract negative hybrid population growth, hybrid zones can be sustained (Levins 1970; Nolte and Tautz, 2010).

Occasionally, introgression can result in adaptive traits in hybrid offspring. This phenomenon, termed adaptive introgression, refers to selective advantages conferred to populations of hybrids thanks to synergistic combinations of ancestral alleles from parent taxa (Theodosopoulos 2019). These benefits can bolster the relative ability of parent and hybrid species to resist or tolerate parasite infections (Jackson and Tinsley, 2002; Joly et al., 2007). A study of skin antimicrobial peptides (AMPs) in hybrid water frogs (*Pelophylax esculentus*) infected with the devastatingly pathogenic chytrid fungus, *Batrachochytrium dendrobatidids* (*Bd*), found that hybrids released greater quantities of AMPs as well as benefited from increased antifungal activity relative to both parent taxa, *P. lessonae* and *P. ridibundus* (Daum et al., 2012). This increased immune diversity compared to parent taxa has far-reaching consequences for mechanisms likely to conserve genetic diversity amid mass extinction events.

Just as hybrid zones can be maintained by a myriad of mechanisms, hybrid zones can also serve as maintainers of species boundaries through hybrid inferiority (Barton 1979). Hybrid inferiority is a term that can be used to describe the outcomes of a list of forces that render populations of hybrids competitively weak or as sinks for genetic information from parent taxa in situations where gene flow is maintained. Applying these concepts to host-parasite systems, the resistance and tolerance of hybrids to parasite infections has been shown to vary widely (Wolinska 2008; Theodosopolous et al., 2019), with some hybrid lineages suffering decreased performance in the presence of disease (Parris 2004). Genetic admixture is often an evolutionary blender of genes that doesn’t always preserve beneficial traits from parent taxa in hybrid offspring, like immune diversity, due to recombination during sexual reproduction (Adavoudi and Pilot, 2022). Consequently, introgression has the potential to undo eons of local adaptation and specialization, dampening competitiveness of hybrids. Just because combinations of traits or alleles are novel doesn’t make them advantageous. The diversity in how hybrids compared to parent species regarding tolerance or resistance to parasites compounded by the likely reshuffling of parasitic diseases under climate change necessitates inquiry into the potential ability of parasite dynamics to influence the extent and stability of hybrid zones.

Some studies have tried to tackle this question in hybridizing host species, but most focus on dynamics involving only one or two parasites (Fritz and Newcombe, 1999; Jackson and Tinsley, 2003; Joly et al., 2008; Daum et al., 2012; Guttel and Ben-Ami, 2014; Roth et al., 2021; Balard and Heitlinger, 2022). Most animals are simultaneously and sequentially infected with diverse communities of parasites (Poulin 1997; Johnson and Haas, 2021; Junker et al., 2023; Starkloff and Galen, 2023) that vary with space (McNew et al., 2021) and seasonality (Rice et al., 2021; Odom et al., 2025). Some studies have compared parasite species richness between hybridizing host species (Baird et al., 2012), but richness patterns don't capture consequential changes in community composition since two communities may vary in species identity while maintaining the same richness.

Patterns in parasite co-occurrence and community assembly can directly impact host fitness (Griffiths et al., 2011; Johnson and Hoverman, 2012), but many studies assume that measures of parasite pressure translate to fitness costs in infected hosts. This assumption is unsafe, as many species evolve tolerance to parasite infections without experiencing extreme fitness costs (Balard and Heitlinger, 2022). Furthermore, observations of apparent bias in parasite distributions could be more related to environmental factors than tight evolutionary host-parasite relationships. Parasite transmission relies on aspects of both the host and the environmental conditions outside of the host, such that ecological interactions between parasites and their species may be idiosyncratic (Maestri et al., 2020; Johnson and Haas, 2021; Starkloff and Galen, 2023). Hybridizing species could differ fundamentally in behavioral or habitat traits that shape the distribution of parasites (Hiadlovska et al., 2013; Theodosopoulos et al., 2019; Roth et al., 2021; Sartor et al., 2021). A study of *Haemoproteus spp.* infections in hybridizing warblers found that the relationship between elevation and the likelihood of infection was idiosyncratic between parent and hybrid taxa, where infections increased with elevation in one species and decreased with the other as well as in hybrids of the two (Cozzarolo et al., 2018).

Although parasite mediated competition may be an important factor in shaping the future of some hybrid zones, informed predictions require investigation into how hybridizing host species share parasites, and how infection dynamics operate at the parasite community scale. We also need to tease apart the relative importance of host and environmental factors for determining the distribution of parasite species along ecotones structured hybrid zones, and to measure the relative impact of parasitism on actual components of host fitness like body condition and reproductive output.

In this dissertation, I leverage 76 years of preserved specimens from natural history collections at The University of Georgia to determine if parasite mediated competition is a potential mechanism influencing the extent of the hybrid zone between the lungless salamander species, *Plethodon shermani* and *Plethodon teyahalee,* asking 1.) How do host and environmental factors influence parasite community composition? 2.) Do host traits, environmental traits, or some combination of both better predict individual parasite occurrence, infection intensity and overall parasite species richness? and 3.) Do infections with shared parasites differentially influence host fitness between hybrids and parent taxa?

STUDY SYSTEM AND BACKGROUND

Plethodon in the Southern Appalachian Mountains commonly hybridize along elevational gradients, where, broadly, populations of montane salamanders like *P. shermani* occupy higher elevational sites and foothill species like *P. teyahalee* are found at lower elevations (Hairston 1949; Gifford and Kozak 2012). Intermediate elevations are occupied by populations of salamanders of hybrid ancestry, displaying phenotypic traits found in both parent taxa, notably *P. shermani's* red legs, and *P. teyahalee's* white spots (Hairston 1950; Hairston et al., 1992; Carter 2023). The climate-tied dynamics of mixing and separating of lineages in groups like *Plethodon* have precipitated gradual diversification of salamanders in the region (Highton 1995; Newman & Austin, 2015), resulting in the emergence of the greatest concentration of salamander species in the world (Petranka 1998; Hocking et al., 2021). The diversity of terrestrial and stream habitats in the Southern Appalachian Mountains paired with cyclical elevational range shifts of species tracking glacial advance and retreat have resulted in a sky-island archipelago characterized by reproductively isolated montane salamander species, like *P. shermani,* restricted to glacial refugia in high elevation sites due to climatic niche conservatism and bounded at their lower elevational extents by species like *P. teyahalee* (Lyons and Kozak, 2020; Marsh et al., 2020).

This hybrid zone displays patterns reflective of the bounded-superiority hypothesis, where the spatial distribution of populations of hybrid descent tracks the ecotone upon which the zone of introgression is structured (Moore & Buchanan 1985; Lowe 2016). Although no longer hybridizing as a consequence of sympatry between *P. teyahalee* and *P. shermani,* a continuous genomic gradient of introgression between these species has persisted for at least the last several hundred thousand years (Weisrock et al., 2005; Lowe 2016), meaningful inferences of hybrid performance regarding resistance or tolerance to disease have arisen from studies of species considered to be of hybrid lineage (Daum et al., 2012). Many have sought to disentangle the eco-evolutionary factors responsible for the contemporary spatial extent of these species and the stability of this and other tension zones between *Plethodon* in the region (Chatfield et al., 2010; Ryan et al., 2024) and predict its future dynamics with mixed consensus (Hairston 1980; Jaeger et al., 1998). Although no longer actively hybridizing, the distribution of populations of *P. shermani x P. teyahalee* hybrids in the region are fundamentally tied to climate, and there are ongoing debates as to whether the distribution of the hybrid zone between these species may be shifting in relation to anthropogenic disturbances. Some studies have found that the hybrid zone at Coweeta is evolutionarily active, with bias toward *P. teyahalee* traits driven by changes in temperature (Hairston et al., 1992; Walls 2009), and others have suggested that the hybrid zone is stable (Lowe 2016). However, recent work by Carter (2023) found that the hybrid zone between *P. teyahalee* and *P. shermani* was evolutionarily active, with selection favoring alleles of both parent lineages with biased introgression favoring *P. teyahalee* alleles, proposing that changes in rainfall patterns may be partially responsible.

So far, parasite mediated competition has not been considered as a potential mechanism behind observed patterns of introgression of *P. teyahalee* alleles but could be an important factor since temperature and moisture availability have been shown to influence the diversity and abundance of parasites in communities of amphibian hosts (Mihaljevic et al., 2018). Historical observations of gastrointestinal helminth infections of salamanders in the Southern Appalachians have reflected these trends, and specifically, some gastrointestinal parasite species vary in abundance along ecological gradients that mirror those experienced by *P. shermani* and *P. teyahalee*. For instance, the ciliated protozoan *Cepedietta michiganensis* is a gastrointestinal parasite observed in *Plethodon* salamanders in the region and is confined to sites with lower elevational extents (Joy and Tucker, 2001; Davis and Golladay, 2019). The abundance of the nematode, *Cosmocercoides variabilis,* is known to vary tightly with seasonal patterns of rainfall (Bolek 1997; Bolek, 2011). Both parasite species have been documented in *P. shermani* and *P. teyahalee* in the region (Rankin 1937). It is possible that the ecological heterogeneity that characterizes this hybrid zone could shape the parasite communities to which each species is exposed as well as ecological interactions between hybridizing host species and their parasites (Maestri et al., 2020; Johnson and Haas, 2021; Starkloff and Galen, 2023). This would result in heterogeneity in disease pressure between host groups, and consequently, parasite-mediated competition.

In Chapter 2, I explore how parasite community composition and assembly transform across the hybrid zone between *P. teyahalee* and *P. shermani* at the Coweeta Hydrological Station. I dissect preserved *Plethodon* salamanders from the Coweeta Basin from natural history collections at The University of Georgia and document the gastrointestinal parasite diversity and abundance found within each individual host. I employ the Elements of Metacommunity Structure framework (EMS) to characterize the parasite metacommunity structure within *P. teyahalee,* hybrids, and *P. shermani* and investigate the degree to which parasite taxa infecting *Plethodon* at this locality replace each other between host species. I also incorporate a probabilistic species co-occurrence analysis, investigating the within-host competitive interactions between co-infecting parasites to determine if interspecific competition between parasites is an important factor influencing parasite community assembly. Finally, I leverage data on the volume of prey in infected hosts to reveal relationships between parasite infections and salamander prey acquisition. I predicted that parasite community composition would be closely tied to environmental gradients like average monthly rainfall and temperature as well as host species and body size.

Chapter 3 investigates the degree to which host factors such as body size and degree of introgression along with environmental conditions like rainfall influence the distribution of individual parasite species among *P. teyahalee,* hybrids, and *P. shermani* in the Coweeta Basin. I use locality data and the date of capture for each salamander to gather climate data for the month, year, and locality in which each specimen was collected from the field and preserved. I next use generalized additive models, incorporating both host and environmental traits, to identify factors predictive of parasite richness, prevalence, and infection intensity for the 4 most common parasites infecting *Plethodon* at this locality. I predicted that rates of parasitism would be highest in hybrids and *P. shermani,* due to their habitats and behavior being more conducive to gastrointestinal parasite transmission.

In Chapter 4, I incorporate preserved specimens from field surveys of gravid female *Plethodon* at Coweeta conducted between 2015 and 2016 by Howard and Maerz (2022) to estimate the degree to which parasite infections might influence host fitness. I incorporate wet mass and snout vent length measured at time of capture to estimate the body condition of gravid females using scaled mass index. I then measure the degree to which infections with the ciliated protozoan parasite, *Cepedietta michiganensis* influence host body condition as well as reproductive investment *P. teyahalee,* hybrids, and *P. shermani* measured as the number of eggs in a gravid female. I predicted that measures of host fecundity would vary with rates of parasitism, as both increases in reproductive investment as well as fecundity costs have been documented in parasite infections in amphibians (Brannelly et al., 2016). As parasitism is generally considered to be costly to hosts, I further predicted that body condition would decrease with parasite species richness and parasite infection intensity.

Lastly, in Chapter 5 I combine insights from trends in parasite community composition and assembly, patterns in host and environmental associations with parasite pressure, and costs of fitness associated with parasite infections to provide commentary on the likely mechanisms influencing the contemporary extent and long-term stability of the hybrid zone between *P. teyahalee* and *P. shermani* at Coweeta Hydrological Station.

CHAPTER 2

INFECTION BY A CILIATED PROTOZOAN ALTERS PARASITE COMMUNITY STRUCTURE IN HYBRIDIZING SALAMANDERS[1]

[1]Odom, T.L., Altizer, S.A., Park, AW. To be submitted to *Biology Letters*.

ABSTRACT

Animal hybrid zones often form across ecological gradients like elevation, latitude, and habitat type, with hybrids experiencing a diversity of conditions relative to either parent taxa. The gradients along which hybrid zones form also influence the diversity and abundance of communities of parasites to which hybridizing host populations are exposed. We leveraged 238 preserved specimens of two *Plethodon* salamanders, *P. shermani* and *P. teyahalee,* and their hybrids from natural history collections at The University of Georgia to determine if hybridizing *Plethodon* at Coweeta Hydrological Station, Otto, NC, USA are host to distinct parasite communities and if factors related to host traits, environmental conditions, or co-infection dynamics better predict parasite community composition. Our analyses revealed that negative interactions between shared parasites better predicted parasite community structure than measures of host or environmental traits. Differences in infection between parasite species were associated with infections by the ciliated protozoan, *Cepedietta michiganensis*, which appears to reduce host foraging activity in *P. teyahalee* and could therefore limit exposure to other parasite species. This form of parasite-mediated enemy release could underlie the reduced parasite species richness and lower parasite prevalence in *P. teyahalee* and hybrids relative to *P. shermani*, potentially altering the outcome of competition among salamander hosts.

INTRODUCTION

The field of disease ecology increasingly recognizes that most parasites are generalists and co-infection is common, leading to multi-host, multi-parasite communities (Rigaud et al. 2010; Stewart Merrill et al. 2022). Further, global change is resulting in high occurrence of animal redistributions, with the potential for new species assemblages to form (Pecl et al., 2017). Among such phenomena, climate tracking species can create new species contact zones (Urban 2020), with hybridization being a growing consequence (Chunco 2014). While many ecological forces ultimately determine the outcome of novel species assemblages and their hybrids, the role of parasites is relatively unexplored (Theodosopolous et al., 2019; Baird and de Bellocq, 2019).

Among related species, parasite sharing is often relatively high (Davies and Pedersen, 2008; Huang et al., 2013; Schatz and Park, 2021). However, animal hybrid zones often form over ecological clines like elevation, latitude, and habitat type, with hybrids often experiencing distinct conditions relative to either parent taxa (Walls 2009; Culumber et al., 2012; Davis et al., 2016; Sartor et al., 2021). Since parasite transmission is influenced by both host and environmental factors, ecological interactions between hybridizing host species and their parasites may be idiosyncratic (Maestri et al., 2020; Johnson and Haas 2021; Starkloff and Galen 2023), and parasite pressure may vary across host groups, contributing to the net effects of competition. The resistance and tolerance of hybrids to parasite infections has been shown to vary widely (Moulia 1999; Fritz et al., 1999; Wolinska 2008; Theodosopolous et al., 2019). In some cases introgression is beneficial, resulting in hybrid resistance to parasites (Jackson and Tinsley, 2003; Joly et al., 2008). The opposite has also been seen, where hybrids suffer decreased performance in the presence of disease (Parris 2004).

Studies of parasite infection in animal hybrid zones generally focus on the impacts of one or two parasite taxa on the fitness of hybrid hosts (Fritz and Newcombe 1999; Jackson and Tinsley 2003; Joly et al., 2008; Daum and Woodhams 2012; Guttel and Ben-Ami 2014; Roth et al., 2021; Balard and Heitlinger, 2022). However, most animals are simultaneously and sequentially infected with diverse communities of parasites (Poulin 1996; Johnson and Haas, 2021; Junker et al., 2023; Starkloff and Galen, 2023), and the processes that shape parasite co-occurrence and community assembly can have demonstrable consequences for host fitness (Griffiths et al., 2011; Johnson & Hoverman, 2012; Ramsay and Rohr, 2021). In hybrid zones, introgression between closely related species is likely to be a crucial mechanism for the conservation of genetic diversity under projected anthropogenic climate change (Chunko 2014), emphasizing the need for studies investigating how parasite community assembly operates across ecological and host genetic gradients and how processes at the parasite community scale may impact the fitness of hybridizing host species (Daum et al., 2012; Guttel et al., 2014).

An important consideration, in addition to host genetic diversity and environmental variation, is that among a community of shared parasites, pairwise interactions within host individuals, which include direct competition (i.e., for space, cells, and tissue) and apparent (immune-mediated) competition as well as facilitation (Pedersen and Fenton, 2007; Telfer et al., 2010), may also influence the patterns of infection (Torchin et al., 2015). Consequences of infection by certain parasite species, including those that change host behaviors, have the potential to transcend pairwise interactions by altering the infection probability of several parasite species through changes in exposure (Wood and Johnson, 2015). Consequently, in hybrid zones where parasite sharing is likely to be high, there is a large set of potential outcomes of infection which may manifest differentially in parental species and their hybrids.

Plethodon shermani and *Plethodon teyahalee*, two hybridizing lungless salamanders found in the southern Appalachian Mountains, provide a unique opportunity to investigate the role of parasite communities in a hybrid zone. At present, populations of *P. shermani* are generally confined to cool, moist, high elevation sites, and *P. teyahalee* generally occupy warmer, low elevation sites (Hairston 1949; Gifford and Kozak, 2012). At intermediate elevations where their distributions overlap populations of hybrid ancestry display phenotypes of either parent species to varying extents, the red legs of *P. shermani* and the white spots of *P. teyahalee* (Hairston 1950; Hairston 1992; Lowe 2016; Carter 2023). The result of this genetic stratification is a continuous gradient of host genetics and environmental conditions that could influence the diversity and abundance of parasites. The prevailing consensus is that the lower elevational extent of high elevation *Plethodon* (like *P. shermani*) that hybridize with *P. teyahalee* is set by physiological constraints associated with evaporative water loss and temperature (Lyons and Kozak, 2020; Marsh et al., 2020). The upper extent of *P. teyahalee* alleles are limited by selection favoring *P. shermani* alleles at higher elevations (Gifford and Kozak, 2012).

Decades of legacy work in this hybrid salamander system investigating the evolutionary and ecological drivers of the extent and long-term stability of this hybrid zone have been conducted at Coweeta Hydrological Laboratory and have resulted in the collection, liquid preservation, and deposition of hundreds of *P. shermani*, *P. teyahalee*, and hybrids into collections at The Georgia Museum of Natural History. In this study, we leveraged preserved salamanders from this site to determine if *P. shermani, P. teyahalee*, and hybrids host communities of shared gastrointestinal parasites and to identify the host and environmental factors influencing parasite community composition and assembly. We predicted that parasite community composition would be closely tied to environmental gradients like average monthly rainfall and temperature as well as host species and body size. We instead found that parasite community composition was heavily influenced by interspecific interactions between parasites that differed in strength between *P. shermani*, hybrids, and *P. teyahalee*. These were largely associated with infections with a ciliated protozoan, *Cepedietta michiganensis*, which appears to reduce host foraging activity and thereby exposure to other parasite species - a form of parasite-mediated enemy release (Hatcher et al., 2006).

METHODS

Preserved specimen selection

We dissected 238 preserved salamanders collected from Coweeta Hydrological Station, Otto, NC, USA and deposited in the Georgia Museum of Natural History and herpetological collections from the Warnell School of Forestry, University of Georgia, USA, consisting of observations ranging from 1946 to 2022. Specimens were selected based on field identification of species and the availability of specific spatiotemporal data including lat/lons of point of collection and date of field collection. Observations were limited to specimens fixed in formalin and stored in EtOH with measurable phenotype scores (red legs and white spots). We further limited observations to those collected between May and October, the time of year in which Southern Appalachian plethodontid salamanders are most surface active (Gade et al., 2020).

Parasite collection

A single incision spanning from the thorax to the cloaca was made using a scalpel and other dissection tools. Following dissection, the GI tract of each salamander was removed by severing the terminal ends of the GI tract from the esophagus and cloaca respectively. Following excision from the salamander host, each GI tract was elongated in a petri dish containing 2 ml of 75% EtOH. A single incision was made along the length of the GI tract, being sure to only open one side of the GI tract to prevent damaging intact helminths in the gut. Using the end of a microscope slide, the inside of the GI tract was gently scraped to dislodge helminths embedded in the lacteals and villi of the gut while minimizing damage to diagnostic parasite features (Galbreath et al., 2019). The contents of each GI tract were sorted under a dissecting microscope, separating the endoparasites from each salamander based on their higher organization (nematodes, trematodes, and cestodes). Parasites were identified to species with dichotomous keys and parasite species descriptions. Following identification, parasites were preserved in either Canada balsam or glycerol.

Parasite identification

For morphological identification of cestodes and digeneans, the internal structures of each worm were stained using Simichon's Aceto-Carmine. Next, worms were moved from the stain to an acid-EtOH bath for regressive staining, and trematodes and cestodes were subjected to a gradient alcohol dehydration series. Dehydrated worms were then placed in xylene to clear the tegument enabling measurement and differentiation of internal structures such as testes, ovaries, and other identifying features. Parasites were moved from xylene and mounted on microscope slides using Canada balsam suspended in xylene and placed on a slide warmer (Premier Model XH 2001) at 40C for 3 days or until the Canada balsam was fully dry.

Parasites identified as nematodes were placed in a lactophenol-alanine blue staining and clearing solution for ~20 minutes, with the time spent in the stain varying based on size of each worm. Following staining and clearing, nematodes were placed on a temporary wet mount for identification via light microscopy before storage in glycerine-alcohol. Parasites were identified to the greatest taxonomic resolution using dichotomous keys, parasite species descriptions, and published parasite assemblages for reference, with newer keys and taxonomic classifications taking precedence (Rankin 1937; Yamaguti 1961; Gibson 2002).

Environmental variables

With the collection dates and coordinate data associated with each specimen, we leveraged the 800m gridded Parameter-Elevation Regressions on Independent Slopes Model (PRISM) database to access measures of environmental conditions at the time of field collection of each specimen. Metrics like rainfall in the months prior to sampling (Shearer and Ezenwa, 2020) and average temperature and rainfall during the month-year combination that each specimen was collected from the field (Odom et al., 2025) have all been successfully tied to host-parasite dynamics in parasites with environmental stages. However, elevation, temperature, and rainfall are tightly confounded in this system. Elevation has been used as a climate proxy, but we argue that ecological traits like rainfall are most related to habitat moisture availability are more reflective of eco-evolutionary pressures shaping *Plethodon* biology (Feder et al., 1983). As such, we elected to use measures of rainfall during the month of capture as a covariate for quantifying interactions between host, parasites, and climate. Odom et al., (2025) cited host pace of life as a justification for using rainfall during the month of capture to predict seasonal turnover in parasite communities and demonstrated success with this metric. We justify this choice here for comparable reasons but operating at the other end of the pace of life spectrum. *Plethodon* from dryer low elevation sites can vary in their frequencies of surface activity, with some individuals only surface active a couple of times a year (Carter 2023). As such, the only measures of rainfall we can safely speculate as associated with host-parasite dynamics in this case are those paired with observations of surface-active hosts.

Elements of Metacommunity Structure (EMS) Analysis

The Elements of Metacommunity Structure framework (EMS) provides a unique tool for detecting and characterizing communities of organisms structured along ecological gradients. The classical site-by-species structure of a community can be applied to infectious disease data, with hosts and parasites taking the role of 'sites' and 'species', respectively. This approach identifies likely interactions shaping parasite community composition and assembly (Presley et al., 2010; Presley et al., 2019; Odom et al., 2025). Using the R package `metacom` (Dallas 2014), we tested if the infracommunities infecting individuals of the species *P. shermani*, *P. teyahalee*, and their hybrids were non-random and if so, what latent gradients explained patterning. We began by constructing an interaction matrix where rows are individual hosts and columns are parasite species detected during dissection, coding parasite presence-absence as a binary variable (1 or 0). We then ordinated this matrix via reciprocal averaging, generating a score for each host. Individuals were then sorted based on these scores, effectively ranking individual hosts based on parasite infracommunity similarity. Scores obtained from reciprocal averaging were then related to variables hypothesized to be predictive of parasite community composition (Gauch 1982; Presley et al., 2010).

Boosted Regression Trees

We used boosted regression trees (GBM) to identify likely drivers of gastrointestinal parasite community composition, measured as metacommunity score generated via reciprocal averaging of a host-parasite presence-absence matrix (Dallas and Presley 2014). Variables comprised host species, snout-vent length (SVL), average precipitation, and maximum temperature (Cooper and Hollingsworth, 2018) for the month in which each salamander specimen was collected from the field using the R package `gbm` (Elith et al., 2008; Ridgeway, 2013). We also included collection year to account for any variation attributable to specimen age or site changes over time. The reciprocal averaging generates host scores that are both positive and negative, so we added the minimum score to each observation, resulting in a new minimum score of zero. We square-root transformed host scores so the response variable was approximately normally distributed prior to analysis. We determined the appropriate number of trees via cross-validation using 70% training and 30% test data, and evaluated model performance through root mean squared error.

Probabilistic co-occurrence and C-score analysis

We used the R package `cooccur` to conduct a probabilistic species co-occurrence analysis to determine if pairs of parasites co-infect or exclude each other more often than would be expected by chance. This test is motivated by the assumption that if two pathogens do not influence the infection success of each other, then the probability of co-infection should not differ significantly from the product of their prevalences. Facultative relationships result in greater frequency of co-infection than would be expected by chance, and antagonistic interactions should lead to significant reductions in the frequency of co-infections (Veech 2013; Griffith et al., 2016).

To quantify the relative degree to which parasite communities, as a whole, are characterized by antagonistic vs facultative interactions within each species, we conducted a C-score analysis using the R package `bipartite` (Dorman et al., 2009). The analysis compares the host-parasite presence-absence matrix for each host species to a null distribution and calculates a C-score. C-score values range from 0 to 1, with higher values indicating greater degrees of antagonism between parasites. Intermediate values (~0.5) indicate random co-occurrence patterns, and values close to 0 indicate a more aggregated parasite community characterized by facultative interactions (Gotelli 2000; Gotelli and Ulrich, 2012). We then compared mean C-score values between host groups using a one-way ANOVA.

RESULTS

We identified 8 parasite species infecting Plethodon salamanders in this locality. Specifically, 1 ciliated protozoan (*Cepedietta michiganensis* (Woodhead 1928)), 1 digenean (*Brachycoelium hospitale* (Stafford, 1900; Cheng 1959; Cheng 1960)), 1 cestode (*Crepidobothrium cryptobranchi* (La Rue 1914; Brooks 1978)), 5 nematodes (*Cosmocercoides variabilis* (Harwood 1930)*, Batracholandros salamandrae* (Schad, 1960)*, Oswaldocruzia pipiens* (Walton 1929; Baker 1977), *Amphibiocapillaria tritonispunctati* (Moravec 1986; Moravec and Huffman, 2000)), and *Porrocaecum sp* larvae (Railliet & Henry, 1912) in sacs found in the body cavities of some individuals.

We found that the gastrointestinal parasites infecting *Plethodon* salamanders at this site formed a coherent metacommunity with significant parasite turnover between infected individuals (Table 2.1). Results from our boosted regression analysis revealed no clear relationship to a latent gradient tied to any host or environmental traits that we measured (R^2=0.05; RMSE = 0.52) indicating substantial overlap between the parasite infracommunities of each host species (Figure 2.1A). Indeed, six of the eight parasite species we observed were found in all three host groups, and the only parasites not found in all three hosts, *Porrocaecum sp larvae* and *A. tritonispunctati* were among the most rare (Figure 2.1B.).

We detected significant negative co-infection probabilities between the ciliated protozoan *C. michiganensis*, and four other helminth taxa: *C. variabilis, C. cryptobranchi, B. salamandrae,* and *B. hospitale*. One of these interactions (*C.*

michiganensis and *C. variabilis*) is likely to include competition for space as both parasite species were observed infecting the large intestine. We also detected a significant positive association between *O. pipiens* and *Porrocaecum spp. larvae* (Figure 2.2; Table S2.1).

When comparing the degree to which each parasite community was characterized by more antagonistic interactions between parasites vs facultative or random interactions, we found that *P. shermani* and hybrids were host to parasite communities with near random frequencies of pairwise parasite interactions (C-score = 0.46; 95%CI = 0.38 - 0.54) and (C-score = 0.58; 95%CI = 0.52 - 0.64) respectively. In *P. teyahalee*, antagonistic interactions were more common, reflecting a highly segregated parasite community with greater strength of parasite interspecific competition than *P. shermani* and hybrids (C-score = 0.86; 95%CI = 0.72 - 0.99) (Table S2.2; Figure S2.1).

Relatedly, we found that parasite species richness, i.e., the number of parasite taxa infecting a given host individual, varied significantly between host groups (ANOVA, $F(2,150) = 9.63$, $p < .001$). Tukey's Test revealed that *P. teyahalee* was host to parasite infracommunities that were more species-poor than those infecting hybrids or *P. shermani*. Richness was greatest in *P. shermani*, and hybrids were host to infracommunities of intermediate richness (Table S2.3, Figure S2.2).

We observed that many individuals with *C. michiganensis* infections also had GI tracts void of prey items and other parasites. There were no differences in the likelihood of either species having empty GI tracts overall (ANOVA, $F(2,36) = 0.17$, $p = 0.85$), but *P. teyahalee* with *C. michiganensis* infections were more likely to have empty GI tracts than uninfected *P. teyahalee* ($F(10,111) = -0.31$, $p = 0.04$). Among infected *P. teyahalee*, the probability of having an empty GI tract scaled positively with *C. michiganensis* infection intensity ($F(10,111) = -0.004$, $p < .001$) (Figure 2.3).

DISCUSSION

Metacommunity analysis of *Plethodon* salamanders from across the basin revealed host-parasite interactions that result in non-random parasite community structure, but parasite community composition was difficult to predict in terms of host genetic status or environmental features. These results indicate that *Plethodon* salamanders at this locality are host to a shared parasite species pool of generalist gastrointestinal parasites with strong host specificity playing a minimal, if any, role in the structuring of parasite communities. The detection of significant, non-random metacommunity structure paired with the apparent absence of a shared latent gradient upon which the metacommunity is structured implicates interspecific interactions between parasites as a potential structuring mechanism behind the parasite communities infecting *Plethodon* salamanders at this locality.

The ciliated protozoan parasite, *C. michiganensis*, stood out for two reasons. First, its patterns of occurrence in host individuals formed negative pairwise associations with several GI helminths, and the bulk of negative interspecific interactions between parasites was greater by far in *P. teyahalee*, whose individual hosts exhibited a more 'checkerboard' patterning across parasite species. Second, infection of *P. teyahalee* individuals with this parasite, especially high intensity infection, was associated with GI tracts that were completely void of prey items or other parasites. This pattern suggests a potential mechanism by which *C. michiganensis* could compete with other parasites, in addition to the direct competition for space in the large intestine, likely governing competition with *C. variabilis*. If heavy *C. michiganensis* infections result in one of several outcomes that reduce prey acquisition such as reduced predation success, lethargy, reductions in surface activity, or appetite suppression, then the presence of parasites that are trophically or environmentally transmitted during surface activity should decrease through disease induced reductions in exposure alone, resulting in detectible antagonistic interactions with several other parasite taxa and the observed suppression of parasite species richness in *P. teyahalee*. We observed *C. michiganensis* infecting the gall bladder of some individuals, suggesting that infection virulence related to interference of digestive processes are a likely mechanism behind reductions in prey acquisition (Oldham-Ott and Gilloteaux, 1997). Although costly, infections likely impact host fitness minimally. These host species have evolved life histories tied closely to the habitat conditions in which they occur. Consequently, *P. teyahalee* has adapted to greater periods of time between foraging events by increasing age and size at sexual maturity (Feder 1983; Carter 2023). It is likely that *P. shermani* experiences greater costs associated with parasitism, as other work in amphibians found that species that develop more quickly and metamorphose at smaller body sizes were more likely to contract parasite infections as well as experience pathology associated with infection (Johnson et al., 2012).

Plethodon teyahalee is demographically weaker than *P. shermani* and other montane *Plethodon* in the region, and studies have demonstrated antagonism between *P. teyahalee* and both *P. shermani* and *P. jordani,* with *P. teyahalee* population growth only observed in the absence of *P. shermani* (Hairston 1980). Still, there is evidence that the hybrid zone between *P. teyahalee* and *P. shermani* is under active selection, with bias towards introgression of alleles characteristic of *P. teyahalee* (Carter 2023). Here, we propose parasite-mediated enemy release as a potential mechanism influencing the relative fitness of these host species. Parasite coinfections are generally considered to be more costly than infections with a single parasite, and infections with less virulent parasites like *C. michiganensis* might interact with existing host habitat heterogeneity to idiosyncratically influence the frequency of exposure to parasites by each host species, amounting to the net effects of parasite-mediated enemy release.

CONCLUSION

Our analyses revealed that interspecific interactions between shared parasites were more reflective of parasite community dynamics than direct measures of host or environmental traits. Differences in parasite pressure between species were largely associated with infections with a ciliated protozoan, *C. michiganensis*, which appears to reduce host foraging activity in *P. teyahalee* and thereby exposure to other parasite species. Future work should measure factors associated with parasite abundance for individual parasite species to gain further insight into the host-environment interactions shaping parasitism likely masked by interspecific interactions at the parasite community scale.

TABLES

Table 2.1: Results of analysis of host-parasite occupancy matrix via the elements of metacommunity structure (EMS). Coherence represents non-random occupancy patterns between hosts and parasites, turnover represents parasite species replacement between individuals, and boundary clumping measures the degree to which species boundaries are coincidental or idiosyncratic. The “stat” column reports the test statistic for each measure, and p-value indicates statistical significance.

Test	**Stat**	**p-value**
Coherence	z-score = 7.97	>.001
Turnover	z-score = 1.77	>.01
Boundary clumping	Morisita’s index = 0	0.25

FIGURES

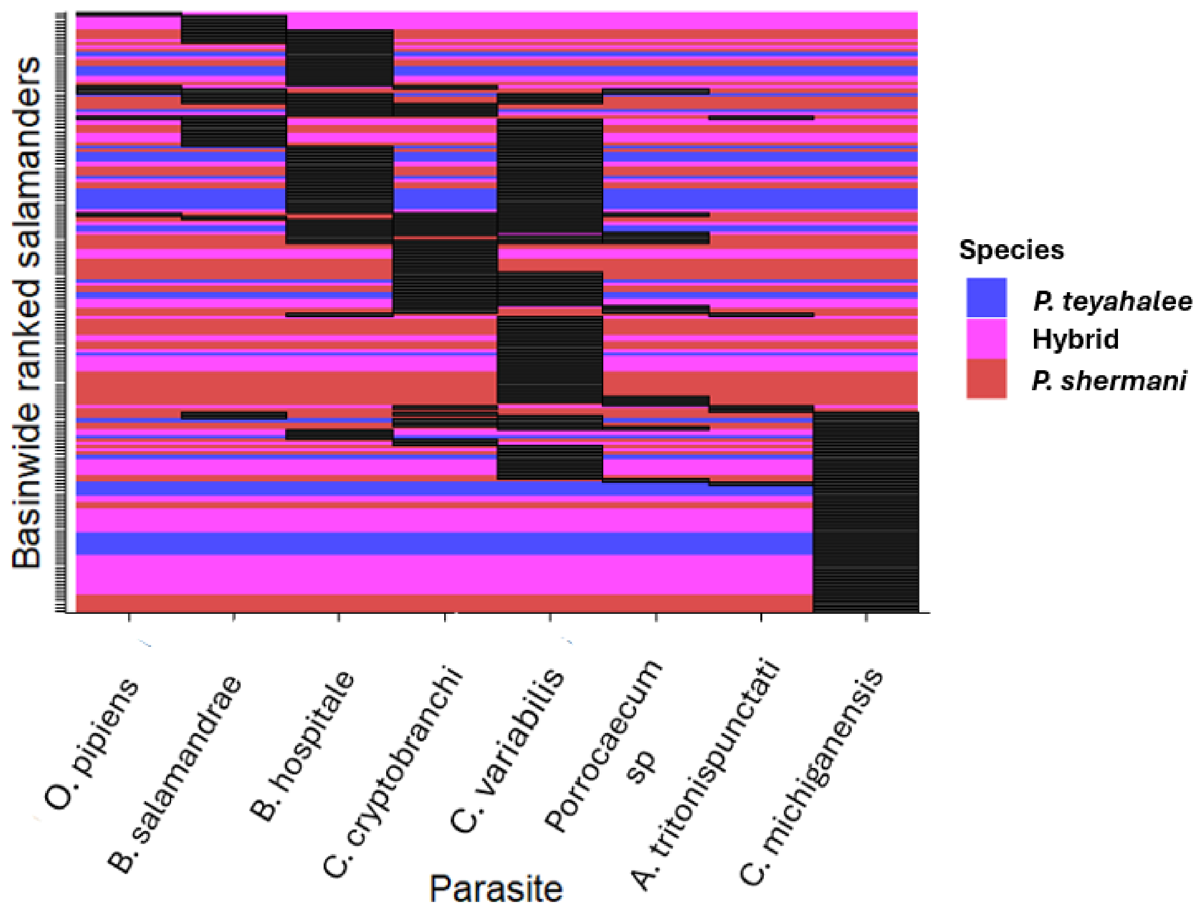

Fig. 2.1a) Parasite distributions (columns) among host (rows) sampled. A black rectangle within the matrix indicates presence of that particular parasite species in the corresponding host. Hosts on the y-axis are ordered according to parasite infection similarity and parasites on the x-axis are ordered based on similarity in infected hosts. Row color indicates the host species for that observation.

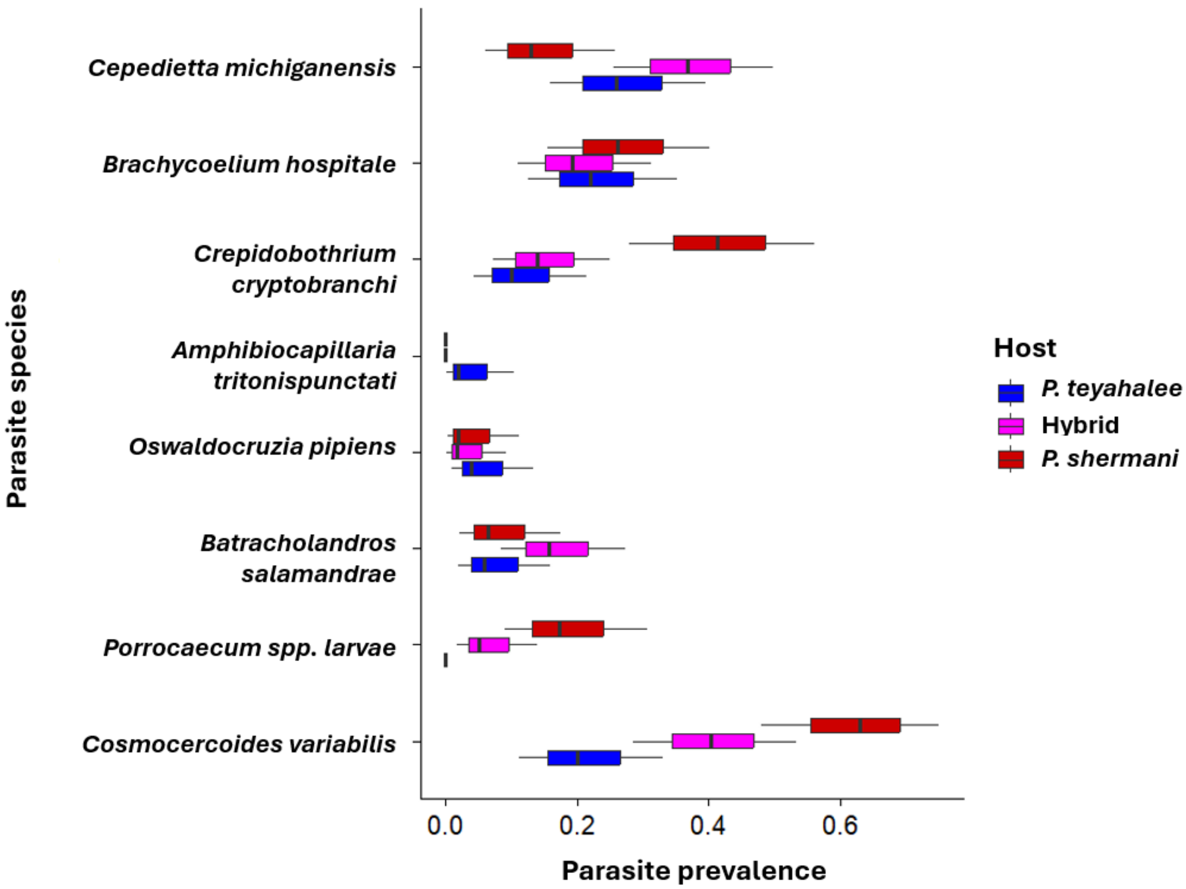

Fig. 2.1b) Box and whisker plot of parasite infection prevalence for all parasites found in Plethodon sp. at Coweeta Hydrological Station. Box color corresponds to host species: *P. teyahalee* (blue), *P. shermani* (red), and hybrids (purple). Centre lines of boxes correspond to the median value; lower and upper hinges represent first and third quartiles (25th and 75th percentiles), respectively. Whiskers extend to the minimum and maximum values no farther than 1.5 × IQR from the lower and upper hinges, respectively (IQR = interquartile range, the distance between hinges).

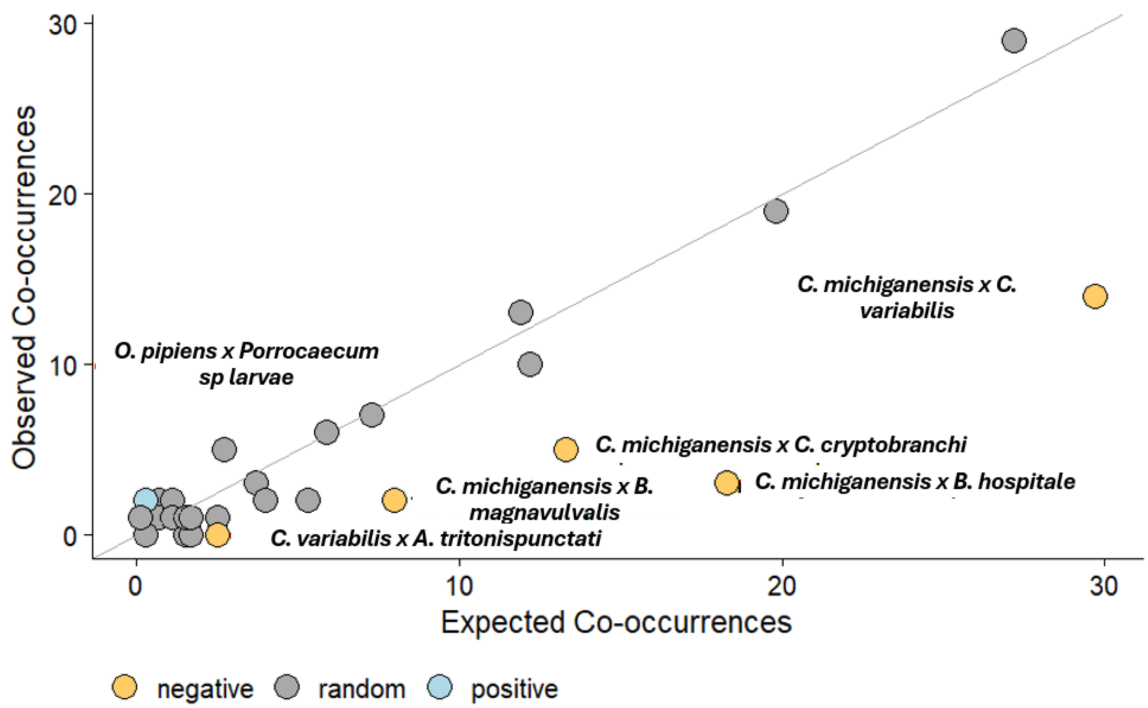

Fig. 2.2: Scatterplot showing the observed number of parasite pair co-occurrences across hosts (y-axis) plotted against the expected number of co-occurrences (x-axis). Points (gray) close to the 1:1 line represent random parasite pairwise associations. Points above and below the line are pairwise associations with statistically significantly higher (blue) or lower (yellow) observations compared to expectations.

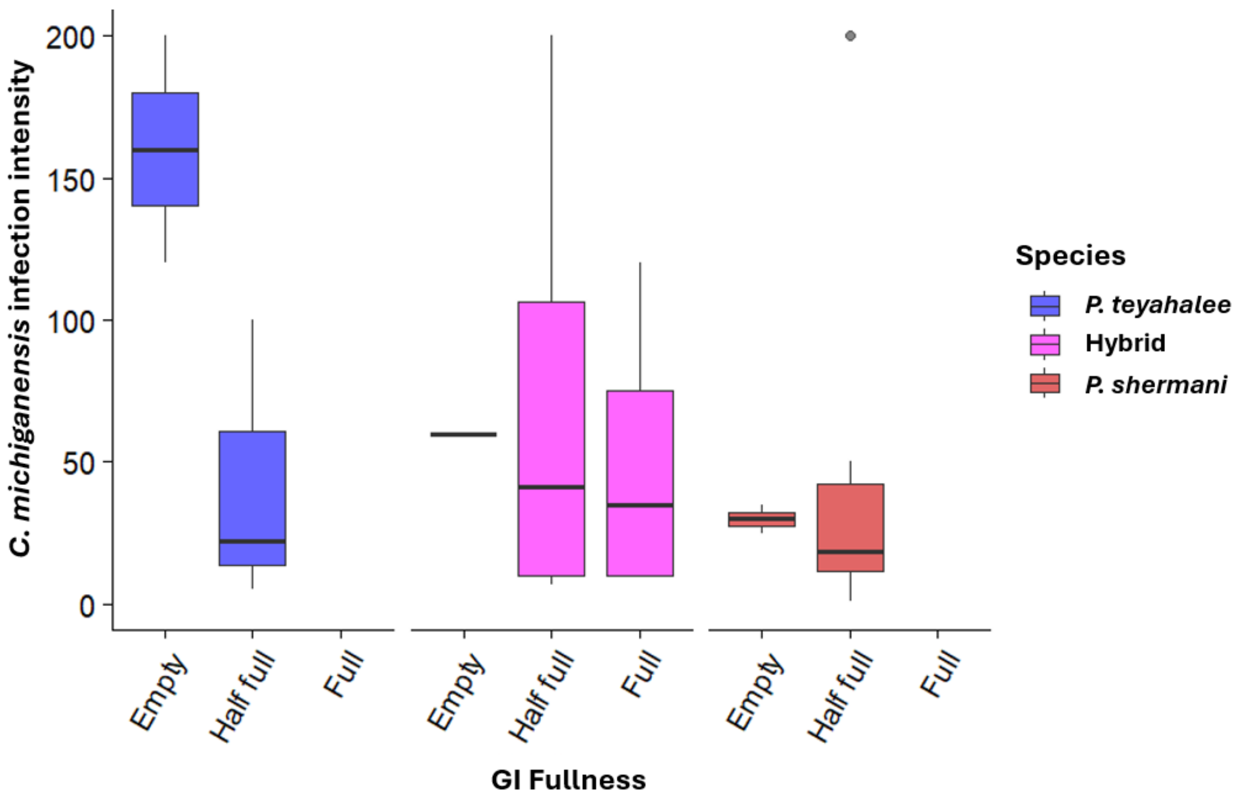

Fig. 2.3: A box and whisker plot of intensity of *C. michiganensis* infections across host species as a function of GI fullness, measured as Empty, Half full, and Full. Box color corresponds to host species: *P. teyahalee* (blue), *P. shermani* (red), and hybrids (purple). Centre lines of boxes correspond to the median value; lower and upper hinges represent first and third quartiles (25th and 75th percentiles), respectively. Whiskers extend to the minimum and maximum values no farther than 1.5 × IQR from the lower and upper hinges, respectively (IQR = interquartile range, the distance between hinges).

CHAPTER 3

HOST INTROGRESSION AND BODY SIZE PREDICT PARASITE INFECTION IN PRESERVED SPECIMENS OF TWO HYBRIDIZING SALAMANDERS[2]

[2]Odom, T.L., Altizer, S.A., Park, AW. To be submitted to *Journal of Parasitology*.

ABSTRACT

Animal hybrid zones reflect complex interactions between host genetics and host environment and can influence adaptive potential and species interactions in wildlife populations. Parasite sharing between parent taxa and hybrids can influence competitive interactions between hybridizing host species and shape spatial distributions of hybrid zones through parasite mediated competition. In this study, we leveraged natural history collections to investigate the distribution of 4 endoparasites in two competing salamanders, *Plethodon shermani* and *Plethodon teyahelee,* and their hybrids across Coweeta Hydrological Station, Otto, NC, USA to determine if these host species experience disparate burdens of shared parasites. We used generalized additive models (GAMs) to identify factors related to the prevalence, infection intensity, and parasite species richness of gastrointestinal parasites infecting salamanders at this locality. We found that infection prevalence for 3 out of 4 parasites and parasite species richness was greatest in hybrids and *P. shermani* (HI values closer to 1), and this pattern was correlated with rainfall during the month of capture. Taken together, results here indicate that parasites could influence host population dynamics in a manner favoring *P. teyahalee,* associated with its adaptations to diminished surface activity and likely enemy release from parasites more commonly seen in *P. shermani* and hybrids.

INTRODUCTION

When distinct lineages can trade genetic material, hybridization works in some cases as an alternative solution for bolstering the adaptive potential of a system. A study of skin antimicrobial peptides (AMPs) in hybrid water frogs (P*elophylax esculentus*) infected with the devastatingly pathogenic chytrid fungus, *Batrachochytrium* *dendrobatidids* (Bd), found that hybrids released greater quantities of AMPs and benefited from increased antifungal activity relative to both parent taxa, *P. lessonae* and *P. ridibundus* (Daum et al., 2012). The success of hybrids relative to parent taxa in resisting or tolerating shared pathogens has been shown to influence the maintenance or degradation of species barriers across several host groups including birds, fish, mammals, and amphibians (Jackson and Tinsley 2003; Joly et al., 2008; Baird and de Bellocq, 2019; Theodosopoulos et al., 2019; Roth et al., 2021; Balard and Heitlinger, 2022). There are also cases where host introgression is far less important than environmental traits in structuring endosymbiont communities (Grieneisen et al., 2019; Rice et al., 2021). When species hybridize in areas of spatial overlap, the potential arises for heterogeneity in both habitat suitability and immune diversity modified by introgression to result in disparate disease burdens and ensuing costs to fitness between hybridizing species.

This may be especially true in climate-sensitive amphibian hosts, whose distributions are reliant on environmental features such as temperature and moisture availability that are increasingly unstable (Semlitsch et al., 2009; Garcia et al., 2020). In terrestrial direct-developing salamanders like *Plethodon shermani* and *Plethodon teyahalee,* two lungless salamander species found in the Southern Appalachian Mountains, both temperature variation and moisture availability are directly correlated with host fitness and frequency of surface activity and are destabilizing under climate change (Feder 1983; Gifford and Kozak, 2012; Caldwell et al., 2016). *Plethodon shermani* occupies higher elevation sites characterized by lower temperatures and greater rainfall on average. *P. teyahalee* occupies lower elevation sites along the same slopes as *P. shermani* that are characterized by warmer, drier habitat conditions outside of microhabitats near streams. Intermediate elevations are occupied by hybrids of *P. teyahalee* and *P. shermani* (Hairston et al., 1992).

Decades of legacy work in this hybridizing salamander system at Coweeta Hydrological Laboratory, a long-term ecological research station in Otto, North Carolina, USA have sought to disentangle the eco-evolutionary factors responsible for the contemporary spatial extent of these species and the stability of this hybrid zone, along with other tension zones between *Plethodon spp*. in the region (Chatfield et al., 2010; Ryan et al., 2024) and predict its future dynamics with mixed consensus (Hairston 1980; Jaeger et al., 1998). Some studies have found that the hybrid zone at Coweeta is evolutionarily active, with introgression of *P. teyahalee* alleles occurring at greater rates than *P. shermani* (Hairston et al., 1992; Walls 2009; Carter et al., 2023), but others have suggested that the hybrid zone is stable (Lowe 2016).

These species experience distinct ecological conditions that shape their relative frequency and duration of exposure to both trophically and environmentally transmitted parasites, but parasitism has not yet been considered as a potential mechanism influencing hybrid zone extent. Recent work in the hybrid zone at this locality has found that *P. shermani, P. teyahalee,* and hybrids experience different realized rates of surface activity, largely attributable to disparity in rainfall levels experienced by each host group (Lowe 2016; Carter 2023). Temperature and moisture availability have been shown to influence the diversity and abundance of parasitic diseases infecting amphibian communities structured along elevational gradients (Mihaljevic et al., 2018), so it is possible that the conditions less favorable for surface activity found at dry, lower elevation sites could result in reductions in parasitism in *P. teyahalee* relative to hybrids and *P. shermani* due to simultaneous reductions in parasite habitat suitability and host exposure, particularly if *P. teyahalee's* greater body size confers resilience to extended periods of time between foraging events. These disparate distributions of disease could confer a fitness advantage in *P. teyahalee* and could explain, at least in part, observations of selection bias for *P. teyahalee* alleles (Hairston et al. 1992; Walls 2009; Lowe 2016; Carter et al., 2025).

Here, we leveraged historical climate data and preserved specimens of *P. shermani, P. teyahalee,* and hybrids collected from Coweeta Hydrological Station between 1943 and 2022 to determine if *P. teyahalee, P. shermani,* and hybrids experience different disease burdens attributable to factors such as host genetics (hybrid index), snout-vent length (SVL), and environmental conditions when/where hosts occur. We used observations of gastrointestinal macroparasite infections in a generalized additive model framework to ask 1. How does parasite richness change across host genomic and ecological gradients? And 2. How do host traits, environmental factors, and host-environment interactions influence the prevalence and infection intensity of individual parasite species across *P. shermani, P. teyahalee,* and hybrids? We predicted that rates of parasitism would be highest in hybrids and *P. shermani,* due to their habitats and behavior being more conducive to gastrointestinal parasite transmission. We found that individuals with HI values indicative of *P. shermani* experienced greater prevalence and intensity of parasite infections as well as greater parasite species richness than *P. teyahalee,* with hybrids experiencing intermediate rates of parasitism in most cases. Prevalence and richness patterns varied with relatively recent rainfall, suggesting that moisture availability may influence the distribution and abundance of parasites at this site as well as differential rates of parasite exposure tied to host surface activity.

METHODS

Specimen selection

We dissected 238 salamanders from the Georgia Museum of Natural History and herpetological collections from the Warnell School of Forestry, University of Georgia, USA, with observations ranging from 1946 to 2022. Specimens were selected based on field identification of species, the availability of specific spatiotemporal data including lat/lons of point of collection and date of field collection). Observations were limited to specimens with measurable phenotype scores (red legs and white spots), and those collected between May and October, the time of year in which Southern Appalachian plethodontid salamanders are most surface active (Gade et al., 2020).

Calculation of Genomic Hybrid Index

Historical investigations of the extent of hybrid zones in this system have relied on categorical scoring of phenotypic traits attributable to either *P. shermani* or *P. teyahalee,* with hybrids presenting phenotypes of both parent taxa to varying degrees (Hairston et al., 1992, Walls 2009). This metric is limited in its usefulness and can underestimate spatial extent of hybrid zones, since individuals can display phenotypes signaling pure ancestry such as only white spots or red legs when they're truly of hybrid genetic descent (Lowe 2016; Carter 2023). Recent work at these sites has generated estimates of a site-wide, continuous hybrid index (HI) between *P. shermani* and *P. teyahalee,* ranging from zero to one, with values close to zero indicating individuals of *P. teyahalee* ancestry, intermediate values indicating hybrid individuals, and values close one indicating individuals of *P. shermani* ancestry (Baird et al., 2012; Carter 2023). We used generalized linear models (GLMs) to calculate the HI of preserved museum specimens from Coweeta, incorporating the covariates identified in Carter (2023) as being predictive of *Plethodon* HI, consisting of phenotype scores reflecting the intensity and extent of *P. shermani* traits (red legs), *P. teyahalee* traits (white spots), and 30 year normals for maximum vapor pressure deficit (hPa) at site of collection.

Parasite collection

A single incision spanning from the thorax to the cloaca was made using a scalpel and other dissection tools. Following dissection, the GI tract of each salamander was removed by severing the terminal ends of the GI tract from the esophagus and cloaca respectively. Following excision from the salamander host, each GI tract was elongated in a petri dish containing 2 ml of 75% EtOH. A single incision was made along the length of the GI tract, being sure to only open one side of the GI tract to prevent damaging intact helminths in the gut. Using the end of a microscope slide, the inside of the GI tract was gently scraped to dislodge helminths embedded in the lacteals and villi of the gut while minimizing damage to diagnostic parasite features (Galbreath et al., 2019). The contents of each GI tract were sorted under a dissecting microscope, separating the worms from each salamander based on their higher organization (nematodes, trematodes, and cestodes).

Parasite identification and preservation

For morphological identification of cestodes and digeneans, the internal structures of each worm were stained using Simichon's Aceto-Carmine. Next, worms were moved from the stain to an acid-EtOH bath for regressive staining, and trematodes and cestodes were subjected to a gradient alcohol dehydration series. Dehydrated worms were then placed in xylene to clear the tegument enabling measurement and differentiation of internal structures such as testes, ovaries, and other identifying features. Parasites were moved from xylene and mounted on microscope slides using

Canada balsam suspended in xylene and placed on a slide warmer (Premier Model XH 2001) at 40C for 3 days or until the Canada balsam was fully dry.

Parasites identified as nematodes were placed in a lactophenol-alanine blue staining and clearing solution for ~20 minutes, with the time spent in the stain varying with the size of each worm. Following staining and clearing, nematodes were placed on a temporary wet mount for identification before storage in glycerine-alcohol. Parasites were identified to the greatest taxonomic resolution using dichotomous keys, parasite species descriptions, and published parasite assemblages for reference, with newer keys and taxonomic classifications taking precedence (Rankin 1937; Yamaguti 1961; Gibson 2002).

Environmental Variables

Elevation, temperature, and rainfall are all tightly confounded in this wet, montane system, and the greatest predictor of salamander surface activity is recent rainfall in the immediate days prior to observation (Gade et al., 2020; Carter 2023). *Plethodon spp.* can spend as much as 80% of the year underground in burrows (Carter 2023), so rainfall in the months leading up to capture may not be as predictive of parasite presence and abundance compared to similar studies of, for example, ungulates in a semi-arid savannah (Shearer and Ezenwa, 2020). Consequently, we used rainfall at the site of collection during month and year of capture, as rainfall shapes both host and parasite biology and has been associated with parasite prevalence in host-parasite systems with smaller host species utilizing comparable prey communities (Odom et al., 2025). All environmental variables, including the 30 year normals for vapor pressure deficit (VPD) used to calculate HI, were gathered using the PRISM (Parameter-elevation Relationships on Independent Slopes Model) climate database, gridded to 800 m.

Generalized Additive Models (GAMs)

We were interested in measuring the relative influence of host introgression and host environment on parasite infection measures reflective of disease distributions in populations of hosts: specifically, infection prevalence, infection intensity, and parasite species richness. We constructed 31 generalized additive models (GAMs) incorporating all possible combinations of variables included in this study as well as interactions between them consisting of precipitation (in) for the month and year of specimen collection, snout-vent length (SVL), hybrid index (HI). We screened GAMs by iteratively dropping covariates and selecting models based on model fit via comparison of AIC and REML with the R package `mgcv` (Wood, 2011) (Table S3.1-S3.3). We used Poisson GAMs with a log link function to assess parasite species richness as well as parasite infection intensity, including only infected individuals for assessment of infection intensity (Bush et al., 1997). To measure predictors of parasite presence/absence within a host, we used binomial GAMs with a logit link function. All GAMs were fit using default smoothing parameters, and model covariates were standardized prior to analysis.

RESULTS

In all, we dissected 238 salamanders with sample HI values ranging from 0.005 to 0.999 (Figure S1). We identified 8 parasite species infecting Plethodon salamanders in this locality. Specifically, 1 ciliated protozoan (*Cepedietta michiganensis* (Woodhead 1928)), 1 digenean (*Brachycoelium hospitale* (Stafford, 1900; Cheng 1959; Cheng 1960)), 1 cestode (*Crepidobothrium cryptobranchi* (La Rue 1914; Brooks 1978)), 5 nematodes (*Cosmocercoides variabilis* (Harwood 1930)*, Batracholandros salamandrae* (Schad, 1960)*, Oswaldocruzia pipiens* (Walton 1929; Baker 1977), *Amphibiocapillaria tritonispunctati* (Moravec 1986; Moravec and Huffman, 2000)), and *Porrocaecum sp* larvae (Railliet & Henry, 1912) in sacs found in the body cavities of some individuals. Parasites detected here were largely environmentally transmitted with no intermediate hosts, although two taxa, the tapeworm *Crepidobothrium cryptobranchi* and the digenean *Brachycoelium hospitale,* utilize planktonic crustaceans and terrestrial mollusks as intermediate hosts, respectively (Cheng 1960; Robello et al., 2023). The nematode, *Cosmocercoides variabilis*, was the most prevalent parasite, infecting ~40.5% of hosts, and the nematode *Amphibiocapillaria tritonispunctati* was the most rare, infecting only ~0.7% of hosts (Figure 3.1). As noted in Chapter 2, nearly all parasites have been observed to cause at least some infection in *P. shermani*, *P. teyahalee* and their hybrids, except for *A. tritonispunctati*, which was only observed to infect *P. teyahalee*, and *Porrocaecum spp*. larvae, which was not observed to infect *P. teyahalee*.

Parasite prevalence

The best model for *C. variabilis* infection prevalence identified HI, rainfall, and an interaction between HI and rainfall as being significant predictors, while controlling for SVL (Table 3.1). Infection prevalence was positively associated with both HI and SVL, but tended to decrease when monthly rainfall values were greater than 5 inches. This relationship was most apparent in individuals with HI less than 0.25 (Figure 3.2). *B. hospitale* infection prevalence was more associated with SVL and rainfall than HI (Table 3.1), with intermediate rainfall values containing the greatest prevalence. HI was not a significant predictor of *B. hospitale* infection prevalence. The presence of *C. cryptobranchi* was most associated with HI values greater than 0.5, while controlling for non-significant trends of increased prevalence in smaller individuals at lower HI values (Table 3.1). *C. michiganensis* infection prevalence did not vary significantly with HI. Instead, the best GAM for *C. michiganensis* infection prevalence found that infections were more common in larger individuals captured during lower rainfall months (Table 3.1). Parasite co-infections were less common in salamanders with HI values closer to zero *(P. teyahalee),* and parasite species richness increased linearly with HI as well as SVL (Table 3.2; Figure 3.3).

Parasite infection intensity

The best GAM for *C. variabilis* infection intensity included HI, SVL, rainfall, and an interaction between HI and SVL. There was a non-linear relationship between *C. variabilis* infection intensity and HI, where individuals with higher HI values generally had more worms per infected host with less variation in infection intensity than seen in individuals with lower HI values. Infection intensities were lowest in salamanders with HI values closer to 0.5, and were positively associated with host body size (Table 3.3; Figure 3.4A). *B. hospitale* infection intensity varied significantly with HI and SVL, with intermediate and higher HI values corresponding to more intense infections (Table 3.3). Individuals with smaller body sizes (SVL) tended to have less intense infections with *B. hospitale* than larger individuals (Figure 3.4B). The best model for *C. cryptobranchi* infection intensity revealed that intensity increased non-linearly with SVL, with no significant difference in infection intensity across HI values when *C. cryptobranchi* was present (Table 3.3; Figure 3.4C). *C. michiganensis* infection intensity was best predicted by the additive effects of HI, SVL, and rainfall, along with interactions between HI and SVL. Interactions between HI and rainfall were also included in the best model but showed no clear trends (Table 3.3). We found that *C. michagenensis* intensities were greatest in individuals with lower HI values and showed a concave relationship with SVL where infection intensities decreased at intermediate SVL values and increased dramatically in individuals with SVL greater than ~ 75 mm (Table 3.3; Figure 3.4D).

DISCUSSION

Our study investigates the degree to which host factors such as body size and degree of introgression along with environmental conditions like rainfall influence the distribution of individual parasite species among *P. teyahalee,* hybrids, and *P. shermani* in the Coweeta Basin. Infections with *C. variabilis* were more prevalent and more intense on average in individuals with intermediate or higher HI values than those with lower ones. These associations likely reflect differences in habitat suitability for *C. variabilis* in sites occupied by salamanders with greater proportions of *P. shermani* alleles (higher HI values), as abundance of *C. variabilis,* as well as other *Cosmocercoides spp.* in amphibian communities occupying elevational gradients, is positively associated with moisture availability and host density (Hossack et al., 2013). *P. shermani* experiences greater habitat moisture on average, outside of *P. teyahalee* occupying microhabitats near streams at lower elevations, as well as greater host population densities at their upper elevational extents compared to hybrids and *P. teyahalee* (Hocking et al., 2021). In general, host density and the frequency of contact with infectious parasite stages are positively correlated with abundance of macroparasites (Hudson et al., 1992), which would partly explain the patterns seen in P. *shermani*. Furthermore, the sky-island like ecology of the Southern Appalachians has facilitated the long-term isolation of *P. shermani* populations in glacial refugia, compared to more connected populations of *P. teyahalee* (Weisrock et al., 2005; Gade et al., 2020). This evolutionary confinement of hosts with more connected populations of generalist parasites, further compounded by the features of *P. shermani* ecology that inherently increase macroparasite abundance, suggest that *P. shermani* have evolved tolerance to infections to *C. variabilis,* particularly since infections with this parasite in lungless salamanders are relocated to the large intestine, a site not typically associated with virulent macroparasite infections in amphibians. Land use changes known to disturb habitat hydrology, like fire severity and frequency, have also been tied to *C. variabilis* abundance (Hossack et al., 2013), and a major fire occurred at high elevation *P. shermani* sites at Coweeta Hydrological Laboratory in 2016. The drop in infection prevalence observed during months with extreme rainfall could reflect disturbance regimes like flooding that would result in the washing out of infective stages in the soil. Future studies should investigate interactions between the abundance of rainfall, slope steepness, soil type, and vegetation cover where hosts occur and their impacts on the prevalence and intensity of soil-transmitted helminths like *C. variabilis*.

Brachycoelium hospitale infection prevalence was more associated with SVL and rainfall than HI (Table 3.1), with salamanders collected during periods of intermediate rainfall being more frequently infected. HI was not a significant predictor of *B. hospitale* infection prevalence, but was predictive of infection intensity, with individuals of intermediate or high HI values hosting the most intense infections. Regardless of HI, individuals with smaller body sizes (SVL) tended to have less intense infections with *B. hospitale* than larger individuals (Figure 3.4B). Macroparasite infection intensity is often tied to host body size due to greater host resources (space, surface area of GI tract) afforded by larger hosts. Bigger salamanders consume a larger volume of prey during surface activity, food consumption is tied to the potential for parasite exposure, and *B. hospitale* is transmitted via the consumption of infected land snails of the species *Ventridens ligera* (Cheng 1960). Bigger salamanders can eat bigger intermediate hosts which in turn support a greater number of infectious larvae than smaller ones, which could partially explain the positive relationship between SVL and intensity in *B. hospitale*.

The presence of *C. cryptobranchi* was most associated with HI values greater than 0.5 (Table 1; Figure S2). These patterns could represent differences in prey community accessibility between *P. teyahalee* and *P. shermani*. The genus *Crepidobothrium* has primarily evolved to infect fish and reptiles (Magath 1929), but *C. cryptobranchi* has adapted its life cycle to infect terrestrial, direct developing salamanders by using terrestrial copepods as intermediate hosts, although the exact copepod species in terrestrial life cycles is currently unknown (de Luna et al., 2023). Terrestrial copepods in forest habitats are confined to wet soils with damp leaf litter, conditions most common in habitats occupied by hybrids and *P. shermani*. The negative relationship between body size and infection intensity further points to heterogeneity in the frequency and identity of prey consumed as potentially important for transmission, as terrestrial copepods are small and more likely to be utilized by smaller host individuals as a prey source.

Cepedietta michiganensis infection prevalence did not vary significantly with HI. Instead, the best GAM for *C. michiganensis* infection prevalence found that infections were more common in larger individuals captured during lower rainfall months (Table 3.1). These results suggest that host genetics may be less important than habitat conditions where hosts occur for determining the prevalence of *C. michiganensis*. We did, however, detect a non-linear, negative relationship between HI and infection intensity as well as a clear, concave relationship between infection intensity and host SVL where intensity decreased rapidly as SVL increased, then increased rapidly again as individuals became very large (Figure 3.4D). *C. michiganensis* transmission occurs through the consumption of eggs passed in salamander feces, but the rubbing of the snout in fecal matter of other conspecifics, a behavior that likely happens in burrows as well as during surface activity, may facilitate transmission even in the absence of intentional foraging on behalf of the host. Although not a direct replacement of host age in salamanders (Staub 2016), the relationships observed between SVL and both infection prevalence and intensity could be related to age associated infection dynamics mediated by acquired immunity (Raffel et al., 2009).

We found that parasite species richness generally increased with HI, indicating that hybrids and *P. shermani* experience more frequent parasite infections and higher parasite species richness at the individual host level than *P. teyahalee,* even when accounting for differences in body size (Table 3.2) (Figure 3.2). Notably, the predictors of the presence of a given parasite were not necessarily predictive of infection intensity as seen in other host-parasite systems (Correa et al., 2021), even though for every parasite but *C. michiganensis* each observed worm reflects an independent transmission event, with the absence of within host replication (Woodhead 1928). Individuals with higher HI values (hybrids and *P. shermani*) tended to have greater prevalence of most parasites (Figure 3.1), which could explain in part the positive relationship between HI and parasite species richness, but following infection of a given parasite, SVL was the most common predictor of infection intensity in most cases.

We observed significant trends in parasitism associated with HI, but it is important to note that although heterogeneity in parasite prevalence and intensity between hosts has been used to infer relative host fitness (Theodosopoulos et al., 2019), disparity between hosts in tolerance to parasite infections may blur associations between parasite load and host fitness (Baird and de Bellocq, 2019; Roth et al., 2021). Furthermore, most of the parasites observed here are generalists within amphibians and are observed across several amphibian host systems, both aquatic and terrestrial. *C. variabilis, Oswaldocruzia spp.,* and *C. michiganensis* have all been observed to infect other salamander genera, frogs, and toads, including *Desmognathus* and *Eurycea spp.* in the region, of which both host genera have aquatic larval stages (Rankin 1937; Joy and Bunten 1997; McAllister and Bursey, 2004). We know little of the abundance of these parasites in salamanders sympatric with *Plethodon spp.* at Coweeta Hydrological Station, and the diversity and abundance of available hosts in a community can impact the abundance and spread of parasites (Orlofske et al., 2012; Bielby et al., 2015; McClure et al., 2020). Consequently, inferences about the strength of host-parasite associations made without knowledge of the abundance and suitability of other sympatric hosts should be made with caution.

CONCLUSION

We found that individuals with higher HI (hybrids and *P. shermani*) experienced greater prevalence and intensity of parasite infections as well as more species-rich parasite communities than *P. teyahalee,* with hybrids experiencing intermediate rates of parasitism in most cases. These patterns varied with rainfall, suggesting that moisture availability may influence distribution and abundance of some parasites at this site through mechanisms like reduced parasite exposure associated with hosts in dry conditions or variability in environmental suitability for parasite stages outside of the host. Parasite infections are, by nature, costly to their hosts, and we observed relatively diminished parasitism in *P. teyahalee*. These results suggest that gastrointestinal parasites infecting *Plethodon* at this locality are more abundant in *P. shermani* and hybrids, reinforcing the potential for parasitism to influence the relative competitiveness of each host species. Future work should measure the direct impact of parasitism on components of host fitness to quantify heterogeneity in the distribution and magnitude of infection costs between *P. shermani,* hybrids, and *P. teyahalee,* and identify pathogens most likely to exert selective pressures on the distribution of host alleles.

TABLES

Table 3.1: Results of GAM for infection prevalence with the four most abundant parasites observed in *P. teyahalee, P. shermani,* and hybrids. Terms with "s()" indicate nonlinear relationships, and "ti()" represents a tensor interaction between covariates.

Model	**Terms**	**edf**	**Rf.df**	**Chi.sq**	**p-value**	**R^2**	**Dev. exp**
C. variabilis prevalence	s(HI) + s(SVL) s(Rainfall) + ti(HI*Rainfall)					0.09	9.6%
	s(HI)	1	1	8.052	0.005		
	s(SVL)	1	1	0.966	0.326		
	ti(Rainfall)	2.744	3.44	11.04	0.016		
	ti(HI*Rainfall)	1.128	1.24	4.996	0.026		
B. hosptale prevalence	s(SVL) + s(Rainfall)					0.1	10.6%
	s(SVL)	1.364	1.648	0.779	0.700		
	s(Rainfall)	5.125	6.236	20.571	0.003		
C. cryptobranchi prevalence	s(HI) + ti(HI*SVL)					0.11	18.4%
	s(HI)	2.713	3.346	7.198	0.086		
	ti(HI*SVL)	6.018	7.900	11.713	0.174		
C. michagenensis prevalence	S(SVL) + s(Rainfall) + ti(HI*Rainfall)					0.171	16.9%
	s(SVL)	1.941	2.456	7.276	0.037		
	s(Rainfall)	5.312	6.408	21.771	0.002		
	ti(HI*Rainfall)	1	1	1.358	0.244		

Table 3.2: Results of GAM for parasite species richness. Terms with "s()" indicate nonlinear relationships, and "ti()" represents a tensor interaction between covariates.

Model	Formulae	edf	Ref.df	Chi.sq	p-value	R^2	Dev. exp
Parasite species richness	s(HI) + s(SVL)					0.05	4.59%
	s(HI)	1	1	8.198	0.004		
	s(SVL)	1	1	1.511	0.219		

Table 3.3: Results of GAM for infection prevalence with the four most abundant parasites observed in *P. teyahalee, P. shermani,* and hybrids. Terms with “s()” indicate nonlinear relationships, and “ti()” represents a tensor interaction between covariates.

Model	**Formula**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**R^2**	**Dev. exp**
C. variabilis intensity	s(HI) + s(SVL) + s(Rainfall) + ti(HI*SVL)					0.129	37%
	s(HI)	4.484	5.414	14.466	0.017		
	S(SVL)	4.117	5.034	12.946	0.021		
	s(Rainfall)	2.674	3.308	7.725	0.087		
	ti(HI*SVL)	7.432	9.152	27.306	0.001		
B. hospitale intensity	s(HI) + s(SVL)					0.051	23.5%
	s(HI)	7.135	8.119	43.672	<.001		
	s(SVL)	2.162	2.699	9.165	0.027		
C. cryptobranchi intensity	S(SVL)					0.077	19.6%
	s(SVL)	3.579	4.42	9.677	0.079		
C. michiganensis intensity	s(HI) + s(SVL) + s(Rainfall) + ti(HI,SVL) + ti(HI, Rainfall)					0.605	95.5
	s(HI)	8.823	8.897	372.6	<.001		
	s(SVL)	8.757	8.887	382.6	<.001		
	s(Rainfall)	7.893	8.046	241.8	<.001		
	ti(HI, SVL)	14.793	15.068	758.0	<.001		
	ti(HI, Rainfall)	13.942	14.236	453.0	<.001		

FIGURES

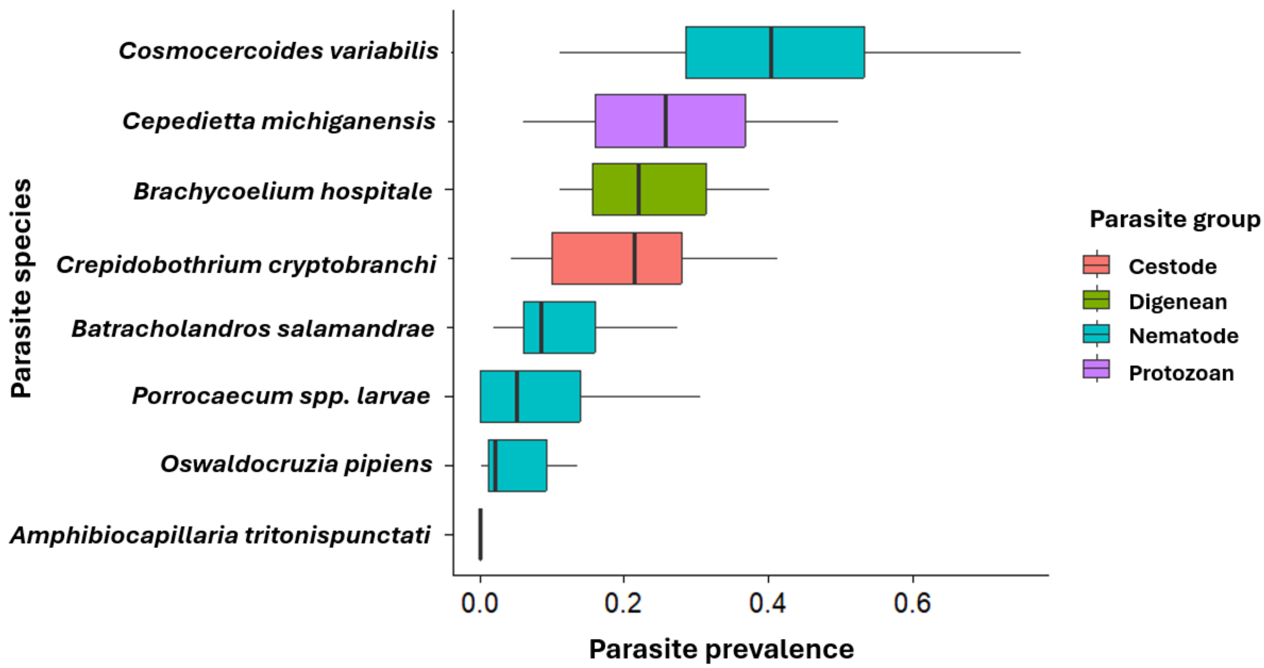

Figure 3.1: Box and whisker plot of parasite infection prevalence. Parasites are ranked from most to least common on the y-axis. Box color corresponds to parasite type. Bars indicate mean values and whiskers indicate 95% CI.

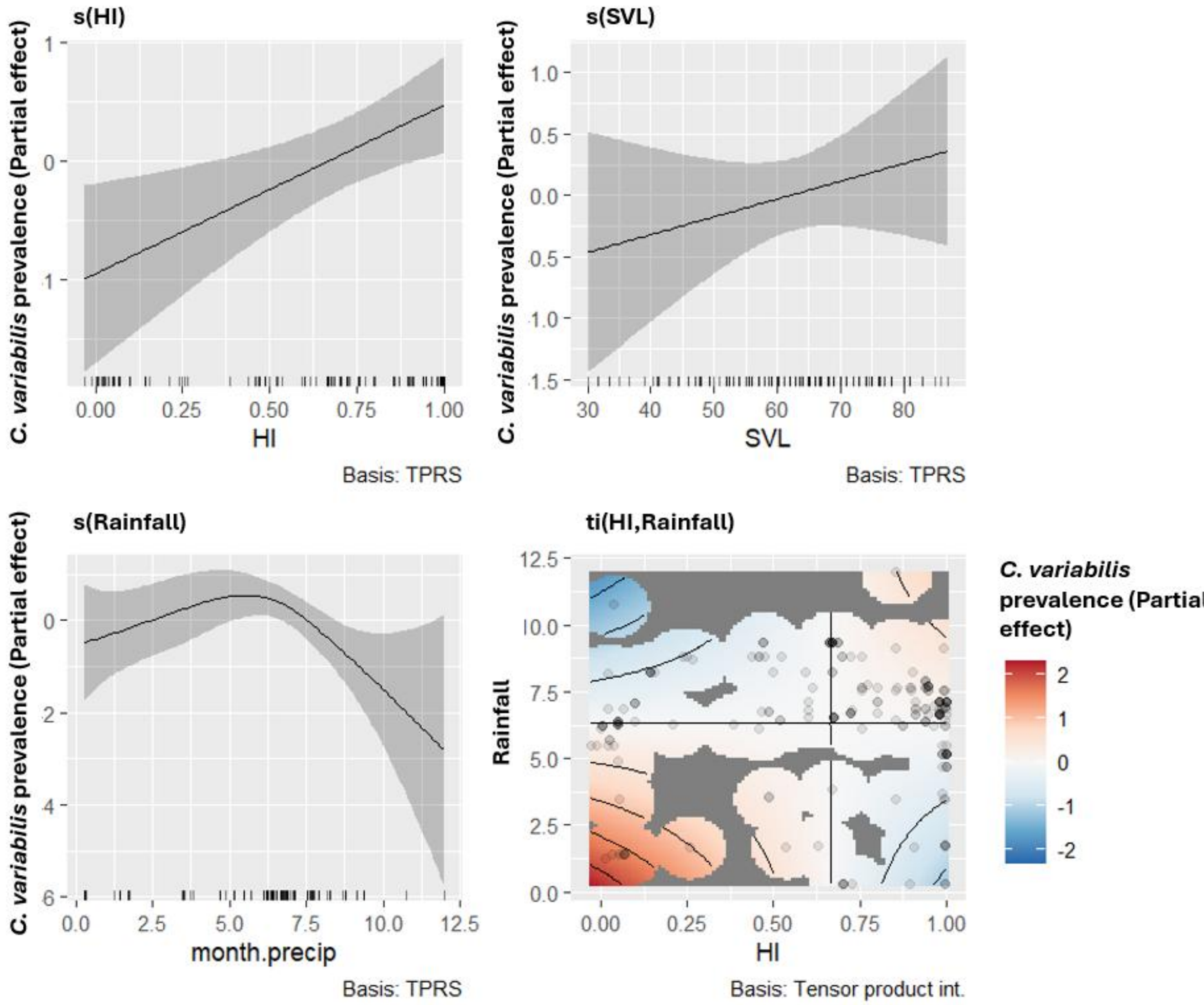

Figure 3.2: Partial dependency plots for generalized additive model of *C. variabilis* prevalence, the most prevalent parasite overall. "s(variable)" indicates that a smoothing parameter has been applied to the respective variable, and "t(variable1, variable2)" indicates an interaction between model covariates. Heatmap colors in interaction plot in bottom right represent the magnitude and directionality of the partial effect of the interaction on *C. variabilis* prevalence as it relates to HI and rainfall in the month of capture. Grey regions represent data deficiency.

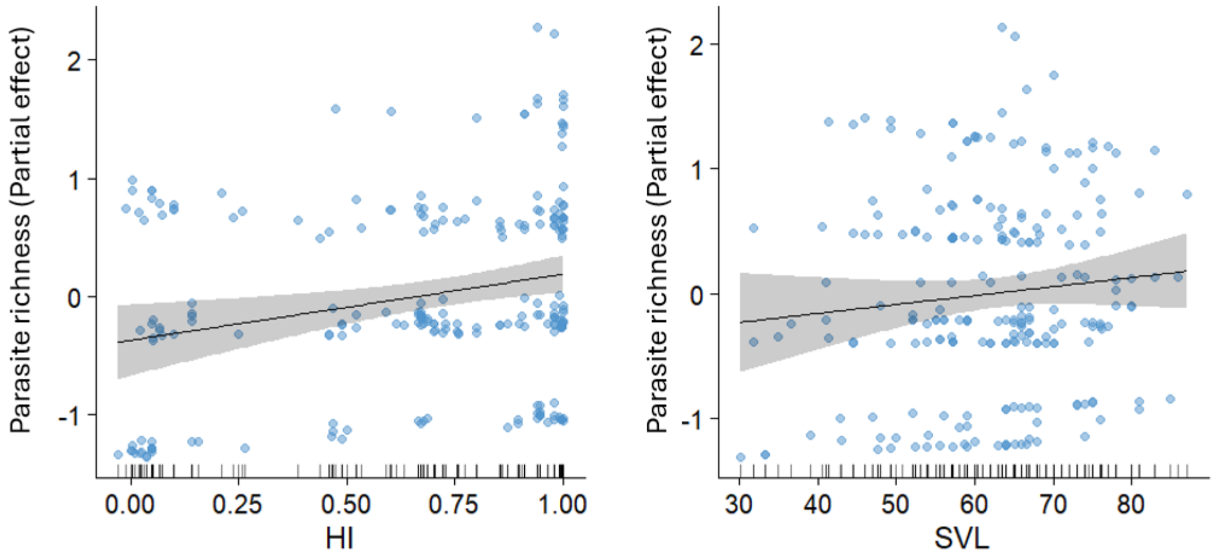

Figure 3.3**:** Partial dependency plots for covariates of the best generalized additive model for parasite species richness.

CHAPTER 4

PARASITE INFECTIONS PREDICT HOST FECUNDITY IN HYBRIDIZING *PLETHODON*[3]

[3]Odom, T.L., Altizer, S.A., Park, AW., Maerz, J.C. To be submitted to *Journal of Herpetology*.

ABSTRACT

Heterogeneity in within-host processes between host species that share parasites can result in disparities in costs of fitness associated with infection that alter competitive outcomes. We measured the impacts of gastrointestinal parasite infections on host fitness, considering both host body condition measured as scaled-mass index (SMI) and measures of energetic investment into reproductive processes such as average follicle diameter in gravid females, number of follicles greater than 0.5 mm, and a model predicted final clutch size. We observed differences between host species in the effects of parasites on the components of host fitness that influence the final clutch size, and that female *P. teyahalee* infected with the ciliated protozoan, *Cepedietta michiganensis,* contained more follicles on average than uninfected females, a pattern not seen in hybrids or *P. shermani*. These differences have the potential to influence relative host population growth rates and implicate parasite mediated competition as a potential mechanism behind observations of bias toward *P. teyahalee* alleles in the *Plethodon* hybrid zone at Coweeta Hydrological Station.

INTRODUCTION

The impact of parasitism on the extent and stability of the hybrid zone between *P. teyahalee* and *P. shermani* at Coweeta Hydrological Station, Otto, NC, USA is poorly understood. Recent work by Carter (2023) found that the hybrid zone between *P. shermani* and *P. teyahalee* was evolutionarily active and may be undergoing adaptive introgression with biased introgression of *P. teyahalee* alleles. The exact mechanism behind the observed selection bias of *P. teyahalee* alleles as well as phenotypic traits attributable to *P. teyahalee* (Hairston et al., 1992; Walls 2009) is still a topic of discussion (Lowe 2016; Carter 2023). So far, parasitism hasn't been considered as a potential mechanism influencing the relative fitness of parent and hybrid taxa in this system.

We found that *P. teyahalee* typically experiences less diverse and less frequent parasite infections than hybrids and *P. shermani* (Chapters 2 & 3). The disparity in disease burdens between host species suggests that the relative fitness of hybrids and parent taxa may be shaped, at least in part, by parasite mediated competition. However, many helminths that infect salamanders in the Southern Appalachians are generalist parasites with relatively low virulence (Goater et al., 1987), so the assumption that heterogeneity in disease distributions indicates parasite mediated competition is incomplete without a demonstrable connection to host fitness. In fact, this mechanism could theoretically operate in systems where competing hosts don't differ in richness, prevalence, or intensity of shared parasites provided the fitness costs experienced by host species differ as a result of infection. However, the most compelling studies demonstrating parasite mediated competition have all included heterogeneity in disease distributions between parasite sharing hosts and measurable differences in relative costs to fitness associated with infections with shared parasites (Greenman and Hudson, 2000; Tompkins et al., 2000; Ortega et al., 2022), with the key evidence required to support the parasite mediated competition hypothesis being disparity in fitness costs that result from infection with shared parasites. Therefore, measures of the relative fitness costs between hosts associated with parasite infections and demonstration of unequal disease distributions across species promise robust support for parasite mediated competition.

A series of hurdles arise when tackling these questions using natural history collections. Although museum specimens can be good tools for measuring parasite diversity and abundance, measuring correlations between parasite infections and components of host fitness like reproductive output and body condition in preserved specimens remains a challenge. Furthermore, point observations of individuals collected over large time spans are difficult to connect to reproductive success or population level mortality rates which could tell us more about the burden of a pathogen on a population of organisms. This is especially difficult in host species like fish and amphibians, that are typically preserved and stored in liquids like formalin and ethanol and who's metrics of body condition conventionally require both snout-vent length (SVL) and wet mass (Warton et al., 2012; Hinderer et al., 2025). Snout-vent length (SVL) and phenotype data can be measured in preserved specimens (Pierson et al., 2020), but unless metadata containing the wet mass of individuals collected in the field is available, true measures of body condition remain elusive.

Inferences of reproductive output may be made through counting the number of follicles in gravid female salamanders, but recent work by Howard and Maerz (2022) found that the number of follicles in the ovaries of female *Plethodon spp*. salamanders at Coweeta Hydrological Station are not necessarily predictive of final clutch size. They collected gravid female *Plethodon* hybrid females from sites encompassing the Coweeta Basin and found that follicle counts were only a component of the several host traits that together predict final clutch size, which include the number of follicles >0.5 mm, average follicle diameter, and SVL. Following their fecundity study, Howard and Maerz (2022) preserved and stored the salamanders they collected along with associated metadata, including measures of host wet mass (g) and the predictors of final clutch size listed above.

The availability of preserved specimens with intact GI tracts, along with their associated metadata, have enabled us to investigate if parasite mediated competition may be a mechanism influencing hybrid zone stability at Coweeta Hydrological Station by tying infection patterns to components of host fitness. In this study, we dissected 89 preserved gravid female *Plethodon spp*. collected by Howard and Maerz (2022) to ask 1. How do parasite infections relate to components of host fitness including reproductive output and body condition? and 2. Are these impacts consistent between parent taxa and hybrids? We predicted that measures of host fecundity would vary with rates of parasitism, as both increases in reproductive investment as well as fecundity costs have been documented in parasite infections in amphibians (Brannelly et al., 2016). As parasitism is generally considered to be costly to hosts, we predicted that body condition would decrease with parasite species richness and parasite infection intensity. We instead found that there were no reductions in host body condition associated with infections with any parasite that we measured. We also found that salamanders infected with *C. michiganensis* had larger final clutch sizes on average than uninfected ones, and infected *P. teyahalee* showed signs of increased energetic investment in traits tied to fecundity. This effect combined with the absence of decreased body condition associated with infections suggests relative fitness increase in *P. teyahalee,* and parasite-mediated competition shaped by infections with the ciliated protozoan parasite, *C. michiganensis*.

METHODS

Salamander field methods

Howard and Maerz (2022) modeled large *Plethodon shermani – teyahalee* hybrid clutch sizes as a function of female body size (snout-vent length [SVL]), body condition, and average follicle diameter. They collected adult and subadult salamanders from Coweeta Hydrologic Laboratory, in Macon County, North Carolina, USA (35.0583978N, -83.4374128W) from April 2015 to October 2016. Salamanders were weighed and measured in the field prior to preservation through fixing in 10% buffered formalin and storage in 70% ethanol until dissection. Researchers counted and measured all ovarian follicles found to be greater than 0.5mm in diameter. They also calculated an estimated "cycle day" based on capture month and measured follicle diameters to determine where in the two-year reproductive cycle of assumed *Plethodon* gravid females were (Howard and Maerz 2022).

Calculation of hybrid index and specimen selection

There is significant variation in the degree to which hybrid *Plethodon spp*. at this locality display phenotypes attributable to *P. shermani* (red legs) and *P. teyahalee* (white spots). Substantial surveys of the spatial distribution of phenotypic traits at Coweeta have concluded that most salamanders at this locality are hybrids of the two species (Hairston et al., 1992). This conventional knowledge of the natural history of the site paired with the lack of knowledge gleaned from the model for HI designed by Carter (2023) prompted the assumption of Howard and Maerz (2022) that the salamanders they collected were all hybrids. We investigated if the genomic spread of individuals in their study was sufficient to categorically classify individuals as *P. teyahalee, P. shermani,* and hybrids. We leveraged the models of hybrid index (HI) from Chapter 3 to calculate the HI for each salamander, incorporating phenotype scores from the metadata associated with each individual salamander and the 30 year norms for maximum vapor pressure deficit (vpK) at the locality of collection (PRISM (Parameter-elevation Relationships on Independent Slopes Model) climate database, gridded to 800 m.). We designed a 3-bin system to relocate individuals to species as follows: HI < 0.25 = *P. teyahalee,* 0.25 < HI < 0.75 = hybrids, HI > 0.75 = *P. shermani.* I found that a large number of the 307 *Plethodon spp.* in their study were indeed hybrids, but a significant proportion of individuals were classifiable as nearly pure *P. shermani* or *P. teyahalee.* Of the 307 individuals collected for their study, 120 individuals were gravid females. We obtained and dissected 89 gravid females surveyed in the Howard and Maerz (2022) fecundity modeling study including 45 hybrids, 29 *P. shermani,* and 17 *P. teyahalee.*

Parasite identification methods

We reopened the existing incisions from surveys of eggs in the ovaries and removed the gastrointestinal tracts from the salamanders, placing them in a watch glass under a dissecting microscope. Parasites were identified to species using morphological diagnostic features observed through classical parasitological techniques and microscopy, referencing dichotomous keys and parasite species descriptions (Chapters 2 & 3).

Parasitism and Host Fitness

We estimated salamander body condition independently for each species, calculating a scaled mass index (SMI) of SVL regressed on wet mass in the R package `smatr` (Warton et al., 2012; Hinderer et al., 2025). We used two-factor ANOVA to determine if infected and uninfected individuals in each host species differ in body condition. We measured this effect for each of the four most common parasites. We next used GAMs to determine if body condition varied with infection intensity of each parasite as well as parasite species richness. We also measured relationships between reproductive output and body condition to account for inherent energetic demands associated with reproductive effort and to capture indirect effects of parasitism on body condition through changes in host reproductive investment.

We used one-way ANOVA to determine if *Plethodon spp.* and their hybrids in the Coweeta Basin differ in their model predicted final clutch size, as well as individual components of fitness that impact final clutch size, measured as SVL, average egg diameter, and total eggs (Howard and Maerz 2022). We used two-factor ANOVA to determine if infected and uninfected individuals in each host species differ in reproductive output. We measured this effect for each of the four most common parasites, as identified in Chapters 2 and 3. We used regression analyses to determine if measures of reproductive effort vary with parasite infection intensity as well as the richness of parasite infections using the R package, `mgcv` (Wood 2011).

RESULTS

Predictors of final clutch size between host species

We found heterogeneity in the predictors of final clutch size between *P. teyahalee, P. shermani* and hybrids, regardless of infection status. Average egg diameter was consistent between females of each species, but there were differences in average body size ($F(2,80) = 20.15$, $p < .001$), with *P. teyahalee* possessing greater SVL than both hybrids and *P. shermani*. Although smaller than *P. teyahalee,* hybrids were larger on average than *P. shermani* (Table S4.1; Figure 4.1). We also found differences between host species in the number of eggs in the ovaries of gravid females ($F(2, 80) = 4.07$, $p = .021$). Female *P. teyahalee* tended to have a greater number of eggs than in hybrids or *P. shermani* (Figure 4.1; Table S4.2). Differences in SVL and the number of eggs per female were reflected in their model predicted final clutch sizes, where average final clutch size differed among *P. teyahalee, P. shermani,* and hybrids ($F(2, 79) = 8.27$, $p <.001$). A Tukey post-hoc test revealed larger final clutch sizes on average in *P. teyahalee* compared to both hybrids and *P. shermani* (Figure 4.1; Table 4.1).

Parasitism and body condition

We found no correlation between infections with *C. michiganensis* ($F(2,77) = 0.015$, $p = 0.98$)*, C. variabilis* ($F(2,77) = 0.06$, $p = 0.94$)*, B. hospitale* ($F(2,77) = 0.84$, $p = 0.44$)*,* or *C. cryptobranchi* ($F(1,78) = 0.13$, $p = 0.72$) and host body condition (SMI) for any host species.

Parasitism and fecundity

There were no relationships between SVL, follicle counts, follicle diameter, or predicted final clutch size and infection with *C. variabilis, B. hospitale, or C. cryptobranchi* in any host species (Table 4.2; Figures S4.3-S4.6). For infections with *C.*

michiganensis, we found that there were no differences in average follicle diameter between infected and uninfected individuals in either host species (Table 4.3). The number of follicles per gravid female did not differ between infected and uninfected hybrids or *P. shermani,* but there was a trend of a greater number of eggs in the ovaries of infected *P. teyahalee* than in uninfected *P. teyahalee* with an average difference of 17 eggs per female (Figure 4.2; Table 4.3). Only *P. teyahalee* infected with *C. michiganensis* differed in the number of follicles, with uninfected *P. teyahalee* showing no difference in egg counts from other host species, regardless of infection status (Table 4.3). On average, salamanders infected with *C. michiganensis* had larger predicted final clutch sizes than uninfected ones ($F(1,76) = 4.224$, $p = 0.043$). *P. teyahalee* infected with *C. michiganensis* had the largest predicted final clutch size on average (Figure 4.2; Table S4.1), and we identified no relationships between parasite species richness and measures of fecundity (Table 4.2).

Fecundity and body condition

When measuring the effects of reproductive effort on host body condition, we found no significant relationship between host body condition and average egg diameter (estimate = 0.881, se = 0.900, $p = 0.331$), but final clutch size was positively associated with host body condition (estimate = 8.077, se = 0.372, $p = <.001$). The GAM for eggs per gravid female, in the absence of outlier females from *P. teyahalee* with greater than 75 eggs, revealed a negative relationship between the number of eggs in a female's ovaries and host body condition (estimate = -34.02, se = 14.94, $p = 0.025$). This relationship was comparable between host species, but less evident in hybrids (Figure 4.3; Figure S4.4).

DISCUSSION

Our study addressed how hybridizing host species that share parasites may differ in the degree to which parasitism influences host fitness. We found that host species didn't differ in average egg diameter, but *P. teyahalee* trended towards having more eggs per gravid female than hybrids or *P. shermani*. This relationship was only evident in *P. teyahalee* infected with *C. michiganensis* and absent in the other host species. In the absence of *C. michiganensis, P. teyahalee* did not differ from the other species in the number of eggs per female (Figure 4.2). The differences in final clutch size observed between host species is most likely related to differences in host body size (Wise and Jaeger, 2021), since *P. shermani, P. teyahalee,* nor hybrids differ in measures of fitness that reflect direct energetic investment in reproductive processes in the absence of disease. This study adds further clarity to individual traits predictive of final clutch size between *P. shermani,* hybrids, and *P. teyahalee,* demonstrating that final clutch size emerges as a distinct trait between *P. shermani* and *P. teyahalee.*

Reproductive investment may come at an energetic cost, regardless of host species, as we found a negative relationship between the number of follicles > 0.5 mm in a gravid female and body condition (Figure 4.3). However, observations of host traits and reproductive output in this system and *Plethodon cinereus* (Wise and Jaeger, 2021), have found that reproductive output varies with host body size and hasn't yet been shown to negatively impact body condition. It is possible that body condition may decrease as salamanders become closer to egg deposition, but follicle diameter has been shown to vary more with cycle day than follicle counts (Howard and Maerz, 2022). There were no relationships between host body condition and parasite infection status or infection intensity for either parasite species. One important thing to consider may be the use of SMI to measure body condition. The ratio of mass to SVL may vary with changes in egg mass in females as time of deposition approaches. These deviations in host body mass without increasing host SVL may introduce bias into calculations of SMI overestimating body condition of gravid females.

Parasite infections are, by definition, costly (Hicks et al., 2018). However, our findings that parasitism doesn't influence host SMI, an indicator of fitness, is reflected in other studies (Peig and Green, 2009). Earlier work in Southern Appalachian desmognathine salamanders found that parasite infracommunities were, in general, not host specific and characterized by generalist helminths (mainly nematodes with direct life cycles) with low virulence (Goater et al., 1987). Our results from terrestrial plethodontids echo their findings, with most helminth species observed across *P. shermani, P. teyahalee,* and hybrids showing no apparent changes in body condition associated with parasite infections, underscoring that parasite effects on host fitness are manifold and sometimes cryptic.

We found that individuals with *C. michiganensis* infections had larger predicted final clutch sizes than uninfected ones, and this pattern was most evident in *P. teyahalee*. Only in *P. teyahalee* did we observe differences in egg counts between infected and uninfected individuals, a trend likely to reflect increased energetic investment in reproduction by infected individuals. This reallocation of host resources could be tied to trade-offs in immunity and reproduction, and several studies have documented increased fecundity in amphibians as a result of parasite infections. A study of gametogenesis in frogs found that infections with *Batrachochytrium dendrobatidis (Bd)* resulted in increases in the number of follicles in the ovaries of infected females, and implicated terminal investment as the likely reason for their observations

(Brannelly et al., 2016). Another study investigated trends of malaria co-infections in house martins and found that infections were negatively associated with host survival and that individuals with co-infections had higher reproductive success (Marzal et al., 2008). Our observations could be reflective of terminal investment, but we saw no changes in host body condition associated with parasite infections in any host group. Although we found negative associations between follicle counts and host body condition, it is unlikely that the observed increase in follicles of infected *P. teyahalee* is sufficient to result in meaningful indirect effects of parasitism that might result in decreased body condition.

Larger clutch sizes may help compensate for the reduced frequency of surface activity and delayed age at first reproduction observed in *P. teyahalee*. Although, some studies suggest that other high elevation *Plethodon* in the region like *P. jordani* are simultaneously decreasing in average body size and increasing in age at first reproduction in response to anthropogenic climate change. *P. teyahalee* generally have greater adult survival, and the strength of the potential fitness advantage associated with infections with *C. michiganensis* may increase under future climate scenarios if patterns observed in other *Plethodon* emerge in *P. shermani*. Particularly since the distribution of *C. michiganensis* is known to be limited to lower elevation sites (Joy and Tucker, 2001). These differences could reshape relative population growth rates in *Plethodon* at Coweeta Hydrological Station, bringing into question once again the future of high elevation endemic *Plethodon* in the Southern Appalachians.

CONCLUSION

In systems where competing host species share parasites, heterogeneity in within-host processes following infection can result in parasite-mediated competitive outcomes. We found that *P. teyahalee* differed from hybrids and *P. shermani* in the number of follicles in females infected with the ciliated protozoan *C. michiganensis*. We observed differences between host species in the effects of parasites on the components of host fitness that influence the final clutch size. The presence of increased reproductive effort in *P. teyahalee* paired with the absence of costs to host body condition suggest a relative fitness increase in *P. teyahalee* associated with *C. michiganensis* infections not seen in hybrids or *P. shermani*. These differences could influence relative host population growth rates and implicate parasite mediated competition as a potential mechanism behind observations of bias toward *P. teyahalee* alleles in the *Plethodon* hybrid zone at Coweeta Hydrological Station.

TABLES

Table 4.1: Results of Tukey post-hoc test for difference in average final clutch size between *P. teyahalee, P. shermani,* and hybrids.

Host species – Final clutch size	diff	lwr	upr	p-adj
hybrid – *P. teyahalee*	-2.582	-4.824	-0.340	0.020*
P. shermani – *P. teyahalee*	-4.143	-6.577	-1.709	<.001***
P. shermani – hybrid	-1.561	-3.443	0.322	0.124

Table 4.2: Results of GAM for the influence of parasite infection intensity and parasite species richness on follicle counts, average follicle diameter, and estimated final clutch size for each host species.

	P. shermani				Hybrid				*P. teyahalee*			
Follicle count												
	Est.	Std. Error	t-value	Pr(>|t|)	Est.	Std. Error	t-value	Pr(>|t|)	Est.	Std. Error	t-value	Pr(>|t|)
C. variabilis	0.5	5.127	0.098	0.925	1.214	1.032	1.176	0.259	1.655	1.203	0.915	0.427
B. hospitale	-0.017	2.024	-0.009	0.994	0.616	0.324	1.091	0.087	-	-	-	-
C. michiganensis	0.016	0.056	0.286	0.781	-0.031	0.034	-0.943	0.361	-0.178	0.111	-1.609	0.158
C. cryptobranchi	-	-	-	-	-7.5	2.11	-3.555	0.038	-	-	-	-
PSR	1.44	2.528	0.453	0.655	-0.031	0.034	-0.943	0.361	3	6.33	0.474	0.643
Follicle diameter												
	Est.	Std. Error	t-value	Pr(>|t|)	Est.	Std. Error	t-value	Pr(>|t|)	Est.	Std. Error	t-value	Pr(>|t|)
C. variabilis	0.145	0.179	0.804	0.448	0.384	0.561	0.686	0.504	-0.054	0.044	-1.218	0.31
B. hospitale	-0.063	0.067	-0.93	0.451	-0.018	0.022	-0.838	0.422	-	-	-	-
C. michiganensis	-0.005	0.002	-1.173	0.122	0	0.002	0.25	0.806	0.001	0.001	1.311	0.237
C. cryptobranchi	-	-	-	-	0.211	0.364	0.582	0.601	-	-	-	-
PSR	-0.043	0.159	-0.273	0.787	0.174	0.222	0.784	0.446	0	0.09	0.008	0.994
Final clutch size												
	Est.	Std. Error	t-value	Pr(>|t|)	Est.	Std. Error	t-value	Pr(>|t|)	Est.	Std. Error	t-value	Pr(>|t|)
C. variabilis	0.601	1.151	0.522	0.618	0.487	0.369	1.321	0.207	0.85	0.723	1.174	0.325
B. hospitale	-0.203	0.486	-0.416	0.718	0.173	0.112	1.539	0.155	-	-	-	-
C. michiganensis	-0.022	0.014	-1.52	0.167	-0.002	0.014	-0.184	0.857	-0.032	0.008	-3.557	0.016
C. cryptobranchi	-	-	-	-	-2.719	1.828	-1.488	0.234	-	-	-	-
PSR	0.75	0.589	1.273	0.216	2.475	1.324	1.869	0.082	0.442	1.046	0.423	0.679

Table 4.3: Results of Tukey post-hoc test for difference in average follicle per female between *P. teyahalee, P. shermani,* and hybrids with (1) and without (0) *C. michiganensis* infections. Stars and dashes indicate statistical significance.

	diff	lwr	upr	p-adj
P. hybrid:0-P. teyahalee:0	-1.205	-15.377	12.967	1.000
P. shermani:0-P. teyahalee:0	-1.192	-16.466	14.082	1.000
P. teyahalee:1-P. teyahalee:0	17.000	-0.444	34.444	0.060-
P. hybrid:1-P. teyahalee:0	-1.949	-16.907	13.010	0.999
P. shermani:1-P. teyahalee:0	-0.225	-16.774	16.324	1.000
P. shermani:0-P. hybrid:0	0.013	-11.381	11.408	1.000
P. teyahalee:1-P. hybrid:0	18.205	4.033	32.377	0.004**
P. hybrid:1-P. hybrid:0	-0.744	-11.711	10.224	1.000
P. shermani:1-P. hybrid:0	0.980	-12.074	14.034	1.000
P. teyahalee:1-P. shermani:0	18.192	2.918	33.466	0.010*
P. hybrid:1-P. shermani:0	-0.757	-13.116	11.602	1.000
P. shermani:1-P. shermani:0	0.967	-13.276	15.210	1.000
P. hybrid:1-P. teyahalee:1	-18.949	-33.907	-3.990	0.005**
P. shermani:1-P. teyahalee:1	-17.225	-33.774	-0.676	0.036*
P. shermani:1-P. hybrid:1	1.724	-12.180	15.628	0.999

FIGURES

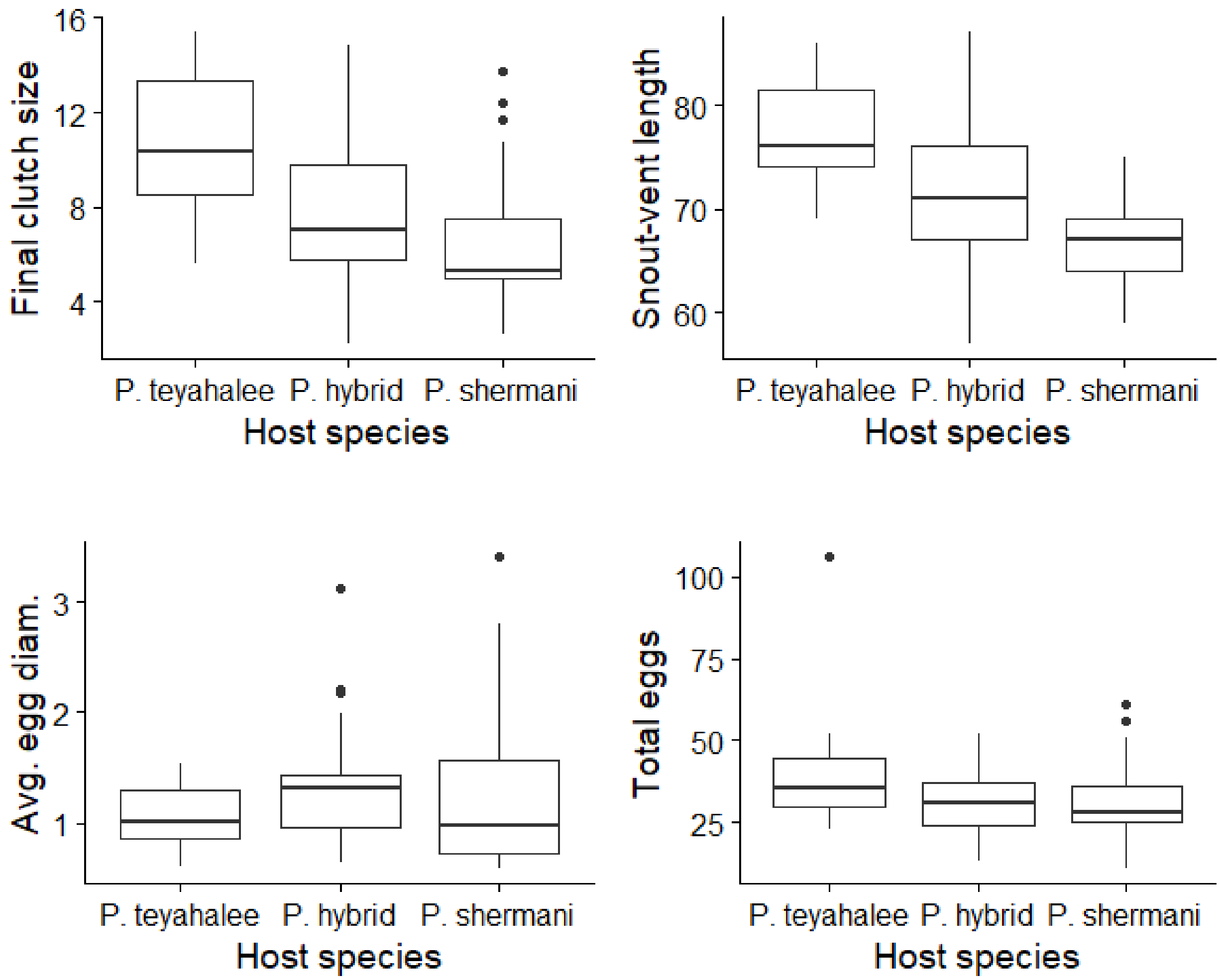

Figure 4.1: Box and whisker plot of average final clutch size as well as predictors of final clutch size including average egg diameter, total number of eggs, and snout-vent length. Black bars represent mean values. Whiskers represent 95% CI, and black dots represent outliers.

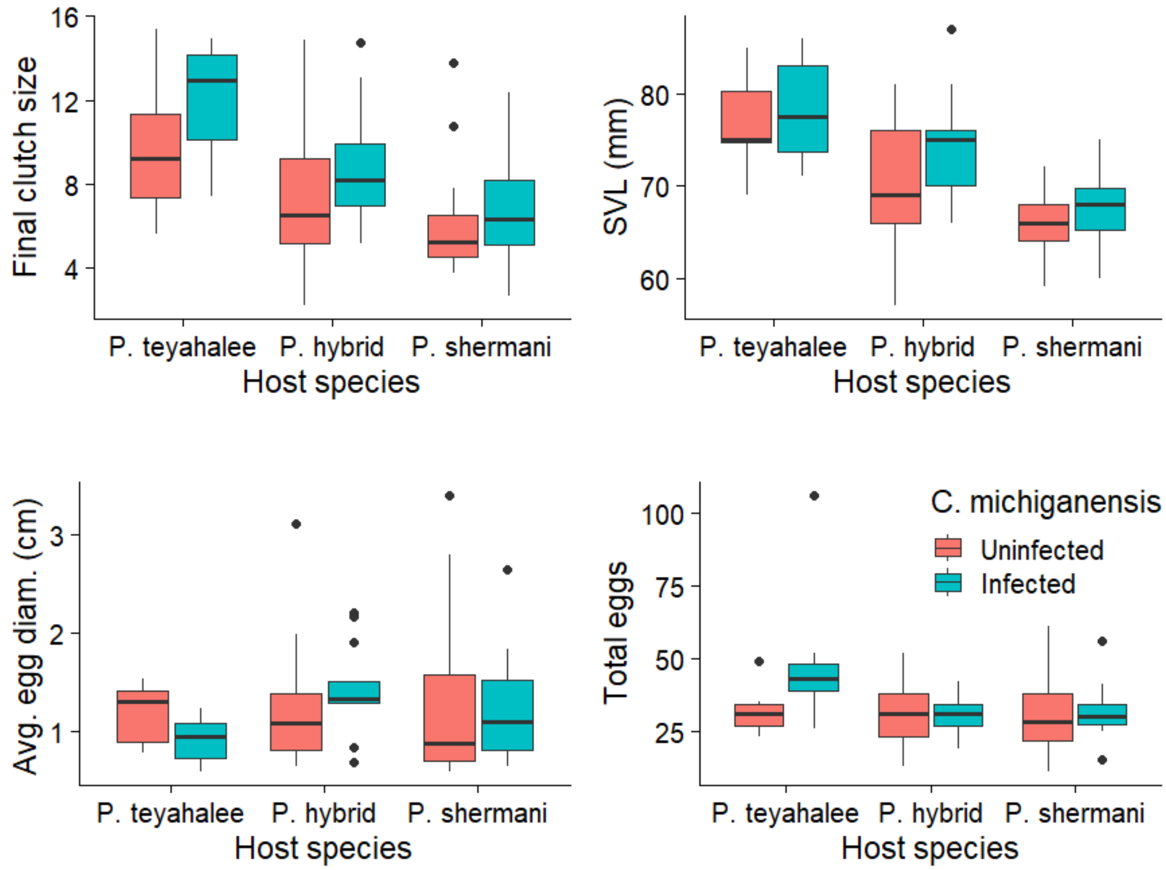

Figure 4.2: Box and whisker plot of model predicted final clutch size as well as predictors of final clutch size including average egg diameter, total number of eggs, and snout-vent length for individuals infected with and uninfected with *C. michiganensis* in each host species. Black bars represent mean values. Whiskers represent 95% CI, and black dots represent outliers.

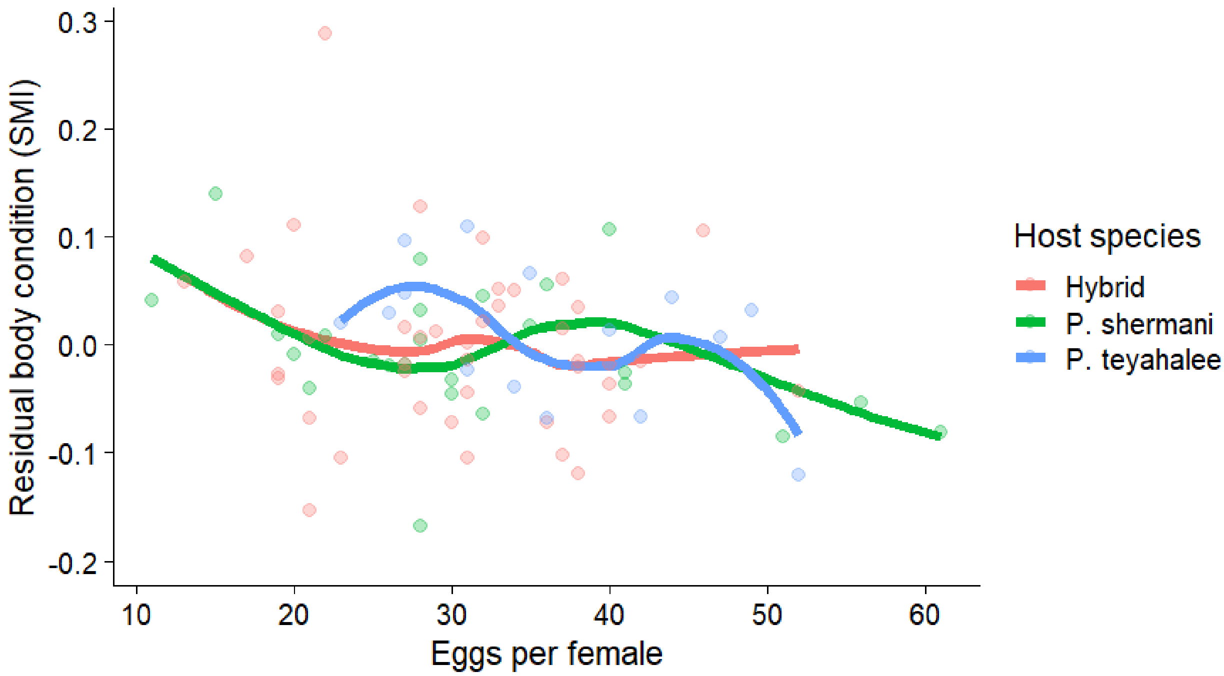

Figure 4.3: Smoothed line plot (GAM) of the relationship between eggs per female >0.5mm (x-axis) and host body condition (y-axis), measured as residual scaled mass index (SMI). Line color represents host species.

CHAPTER 5

CONCLUSIONS

OVERVIEW

The goal of this dissertation is to investigate the degree to which parasite mediated competition may contribute to the suite of mechanisms shaping the spatial extent and stability of the hybrid zone between *P. shermani* and *P. teyahalee* at Coweeta Hydrological Station. The results of this work provide compelling evidence that these host species experience disparate burdens of disease and that differences in parasitism between *P. teyahalee, P. shermani,* and hybrids differentially impact host fitness, amounting to the net effects of parasite mediated competition.

In Chapter 2, I demonstrate that parasite community composition is heavily influenced by interspecific interactions between parasites that differed in strength between *P. shermani*, hybrids, and *P. teyahalee*. Differences between species were largely associated with infections with a ciliated protozoan, *Cepedietta michiganensis*, which appears to reduce host foraging activity and thereby exposure to other parasite species - a form of parasite-mediated enemy release (Hatcher et al., 2006). Regardless, parasitism is costly, so reductions in parasite prevalence and richness in *P. teyahalee* may amount to a net benefit. A greater proportion of individuals in *P. teyahalee* were uninfected with any parasites altogether, which could be explained by both demography of *P. teyahalee,* the frequency of their surface activity, even in the absence of disease, and the physiological traits of *P. teyahalee* that enable them to go longer periods of time between foraging events. These results demonstrate that parasite community dynamics can be heterogeneous across hybrid zones that straddle ecotones, supporting the need for considerations of host-parasite interactions at the parasite community scale for making meaningful comparisons of parasitism between hybridizing host species. Compared to other animal groups, amphibian parasite communities are relatively understudied (Martins et al., 2021). Future work should survey a greater number of sympatric host species to truly quantify the degree of specialism or generalism of a parasite and to determine which host species are likely responsible for the maintenance of parasites in the host community.

In Chapter 3, I found that individuals with HI values reflective of hybrids or *P. shermani* experienced greater prevalence and intensity of parasite infections as well as richer co-infections than *P. teyahalee,* with hybrids experiencing intermediate rates of parasitism in most cases. Prevalence and richness patterns varied with rainfall, suggesting that moisture availability may influence the distribution and abundance of parasites at this site as well as differential rates of parasite exposure tied to host surface activity. Individual parasite prevalence and parasite species richness models reflected patterns we observed at the parasite community scale, where predictors of parasite prevalence like HI and rainfall were also predictors of parasite richness, although the relative importance of any one factor differed across parasite species. These results suggest that individual parasite species shared by hybridizing hosts may vary in the degree to which host genetics and environmental traits shape their distributions.

I demonstrate in Chapter 4 that heterogeneity in parasite infections between hybridizing host species can be directly tied to measures of host fitness in liquid preserved specimens. Generally, individuals infected with *C. michiganensis* were found to have larger predicted final clutch sizes than those uninfected. *P. teyahalee* differed from hybrids and *P. shermani* in the number of follicles in females infected with *C. michiganensis,* where infected *P. teyahalee* alone had more follicles > 0.5 mm in diameter than their uninfected counterparts. We found no changes in host body condition associated with infections with the four most common shared parasites, supporting previous hypotheses that gastrointestinal parasite communities infecting amphibians are most often generalist parasites with generally low virulence. However, there may be an eco-evolutionary history of virulent infections of *C. michiganensis* in *P. teyahalee* not shared with *P. shermani* that would result in increased allocation of resources to reproductive output. Increases in host reproductive effort following parasite infection is generally thought to be an adaptation to extreme virulence, since species should increase investment in current progeny if the probability of future survival is low. The presence of increased reproductive effort during infection paired with the absence of an effect on host body condition suggests a relative fitness increase in *P. teyahalee* associated with *C. michiganensis* infections compared to hybrids or *P. shermani*.

Although we didn't set out to expressly test this hypothesis, we found no evidence of hybrid vigor or hybrid unviability concerning resistance or tolerance to intestinal parasites. In nearly every case, salamanders with intermediate HI values (hybrids) experienced intermediate disease states as well as reproductive output relative to *P. shermani* and *P. teyahalee,* suggesting additive dominance, where parent species vary in the abundance of parasites, but the resistance of hybrids is intermediate to either parent species (Wolinska et al., 2008; Theodosopoulous 2019). This may be related to the fact that hybrids in this system inherit intermediate host behavioral and ecological traits (Roth et al., 2021) and experience habitat conditions that positively impact parasite fitness relative to their parent taxa at this site.

REFLECTIONS

Taken together, these results provide robust evidence that parasite mediated competition could be a factor influencing observations of bias toward introgression of *P. teyahalee* alleles. We identified two separate mechanisms by which parasites, specifically *C. michiganensis,* might increase the relative fitness of *P. teyahalee*: parasite mediated enemy release and infection induced increases in reproductive output. Whether these mechanisms are enough to drastically impact the future distribution of this hybrid zone remains uncertain. Although *P. teyahalee* experiences fewer and less rich parasite infections than hybrids and *P. shermani,* we found in Chapter 4 that parasite infections with any of the parasites we measured, *Cosmocercoides variabilis, C. michiganensis, Brachycoelium hospitale,* and *Crepidobothrium cryptobranchi* did not result in changes in host body condition. We also found no relationships between infection intensity with any parasite or parasite species richness and host body condition, reducing the probability that release from gastrointestinal helminth infections, which occur more commonly in hybrids and *P. shermani,* results in a substantial fitness advantage. For parasite mediated enemy release to genuinely shape the relative fitness of a host, the costs associated with reduced foraging would need to be less than the costs associated with infections with other parasites in the community. That being said, deleterious effects to hosts are usually exacerbated with growing numbers of co-infecting parasites (Herczeg et al., 2021). Populations of foothill species like *P. teyahalee* are known to have greater adult survival rates than montane *Plethodon* like *P. shermani,* which could be tied to costs of parasite infections that accumulate more rapidly over time in *P. shermani* and hybrids compared to *P. teyahalee*. Exposing *Plethodon spp. to C. michagenensis* infections in a controlled laboratory setting would enable us to experimentally determine if *C. michagenensis* infections influence host behavior and the likely mechanisms responsible.

We observed increases in reproductive output in individuals infected with *C. michiganensis,* and this pathogen is most abundant in *P. teyahalee*. *P. teyahalee* also has larger final clutch sizes, even in the absence of parasites. More frequent increases in reproductive investment by a species characterized by inherently larger clutch sizes on average and greater adult survival could be incredibly consequential for shaping the distribution of host alleles over time. The net outcome of this phenomenon remains to be seen, as *P. teyahalee* has a greater age at first reproduction by as much as 5 years, and *P. shermani* and hybrids have more frequent opportunities for mate access afforded by conditions more frequently suitable for surface activity. Furthermore, predictions of the impact of parasitism on host population dynamics require that we understand not only how species differ in age at first reproduction and clutch size but also the relative frequency at which *P. teyahalee, P. shermani,* and hybrids deposit said clutches. We suggest that future work quantify heterogeneity in pace of life between *P. teyahalee, P. shermani,* and hybrids to parameterize dynamical equation models of host population growth to determine if *C. michiganensis* has the potential to shape host population growth rates and predict the timescales over which changes are most likely to occur.

The dynamics of parasite communities we observed in this system are unlikely to change in the near future. Models of contemporary extent of plethodontids and their historical distribution suggest that species have persisted under comparable climate conditions as well as those predicted under forecasts of the next 50 years of environmental change (Lyons and Kozak, 2019). The evolutionary trajectories of parasites are closely tied to the selective pressures influencing the distribution of their hosts, so in the absence of changes in host demography, future climate conditions will likely not deviate from conditions tolerable by gastrointestinal parasites infecting *Plethodon* at Coweeta.

LIMITATIONS

There were a few limitations to this dissertation, many of which are associated with using preserved specimens for asking questions in disease ecology. The first is that it is difficult to know how long individuals were held prior to euthanasia and preservation. Measures of infection intensities of parasites like *C. variabilis* that infect the large intestine may be lost if hosts pass fecal material between capture and preservation. The second is that not every salamander preserves equally, and this factor influences both the clarity of phenotype patterns used to quantify host genetic ancestry as well as the preservation of parasite morphological features used for diagnosis.

The specificity of locality data associated with preserved specimens also presents a challenge, as geographical coordinates are necessary to extract historical climate data and the metadata of many individuals contain only sparse descriptions of where each specimen was collected. Challenges reported in obtaining *Plethodon* specimens from dry sites also manifested in the availability of *P. teyahalee* in natural history collections (Bailey et al. 2004). This resulted in difficulty acquiring a large number of preserved *P. teyahalee* and a potential bias in our dataset toward individuals with higher HI values from wetter sites.

Modeling disease dynamics in host systems like *Plethodon* where host exposure is difficult to predict presents a challenge, as infected individuals that are surface active are almost certainly not the only infected individuals in a population. Indeed, some individuals spend as much as 80% of the year in burrows underground (Highton and Henry 1970). We still don't fully understand how parasitism might influence rates of host surface activity. There may be an observation bias regarding the detection of infected *P. teyahalee,* as parasite infections are known to decrease thermal tolerance in amphibians (Sherman 2008; Greenspan et al., 2017). This might result in the emergence of sickness behaviors in infected individuals that decrease the probability of surface activity and, therefore, detection. Finally, it is difficult to tell if the absence of relationships between parasitism and body condition in gravid females is a true signal, as scaled-mass index incorporates measures of wet mass, which may increase with egg diameter as females approach time of egg deposition, effectively masking changes in body condition.

Another limitation pervasive throughout this dissertation is our failure to incorporate measures of error propagation in estimates of the continuous genomic hybrid index (HI) (Carter 2023). This model predicted value is generated using covariates with known degrees of error (mean vapor pressure deficit (VPD), phenotype scores) that when combined influence estimates of confidence in HI values (Alsadik 2019; Carter 2023), which we did not account for in our analyses. We plan to remedy this by incorporating Monte Carlo procedures for calculating estimations of variance in predicted HI values due to model error (Roxburgh and Paul 2025). Although less likely to impact results in chapters 2 and 4, where HI values were used to categorize individuals into bins of host species, results from chapter 3 using model predicted HI as a covariate should be interpreted with caution. Future work includes revisiting this analysis incorporating Monte Carlo methods to assess error propagation.

FINAL THOUGHTS

This dissertation makes meaningful contributions to the growing body of work investigating how hybridizing species share parasites and the potential impacts of parasite sharing on the extent and stability of animal hybrid zones. Our work has facilitated the first study of parasite gastrointestinal parasite community dynamics in amphibian hybrid zones as well as the first ever survey of gastrointestinal parasites from amphibians in historical collections. Findings here provide proof of concept that natural history collections represent a vastly underutilized resource for measuring host-parasite dynamics, particularly in charismatic species like salamanders, where lethal sampling for helminth communities is generally a tough sell. The threatened status of many climate-sensitive host species requires that we tease apart the relative importance of host and environmental factors on shaping parasite pressure and directly measure effects of parasitism on factors that impact host population growth dynamics in order to implement the most informed disease management strategies. Understanding the disease associated mechanisms shaping the relative fitness of hybridizing host species is likely to be a crucial factor in predicting wildlife disease dynamics and the future of global genetic diversity under climate change. This work introduces a novel suite of hypotheses to the growing list of mechanisms proposed to be shaping the *P. shermani* x *P. teyahalee* hybrid zone at Coweeta, once again bringing into question the future of high elevation endemic *Plethodon* in the Southern Appalachians.

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APPENDIX A:

SUPPLIMENTARY TABLES AND FIGURES

Table S2.1: Results of a Tukey's-Test of pairwise comparisons of average parasite richness between infracommunities within each host species including average difference in richness and adjusted p-values.

Species comparison	**diff**	**p adj**
P. hybrid – P. teyahalee	0.433	0.035*
P. shermani – P. teyahalee	0.795	>.001***
P. shermani – P. hybrid	0.362	0.103

Table S2.2: Results of probabilistic species cooccurrence analysis for each possible parasite pair. Stars next to p-values indicate significance.

Parasite 1	Parasite 2	Incidence of Parasite 1	Incidence of Parasite 2	Observed Cooccurence	Expected Cooccurence	P Less Than Expected	P Greater Than Expected
Cosmocercoides	Batracholandros	89	24	13	11.9	0.762	0.39
Cosmocercoides	Porrocaecum spp	89	12	6	5.9	0.632	0.601
Cosmocercoides	Oswaldocruzia	89	5	1	2.5	0.191	0.968
Cosmocercoides	Amphibiocapillaria	89	5	0	2.5	0.031*	1
Cosmocercoides	Crepidobothrium	89	40	19	19.8	0.46	0.676
Cosmocercoides	Brachycoelium	89	55	29	27.2	0.772	0.336
Cosmocercoides	Cepedietta	89	60	14	29.7	<.001***	1
Batracholandros	Porrocaecum spp	24	12	1	1.6	0.505	0.83
Batracholandros	Oswaldocruzia	24	5	2	0.7	0.982	0.132
Batracholandros	Amphibiocapillaria	24	5	1	0.7	0.867	0.515
Batracholandros	Crepidobothrium	24	40	2	5.3	0.06	0.986
Batracholandros	Brachycoelium	24	55	7	7.3	0.54	0.646
Batracholandros	Cepedietta	24	60	2	8	0.003**	0.999
Porrocaecum spp	Oswaldocruzia	12	5	2	0.3	0.997	0.037*
Porrocaecum spp	Amphibiocapillaria	12	5	0	0.3	0.705	1
Porrocaecum spp	Crepidobothrium	12	40	5	2.7	0.972	0.098
Porrocaecum spp	Brachycoelium	12	55	3	3.7	0.472	0.769
Porrocaecum spp	Cepedietta	12	60	2	4	0.172	0.951
Oswaldocruzia	Amphibiocapillaria	5	5	1	0.1	0.994	0.133
Oswaldocruzia	Crepidobothrium	5	40	2	1.1	0.926	0.308
Oswaldocruzia	Brachycoelium	5	55	0	1.5	0.157	1
Oswaldocruzia	Cepedietta	5	60	0	1.7	0.128	1
Amphibiocapillaria	Crepidobothrium	5	40	1	1.1	0.691	0.719
Amphibiocapillaria	Brachycoelium	5	55	1	1.5	0.515	0.842
Amphibiocapillaria	Cepedietta	5	60	1	1.7	0.459	0.872
Crepidobothrium	Brachycoelium	40	55	10	12.2	0.254	0.855
Crepidobothrium	Cepedietta	40	60	5	13.3	0.001**	0.999
Brachycoelium	Cepedietta	55	60	3	18.3	0	1

Table S2.3: Results of 2-way ANOVA for differences in C-score values for *P. teyahalee, P. shermani,* and hybrids.

Pairwise difference	**Estimate**	**95% CI**	**p-value**
P. shermani - P. hybrid	0.4	0.36-0.44	0.349
P. hybrid - P. teyahalee	0.46	0.41-0.48	0.031*
P. shermani - P. teyahalee	0.52	0.48-0.55	0.006**

Table S3.1: Model formulae for the 31 generalized additive models fit for parasite prevalence and specie richness.

Model	Model formulae
1	HI
2	SVL
3	Precipitation
4	HI*SVL
5	HI*Precipitation
6	HI+SVL
7	HI + Precipitation
8	HI + HI*SVL
9	HI + HI*Precipitation
10	SVL + Precipitation
11	SVL + HI*SVL
12	SVL + HI*Precipitation
13	Precipitation + HI*SVL
14	Precipitation + HI*Precipitation
15	HI*SVL + HI*Precipitation
16	HI + SVL + Precipitation
17	HI + SVL + HI*SVL
18	HI + SVL + HI*Precipitation
19	HI + Precipitation + HI*SVL
20	HI + Precipitation + HI*Precipitation
21	HI + HI*SVL + HI*Precipitation
22	SVL+ Precipitation + HI*SVL
23	SVL + Precipitation HI*Precipitation
24	SVL + HI*SVL HI*Precipitation
25	Precipitation + HI*SVL HI*Precipitation
26	HI + SVL + Precipitation + HI*SVL
27	HI + SVL + Precipitation + HI*Precipitation
28	HI + Precipitation + HI*SVL + HI*Precipitation
29	SVL + Precipitation + HI*SVL + HI*Precipitation
31	HI + SVL + Precipitation + HI*SVL + HI*Precipitation

Table S3.2: Results of the three best generalized additive models for *C. variabilis prevalence*. The best model is placed in the first column.

	Mod29				Mod27				Mod25			
Terms	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	-0.9815	0.358	-2.74	0.006	-0.991	0.3366	-2.94	0.003	-1.105	3.178	-3.48	<.001
	edf	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Precipitation	2.876	3.413	10.39	0.0218	2.795	3.323	9.601	0.029	3.519	4.147	18.95	0.001
HI	1	1	9.10	0.0026	1	1	9.28	0.002	--	--	--	--
SVL	--	--	--	--	1.92	2.443	3.28	0.306	--	--	--	--
HI * Precipitation	2.388	2.768	10.24	0.013	2.341	2.714	10.21	0.012	2.575	3.033	13.70	0.003
HI*SVL	1.043	1.084	1.54	0.217	--	--	--	--	1	1	2.84	0.092
	AIC	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**
	175.3444	81.275	0.218	22.70%	176.009	83.63	0.229	23.60%	176.323	83.229	0.199	21.90%

Table S3.3: Results of the three best generalized additive models for *Brachycoelium hospitale* prevalence. The best model is placed in the first column.

	Mod18				Mod21				Mod28			
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	-0.391	0.337	-1.59	0.247	-0.394	0.339	-1.16	0.246	-0.3479	0.359	-0.97	0.333
	edf	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Precipitation	--	--	-	--	--	--	--	--	--	--	--	--
HI	3.185	3.996	9.79	0.043	3.208	4.033	9.54	0.05	3.24	4.07	9.53	0.051
SVL	3.241	4.04	4.76	0.325	--	--	--	--	3.294	4.104	4.99	0.312
HI * Precipitation	3.595	3.875	21.02	<.001	3.61	3.885	17.21	0.001	3.597	3.876	18.393	<.001
HI*SVL	--	--	--	--	1	1	0.03	0.861	1	1	0.05	0.819
	AIC	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**
	136.9451	66.754	0.269	29.90%	137.7721	64.527	0.237	25.60%	138.6059	64.456	0.268	30.30%

Table S3.4: Results of the three best generalized additive models for *Crepidobothrium cryptobranchi* prevalence. The best model is placed in the first column.

	Mod27				Mod22				Mod28			
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	-2.414	0.487	-4.96	<.001	-2.544	0.588	-4.33	<.001	-2.125	0.4	-5.31	<.001
	edf	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Precipitation	2.188	2.661	3.00	0.328	5.992	6.798	10.832	0.125	--	--	--	--
HI	1.461	1.792	4.37	0.109	--	--	--	--	1	1	7.10	0.008
SVL	5.607	6.659	9.64	0.198	5.614	6.617	9.09	0.232	5.634	6.696	8.84	0.219
HI * Precipitation	1	1	10.56	0.001	--	--	--	--	1	1	10.23	0.001
HI*SVL	--	--		--	2.108	2.585	3.18	0.347	1.422	1.724	0.58	0.769
	AIC	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**
	136.8307	64.678	0.22	27.90%	137.011	71.401	0.257	32.80%	137.9395	62.822	0.197	24.90%

Table S3.5: Results of the three best generalized additive models for *Cepedietta michiganensis* prevalence. The best model is placed in the first column.

	Mod8				Mod15				Mod11			
Terms	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	-1.063	0.189	-5.61	<.001	-1.025	0.188	-5.46	<.001	-1.042	0.188	-5.53	<.001
	edf	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Precipitation	--	--	--	--	--	--	--	--	--	--	--	--
HI	2.087	2.604	2.35	0.372	--	--	--	--	--	--	--	--
SVL	--	--	--	--	--	--	--	--	1.95	2.478	2.88	0.36
HI * Precipitation	--	--	--	--	1	1	0.67	0.057	--	--	--	--
HI*SVL	1	1	1.31	0.252	1	1.001	1.75	0.187	1.001	1.001	2.55	0.11
	AIC	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**
	176.1509	84.492	0.0121	3.23%	176.1527	82.464	0.00159	1.37%	176.1727	84.644	0.0151	3.07%

Table S3.6: Results of the three best generalized additive models for *Cosmocercoides variabilis* intensity. The best model is placed in the first column.

	Mod19				Mod29				Mod31			
Terms	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	0.996	0.0931	10.70	<.001	0.968	0.147	6.60	<.001	0.8404	0.241	3.49	<.001
	edf	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Precipitation	4.073	5.663	29.08	<.001	3.951	4.604	24.721	<.001	3.799	4.475	13.15	0.009
HI	4.312	5.145	30.69	<.001	4.227	5.028	26.31	<.001	4.354	5.13	22.21	<.001
SVL	--	--	--	--	--	--	--	--	3.769	4.566	11.68	0.024
HI * Precipitation	--	--	--	--	1.001	1.001	0.06	0.805	2.271	2.62	2.46	0.379
HI*SVL	9.105	10.632	38.67	<.001	9.041	10.563	38.22	<.001	3.623	4.506	23.32	<.001
	AIC	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**
	278.2144	150.1	0.294	55.40%	280.0431	147.88	0.279	55.30%	285.1547	146.81	0.219	52.40%

Table S3.7: Results of the three best generalized additive models for *Brachycoelium hospitale* intensity. The best model is placed in the first column.

	Mod4				Mod7				Mod2			
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	1.362	0.093	14.59	<.001	1.358	0.094	14.50	<.001	1.395	0.09	15.36	<.001
	edf	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Precipitation	--	--	--	--	1.001	1.001	0.481	0.488	--	--	--	--
HI	2.522	3.086	8.31	0.042	2.588	3.153	8.60	0.039	--	--	--	--
SVL	7.507	8.41	31.55	<.001	7.575	8.459	32.52	<.001	7.572	8.468	36.71	<.001
HI * Precipitation	--	--	--	--	--	--	--	--	--	--	--	--
HI*SVL	--	--	--	--	--	--	--	--	--	--	--	--
	AIC	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**
	200.3625	109.2	0.102	41.70%	201.1115	110.36	0.07	42.80%	205.1805	111.04	0.0907	33.50%

Table S3.8: Results of the three best generalized additive models for *Crepidobothrium cryptobranchi* intensity. The best model is placed in the first column.

	Mod7				Mod5				Mod2			
	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	1.01	0.112	9.07	<.001	0.984	0.112	8.76	<.001	1.1071	0.106	10.10	<.001
	edf	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Precipitation	3.14	3.601	6.62	0.082	3.182	3.64/8	8.612	0.037	--	--	--	--
HI	3.66	4.53	10.13	0.049	3.45	4.283	11.65	0.026	--	--	--	--
SVL	3.08	3.876	8.09	0.069	--	--	--	--	2.096	2.63	2.38	0.447
HI * Precipitation	--	--	--	--	--	--	--	--	--	--	--	--
HI * SVL	--	--	--	--	--	--	--	--	--	--	--	--
	AIC	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**
	140.936	73.394	0.055	37.00%	141.6766	73.855	0.104	36.90%	145.236	72.823	-0.018	7.25%

Table S3.9: Results of the three best generalized additive models for *Cepedietta michiganensis* intensity. The best model is placed in the first column.

	Mod7				Mod4				Mod6			
Terms	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	3.5207	0.033	104.10	<.001	3.622	0.033	112.20	<.001	3.705	0.029	124.90	<.001
	edf	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Precipitation	8.925	8.991	565.20	<.001	--	--	--	--	8.896	8.995	386.20	<.001
HI	8.989	8.987	609.70	<.001	8.929	8.998	513.90	<.001	--	--	--	--
SVL	8.888	8.993	831.70	<.001	8.903	8.996	900.00	<.001	8.848	8.991	483.20	<.001
HI * Precipitation	--	--	--	--	--	--	--	--	--	--	--	--
HI*SVL	--	--	--	--	--	--	--	--	--	--	--	--
	AIC	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**
	525.152	351.79	0.826	88.60%	1097.956	466.48	0.873	96.10%	1297.113	696.7	0.063	54.40%

Table S3.10: Results of the three best generalized additive models for parasite species richness. The best model is placed in the first column.

	Mod18				Mod28				Mod27			
Terms	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	0.157	0.08	1.91	0.056	0.516	0.08	1.95	0.052	0.201	0.062	3.25	0.002
	edf	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**	**edf**	**Ref.df**	**Chi.sq**	**p-value**
Precipitation	--	--	--	--	--	--	--	--	--	--	--	--
HI	1	1	19.11	<.001	1	1	18.92	<.001	1	1	1.09	0.296
SVL	1	1	3.48	0.0622	1	1	3.14	0.0762	--	--	--	--
HI * Precipitation	1	1	4.51	0.034	1	1	4.62	0.0316	1	1	4.13	0.042
HI*SVL	--	--	--	--	1	1	0.17	0.679	1.215	1.39	3.42	0.138
	AIC	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**	**AIC**	**REML**	**R-sq. (adj)**	**Dev. Exp**
	390.649	191.51	0.23	20.40%	392.478	194.56	0.221	20.50%	392.873	196.73	0.246	23.00%

Table S4.1: Results of Tukey post-hoc test for difference in average SVL gravid female between *P. teyahalee, P. shermani,* and hybrids.

Host species – SVL	**diff**	**lwr**	**upr**	**p-adj**
hybrid – *P. teyahalee*	-10.006	-18.648	-1.364	0.020*
P. shermani – P. teyahalee	-9.305	-18.723	0.443	0.054-
P. shermani – hybrid	0.709	-6.730	8.131	0.972

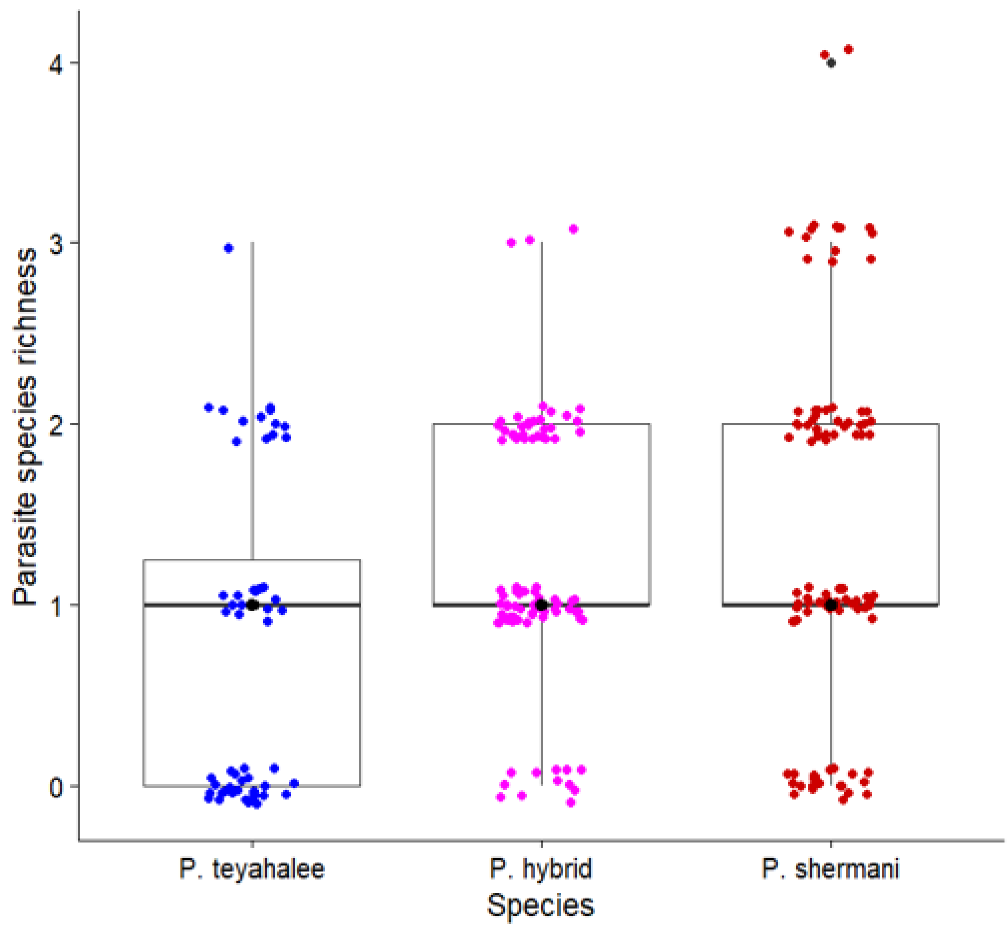

Fig. S2.1: Box and whisker plot of parasite species richness in *P. shermani*, *P. teyahalee* and hybrids. Black bars indicate median richness values.

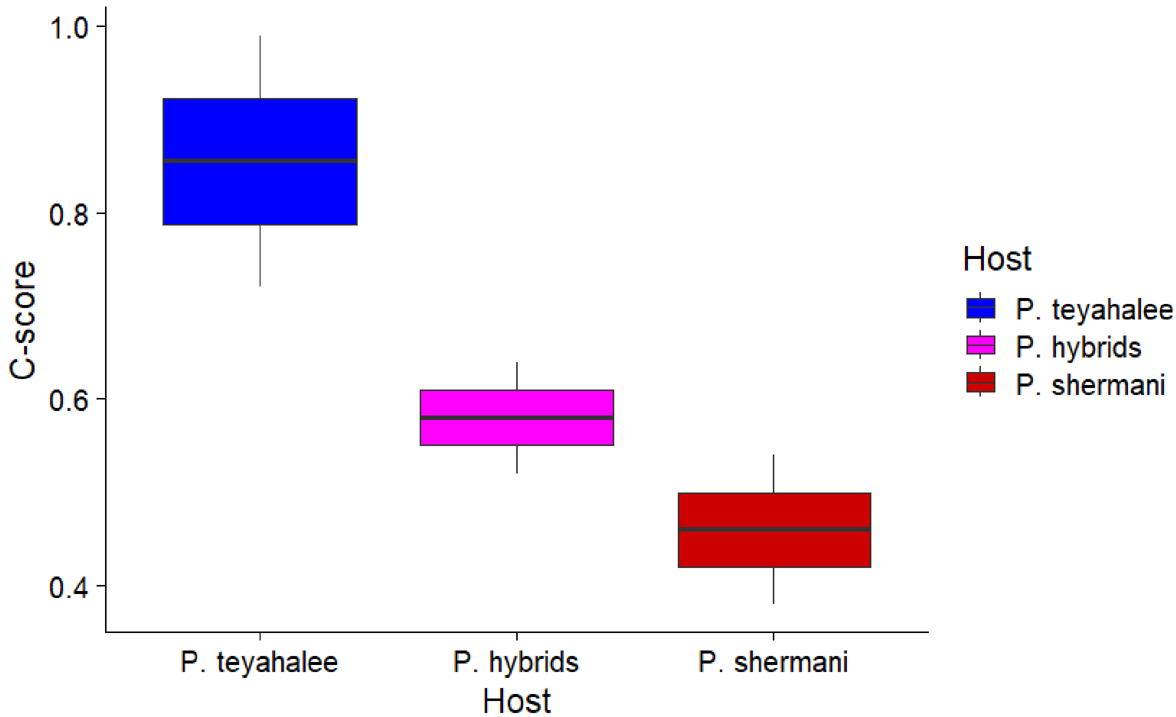

Fig. S2.2: Box and whisker plot of the degree of antagonistic pairwise interactions (C-score) between parasites infecting each host group. Color corresponds to host species. Black bars represent mean values, and whiskers indicate 95% CI.

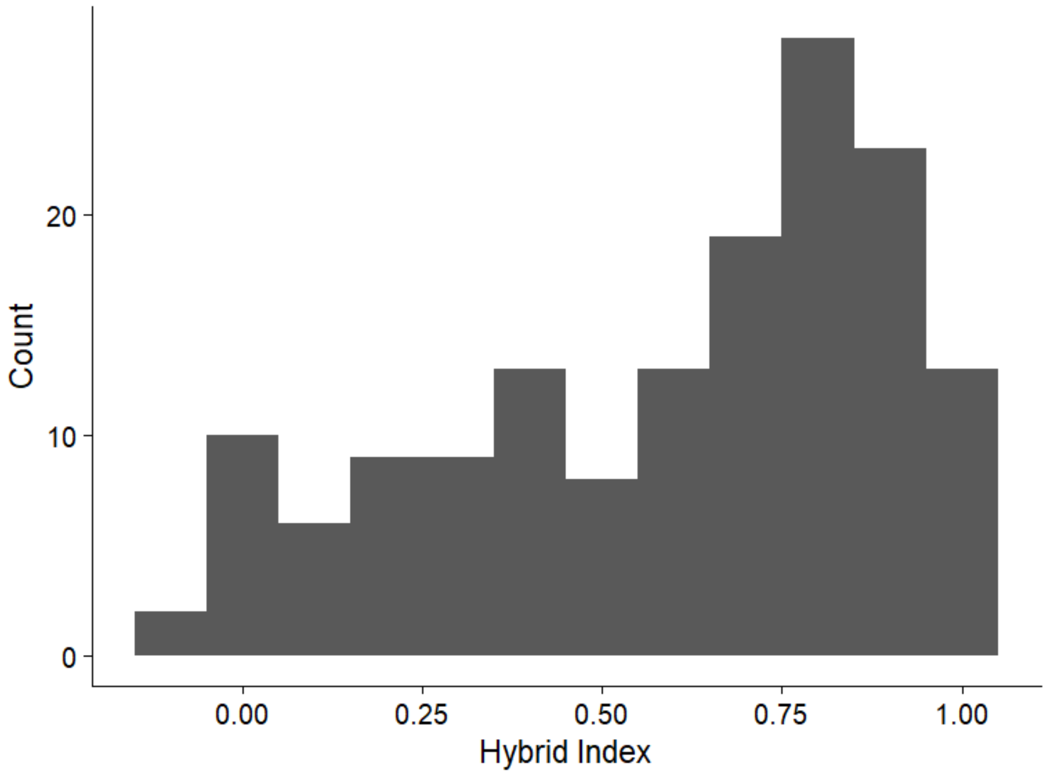

Figure S3.1**:** A histogram of the distribution of hybrid index (HI) among dissected salamanders. X-axis corresponds to HI, y-axis represents the frequency of observations.

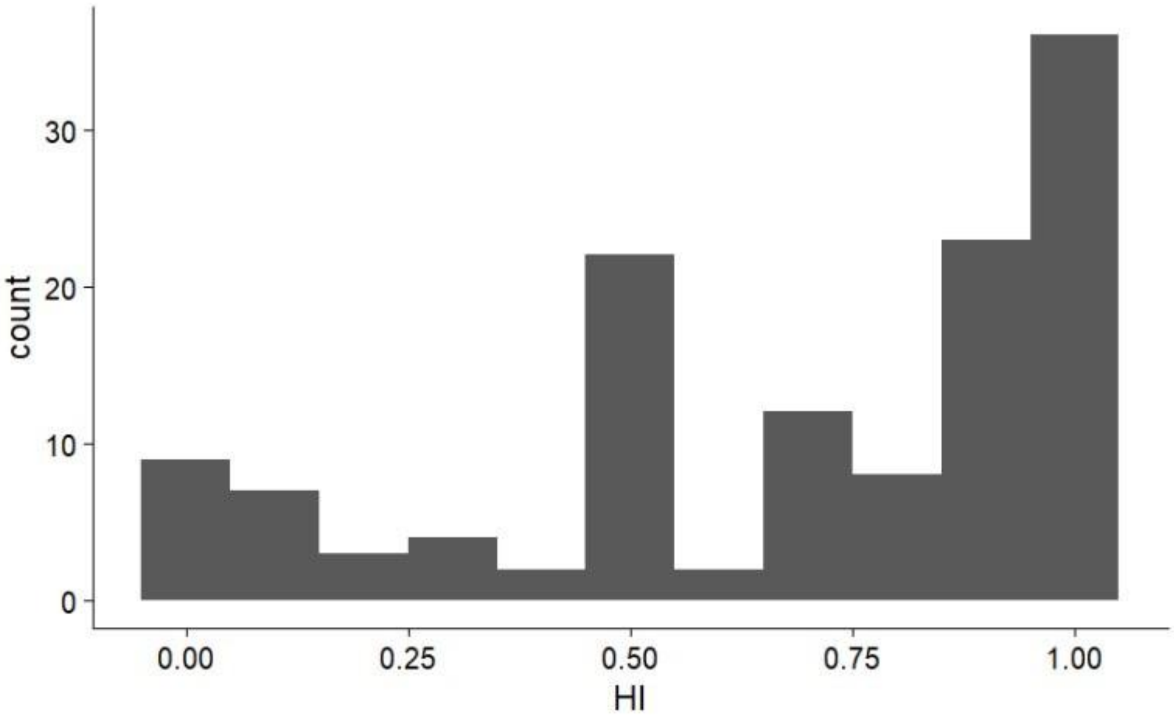

Figure S4.1: Histogram of the distribution of hybrid index values for specimens collected by Howard and Maerz (2022) and screened for parasites. Values close to zero indicate *P. teyahalee,* values close to 1 indicate *P. shermani,* and intermediate values (0.25-0.75) were categorized as hybrids.

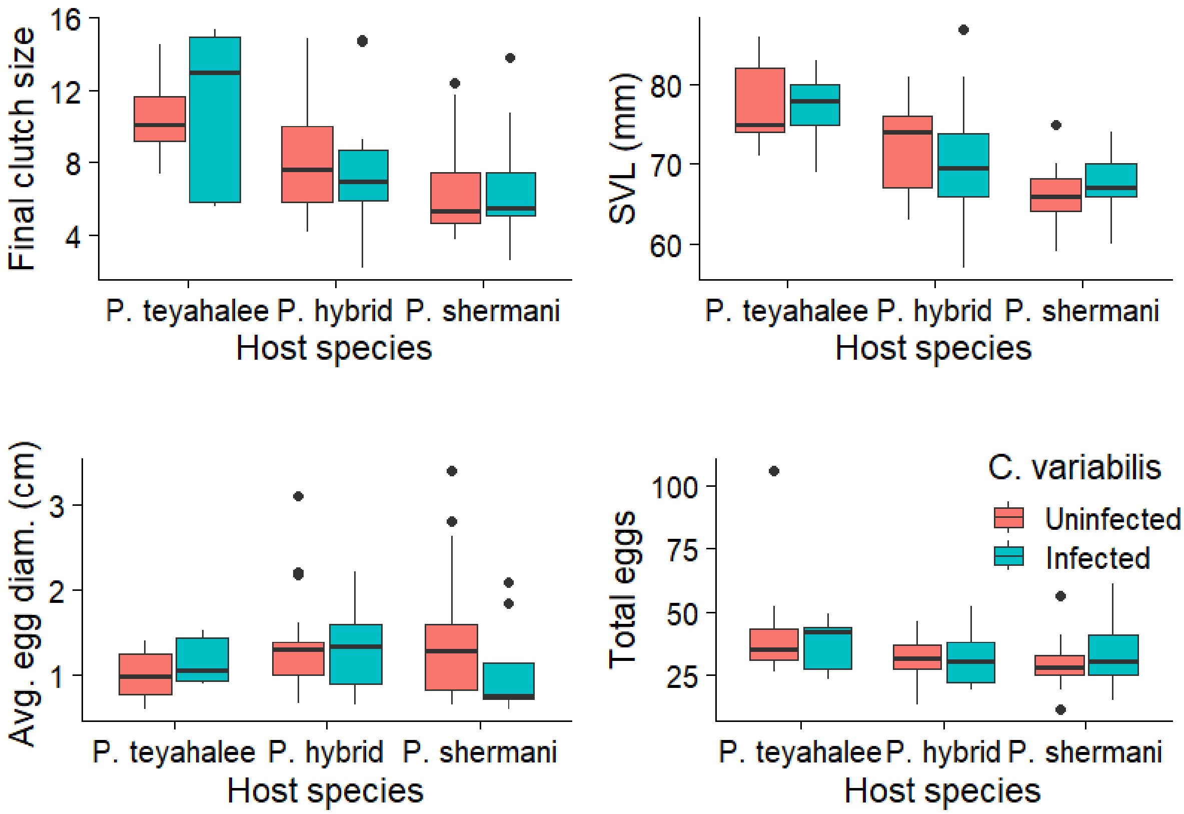

Figure S4.2: Box and whisker plot of model predicted final clutch size as well as predictors of final clutch size including average egg diameter, total number of eggs, and snout-vent length for individuals infected with and uninfected with *C. variabilis* in each host species. Black bars represent mean values. Whiskers represent 95% CI, and black dots represent outliers.

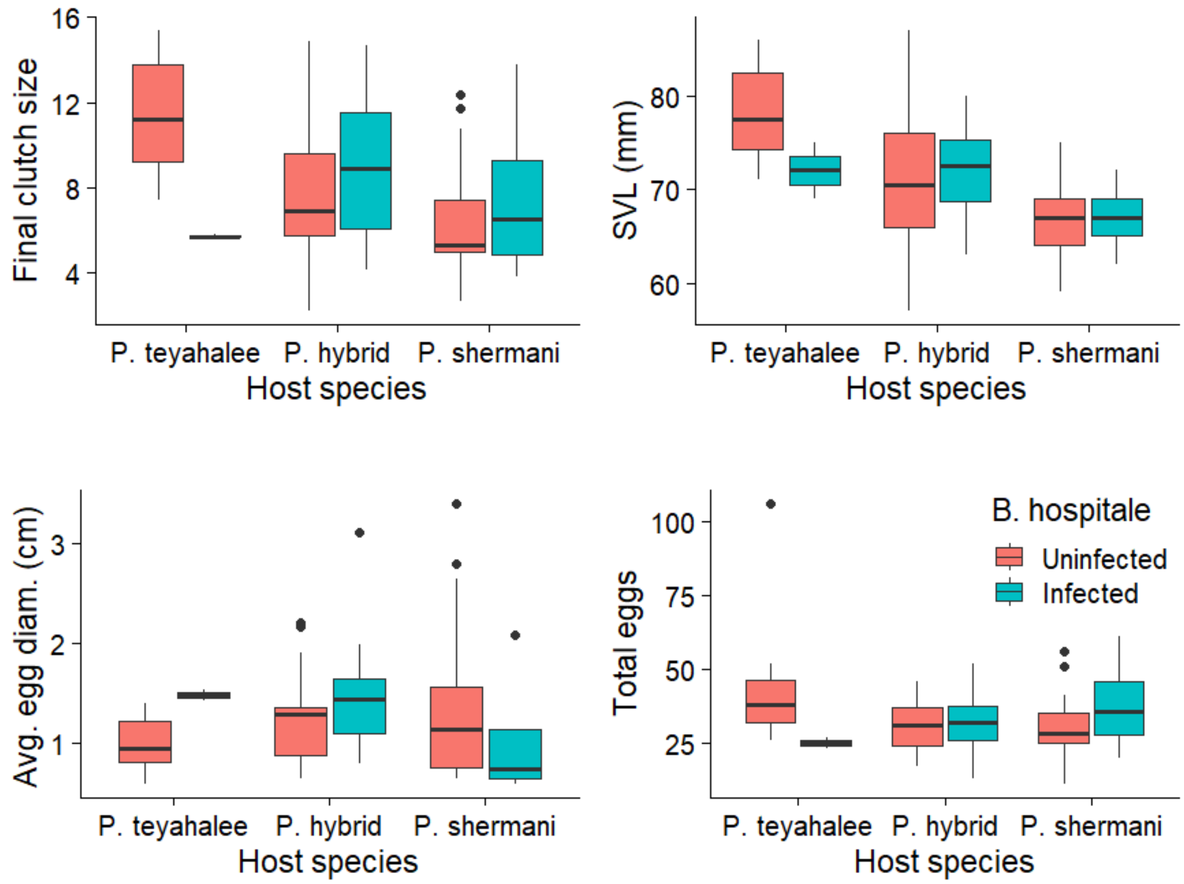

Figure S4.3: Box and whisker plot of model predicted final clutch size as well as predictors of final clutch size including average egg diameter, total number of eggs, and snout-vent length for individuals infected with and uninfected with *B. hospitale* in each host species. Black bars represent mean values. Whiskers represent 95% CI, and black dots represent outliers.

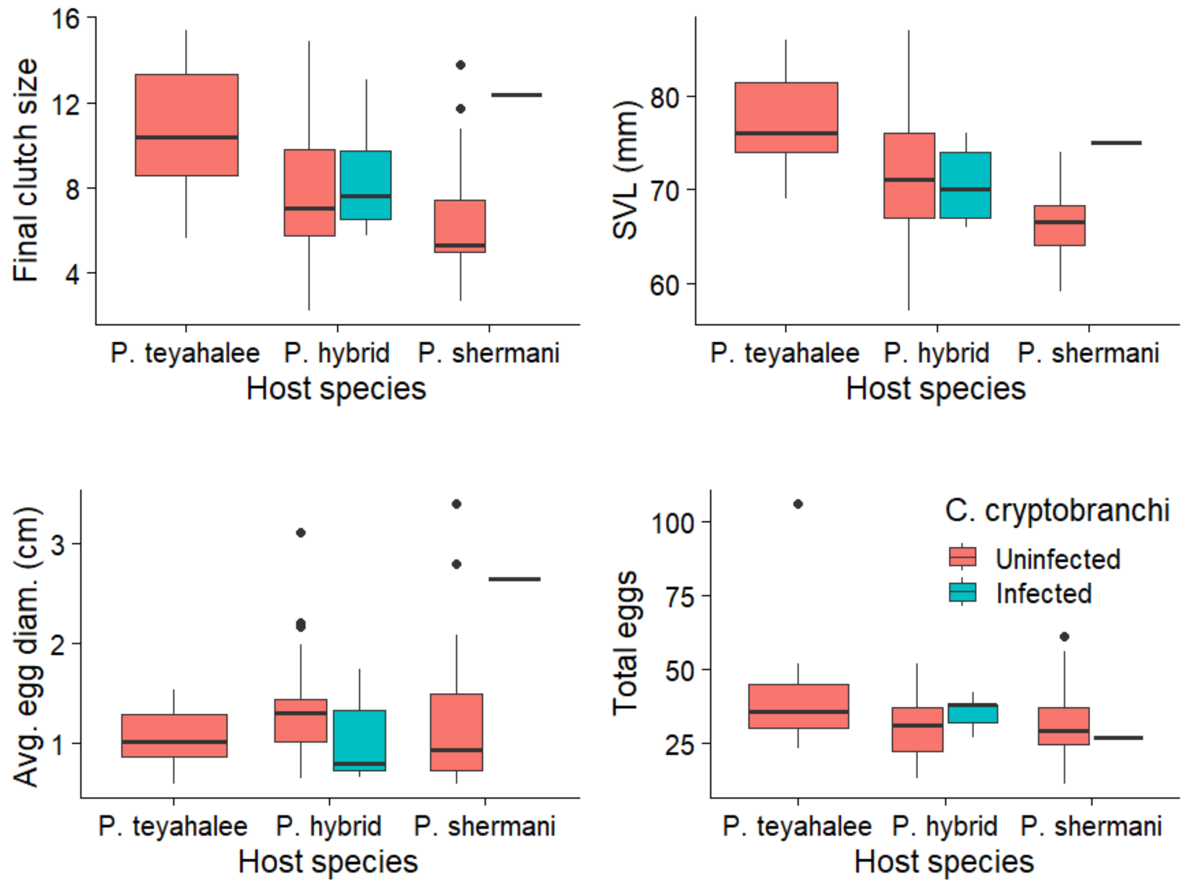

Figure S4.4: Box and whisker plot of model predicted final clutch size as well as predictors of final clutch size including average egg diameter, total number of eggs, and snout-vent length for individuals infected with and uninfected with *C. cryptobranchi* in each host species. Black bars represent mean values. Whiskers represent 95% CI, and black dots represent outliers.

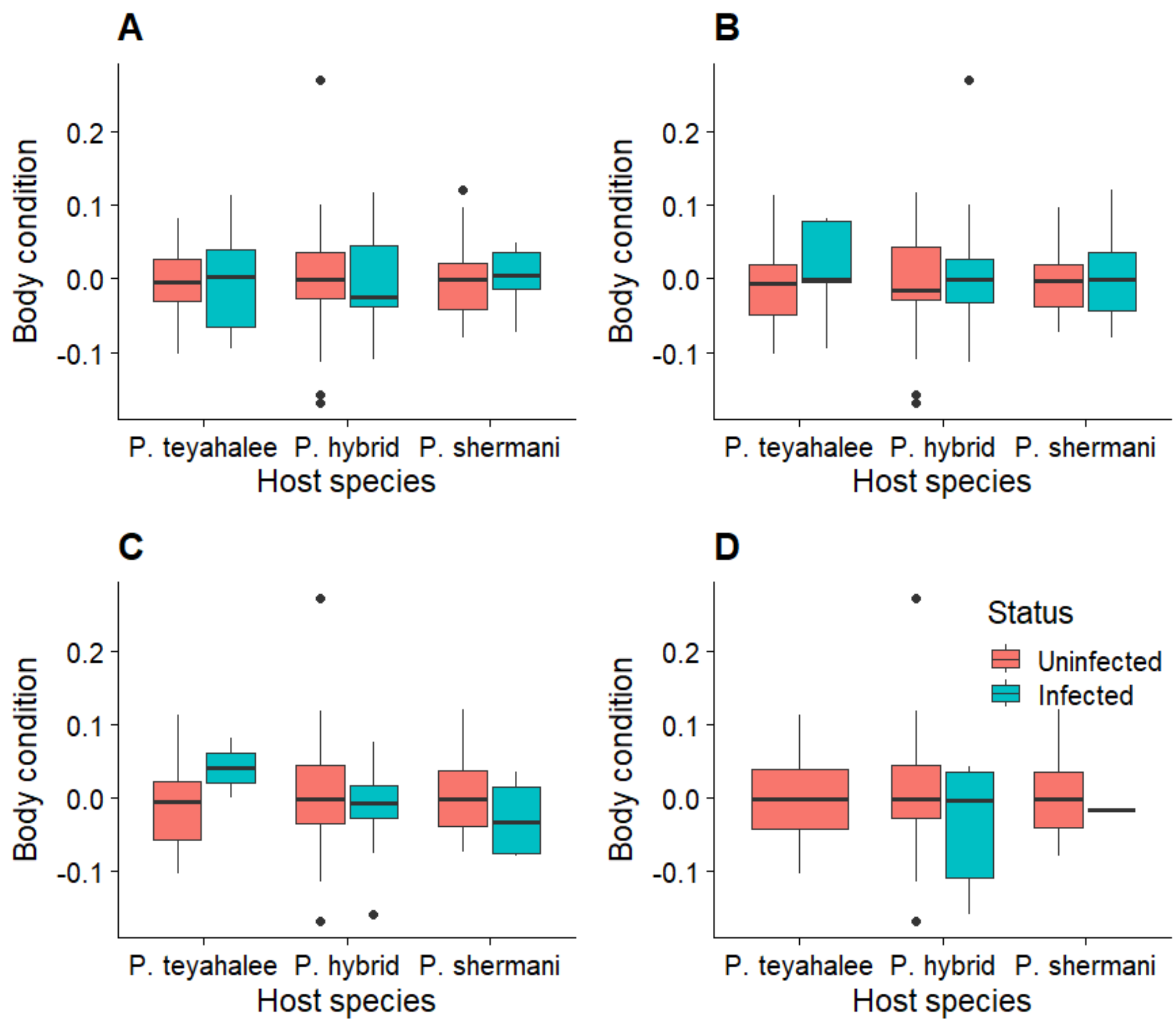

Figure S4.5: A four paneled box and whisker plot showing average body condition (y-axis) betwen infected and uninfected individuals for each host species (x-axis) and each parasite: A = *C. michiganensis,* B =*C. variabilis,* C = *B. hospitale,* and D = *C. cryptobranchi.*

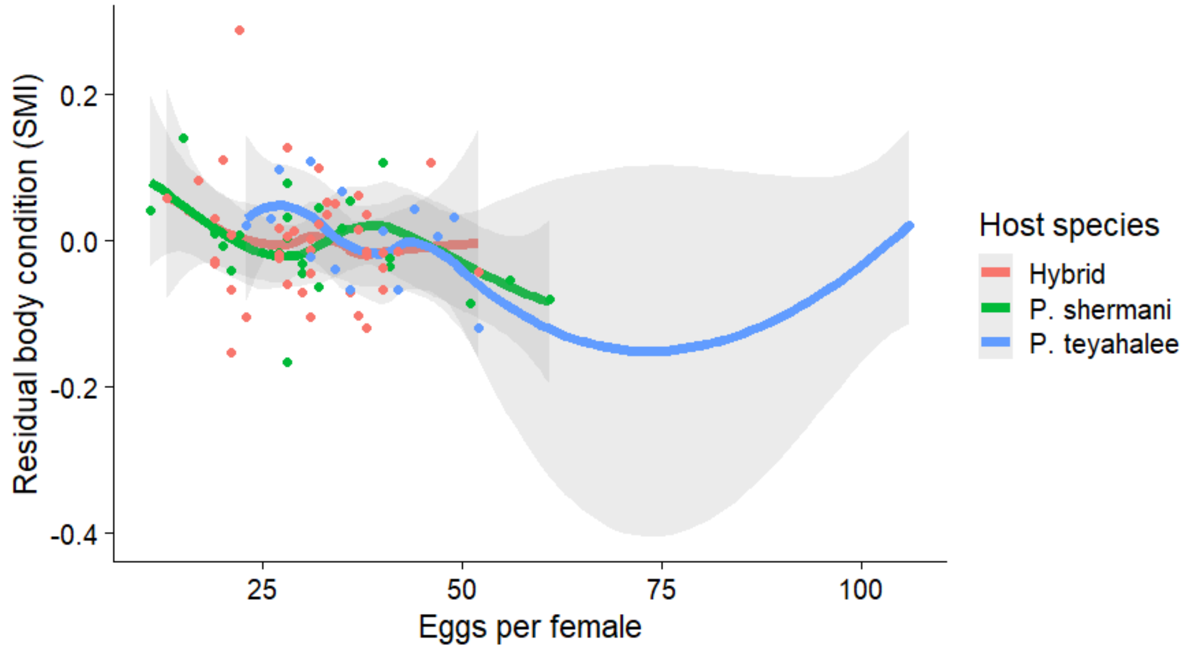

Figure S4.6: Line plot of the relationship between eggs per female >0.5mm (x-axis) and host body condition (y-axis), measured as residual scaled mass index (SMI). Line color represents host species.