THESIS

WILDFIRE EFFECTS ON HOST-PARASITE INTERACTIONS IN FRESHWATER STREAMS

Submitted by

Emma C. Svatos

Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Masters of Science

Colorado State University

Fort Collins, Colorado

Spring 2024

Master's Committee:
    Advisor: Daniel Preston

    Sarah Hart
    Caitlin Well

ABSTRACT

WILDFIRE EFFECTS ON HOST-PARASITE INTERACTIONS IN FRESHWATER STREAMS

Wildfires are increasing in intensity and frequency globally, accentuating the need to understand the implications of fire on community interactions. While previous research has focused on fire effects on free-living species, our knowledge of how wildfires influence parasite interactions with hosts and predators remains limited, especially in freshwater ecosystems. This thesis addresses this knowledge gap and presents results from two distinct multi-year observational field studies that explore how wildfires influence interactions among parasites, hosts, and predators in freshwater streams. In Chapter 1, I used a Before-After-Control-Impact design to compare freshwater snail (*Juga plicifera)* host populations and trematode parasite communities in Oregon streams before and after wildfire disturbance. In Chapter 2, I investigated host-parasite-predator interactions involving mermithid nematode parasites (Family Mermithidae), mayfly hosts (Order Ephemeroptera), and trout predators (*Salvelinus fontinalis, Salmo trutta,* and *Oncorhynchus clarkia*) in the southern Rocky Mountains after severe wildfires. Despite substantial changes to stream habitat, snail host populations and trematode infection patterns and community structure remained relatively stable following fire disturbance in Oregon streams; however, I observed subtle taxon-specific responses to fire, suggesting changes in abundance or behavior of definitive hosts. In Rocky Mountain watersheds, mermithid parasite patterns varied considerably over time in burned streams, which coincided with similar responses in mayfly host densities, suggesting that wildfire indirectly affected mermithid infection patterns through host-density changes in the stream. Host attributes also influenced parasite interactions, as intermediate-size Baetidae mayflies experienced the highest probability of infection. Furthermore, infection prevalences of mayflies consumed by trout were positively related to mayflies in the benthos; yet, infection prevalences in trout stomachs were lower on average, potentially due to parasite-induced behavioral changes in infected mayfly hosts that reduced susceptibility to predation. Wildfire did not seem to affect rates of this predator-parasite interaction, as instances of concomitant predation remained consistent in burned streams over time. Comparing two different host-parasite systems sheds light on how environmental variables and host-parasite ecologies mediate wildfire effects on parasite interactions with hosts and predators. Together these findings expand our knowledge of parasite ecology in aquatic macroinvertebrate hosts, offer insights into the role of parasites in energy flow through food webs and as bioindicators of environmental change, and help integrate parasite interactions into our understanding of disturbance ecology in freshwater streams.

ACKNOWLEDGEMENTS

I would like to express my immense gratitude to the remarkable individuals whose support was integral to the completion of this thesis. First, I want to thank my advisor, Dr. Dan Preston, whose thoughtful insights and unwavering assurance propelled my graduate journey. I am also grateful for the expertise and support of my graduate committee members, Dr. Sarah Hart and Dr. Caitlin Wells.

Much of the field and lab work involved in my graduate research would not have been possible without the collaboration of past and current Preston Lab members. I’m infinitely thankful to Landon Falke and Fernando Carvallo, for their company during long field excursions, valuable feedback on manuscripts, and treasured friendships. I’m also greatly appreciative of Erin Crone, Jordan Trujillo, Kim Nichter, Maricela Alaniz, Alyssa Graziano, and Sam Radosevich for surrounding me with such an inspiring community of graduate peers. Special recognition is also due to my dedicated mayfly-dissecting undergraduates, Mia Ter Kuile-Miller, Sophia Rye, and Lukas Keller, whose generous assistance in the lab greatly contributed to my work. I also want to offer my heartfelt thanks to my mentor and friend, Dr. Miranda Strasburg, whose guidance ignited my passion for freshwater science and showed me what an amazing ecologist can be.

I am deeply grateful to my partner and to my loving family, whose steadfast encouragement was constant source of motivation throughout my graduate education and made this achievement possible. Of course, I thank Agatha, for making my days brighter.

Most importantly, thank you, Mom, for always believing in me.

TABLE OF CONTENTS

CHAPTER 1:

DISTURBANCE AND DISEASE: HOST-PARASITE INTERACTIONS IN FRESHWATER STREAMS REMAIN STABLE FOLLOWING WILDFIRE[1]

Chapter Summary

Increases in the intensity and frequency of wildfires highlight the need to understand how fire disturbance affects ecological interactions. Though the effects of wildfire on free-living aquatic communities are relatively well-studied, how host-parasite interactions respond to fire disturbance is largely unexplored. Using a Before-After-Control-Impact design, we surveyed 10 stream sites (5 burned and 5 unburned) in the Willamette River Basin, Oregon and quantified snail host infection status and trematode parasite community structure one year before and two years after historic wildfires. Despite the severity of the wildfires, snail host populations did not show significant shifts in density or size distributions. We detected nine taxa of trematode parasites and overall probability of infection remained consistent over the three-year study period. However, at the taxon-specific level, we found evidence that infection probability by one trematode decreased and another increased after fire. In a larger dataset focusing on the first year after fire (9 burned, 8 unburned sites), we found evidence for subtle differences in trematode community structure, including higher Shannon diversity and evenness at the burned sites. Taken together, host-parasite interactions were remarkably stable for most taxa; for trematodes that did show responses, changes in abundance or behavior of definitive hosts may underlie observed patterns. These results have implications for using parasites as bioindicators of environmental

---

[1] Co-authored by Landon P. Falke and Dan L. Preston

change and suggest that aquatic snail-trematode interactions may be relatively resistant to wildfire disturbance in some ecosystems.

Introduction

Wildfire intensity, frequency, and potential are increasing globally due to anthropogenic influences on climate, fire regime and land-use (Westerling et al. 2006; Liu et al. 2010; Higuera and Abatzoglou 2021). Freshwater ecosystems can be susceptible to fire disturbance, resulting in changes to hydrology and water quality via increases in nutrient loading (Pettit and Naiman 2007; Sherson et al. 2015), peak flows (Saxe et al. 2018), water temperature (Minshall et al. 1997; Ice et al. 2004), turbidity, acidity, and hypoxia (Dahm et al. 2015). Aquatic habitat modification can also result from wildfire, via changes in substrate embeddedness, canopy cover, in-stream woody debris, and habitat heterogeneity (Minshall et al. 1997; Cooper et al. 2015; Vaz et al. 2015). In turn, fire can alter macroinvertebrate abundance and biomass (Musetta-Lambert et al. 2019), and population structure in fish (Rosenberger et al. 2015). Previous reviews have described how fire impacts abiotic factors and free-living (i.e., non-parasitic) biota in freshwater systems (Bixby et al. 2015), but much less is known about how wildfire can affect parasite communities and disease dynamics (Albery et al. 2021).

Fire can alter host-parasite interactions through direct and indirect mechanisms (Scasta 2015; Albery et al. 2021). For instance, wildfires can exert direct effects on transmission, such as rendering the environment unsuitable for the success or survival of mobile parasite life stages (Hossack et al. 2013a; Hossack et al. 2013b; Kaiser et al. 2021). Additionally, fire can have indirect effects on disease transmission via modification to host density or host resistance/tolerance to infection. Fire can cause host density to decline if hosts leave burned areas or experience direct mortality, potentially reducing infection intensity or prevalence for density-dependent parasites that are directly transmitted (see Albery et al. 2021). For example, Hossack et al. (2013a) found that fire reduced parasitic nematode abundance due to declines in host salamander abundance. Alternatively, fire can increase disease risk via changes in host density. For instance, increases in aquatic primary production several years following fire may increase host density of grazing taxa and potentially enhance parasite transmission (Ortega 2018). Wildlife susceptibility to disease can also be influenced by wildfire through the effects of environmental stress on host immune system functioning (Black et al. 2017; Bowen et al. 2015) In general, host-parasite ecology will mediate the mechanisms through which wildfire affects infection. Parasites with complex life cycles and mobile infectious stages are vulnerable to abiotic environmental conditions at multiple life stages (Poulin 2011; Pietrock and Marcogliese 2003), whereas parasites that are directly transmitted between hosts without a mobile stage are most likely to be affected by indirect, host-mediated mechanisms. Previous research (e.g., Scasta 2015 and Albery et al. 2021) has focused primarily on fire effects on terrestrial disease systems, with little evidence for how fire can affect host-parasite interactions in freshwater communities.

Parasite diversity is typically correlated with free-living community diversity at the local scale (Hechinger and Lafferty 2005), therefore host-parasite dynamics can be indicative of environmental disturbance (Vidal-Martinez et al. 2010; Sures et al. 2017). Parasites depend on their host population to survive and reproduce; thus, parasite richness is positively associated with host richness, particularly for complex life-cycle parasites that need multiple host taxa to reproduce (Kamiya et al. 2014; Johnson et al. 2016). Because of this tight connection between multi-host parasites and their host communities, parasites have been considered bioindicators of ecosystem health, with “healthy” ecosystems designated as those with high parasite richness

(Marcogliese 2004; Marcogliese 2005; Hudson et al. 2006). Parasites have also been used as bioindicators for pollution (e.g., reviewed by Vidal-Martinez et al. 2010, Sures et al. 2017) and extreme weather (Aguirre-Macedo et al. 2011), as well as for quantifying the effectiveness of ecosystem restoration (Valtonen et al. 1997; Huspeni and Lafferty 2004; Moore et al. 2020). Parasites are a potentially useful proxy for understanding effects of fire disturbance and subsequent community recovery, yet few studies have explored this potential (e.g., Galán-Puchades et al. 1998).

Our study objective was to quantify the effects of wildfire on trematode parasite communities and host populations in western Oregon streams using a Before-After-Control-Impact (BACI) design. The 2020 wildfire season was historic in burning over 2.5 million hectares in California and the Pacific Northwest (Higuera and Abatzoglou 2021). Wildfires burned 541,444 acres of forest in Oregon in 2020, the largest single year wildfire impact recorded by the Oregon Department of Forestry since its creation in 1911 (Oregon Department of Forestry; Reilly et al. 2022). Western Oregon is within the distribution of the freshwater stream snail, *Juga plicifera*, which hosts a diverse community of trematode parasites (Pratt and McCauley 1961; Preston et al. 2021; Falke and Preston 2022). Trematodes have complex multi-host life cycles, which can span both aquatic and terrestrial environments, and include a molluscan first-intermediate host (Esch et al. 2001; Galaktionov and Dobrovolskij 2003). Within the molluscan host, trematodes reproduce asexually, shedding infective stages (cercariae) into the aquatic environment. The free-swimming cercariae typically infect a second intermediate host and are trophically transmitted to definitive vertebrate hosts, such as fish, amphibians, birds, and/or mammals depending on the trematode species (Pratt and McCauley 1961; Galaktionov and Dobrovolskij 2003). The definitive host then deposits trematode eggs into the aquatic environment through defecation (Galaktionov and Dobrovolskij 2003).

We expected that responses of trematode communities to wildfire would depend on species-specific life-histories. Whether the trematodes have autogenic lifecycles (i.e., only use aquatic hosts) versus allogenic lifecycles (i.e., use aquatic and terrestrial hosts) was expected to influence responses to wildfire. We expected that autogenic taxa would be less responsive than allogenic taxa if fire had strong effects on abundance or space use of terrestrial hosts. For instance, we hypothesized that some terrestrial species, such as small mammals, may be displaced from the local environment by the fire (Fontaine and Kennedy 2012), potentially reducing the prevalence of mammalian-dependent trematodes. Such effects may not occur for trematode taxa that only infect aquatic hosts, particularly if disturbance was not strong enough to cause direct mortality of stream biota (e.g., snails, other invertebrates, fish). We also expected that responses in trematode community structure to wildfire disturbance may depend on timescale, as parasite responses can lag several years behind environmental changes (Huspeni and Lafferty 2004). Specifically, we predicted that snail density may decrease immediately after fire due to direct mortality from disturbance, but then may increase two years following fire due to bottom-up effects from increased light, nutrients, and primary productivity within the stream (Silins et al. 2014). While snail hosts are necessary to support trematodes (i.e., loss of snails would result in loss of trematodes), we did not necessarily expect snail density to correlate with trematode infection prevalence, as a previous study in the same study system found no correlation between these variables (Falke and Preston 2021).

Methods

Survey Locations

We conducted surveys at 10 stream sites located within the Willamette River Basin, Oregon between the months of June and August in the years 2019, 2021, and 2022 (Fig. 1.1).

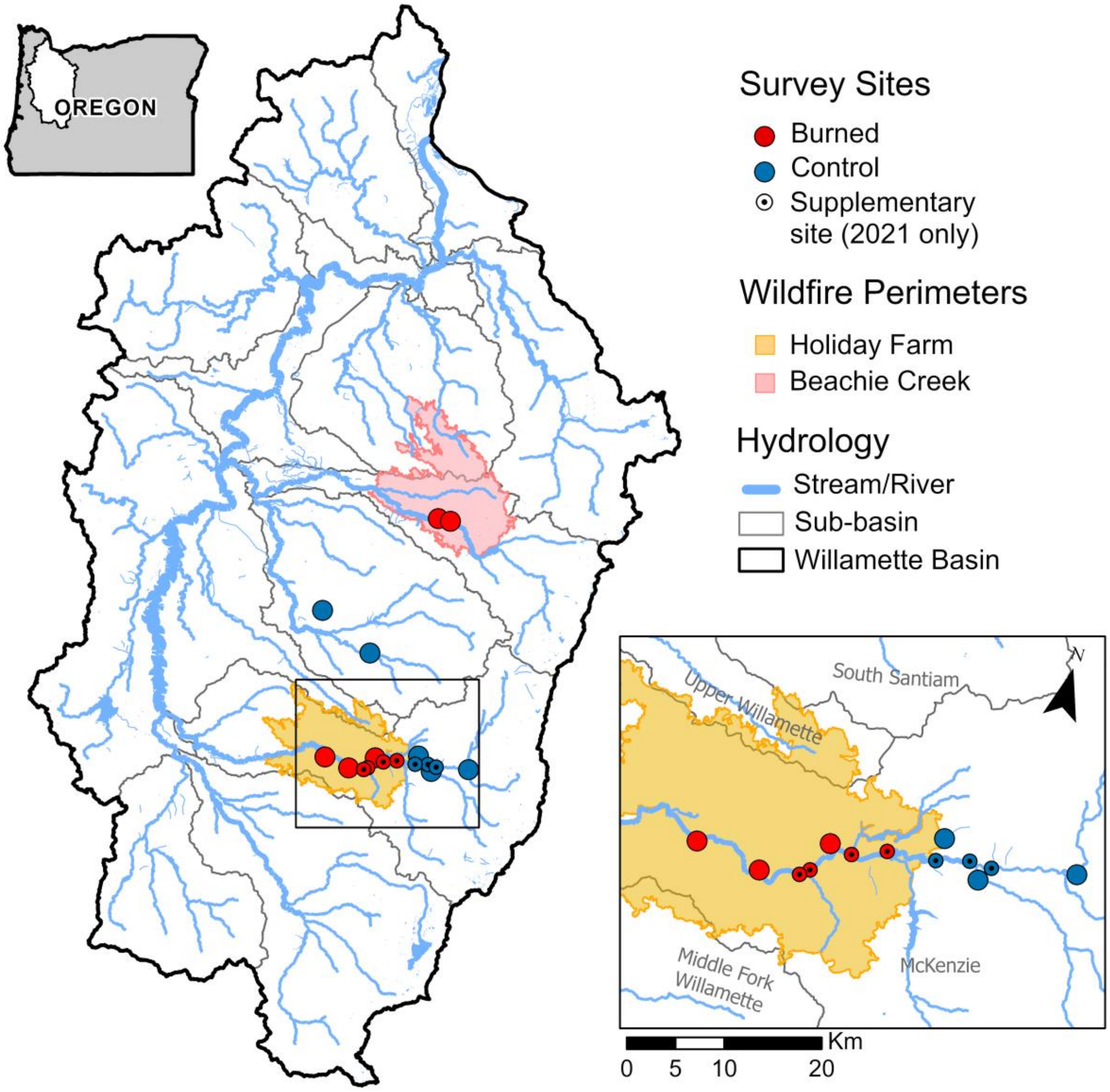

Figure 1.1. Map of the Willamette River Basin, Oregon with survey locations (circles) characterized by burn status (burned in red or unburned in blue) and survey timing (black inner circle indicates that the site was only surveyed in 2021 rather than all three years). Burned areas of the 2020 Holiday Farm (orange) and Beachie Creek (pink) wildfires are indicated by the colored polygons

Our study sites included a mixture of low- to mid-order streams at low-elevations (271-532 m), with relatively dense riparian vegetation and canopy cover prior to the fires. These 10 sites were originally surveyed in 2019 during a watershed-scale study throughout the Willamette River Basin (Falke et al. 2021). In the fall of 2020, the Holiday Farm and Beachie Creek wildfires burned forested areas of the McKenzie River and North Santiam River sub-basins (Reilly et al. 2022). Five of the previously surveyed sites were burned, with three sites in the McKenzie River sub-basin and two sites in the North Santiam sub-basin. We replicated surveys at these five burned sites in 2021 and 2022 along with five unburned reference sites originally surveyed in 2019 (n = 10 total sites surveyed over three years with 5 burned and 5 unburned; Fig. 1.1). The reference sites were selected to have similar stream order and sub-basin location compared to burned sites. Due to low snail densities at potential reference sites in the North Santiam, two reference sites of similar stream order were instead selected from the nearby South Santiam sub-basin. Because we collected data at the same sites before and after the wildfires, we were able to use a Before-After-Control-Impact (BACI) design to analyze the effects of burn status on snail populations and trematode infection responses at these 10 sites (see *Data Analyses* below). To increase sample size, we also surveyed an additional seven sites (4 burned and 3 unburned) in the McKenzie River sub-basin in the summer of 2021 only (Fig. 1.1). This supplementary data set was used to examine spatial effects of fire across all 17 stream sites in the first year after fire only (10 BACI sites + 7 supplementary sites = 17 total sites; 9 burned and 8 unburned).

Field Surveys

At each site, we quantified *Juga* snail population demographics and environmental variables within designated 5-m stream reaches. We quantified snail density by sampling total snail abundance within five 0.25 m^2 quadrats spaced at 1-m intervals along the reach (following Falke and Preston 2021). We measured total shell length of all snails in quadrats to the nearest 0.01 mm and used length-to-dry-mass conversion equations to convert snail size to individual and total dry biomass (g m^{-2}) (Preston et al. 2021). We used a spherical densiometer to measure percent canopy cover (averaged across two measurements taken at the upstream and downstream reach ends). We recorded a measure of relative riparian vegetation density adjacent to the stream reach based on observations of distances between stems of vegetation, on a qualitative scale of 1 (greatest distance between stems) to 5 (shortest distance between stems). We used ArcGIS Pro 3 (ESRI) to determine burn severity in a buffer zone 500-m surrounding each survey site using the "buffer" analysis tool and a map of differenced Normalized Burn Ratios, classified into Unburned-Low-Medium-High burn severity (Monitoring Trends in Burn Severity 2021). The buffer was designated at 500m due to the hypothesized space use of potential terrestrial definitive hosts that would contribute to the trematode lifecycle (Table S1.1).

Quantifying Trematode Infection

To quantify trematode infection, we opportunistically collected 120 *Juga* snails with shell length greater than 10mm from each study site for dissection. Snails below this size are very rarely infected (Falke and Preston 2022). We selected this sample size based on species accumulation curve analyses of infection data from a previous study (see Supplemental Material 1 and Fig. S1.1). For each snail dissection, we measured total shell length using electronic calipers, then fractured the shell using pliers to examine the snail tissue for trematode parasites under a dissecting microscope (8x-35x). We identified trematode taxa based on morphological characteristics of the cercariae and rediae, informed by previous identifications in the Willamette

Basin done with 28S gene sequencing (Preston et al. 2021). Trematodes were identified to genus or species with this approach.

Data Analyses

We first analyzed environmental variables, host snail population responses, and trematode diversity for each site using generalized linear models (GLMs; “stats” package in R). Response variables for the 10 BACI sites were analyzed with the same model structure, which included burn status (burned and unburned), year (2019, 2021, or 2022), their full interaction term, and sub-basin identity (McKenzie or Santiam). If the fires affected the response variable, we expected to see a significant interaction between burn status and year. For riparian vegetation and trematode species richness, we used quasi-Poisson regression because these responses involved right-skewed positive integers and displayed underdispersion. We used negative binomial regressions for canopy cover due to overdispersion. To further characterize trematode communities, we calculated Shannon Diversity (Shannon 1948) and Pielou’s Evenness Index (Pielou 1966; hereby 'evenness') for each site. Because the data were continuous and right-skewed, we specified the Gamma distribution in GLMs for diversity and evenness, as well as the snail host population responses, which included mean snail biomass (g m^{-2}) and mean snail density (snails m^{-2}). We then separately analyzed all environmental, snail population, and trematode diversity response variables for the dataset of 17 sites from 2021 only (one-year post-fire) using the same GLMs as above, except the interaction between burn status and year was replaced by burn status (no interaction term).

We additionally analyzed individual host-level infection status by all trematode taxa combined, as well as for each trematode taxon individually, using binomial generalized mixed effects models (GLMMs, “lme4” package, with a binomial response of infected or uninfected). For the 10 BACI sites, we included the predictor variables burn status (burned and unburned), year (2019, 2021, or 2022), their full interaction term, and sub-basin identity (McKenzie or Santiam), as described above. The GLMMs also included a fixed effect of snail size to account for the higher probability of infection in larger/older snails and a random intercept for site identity to account for non-independence of snails from the same site. We then separately analyzed snail infection status from the dataset of 17 sites from 2021 only (one-year post-fire) using the same binomial GLMMs as above, except omitting the effect of year. All GLMs and GLMMs were assessed for outliers, under or over-dispersion, uniformity, and zero-inflation using the *DHARMa* package (Hartig 2022). Two trematode taxa (*Plagioporus silicus* and *Notocotylus imbricatus*) were too rare to include in the taxon-specific models but were included in the overall infection model.

To evaluate trematode community structure across the 17 sites one-year post-fire, we used non-metric multidimensional scaling (NMDS; Kruskal 1964) with the Bray-Curtis dissimilarity measure and 'burned' or 'unburned' as a categorical grouping factor. We used an Analysis of Similarities (ANOSIM; Clarke 1993) to quantify similarity in community composition between the two groups (n = 9 burned and 8 unburned sites). We did not conduct these trematode community composition analyses (NMDS and ANOSIM) for the primary BACI data due to small sample size and high NMDS stress. The NMDS and ANOSIM models were performed using the “vegan” package (Oksanen et al. 2013). All data analyses were conducted in R version 4.1.1 (R Core Team 2021).

Results

Environmental Variables and Snail Populations

The 2020 wildfires impacted 93.2% of land area within a 500-m buffer surrounding our burned sites, with 17.1% of that area categorized as low burn severity, 47.6% medium severity, and 28.6% high severity. In contrast, only 1.9% of the land area in a 500-m buffer surrounding the unburned sites was categorized as burned (Table S1.2). After the fires, there were decreases in both canopy cover (burn status*year; z=-2.01, p=0.04; Fig. 1.2a) and riparian vegetation (burn status*year; z=-2.65, p=0.01; Fig. 1.2b) at the burned sites. Across all sites in 2021, canopy

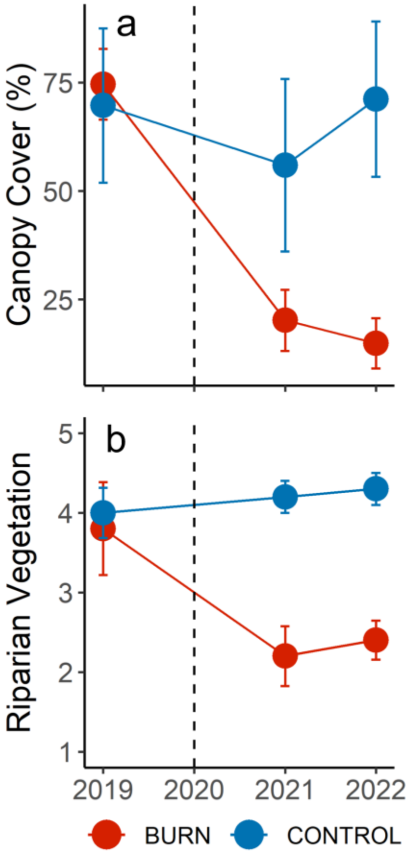

Figure 1.2. Before-After-Control-Impact response of environmental variables to wildfire. Points indicate mean (a) canopy cover and (b) riparian vegetation density across burned (red) and unburned (blue) sites, with the vertical error bar indicating ± one standard error. Timing of wildfires are indicated by the dashed vertical line. Canopy cover is measured as a percentage, with 100% indicating complete canopy cover. Riparian vegetation density is measured on a relative scale of 1 (low) to 5 (high)

cover and riparian vegetation were 70.3% and 42.5% lower respectively at burned sites compared to unburned sites (Table S1.4). Canopy cover also varied with sub-basin identity in the BACI data, with Santiam sites having denser canopy cover than McKenzie sites before and after the fires (z=-2.41, p=0.02; Table S1.3).

There was no evidence that the fires impacted host snail populations, as the interaction between burn status and time was not significant for snail biomass or density (Fig. S1.2; Table S1.3). Similarly, there was no difference in snail biomass or density between burned and unburned sites across all sites in 2021 alone (Table S1.4), but there was a significant effect of sub-basin identity on both snail biomass (z=-0.83, p=0.01) and density (z=-2.18, p=0.05), with body size and density of snails at Santiam sites being lower than McKenzie sites. Snail size distributions were also similar between burned and control sites after the fires (Fig. S1.3).

Snail Host Infection

Across all sites in 2021, 25.4% of dissected snails were infected by at least one trematode taxa, with 1.1% co-infected by more than one. We identified nine unique taxa of trematodes in infected snails: *Acanthatrium* sp. 1*.*, *Acanthatrium* sp. 2*, Metagonimoides oregonensis, Metagonimus* sp., *Notocotylus imbricatus, Cardicola alseae, Plagioporus silicus, Deropegus aspina,* and *Nanophyetus salmincola*. Larger snails were significantly more likely to be infected by at least one trematode in both the BACI (z=19.36, p<0.001) and 2021 alone (z=13.39, p<0.001) data.

There was no effect of wildfire on infection probability in snails (burn status*year; z=1.102, p=0.271; Fig. 1.3a). In general, snail infection was relatively stable throughout the three-year study period and the main effect of year was also nonsignificant (year; z=0.11, p=0.92). At the taxon-specific level, infection probability by *Acanthatrium* sp. 1 decreased after the fires (burn status*year; z=-2.22, p=0.03), but was non-significant for all other taxa that were analyzed (Fig. 1.3b-h; Table S1.5). *Metagonimus* sp. infection probability did not change significantly after fire in the BACI data (burn status*year; z=1.71, p=0.09; Fig. 1.3h), but burned sites had higher probability of *Metagonimus* sp. infection in 2021 than unburned sites (burn status; z=2.57, p=0.01; Table S1.6). Spatial differences were important for some taxa, as *Acanthatrium* sp. 1 infection probability differed across sub-basins in the BACI data (sub-basin; z=-2.27, p=0.02).

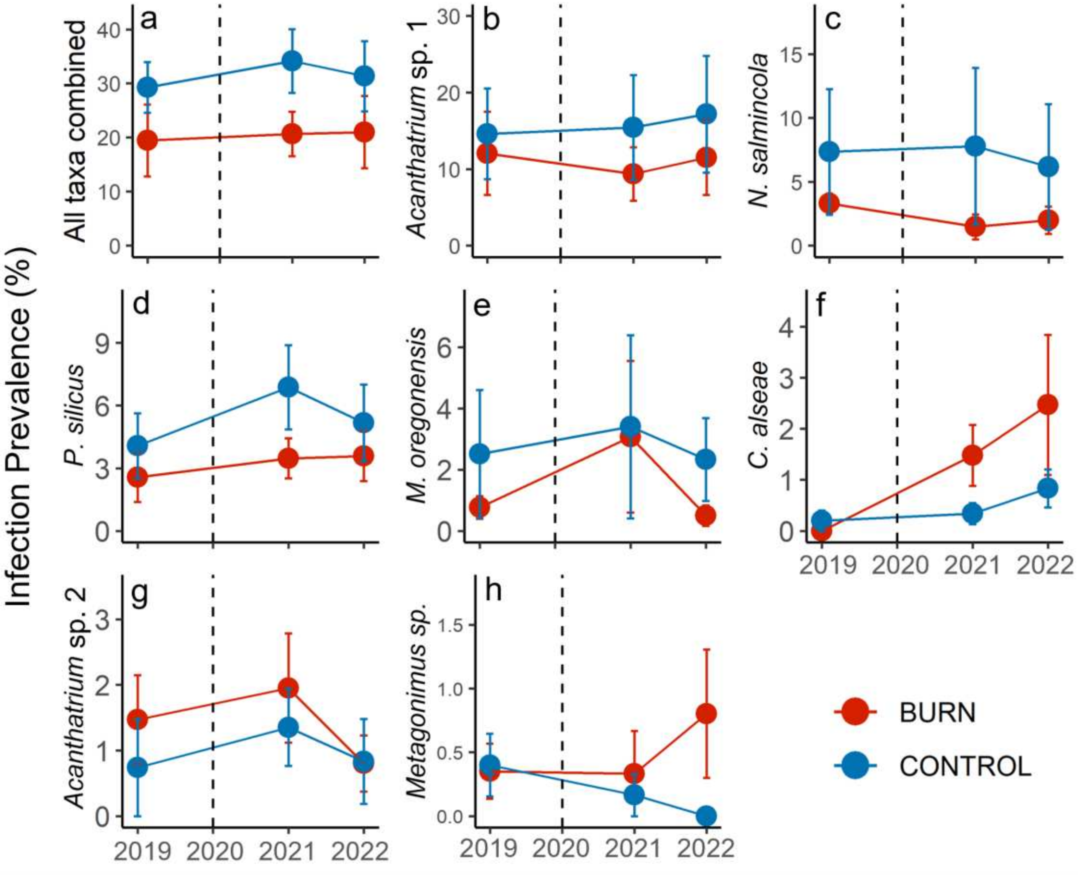

Figure 1.3. Before-After-Control-Impact responses of trematode infection prevalence to wildfire. Points indicate mean infection prevalence across burned (red) and unburned (blue) sites, with the vertical error bar indicating ± one standard error. The timing of the wildfires is indicated by the dashed vertical line. Prevalence is measured as the percent of snails infected out of total snails (>10mm) dissected at each survey site (n = 120 snails per site)

Trematode Community Structure

One year after fire, trematode community structure differed between burned and unburned sites (n=17; Fig. 1.4: NMDS, Stress=0.12; ANOSIM, p=0.05; Fig. S1.4). In support of this finding, trematode communities at burned sites had 41.6% higher Shannon diversity (t=2.68, p=0.02) and 29.4% higher evenness (t=2.90, p=0.01; Fig S1.5) compared to the reference sites.

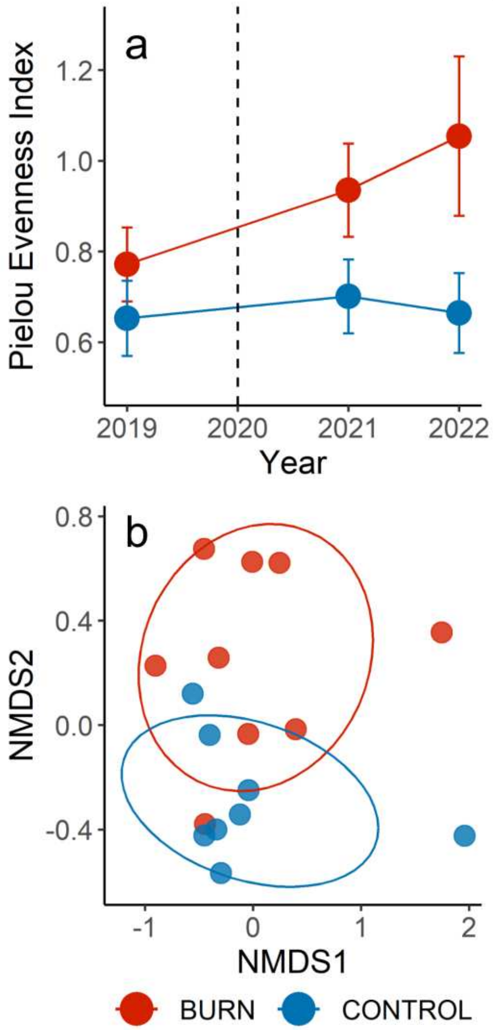

Figure 1.4. Trematode community response to wildfire. (a) Before-After-Control-Impact response of trematode community evenness to wildfire. Points indicate mean Pielou's Evenness Index values across burned (red) and unburned (blue) sites, with the vertical error bar indicating ± one standard error. The timing of the wildfire impact is indicated by the dashed vertical line. (b) Nonmetric multidimensional scaling (NMDS) plot showing trematode community structure one-year post-fire (n = 17 sites). This plot visualizes the trematode community dissimilarity between burned (red) and unburned (sites) in 2021

These community differences may be attributed to an increase in rare trematode taxa, as removal of the three most rare taxa (*Metagonimus* sp*., N. imbricatus, P. silicus*) from the model resulted in non-significant differences in diversity and evenness (Shannon diversity: t=1.98, p=0.07; evenness: t=1.98, p=0.07). Similar trends of increased trematode diversity and evenness were observed in the BACI data (Fig. 1.4), but the interaction between burn status and year was not significant for either response (Shannon diversity: burn status*year; z=0.68, p=0.50) (evenness: burn status*year; z=1.08, p=0.29). The effect of sub-basin was significant for evenness, with lower evenness in trematode communities found in Santiam streams, for both the BACI (sub-basin; z=-3.13, p<0.01) and 2021 data (sub-basin; z=-2.10, p=0.05). Trematode richness did not change significantly after fire in the BACI data (burn status*year; t=-0.02, p=0.98; Table S1.3), nor were there any differences between trematode richness at burned versus unburned sites in the 2021-only data (burn status; t=1.13, p=0.28; Table S1.4).

Discussion

Recent and projected increases in the frequency, intensity, and size of wildfires (Abatzoglou and Williams 2016) highlight the need to understand how fire can influence host-parasite interactions and disease dynamics (Albery et al. 2021). Following a historic wildfire year in the Willamette River basin in Oregon, snail population demographics and host infection probabilities appeared relatively unaffected; however, some taxon-specific changes in infection were observed. We found evidence for differences in trematode community structure at the burned and unburned sites in the first year after fire. The mixed response of trematode parasites to wildfire disturbance could be due to various mechanisms related to life history characteristics of the hosts and/or parasites, resistance of the snail hosts to disturbance, the magnitude of the disturbance, and the temporal scale of the study.

We predicted that host-parasite interactions would be affected by fire via changes in snail host populations. For instance, fire disturbance could cause direct mortality of snail hosts on short timescales due to sedimentation and changes in temperature, dissolved oxygen, and water quality that often cause invertebrate mortality (Bixby et al. 2015). Recent research, however, on the Holiday Farm Fire indicates that stream conditions during and within one year of the fire in low order streams were unlikely to cause significant mortality of biota (Sanders et al. 2022, Swartz and Warren 2022). Fire and associated habitat changes may not have killed snails. Freshwater snails may also be more resilient to fire effects relative to other benthic macroinvertebrates (Munro et al. 2009). Alternatively, fire could drive positive bottom-up effects from increased light and nutrients within a few years after fire disturbance subsides (Minshall 2003; Sillins et al. 2014; Cooper et al. 2015; Sherson et al. 2015; Klose et al. 2015). Bottom-up effects on periphyton due to increased light and temperature have been documented in our study area one year after the Holiday Farm Fire (Swartz and Warren 2022). Yet we did not find evidence for this mechanism increasing snail recruitment or growth, as densities and size distributions were unaffected by fire. The long lifespan of *Juga* snails may limit detection of fire effects in the short-term. Stream macroinvertebrate communities are often altered in the first few years following fire and subsequent floods (Gresswell 1999; Minshall 2003), primarily shifting dominance to early colonizing taxa with short lifespans and high reproduction rates (Anderson 1992; Minshall 2003; Mellon et al. 2008). In contrast, *Juga spp.* are long-lived snails, documented to live five to seven years on average and taking three years to reach sexual maturity (Furnish 1990). If bottom-up effects were occurring*,* *Juga* snail generation times may take longer to “catch-up” to disturbance effects, thus not exhibiting population changes within our study timeframe.

The relative consistency in snail infection before and after fire suggests stability of host-parasite interactions in this study system. Trematode infection typically lasts the lifespan of the snail host once infected (Minchella et al. 1985). This low infection turnover could explain the consistency of infection prevalence observed in our study’s timeframe. Byers et al. (2016) found that trematode infection prevalence in marine snails was surprisingly static over a 10-year study period, even with at least one generational turnover of snail hosts. Similarly, May-tec et al. (2020) found that trematode prevalence in a short-lived snail (~2 years) had relatively high seasonal variability, but only varied between 1% and 5% over nine years. The stability of these interactions at the population level is somewhat remarkable given the complexity of trematode life cycles and their reliance on multiple host taxa in the aquatic and terrestrial environment.

Infection can also be regulated by non-snail host richness and factors that influence trematode transmission. We expected multi-host trematodes to be sensitive to a potential loss of host biodiversity (Lafferty 2012, Johnson et al. 2016) from fire, but our results showed that trematode richness was unchanged following fire. This finding suggests that impacts of wildfire on non-snail hosts were not substantial enough or did not persist on a long enough timescale to affect transmission to snails. It is possible that mobile hosts returned to the burned forests within a short timeframe. In addition to indirect effects via changes in hosts, disturbance can directly affect free-swimming stages of parasites (e.g., miracidia and cercariae for trematodes;(reviewed by Pietrock and Marcogliese 2003). That said, the effects of wildfire on stream conditions may have been relatively brief, minimizing effects on free-living parasite stages. The timing of the fires relative to transmission events from definitive hosts to snails may have also contributed to our results. The hatching time and survival of miracidia, the trematode life stage that infects snails, is positively correlated with temperature (Ménard and Scott 1987). Given that the wildfires in our study occurred in the autumn (mid-August to early December 2020), it is possible that the timing of the fires may have mismatched peak trematode transmission to snails, resulting in minor effects compared to a fire earlier in the season.

Despite the consistency of snail infections overall, we observed some trematode taxon-specific differences. One year following fire, trematode community composition differed between burned sites and unburned sites. Specifically, *Acanthatrium* sp. 1 decreased and *Metagonimus* sp. increased following fire. We expected that trematodes with similar life cycle characteristics (e.g., autogenic or allogenic) would respond similarly to wildfire, but our results did not support this. The life cycles of *Acanthatrium* sp. 1 and *Metagonimus* sp. are both allogenic, meaning that they require hosts from both aquatic and terrestrial environments to complete their life cycles (Knight and Pratt 1955; Izumi 1935). These contrasting responses may be due to fire-induced changes in second intermediate or definitive host populations specific to each taxon. After infecting a snail, *Acanthatrium* infects aquatic insects and then bats (Faust 1919; Alicata 1932; Knight and Pratt 1955). Local abundances of bats may return to baseline or increase in burned forest stands as early as one-year post-fire (Buchalski et al. 2013). On the other hand, abundances of fire-sensitive aquatic insect taxa may decrease immediately following fire (Oliver et al. 2012). Sub-basin affected *Acanthatrium* infection probabilities, which may suggest that other environmental factors are important in regulating abundances of this trematode. Unlike *Acanthatrium, Metagonimus* sp. increased after fire. *Metagonimus* infects fishes and then small mammals (Izumi 1935). Small mammals display mixed species-specific responses to fire, though burns can increase certain small mammal abundances after the initial fire disturbance (Fontaine and Kennedy 2012). Furthermore, some mammalian species may congregate specifically in riparian areas, as "refuge islands" from burned forest (Steenvoorden et al. 2019). This concentration of potential hosts in the riparian corridor could increase trematode transmission and infection observed in aquatic hosts (Ecke et al. 2019), perhaps underlying the increase in *Metagonimus* sp. in burned areas following fire. Quantifying non-snails host responses, including terrestrial vertebrates, would provide additional insights into the mechanisms driving the observed changes in certain trematode taxa.

Our study has several limitations that should be considered in interpreting the results. We surveyed 10 sites using the BACI design, and BACI studies are a powerful means of inference because they control for site-level variation by quantifying responses before and after the event of interest. That said, for responses that were not replicated at the individual snail level, such as trematode richness, our ability (i.e., statistical power) to detect subtle changes may have been somewhat limited. For this reason, we also conducted analyses on a larger set of sites from 2021 only (n = 17 sites). For some responses, the results between the BACI analysis and the larger set of sites differed slightly. This may be due to site-specific effects or differences in statistical power. Additionally, our sample sizes of snails per sites (n = 120) was sufficient to characterize trematode community structure based on prior work, but an even larger sample size may detect extremely rare taxa at the site level (see Fig. S1.1 for species accumulation curves). We do not believe the sample size affected the overall patterns in the study, but it is a consideration when analyzing differences in richness and composition with a small number of taxa (nine trematodes). Finally, our study would have benefitted from additional measures of the magnitude of fire disturbance at each site, such as measurements of nutrients, turbidity, water temperatures and other factors that typically mediate biotic stream responses after fire. Despite these limitations, our study is among the first to quantify aquatic host-parasite responses to fire, and thus provides a major step in motivating further research.

We propose the following future directions to deepen our understanding of wildfire effects on host-parasite interactions in aquatic systems, starting with a (1) longer study timeframe with seasonal variability in sampling. Given the lifespan of *Juga* and the longevity of trematode infections, response to wildfire may not occur for several years after the time of disturbance. For instance, responses of trematode prevalence to a saltwater marsh restoration project were not apparent until six years after the project was completed (Huspeni and Lafferty 2004). We also suggest studying (2) aquatic parasites with differing life history strategies, such as monoxenous (single-host life cycle) vs. heteroxenous (multi-host life cycle). Other parasite life-histories may show unique responses to disturbance (Blanar et al. 2009). Additionally, we suggest research into (3) other aquatic habitat types and fire patterns (timing of fire) to understand how environmental conditions mediate wildfire effects on host-parasite interactions. Finally, we suggest (4) further research directly linking parasite responses to wildfire disturbance to responses of free-living biodiversity. While quantifying all aquatic and terrestrial hosts infected by the trematode taxa we observed was beyond the scope of our study, such efforts are needed to evaluate the extent to which trematodes are useful indicators of broader community changes after wildfire and other environmental disturbances.

More broadly, understanding host-parasite responses to wildfire is imperative as fire patterns shift with climate change (Albery et al. 2021). Attention has focused on studies in terrestrial ecosystems, which have shown wildfire can be effective ecological tools to suppress terrestrial parasites, including ticks, mosquitos, and lice (Scasta 2015; Gallagher et al. 2022). Additional research into aquatic ecosystems is necessary, given that aquatic parasites may respond differently to fire than terrestrial parasites. Our findings suggest that infection in fire-resistant and long-lived hosts creates a mechanism for parasite persistence and may provide a source for re-establishment in disturbed habitat. Considering that water-borne infections are a serious concern for human and wildlife health, this research has implications for the use of fire as disease control for parasites with aquatic life stages. Beyond disease risk, studying host-parasite responses to disturbances is ecologically important because it expands the growing field of environmental parasitology and the use of parasites when monitoring restoration projects of disturbed ecosystems (Sures et al. 2017; Huspeni and Lafferty 2004). Our research contributes to this field by testing for responses of complex life-cycle trematode parasites to wildfire disturbance and motivating future work elucidating underlying mechanisms and the timescales of their effects.

Funding and Support

We thank Colorado State University's Department of Fish, Wildlife, and Conservation Biology, and Graduate Degree Program in Ecology for providing funding and support for this research. We also thank the Oregon Department of Forestry and the Oregon Department of Fish and Wildfire for granting us permits to access our survey sites and collect invertebrate specimens (Permits #314.24384; #26611). We would like to thank F. Carvallo, J. Trujillo, and the Preston Lab for their assistance with manuscript preparation.

References

Abatzoglou JT, Williams AP (2016) Impact of anthropogenic climate change on wildfire across western US forests. Proc Natl Acad Sci USA, 113(42):11770-11775

Albery GF, Turilli I, Joseph MB, Foley J, Frere CH, Bansal S (2021) From flames to inflammation: how wildfires affect patterns of wildlife disease. Fire Ecol, 17(1):1–17

Alicata JE (1932) A new trematode, *Acanthatrium eptesici*, from the brown bat. J Wash Acad Sci, 22(10):271-274

Aguirre-Macedo ML, Vidal-Martinez VM, & Lafferty KD (2011) Trematode communities in snails can indicate impact and recovery from hurricanes in a tropical coastal lagoon. Int J Parasitol, 41(13-14):1403-1408

Anderson NH (1992) Influence of disturbance on insect communities in Pacific Northwest streams. Hydrobiologia, 248:79-92

Bixby RJ, Cooper SD, Gresswell RE, Brown LE, Dahm CN, Dwire KA (2015) Fire effects on aquatic ecosystems: An assessment of the current state of the science. Freshw Sci, 34(4):1340–1350

Black C, Gerriets JE, Fontaine JH, Harper RW, Kenyon NJ, Tablin F, Schelegle ES, Miller LA (2017) Early life wildfire smoke exposure is associated with immune dysregulation and lung function decrements in adolescence. Am J Respir Cell Mol Biol, 56(5):657-666

Blanar CA, Munkittrick KR, Houlahan J, MacLatchy DL, Marcogliese DJ (2009) Pollution and parasitism in aquatic animals: a meta-analysis of effect size. Aquat Toxicol, 93(1):18-28

Bowen L, Miles AK, Kolden CA, Saarinen JA, Bodkin JL, Murray MJ, Tinker MT (2015) Effects of wildfire on sea otter (*Enhydra lutris*) gene transcript profiles. Mar Mamm Sci, 31(1):191-210

Buchalski MR, Fontaine JB, Heady III PA, Hayes JP, Frick WF (2013) Bat response to differing fire severity in mixed-conifer forest California, USA. PloS One, 8(3):e57884

Byers JE, Holmes ZC, Blakeslee AM (2016) Consistency of trematode infection prevalence in host populations across large spatial and temporal scales. Ecology, 97(7):1643-1649

Cooper SD, Page HM, Wiseman SW, Klose K, Bennett D, Even T, Sadro S, Nelso CE, Dudley TL (2015) Physicochemical and biological responses of streams to wildfire severity in riparian zones. Freshw Biol, 60(12):2600-2619

Clarke, KR (1993) "Non-parametric multivariate analyses of changes in community structure. Austral Ecol, 18(1):117–143

Dahm CN, Candelaria-Ley RI, Reale CS, Reale JK, Van Horn DJ (2015) Extreme water quality degradation following a catastrophic forest fire. Freshw Biol, 60(12):2584-2599

Ecke F, Nematollahi Mahani SA, Evander M, Hörnfeldt B, Khalil H (2019) Wildfire-induced short-term changes in a small mammal community increase prevalence of a zoonotic pathogen? Ecol Evol, 9(22):12459-12470

Esch GW, Curtis LA, Barger MA (2001) A perspective on the ecology of trematode communities in snails. Parasitology, 123(7):57-75

Falke LP, Preston DL (2021) Infection prevalence and density of a pathogenic trematode parasite decrease with stream order along a river continuum. Ecosphere, 12(8):e03670

Falke LP, Preston DL (2022). Freshwater disease hotspots: Drivers of fine-scale spatial heterogeneity in trematode parasitism in streams. Freshw Biol, 67(3):487-497

Fontaine JB, Kennedy PL (2012) Meta-analysis of avian and small-mammal response to fire severity and fire surrogate treatments in US fire-prone forests. Ecol Appl, 22(5):1547-1561

Furnish JL (1990) Factors affecting the growth, production, and distribution of the stream snail *Juga silicule*. Ph. D. Dissertation, Oregon State University, Corvallis, Oregon

Galaktionov KV, Dobrovolskij A (2003). The biology and evolution of trematodes: an essay on the biology, morphology, life cycles, transmissions, and evolution of digenetic trematodes. Springer Science & Business Media

Galán-Puchades MT, Fuentes MV, Cerezuela AM, Fons R, Mas-Coma S (1998) Host/parasite postfire responses: the helminths of *Apodemus sylvaticus* (Rodentia, Muridae) as bioindicators of its movements after fire. Misc Zool, 35-43

Gallagher MR, Kreye JK, Machtinger ET, Everland A, Schmidt N, Skowronski NS (2022) Can restoration of fire-dependent ecosystems reduce ticks and tick-borne disease prevalence in the eastern United States?. Ecol Appl, 32(7):e2637.

Gresswell RE (1999) Fire and aquatic ecosystems in forested biomes of North America. Trans Am Fish, 128(2):193-221

Faust EC (1919) A new trematode, *Acanthatrium nycteridis*, nov. gen., nov. spec., from the little brown bat. T Am Microsc, 38(3):209-215

Florian Hartig (2022) DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.6.

Hechinger RF, Lafferty KD (2005) Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proc Royal Soc B, 272(1567):1059-1066

Higuera PE, Abatzoglou JT (2021) Record-setting climate enabled the extraordinary 2020 fire season in the western United States. Glob Chang Bio, 27(1)

Hossack BR, Lowe WH, Honeycutt RK, Parks SA, Corn PS (2013a) Interactive effects of wildfire, forest management, and isolation on amphibian and parasite abundance. Ecol Appl, 23(2):479-492

Hossack BR, Lowe WH, Ware JL, Corn PS (2013b) Disease in a dynamic landscape: Host behavior and wildfire reduce amphibian chytrid infection. Biol Conserv, 157: 293–299

Hudson PJ, Dobson AP, Lafferty KD (2006) Is a healthy ecosystem one that is rich in parasites? Trends Ecol Evol, 21(7):381-385

Huspeni TC, Lafferty KD (2004) Using larval trematodes that parasitize snails to evaluate a saltmarsh restoration project. Ecol Appl, 14(3):795-804

Ice GG, Neary DG, Adams PW (2004) Effects of wildfire on soils and watershed processes. J For, 102(6):16-20

Izumi M (1935) Studies concerning a New Species of *Metagonimus* and its Life Cycle. Kitasato Arch Exp Med, 12(4)

Johnson PT, Wood CL, Joseph MB, Preston DL, Haas SE, Springer YP (2016) Habitat heterogeneity drives the host-diversity-begets-parasite-diversity relationship: evidence from experimental and field studies. Ecol Lett, 19(7):752-761

Kaiser SW, Greenlees MJ, Shine R (2021) Wildfires modify the parasite loads of invasive cane toads. Biol Lett, 17(12):20210470

Kamiya T, O'Dwyer K, Nakagawa S, Poulin R (2014) Host diversity drives parasite diversity: meta-analytical insights into patterns and causal mechanisms. Ecography, 37(7):689-697

Klose K, Cooper SD, Bennett D (2015) Effects of wildfire on stream algal abundance, community structure, and nutrient limitation. Freshw Sci, 34(4):1494-1509

Knight RA, Pratt I (1955) The life-histories of *Allassogonoporus vespertilionis* Macy and *Acanthatrium oregonense* Macy (Trematoda: Lecithodendriidae). J Parasitol, 41(3):248-255

Kruskal JB (1964) Nonmetric multidimensional scaling: A numerical method. Pschometrika, 29:115-129

Lafferty KD (2012) Biodiversity loss decreases parasite diversity: theory and patterns. Philos Trans R Soc Lond B Biol Sci, 367(1604):2814-2827

Lui Y, Stanturf J, Goodrick S (2010) Trends in global wildfire potential in a changing climate. For Ecol Manag, 259:685-697

Marcogliese DJ (2004) Parasites: Small players with crucial roles in the ecological theater. EcoHealth, 1(2):151–64

Marcogliese DJ (2005) Parasites of the superorganism: Are they indicators of ecosystem health? Int J Parasitol, 35(7):705–16

May-Tec AL, Herrera-Castillo NA, Vidal-Martínez VM, Aguirre-Macedo ML (2020) Following the infection dynamics of the tropical trematode *Oligogonotylus mayae* in its intermediate and definitive hosts for 13 years. J Helmintol, 94:e208

Mellon CD, Wipfli MS, Li JL (2008) Effects of forest fire on headwater stream macroinvertebrate communities in eastern Washington, USA. Freshw Biol, 53(11):2331-2343

Ménard L, Scott ME (1987) Hatching and survival characteristics of *Cyathocotyle bushiensis* Khan 1962 (Digenea) eggs and miracidia. Parasitol, 95(2):355-62

Minchella DJ, Leathers BK, Brown KM, McNair JN (1985) Host and parasite counteradaptations: an example from a freshwater snail. Am Nat, 126(6):843-854

Minshall GW, Robinson CT, Lawrence DE (1997) Postfire responses of lotic ecosystems in Yellowstone National Park, USA. Can J Fish Aquat Sci, 54(11):2509-2525

Minshall GW (2003) Responses of stream benthic macroinvertebrates to fire. For Ecol Manag, 178(1-2):155-161

Moore CS, Gittman RK, Puckett BJ, Wellman EH, Blakeslee AMH (2020) If you build it, they will come: Restoration positively influences free-living and parasite diversity in a restored tidal marsh. Food Webs, 25:e00167

Munro NT, Kovac KJ, Niejalke D, Cunningham RB (2009) The effect of a single burn event on the aquatic invertebrates in artesian springs. Austral Ecol, 34(8):837-847

Musetta-Lambert J, Kreutzweiser D, Sibley P (2019) Influence of wildfire and harvesting on aquatic and terrestrial invertebrate drift patterns in boreal headwater streams. Hydrobiologia, 834(1):27-45

Monitoring Trends in Burn Severity Direct Download: Monitoring Trends in Burn Severity assessment of Event ID – or4482112218820200816. (2021, August - last revised). MTBS Project (USDA Forest Service/U.S. Geological Survey). Available online: http://mtbs.gov/direct-download [2022, September 28]

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MH, Wagner H, Oksanen MJ (2013). Package 'vegan'. Community ecology package, version, 2(9):1-295

Oliver AA, Bogan MT, Herbst DB, Dahlgren RA (2012) Short-term changes in-stream macroinvertebrate communities following a severe fire in the Lake Tahoe basin, California. Hydrobiologia, 694:117-30

Oregon Department of Forestry. "ODF FireDB". Web.

Ortega N (2018) Flames and frogs - the impact of environmental disturbances on host-parasite dynamics. PhD Dissertation, Department of Biology, University of South Florida, Tampa, Florida, USA.

Pettit NE, Naiman RJ (2007) Fire in the riparian zone: characteristics and ecological consequences. Ecosyst, 10(5):673-687

Pielou, EC (1966) The Measurement of Diversity in Different Types of Biological Collections. J Theor Biol, 13:131-144

Pietrock M, Marcogliese DJ (2003) Free-living endohelminth stages: at the mercy of environmental conditions. Trends Parasitol, 19(7):293-299

Poulin R (2011) Origins of parasitism and complex life-cycles. In: Evolutionary ecology of parasites, 2nd edn. Princeton university press, Princeton, pp 8-40

Pratt I, McCauley JE (1961) Trematodes of the Pacific Northwest: An annotated catalog, Oregon State University Press, Corvallis

Preston DL, Layden TJ, Segui LM, Falke LP, Brant SV, Novak M (2021) Trematode parasites exceed aquatic insect biomass in Oregon stream food webs. J Anim Ecol, 90(3):766-775

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reilly MJ, Zuspan A, Halofsky JS, Raymond C, McEvoy A, Dye AW, Donato DC, Kim JB, Potter BE, Walker N, Davis RJ (2022) Cascadia Burning: The historic, but not historically unprecedented, 2020 wildfires in the Pacific Northwest, USA. Ecosphere, 13(6):e4070

Rosenberger AE, Dunham JB, Neuswanger JR, Railsback SF (2015) Legacy effects of wildfire on stream thermal regimes and rainbow trout ecology: an integrated analysis of observation and individual-based models. Freshw Sci, 34(4):1571-1584

Sanders AM, Coble AA, Swartz AG, River M, James P, Warren DR (2022) Heat and smoke from wildfires influence water temperature and dissolved oxygen levels in headwater streams. Freshw Sci, 41(4):665-679

Saxe S, Hogue TS, Hay L (2018) Characterization and evaluation of controls on post-fire streamflow response across western US watersheds. Hydrol Earth Syst Eci, 22(2):1221-1237

Scasta JD (2015) Fire and parasites: an under-recognized form of anthropogenic land use change and mechanism of disease exposure. EcoHealth, 12(3):398-403

Shannon, CE (1948) A mathematical theory of communication. Bell Syst Tech J, 27:379–423

Sherson LR, Van Horn DJ, Gomez-Velez JD, Crossey LJ, Dahm CN (2015) Nutrient dynamics in an alpine headwater stream: use of continuous water quality sensors to examine responses to wildfire and precipitation events. Hydrol Process, 29(14):3193-3207

Silins U, Bladon KD, Kelly EN, Esch E, Spence JR, Stone M, Emelko MB, Boon S, Wagner MJ, Williams CH, Tichkowsky I (2014) Five-year legacy of wildfire and salvage logging impacts on nutrient runoff and aquatic plant, invertebrate, and fish productivity. Ecohydrol, 7(6):1508-1523

Steenvoorden J, Meddens AJ, Martinez AJ, Foster LJ, Kissling WD (2019) The potential importance of unburned islands as refugia for the persistence of wildlife species in fire-prone ecosystems. Ecol Evol, 9(15):8800-8812

Sures B, Nachev M, Selbach C, Marcogliese DJ (2017) Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology'. Parasit Vectors, 10:1-19

Swartz A, Warren DR (2022) Wildfire in western Oregon increases stream temperatures, benthic biofilms and juvenile coastal cutthroat trout size and densities with mixed effects on adult trout and coastal giant salamanders. Can J Fish Aquat Sci, (80)3:503-516

Valtonen ET, Holmes JC, Koskivaara M (1997) Eutrophication, pollution and fragmentation: effects on parasite communities in roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in four lakes in central Finland. Can J Fish Aquat Sci, 54(3):572-585

Vaz PG, Merten EC, Warren DR, Durscher K, Tapp M, Robinson CT, Rego FC, Pinto P (2015) Fire meets inland water via burned wood: and then what? Freshw Sci, 34(4):1468-1481

Vidal-Martinzed VM, Pech D, Sures B, Purucker T, Poulin R (2010) Can parasites really reveal environmental impact? Trends Parasitol, 26(1):44-51

Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW (2006) Warming and earlier spring increase western U.S. Forest wildfire activity. Science, 313(5789):940–943

CHAPTER 2:

EFFECTS OF WILDFIRE ON INTERACTIONS AMONG NEMATODE PARASITES, MAYFLY HOSTS, AND TROUT PREDATORS IN ROCKY MOUNTAIN STREAMS[2]

Chapter Summary

Wildfire activity is increasing globally, highlighting the need to understand how fire disturbance affects species interactions. In particular, few studies have examined how fire influences interactions among parasites, hosts, and predators in freshwater streams. We characterized host-parasite and parasite-predator interactions involving nematode parasites (Family Mermithidae), mayfly hosts (Order Ephemeroptera), and trout predators (*Salvelinus fontinalis, Salmo trutta,* and *Oncorhynchus clarkii*) at 8 burned and 8 unburned stream sites in the southern Rocky Mountains for two years following severe wildfires. Mayfly density, infection probability, and density of infected mayflies were all lower at burned sites one year after fire but returned to similar levels compared to unburned sites after two years. Density of infected mayflies increased with overall mayfly density; however, there was no relationship between mayfly density and infection prevalence. Based on dissections of >20,000 mayflies, intermediate-size (4-6mm) mayflies in the family Baetidae had the highest infection probability and were also the most common mayflies found in trout stomachs. Yet, infection prevalences of mayflies consumed by trout were significantly lower than in the benthos, suggesting predator-avoidance behavior by infected mayflies. Wildfire did not affect the number of mermithids consumed per trout. Overall, our results suggest that mermithid responses to fire reflected changes in host density, consistent with the single-host life cycle of mermithids. Taken together,

---

[2] Co-authored by Sara Weinstein, Fernando Carvallo, Mia ter Kuile-Miller, Julia Gray, Jordan Trujillo, Yoichiro Kanno, Matt Fairchild and Dan L. Preston

our results help integrate host-parasite-predator interactions into our understanding of disturbance ecology in freshwater streams, with implications for parasite roles in energy flow through food webs.

Introduction

Freshwater ecosystems can be strongly altered by wildfires, which are increasing in frequency and severity (Liu et al. 2010; Westerling et al. 2016; Higuera and Abatzoglou 2020). Fire can influence freshwater ecosystems by altering riparian vegetation, hydrology, and water quality, which can subsequently affect aquatic communities (reviewed by Bixby et al. 2015). The effects of fire-associated disturbance on stream biota can vary in magnitude and direction over time. For instance, fire and subsequent flooding and erosion can reduce aquatic macroinvertebrate abundance in the first year following fire, whereas the effects of increased light and nutrients can increase primary and secondary productivity 2-10 years post-fire (Silins et al. 2014; Bixby et al. 2015; Swartz and Warren 2022). While considerable research has focused on how fire affects community structure and ecosystem processes in streams, less research has focused on how fire affects cryptic interactions, such as parasitism.

Infectious processes can be substantially modified by disturbances such as wildfire (reviewed by Albery et al. 2021; Donaldson et al. 2023). For instance, fire can directly affect transmission by altering the survival of free-living parasite stages (Ortega 2018; Kaiser et al. 2021; Donaldson et al. 2023). Changes to host density following fire can indirectly alter infection prevalence and abundance, particularly when transmission is density-dependent (Arneberg et al. 1998; Hossack et al. 2013; Lagrue and Poulin 2015). Environmental changes can also alter host phenology, growth, and development, thereby altering the likelihood of successful transmission to certain host life-stages (Cromwell 2018). Parasites are known to alter host behavior and physiology, which can, in turn, affect trophic interactions of the host (Lafferty and Morris 1996; Shaw et al. 2009; Sato et al. 2012a). Disturbances could also influence trophic interactions between parasites and predators, such as concomitant predation – when parasites are inadvertently consumed by a predator alongside the infected host (Johnson et al. 2010; Thieltges et al. 2013). Predation on parasites is prevalent in food webs and can have implications for transmission and energy flow (Johnson et al. 2010; Thieltges et al. 2013); however, concomitant predation on parasites has not been studied in the context of disturbance ecology.

To address this knowledge gap, we examined how wildfire affects host-parasite and parasite-predator interactions, using nematode parasites (Family Mermithidae), mayfly hosts (Order Ephemeroptera), and trout predators (*Salvelinus fontinalis, Salmo trutta,* and *Oncorhynchus clarkii*) in freshwater streams. Mermithidae is a large family of parasitic nematodes that can infect a variety of invertebrate hosts (Nickle 1972; Poinar 1986), but we focused on mayfly hosts due their abundance in Rocky Mountain streams (Preston et al. 2023), major prey contributions to trout diets (Bozek et al. 1994; Preston et al. 2023) and infection prevalences up to 80% (Cromwell 2018). Free-living adult mermithids overwinter in streams, mate, and lay eggs that hatch the following spring (Hominick and Welch 1980). The free-living immature worm infects early-stage mayfly nymphs and influences mayfly physiology and behavior, including sterilization, sex reversal in males, and inducing oviposition behavior in sterile males (Vance 1996; Vance and Peckarsky 1997; Cromwell 2018). Mermithid infection also suppresses drift and swimming behaviors in mayflies, which can result in lower mayfly consumption rates in trout (Cromwell 2018). After maturing, the parasite emerges from the adult mayfly during oviposition, killing its host (Hominick and Welch 1980; Vance 1996). Each mayfly can support only one individual mermithid, which typically fills the entire host abdomen in a mature infection (see Fig. S2.2). The single-host life cycle of mermithids may suggest tight coupling between host and parasite population densities (Lagrue and Poulin 2015).

We hypothesized that wildfire would cause cascading changes in host-parasite-predator interactions in stream communities. We expected mayfly densities and mermithid infection levels would differ between streams in burned and unburned watersheds due to fire-induced disturbances to the stream environment. We predicted that these responses would vary with time, with an initial decrease in mayfly density due to post-fire disturbance (e.g. scouring; Minshall 2003; Preston et al. 2023), followed by an increase in mayfly density over time due to increased stream productivity (i.e. due to canopy loss). Because mermithids have a single-host life cycle, we expected that increased mayfly density would increase mermithid infection density. Alternatively, wildfire could change host-parasite phenology resulting in timing mismatch (MacDonald et al. 2021; MacDonald and Brisson 2022). For instance, elevated stream temperatures due to canopy loss and increased solar insolation (Minshall et al. 1997; Dunham et al. 2007; Chen and Chang 2023) can accelerate mayfly development (Harper and Peckarsky 2006), limiting the time for larval mermithids to encounter early-stage mayflies and thus reducing infection prevalence (Cromwell 2018). We also predicted that, if fire disturbance increased the density of infected mayflies, more mermithids would be found in trout stomachs.

Methods

Field Surveys

We surveyed 16 stream reaches in the Rocky Mountain region of northern Colorado for two years following wildfires that occurred in the summer and fall of 2020 (Fig. S2.1). Eight of the stream reaches were within the Cameron Peak, East Troublesome, and William Fork fires, which together burned over 169,000 hectares of forest in northern Colorado (National Interagency Fire Center 2020). To serve as unburned references, we also surveyed 8 stream reaches that were outside the fire perimeters, were of relatively similar elevation and stream size, and were located near (<13.5 km) the burned sites. All sites were between 2,490 m and 3,220 m in elevation and were 3rd to 4th order stream reaches. These sites were originally surveyed in 2021 as components of two research projects examining effects of wildfire on Rocky Mountain streams, with ten (5 burned and 5 unburned) sites in the Arapaho and Roosevelt National Forest and six (3 burned and 3 unburned) within Rocky Mountain National Park (Fig. S2.1).

In the first summer following the wildfire (2021), we measured environmental variables related to fire severity and stream conditions. Elevation, latitude, and longitude of each site were measured using a handheld GPS unit. Focal stream reaches measured either 50 m or 100 m in total length (see Table S2.1). We measured channel width and maximum depth every 10 m along the reach. We also recorded discharge using the midsection method at a representative point along each reach using a flowmeter (JDC Electronics, Hamm, Belgium). Turbidity was averaged across three locations along the reach using a handheld turbidimeter (Hanna Instruments, Woonsocket, Rhode Island). Water temperature, pH, dissolved oxygen, and conductivity were measured at one location using a YSI Pro 1020 (Yellow Spring Instruments, Yellow Springs, Ohio). To estimate the severity of fire effects on riparian vegetation, we measured canopy cover using a densiometer in the center of the stream width and at evenly distributed intervals along the reach length (every 10 m for 50 m reaches and 20 m for 100 m reaches). Tree mortality percentage (blackened trees killed by fire) was estimated along the riparian corridor within 3 m of the stream reach. We also calculated percentage of upstream watershed area burned using

ArcGIS to compare possible upstream impacts of fire between burned and unburned streams (see Supplemental Material 2 for details).

In 2021 and 2022, we surveyed streams to quantify mermithid nematodes, mayflies, and trout stomach contents. To quantify mermithids and mayflies in the stream benthos, we collected replicate samples of macroinvertebrates from riffle sections of each stream reach using a Surber sampler (0.09 m^2 in area, 248 µm mesh). In 2021, we collected 5 replicate Surber samples at 10 sites and 3 replicate samples at 6 sites (see Table S2.1). In 2022, we collected 5 replicate samples at all sites. Macroinvertebrate samples were preserved in 80% ethanol until processed in the laboratory. To quantify mayflies and mermithids in trout stomach contents, we used a backpack electroshocker to collect 20-30 individual trout (*Salvelinus fontinalis, Salmo trutta,* and/or *Oncorhynchus clarkii* depending on the local composition) from 10 sites in both 2021 and 2022 (5 of which were burned and 5 unburned; Table S1). We anesthetized the fish with Aqui-S and nonlethally lavaged them to collect stomach contents. Gastric lavage involved flushing water into the trout's stomach using a wash bottle with an attached straw for larger fish, or a 60 mL syringe with a blunt 18-gauge needle for smaller fish. Stomach contents were collected onto a coffee filter and preserved in 80% ethanol before processing in the lab. The fish were released back into the stream upon recovering from anesthesia.

Quantifying Mermithid Infection

We screened 16,589 mayflies from Surber samples and 5,093 from trout diet samples for mermithid infection. Mayflies were first identified to family, counted, and measured to the nearest half millimeter from the anterior end of the head (excluding antennae) to the posterior of the abdomen (excluding cerci). Adult mayflies in trout diets were counted and measured but not identified to family due to identification uncertainty when partially digested. Under a microscope (8x-35x), we dissected mayflies using forceps to evaluate presence/absence of mermithids. We dissected all mayflies larger than 1 mm, but after finding that mermithids infected only 0.13% of mayflies <1 mm, we dissected only a subset (n=2,358 out of 4,983 collected) below this size. While processing trout stomach contents, we found mermithids "loose" in the stomachs and not associated with mayflies, either because they had exited a host after being consumed or because they were consumed as a free-living adult in the benthos. We recorded these worms separately from those that were completely inside or partially inside of consumed mayflies. For analyses of mermithids in trout stomach contents, we conservatively included only nematodes that were associated with a mayfly. This likely underestimated infection prevalence in consumed mayflies; however, these underestimates should be consistent across sites, burn status, and years.

We extracted and sequenced a subset of mermithids to determine whether these parasites belonged to a single species and test whether the same taxa occurred in the benthic samples and trout stomachs. A ~1kb fragment of the 18s rDNA gene was amplified from a subset of nematodes from benthic and trout diets following methods in Floyd et al. 2005. Sequences (n=18; 11 benthic samples and 7 diet samples) were compared with existing sequences in the NCBI BLAST database and then used to construct a maximum likelihood tree (Fig. S2.3; see Supplemental Material 2 for detailed methods).

Data Analyses

We first examined how environmental characteristics varied between burned and unburned sites within one year of wildfire. We focused analyses of environmental variables within one year of the wildfires, when we expected responses to fire would be most apparent. We tested whether elevation, upland burned area, canopy cover, canopy mortality, pH, dissolved oxygen, turbidity, channel width, channel depth, water temperature varied between burned and unburned sites within one year of fire using Two-Sample t-tests and Welch's Two Sample t-tests, as appropriate.

We tested whether wildfire altered benthic mayfly density (log-transformed mayflies/m^2) using a linear mixed effect model (LME; "lme4" package in R) with an interaction between burn status (burned or unburned) and sampling year (2021 or 2022), and a random effect of site. We ran models with all mayflies combined, as well as separate models for each of the three most abundant families (Baetidae, Ephemerellidae, and Heptageniidae) using individual Surber samples as replicates. We restricted analyses to mayflies >1 mm because all mayflies above this size were dissected. Analyses incorporating all mayflies showed similar patterns and are reported in Supplemental Material 2.

We then assessed how host and environmental factors influenced mermithid infection in benthic mayflies. We first tested whether infection density (infected mayflies/m^2) differed between burn status and year. We log-transformed infection density to meet normality assumptions and used an LME that included the main effects and interaction between burn status and year, and a random effect of site. Next, we examined which factors predicted individual mayfly infection status (infected/uninfected; "infection probability") from Surber samples. Data from 14,231 individual mayflies were analyzed using a binomial generalized additive mixed model (GAMM, "mgcv" package; Wood and Wood 2015) with fixed effects of mayfly size (nonlinear smoothing term), mayfly family (Baetidae, Ephemerellidae, or Heptageniidae), an interaction between burn status and year, and a random effect of site. We performed a likelihood ratio test on nested models to generate one test statistic (and p-value) for the categorical mayfly family variable. To test whether infection levels increased in response to host density, we used a linear mixed effects model with benthic mayfly density (log-transformed) as a predictor of either infection density or benthic infection prevalence (%), again incorporating site as a random effect.

We next analyzed mayflies and mermithids in trout stomachs. We first tested whether burn status influenced mayfly and infected mayfly consumption using separate Poisson mixed effects models (GLMM) with an interaction between burn status and year and a random effect of site. We examined which factors predicted infection status in mayflies from trout diets, using a binomial GAMM with the same structure as the model used for infection probability in the Surber samples (n=3,548 individual mayflies). To test whether mayfly infection prevalence in stream benthos differed from the trout stomach contents, we used an LME model with site-level infection prevalence in trout diets as the response and site-level infection prevalence in Surbers as the predictor, including a random effect of site. We also tested whether mayfly infection probabilities differed between the stream benthos and trout diets using a binomial GAMM with the fixed effect of sample type (Surber or trout stomach) and mayfly size (nonlinear smoothing term), and the random effect of site (n=17,779 individual mayflies). Because differences in infection prevalence between stomach contents and Surber samples could be due to trout preferentially feeding on certain size classes, we tested whether mayfly size differed by sample type using a GLMM, including site as a random effect and a Gamma distribution to account for skewed size data (n=17,779 individual mayflies). All analyses were performed in R version 4.2.2 (R Core Team 2023) and all LMEs and GLMMs were assessed for model fit, outliers, and dispersion using the "DHARMa" package (Hartig 2022). We used the package "lmerTest" (Kuznetsova et al. 2017) to generate p-values for LMEs and report all mean values as mean ± standard error.

RESULTS

Environmental Variables

The 2020 wildfires impacted 65.7% (± 10.9) of area upland to the burned sites, compared to 7.1% (± 2.8) of the unburned sites (Welch t-test, t=5.19, p<0.001). Within one year of the fires, streams in the burned areas had lower canopy cover (Welch t-test, t=-3.30, p=0.013) and higher canopy mortality (Welch t-test, t=6.67, p<0.001) than unburned sites (Table S2.1). There were no differences in elevation, channel width, maximum channel depth, water temperature, stream turbidity, discharge, pH, or dissolved oxygen between burned and unburned sites within one year of the fires (all p-values >0.05; Table S2.2).

Host Responses

Mayfly density was substantially lower at burned sites (741.8 ± 136.5 mayflies/m^2) compared to unburned sites (1142.2 ± 118.0 mayflies/m^2) in the first year after fire (LME, Burn*Year, t=2.35, p=0.020), but not the second year after fire (Burn: 1,231.7 ± 184.4; Unburned: 1,261.4 ± 189.7 mayflies/m^2). This pattern was driven largely by Baetidae, which had lower densities at burned sites in the first year, but comparable densities two years after fire (Fig. 2.1a; LME, Burn*Year, t=2.99, p=0.003). There was no effect of burn status, year, or their interaction on Ephemerellidae (Fig. 2.1b; LME, Burn*Year, t=0.56, p=0.574) or Heptageniidae density (Fig. 2.1c; LME, Burn*Year, t=1.27, p=0.207). The majority of mayflies in Surbers were baetids (57.4% overall) followed by heptageniids (25.3%), ephemerellids (17.4%), and other rare families (Leptophlebiidae, Ameletidae; <1%). The size distribution of benthic mayflies was strongly right-skewed, regardless of burn status or year (mean 2.8 ± 0.02 mm) (Fig. S2.4b).

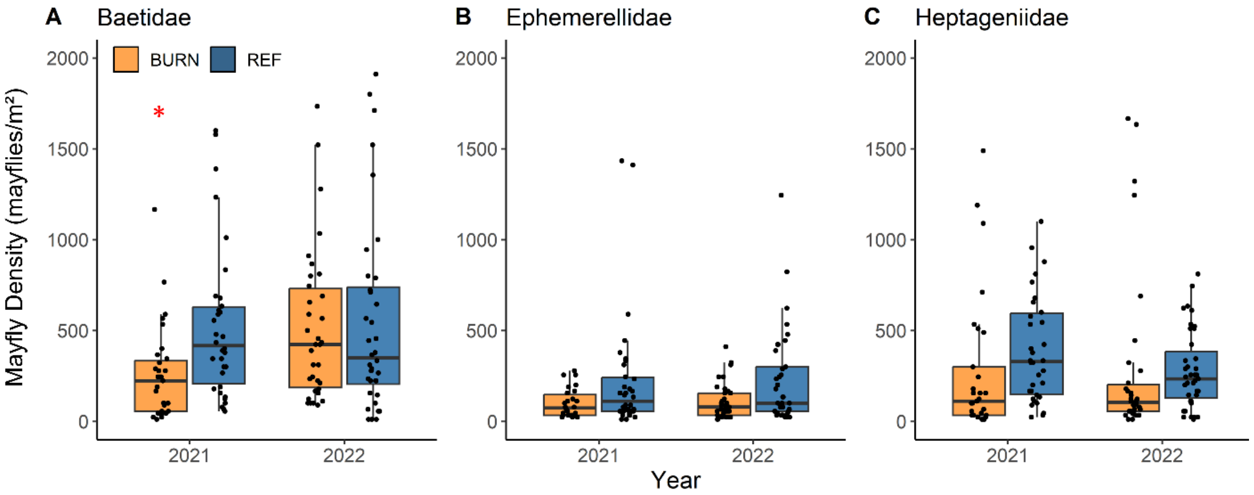

Figure 2.1. Mayfly density of the three most abundant mayfly families, (A) Baetidae, (B) Ephemerellidae, and (C) Heptageniidae at burned (orange) and unburned (blue) sites surveyed one and two years (2021 and 2022) after wildfire. For all boxplots, the thick horizontal lines represent the median, boxes represent interquartile range, and whiskers represent minimum and maximum values of Surber-level density. Red asterisk denotes significance. Outlier values >2000 mayflies/m^2 are not shown on the Baetidae plot. Data do not include mayflies ≤ 1 mm in size.

Infection Patterns in the Benthos

Sequencing of 18 mermithids detected at least five different species across stream sites, with up to three different species at the same site (see Fig. S2.3). Sequencing confirmed that nematodes were mermithids, however, none matched named genera or species in the NBCI BLAST database.

Mermithid infection varied with both mayfly attributes and site factors. Across all sites and years, infection prevalence averaged 5.6% (± 0.8), with a mean of 63.0 (± 6.1) infected mayflies per square meter of streambed. Infection density at burned sites (24.2 ± 0.4 infected mayflies/m^2) was lower than unburned (65.4 ± 1.0 infected mayflies/m^2) sites in 2021 (LME, Burn*Year, t=-3.37, p=0.001), but was similar to unburned sites in 2022 (Fig. 2.2a). Infection density increased with mayfly density (Fig. 2.3a; LME, t=7.80, p<0.001); however, infection prevalence was not related to mayfly density (Fig. 2.3b; LME, t=-0.83, p=0.408).

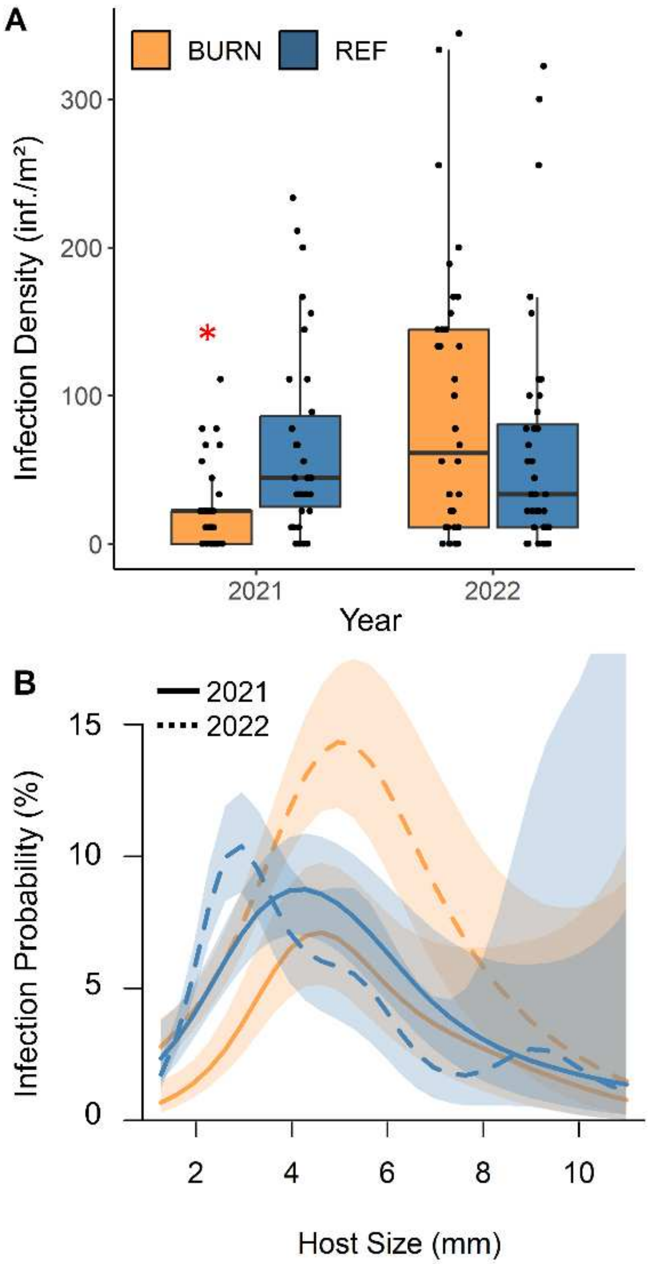

Figure 2.2. (A) Infection density (number of infected mayflies/m^2) within burned (orange) and unburned (blue) streams surveyed one and two years (2021 and 2022) after wildfire. For all boxplots, the thick horizontal lines represent the median, boxes represent interquartile range, and whiskers represent minimum and maximum values of Surber-level density and prevalence values. Red asterisk denotes significance. (B) Infection probability of mayflies collected from burned (orange) and unburned (blue) streams one-year (2021, solid lines) and two-years (2022, dashed lines) after fire by host size (mm). Host size greater >11 mm is omitted from the (B) graph due to low sample size. Data do not include mayflies ≤ 1 mm in size.

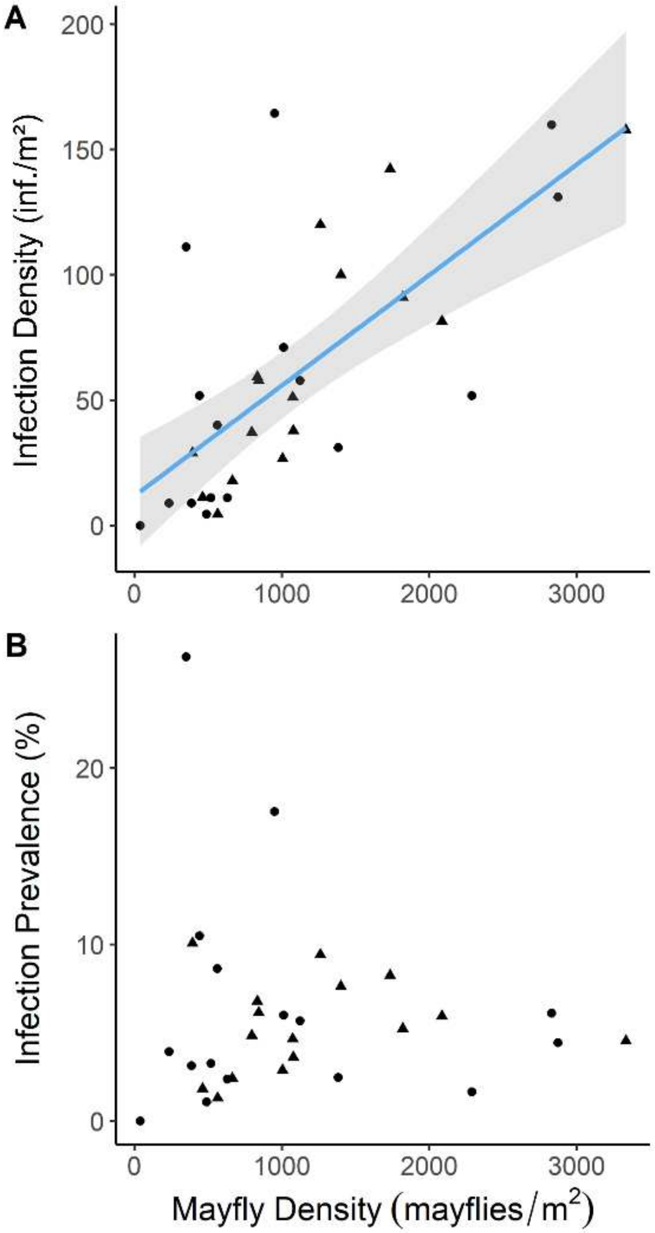

Figure 2.3. Comparison of mermithid infection patterns to site-level mean mayfly density at burned (circles) and unburned (triangle) sites across years. Y-axes of the scatterplots represent site-level means for (A) infection density (number of infected mayflies/m^2) and (B) infection prevalence (percentage of infected mayflies out of total mayflies) at each stream site. A linear regression line with 95% confidence interval is shown for the significant relationship ($p<0.001$) between (A) infection density and log-transformed mayfly density. Data do not include mayflies ≤ 1 mm in size.

At burned sites, infection probability of benthic mayflies was lower in the year following fires but higher after two years (Fig. 2.2b; binomial GAMM, Burn*Year, z=-3.77, p<0.001). Average site-level infection prevalence at burned sites rose from 4.0% (± 0.9) in 2021 to 8.9% (± 1.7) in 2022. At unburned sites, infection prevalence was consistent across years, averaging 5.4% (± 0.7) in 2021 and 5.2% (± 0.7) in 2022. Infection probability differed between mayfly families (Likelihood Ratio Test, F=269.92, p<0.001). Infection prevalence was highest in Baetidae (10.0 ± 1.9%), followed by Ephemerellidae (3.6 ± 0.9%) and Heptageniidae (3.1 ± 0.7%)(Fig. S2.5). Mayfly size was a strong predictor of infection (binomial GAMM, Size, ChiSq=230.80, p<0.001), with infections most common in intermediate-size mayflies (~4-6 mm) (Fig. S2.4a).

Mayflies and Mermithids in Trout Diets

Trout stomachs contained an average of 9.68 (± 4.72) mayflies and there was no difference in the average number of consumed mayflies per trout between burned or unburned sites or across years (Poisson GLMM, Burn*Year, z=1.13, p=0.261). The majority (69.5%) of nymphal mayflies found in trout stomachs were Baetidae and 20.2% of mayflies found in trout stomachs were adults. Sequencing data indicated that mermithids in trout stomachs were the same taxa found in the benthos (Fig. S2.3).

Infection probability of mayflies in trout stomachs did not differ between burned and unburned streams (Fig. 2.4a; binomial GAMM, Burn, z=1.80, p=0.072). Burn status also had no effect on the number of mermithids consumed per trout through concomitant predation (Poisson GLMM, Burn, z=1.59, p=0.112). Overall, infection prevalence of mayflies in trout stomachs was positively associated with infection prevalence of mayflies in benthic samples (Fig. 2.4b; LME, t=2.53, p=0.023); however, infection probability of mayflies in trout diets was significantly lower than in Surber samples (Fig. 2.4c; binomial GAMM, z=10.03, p<0.001). Mean infection

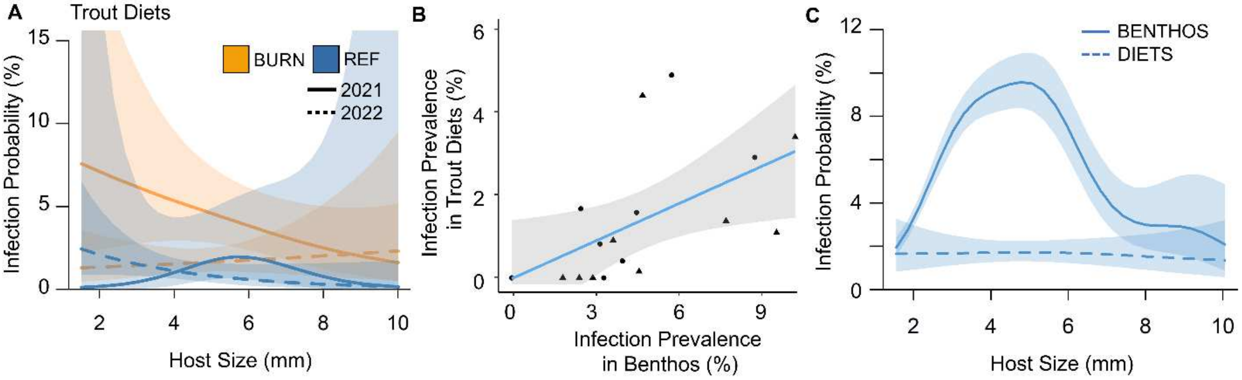

Figure 2.4. (A) Infection probability of mayflies found in trout stomach contents collected from burned (orange) and unburned (blue) streams one-year (2021, solid lines) and two-years (2022, dashed lines) after fire by host size (mm). (B) Comparison of site-level mayfly infection prevalences (percentage of infection mayflies out of total mayflies collected) between trout stomach contents (y-axis) and Surber samples (x-axis) from burned sites (circles) and unburned sites (triangles). A linear regression line with 95% confidence interval is shown representing the positive relationship (p=0.023) between variables. (C) Infection probabilities of benthic mayflies (solid line) compared to those found in trout stomach contents (dashed lines) by host size (mm). Infection probabilities for mayflies > 10 mm are omitted from the figure due to low sample size. Data do not include mayflies ≤ 1 mm in size.

prevalence in trout diets (1.42 ± 0.39%) was 3.2x lower than in the stream benthos (4.63 ± 0.68%). Trout preferentially consumed larger mayflies (Gamma GLMM, t=67.62, p<0.001), with trout diets having normally distributed mayfly sizes centered around intermediate-sized mayflies (5.3 ± 0.03 mm) compared to benthic samples having a right-skewed distribution of mayfly sizes (2.8 ± 0.02 mm)(Fig. S2.4bc).

Discussion

Wildfire activity is increasing globally, especially in the Rocky Mountains (Westerling et al. 2006), underscoring the need to investigate how freshwater communities will be affected in these fire-prone areas. Most research on how wildfires affect stream communities has focused on free-living biota (Bixby et al. 2015), overlooking the parasite interactions that can account for 78% of food web links in aquatic systems (Marcogliese and Cone 1997; Lafferty et al. 2006). We found that fire initially reduced mayfly density, resulting in decreased infection density; however, it did not significantly alter parasite-predator interactions.

Mayfly densities at burned sites initially decreased, but then recovered to levels comparable to unburned sites by two years post-fire. Previous research has shown that aquatic macroinvertebrates in burned stream catchments can recover or even increase as early as one to two years after fire (Caldwell et al. 2013; Vieira et al. 2004; Mellon et al. 2008). Two years after fire, post-fire erosion and flooding could have subsided in frequency or magnitude, allowing mayflies to reestablish. Reestablishment rates appear to be taxon-specific, as we found Baetidae densities recovered within two years, while less abundant Heptageniidae and Ephemerellidae did not. Less common taxa like Ephemerellidae and Heptageniidae may exhibit less predictable recoveries, as smaller populations are more vulnerable to demographic stochasticity (May 1973). More likely, delayed recovery could reflect differences in life-history. Populations of opportunistic aquatic macroinvertebrates that can reproduce and disperse quickly may increase rapidly after disturbance subsides (Minshall et al. 2001; Minshall et al. 2003; Mellon et al. 2008; Malison and Baxter 2010). Baetids, which can reproduce with multiple generations per year (Clifford 1982), can recover or increase in burned streams within a year after fire (Vieira et al. 2004); however, heptageniids and ephemerellids, which typically only have one generation per year (Clifford 1982), can fail to recover for four to eight years after fire (Martens et al. 2019; Vieira et al. 2004). In turn, these host-specific responses to disturbance likely mediated the parasite responses observed in our study.

Wildfire altered mermithid infection over the two-year study period, likely due to changes in host abundance. Mirroring changes in mayfly density, infection density decreased shortly after fire, but then recovered after two years. The strong positive relationship between mayfly density and infection density suggests that changes in mermithid populations are driven by host density. Similar coupling between host population density and parasite density has been documented in other freshwater communities (Lagrue and Poulin 2015). Conversely, we did not find a relationship between infection prevalence and mayfly density. A de-coupling of infection prevalence from host densities may be advantageous to mermithids, as it could stabilize parasite populations regardless of host densities. In a previous study, the prevalence of trematode parasites in long-lived (5-7 years) aquatic snail hosts remained surprisingly stable despite severe wildfires (Svatos et al. 2023). Certain parasite responses to wildfire can be mediated by host-density dependence, as seen with mermithids, but further investigation is warranted into how this response may be contingent on specific host-parasite life history, such as host longevity or parasite life cycle complexity.

At both burned and unburned sites, infection prevalence in Baetidae mayflies was higher than in Ephemerellidae or Heptageniidae. This is consistent with previous studies that found mermithids to be common in baetids but absent from heptageniids (Flecker and Allan 1988). Differing infection levels could be due to differences in host behavior or physical defenses. For example, prevalence of mermithid infection is lower in mosquito species with higher activity levels, thicker cuticles, and physiological resistance to nematode development (Peterson 1975). Increased drifting behavior of Baetidae compared to Heptageniidae and Ephemerellidae (Brewin and Ormerod 1994) might contribute to higher infection prevalence in this family, as this behavior is associated with higher infection in other aquatic insects (Statzner and Bittner 1983;

Wilzbach et al. 1986; Cummins and Wilzbach 1988). Finally, higher infection prevalence in Baetidae could be due to parasite adaption to infect the most abundant host (Lively and Dybdahl 2000), a pattern observed in other nematode-host systems (Arneberg et al. 1998). Without the dilution effect of infrequently-infected mayfly families, streams may be susceptible to increased mermithid loads as Baetidae recolonize and dominate fire-disturbed communities.

The high infection prevalence observed in intermediate-sized mayflies from benthic samples may be due to several mechanisms. Smaller and younger mayflies may have low infection prevalence due to less exposure compared to older individuals (Anderson and Crombie 1982; Pritchard and Zloty 1994). Lower prevalence in larger, older hosts could be from mayflies clearing infections after developing immunity, though this seems unlikely in this system (Anderson and May 1985; Cattadori et al. 2005). Alternatively, parasite-induced host mortality may reduce parasite abundance in older mayflies, as seen in other aquatic hosts (Thomas et al. 1995; Knudsen et al. 2002). Notably, half of mermithid-infected mayflies fail to fully develop, killing both the parasite and mayfly host (Vance and Peckarsky 1996). Another mechanism that may be limiting infection prevalence in large mayflies is that parasite infection itself may limit host body size (Pritchard and Zloty 1994). Parasitized Baetidae mayflies are generally smaller with less flight muscle than unparasitized individuals in late nymphal development stages, due to depletion of host resources (Vance and Peckarsky 1996; Cromwell 2018). Size-specific host infection probability may affect parasite-predator interactions in predators that exhibit size-based consumption of mayflies.

Accounting for size and family, mayflies in trout stomachs were less frequently infected than those in the benthos, suggesting that infected mayflies are less likely to be consumed by trout, consistent with previous field results and proposed mechanisms of predator-avoidance behaviors of infected mayflies (Cromwell 2018). Parasite-induced changes to host behavior or physiology that reduce concomitant predation by trout should increase parasite fitness (Vance and Peckarsky 1997). Mayfly infection probability and number of infected mayflies in trout diets were unaffected by fire, despite decreased mayfly infection in the benthos one year post-fire. The mechanisms underlying this consistency of infected mayfly consumption are unclear, but infection levels of mayflies in the trout diets may have been too low to detect differences between burned and unburned sites. Alternatively, relative trout abundance in streams can decrease following wildfire (Preston et al. 2023), thus the consistency in infected mayflies consumed per trout could be explained by the decrease in prey availability (i.e. mayfly density) being proportional to the decrease in trout predator abundance within the first year after fire. Despite negative consequences for the parasite, parasites can be valuable food resources for stream predators, therefore changes to parasite-predator interactions can potentially affect energy flow within the stream community. Parasites, including nematodes, can be rich in lipids and glycogen (Łopieńska-Biernat et al. 2006; Ponton et al. 2005) and can contribute significant biomass to aquatic food webs, sometimes exceeding the biomass of aquatic insects or top predators (Kuris et al. 2008; Preston et al. 2021). However, predator-avoidance behaviors of mermithid-infected hosts might limit contributions of parasites to trout diets via concomitant predation. For instance, in another study system involving nematomorphs in streams, the availability of nematomorph-infected crickets to trout predators had widespread effects on community and ecosystem-level processes, including altering trout predation on benthic invertebrates, production of benthic algae, and leaf decomposition rates (Sato et al. 2012a). Whether any similar community-level consequences occur due to nematode infection in mayflies is unclear, but deserves further study.

Bottom-up effects of increased stream productivity post-fire (Silins et al. 2014; Bixby et al. 2015; Swartz and Warren 2022) may be contributing to recovery of mayfly and mermithid abundances after two years; however, we did not directly incorporate changes in nutrients, light levels, water temperature, or primary productivity over time in our study. Lagrue et al. (2018) found that riparian tree removal and subsequent canopy opening increased both mayfly density and nematode parasite density within and downstream of the removal area 2-4 years after disturbance. This supports the prediction that bottom-up processes, including increased light and primary production, can underly wildfire effects on mayfly populations. We also predicted that temperature changes in streams could create a mismatch between host phenology and parasite infection. Cromwell et al. (2018) found that elevated stream temperatures accelerated mayfly development, decreasing the window of opportunity for mermithids to infect mayfly hosts, and thus decreasing infection transmission. Fire effects on stream canopy cover and channel morphology can elevate water temperatures in streams for years after fire disturbance (Dunham et al. 2007; Chen and Chang 2023), but we did not find evidence for temperature differences between burned and unburned streams in our study. Our understanding of the relative effects of environmental changes and host-phenology changes on infection outcomes is unclear and would benefit from experimental studies that test these factors in isolation.

Mayflies in both benthic and trout diet samples hosted a diverse assemblage of mermithids. Although we only sequenced 18 nematodes from five sites, we observed five different mermithid species, none of which matched named genera or species in the NCBI database. This finding corroborates previous work, as Sato et al. (2012b) found at least seven mermithid species at two stream sites in Japan, several of which could not be matched to genera or species by molecular sequencing. Clearly, there is vast amount of undescribed mermithid diversity yet to be characterized. This highlights the need for taxonomic- and sequencing-based work, given the high abundance of mermithids in arthropod systems and their impacts on hosts and ecosystem processes (Preston et al. 2006; Sato et al. 2012b), as well as their relevance to biocontrol and integrated pest management (reviewed by Kumar et al. 2023).

Our study improves our understanding of aquatic host-parasite interactions and community-level responses to environmental disturbance. Previous research on parasites with aquatic insect hosts has been narrowly focused on a few host taxa and individual-level host responses, with less than 10% of studies involving parasite interactions with mayflies (Ephemeroptera), stoneflies (Plecoptera), or caddisflies (Trichoptera) hosts and less than 4% of studies measuring population or community level effects of host-parasite interactions (reviewed by Kohler 2008). Parasites serve ecologically important roles in communities, including as regulators of host populations (Kohler and Wiley 1992) and as key members in food webs (Lafferty et al. 2006; Thieltges et al. 2013). As wildfires continue to increase in intensity and frequency globally (Westerling et al. 2006; Lui et al. 2010; Higuera and Abatzoglou 2021), it is necessary to understand how fire effects both free-living and parasitic interactions, as well as how this influences energy flow in stream communities.

Funding and Support

We thank Colorado State University's Department of Fish, Wildlife, and Conservation Biology, Graduate Degree Program in Ecology, the Colorado Water Center, the US Forest Service, Rock Mountain National Park, and the Rocky Mountain Conservancy for providing funding and support for this research. We would also like to thank Caitlin Wells and Sarah Hart for their valuable input on the manuscript draft, Bobbi Peckarsky and Kara Cromwell for their expertise, and Sophia Rye, Lukas Keller, Justice Rogers, Lahrs Quinlan, Erin Crone, Carl Cruz, Landon Falke, Audrey Harris, Alaine Quinn, Abigail Rankin, Kelley Sinning, and Danny White for their assistance collecting data in the field and/or processing samples in the laboratory.

References

Albery, G. F., Turilli, I., Joseph, M. B., Foley, J., Frere, C. H., & Bansal, S. (2021). From flames to inflammation: how wildfires affect patterns of wildlife disease. Fire Ecology, 17(1), 1-17.

Anderson, R. M., & Gordon, D. M. (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology*, *85*(2), 373-398.

Anderson, R. M., & May, R. M. (1985). Anderson, R. M., & Crombie, J. A. (1985). Experimental studies of age-intensity and age-prevalence profiles of infection: Schistosoma mansoni in snails and mice.

Arneberg, P., Skorping, A., Grenfell, B., & Read, A. F. (1998). Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1403), 1283-1289.

Bixby, R. J., Cooper, S. D., Gresswell, R. E., Brown, L. E., Dahm, C. N., & Dwire, K. A. (2015). Fire effects on aquatic ecosystems: an assessment of the current state of the science. *Freshwater Science*, *34*(4), 1340-1350.

Bozek, M. A., DeBrey, L. D., & Lockwood, J. A. (1994). Diet overlap among size classes of Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) in a high-elevation mountain stream. Hydrobiologia, 273, 9-17.

Brewin, P. A., & Ormerod, S. J. (1994). Macroinvertebrate drift in streams of the Nepalese Himalaya. *Freshwater Biology*, *32*(3), 573-583.

Caldwell, C. A., Jacobi, G. Z., Anderson, M. C., Parmenter, R. R., McGann, J., Gould, W. R., ... & Jacobi, M. D. (2013). Prescribed-fire effects on an aquatic community of a southwest montane grassland system. *North American Journal of Fisheries Management*, *33*(5), 1049-1062.

Cattadori, I. M., Boag, B., Bjørnstad, O. N., Cornell, S. J., & Hudson, P. J. (2005). Peak shift and epidemiology in a seasonal host–nematode system. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1568), 1163-1169.

Chen, J., & Chang, H. (2023). A review of wildfire impacts on stream temperature and turbidity across scales. *Progress in Physical Geography: Earth and Environment*, *47*(3), 369-394.

Clifford, H. F. (1982). Life cycles of mayflies (Ephemeroptera), with special reference to voltinism. *Quaest. Entomol.*, *18*(1), 15-90.

Cromwell, K. J. (2018). *Host-Parasite Ecology of Baetis Bicaudatus Mayflies and*

Gasteromermis sp. Nematodes in High-Altitude Streams. (Doctoral dissertation, The University of Wisconsin-Madison.)

Cummins, K. W., & Wilzbach, M. A. (1988). Do pathogens regulate stream invertebrate populations? With 6 figures and 4 tables in the text. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, *23*(2), 1232-1243.

Donaldson, J. E., Holdo, R. M., Anderson, T. M., Morrison, T. A., Hopcraft, J. G. C., McIntyre, J., ... & Ezenwa, V. O. (2023). Direct and indirect effects of fire on parasites in an African savanna. *Journal of Animal Ecology*.

Dunham, J. B., Rosenberger, A. E., Luce, C. H., & Rieman, B. E. (2007). Influences of wildfire and channel reorganization on spatial and temporal variation in stream temperature and the distribution of fish and amphibians. *Ecosystems*, *10*(2), 335-346.

Flecker, A. S., & Allan, J. D. (1988). Flight direction in some Rocky Mountain mayflies (Ephemeroptera), with observations of parasitism. *Aquatic Insects*, *10*(1), 33-42.

Floyd, R. M., Rogers, A. D., Lambshead, P. J. D., & Smith, C. R. (2005). Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Molecular Ecology Notes*, *5*(3), 611-612.

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., ... & Heiberger, R. (2012). Package 'car'. *Vienna: R Foundation for Statistical Computing*, *16*.

Graham, A. L. (2003). Effects of snail size and age on the prevalence and intensity of avian schistosome infection: relating laboratory to field studies. *Journal of Parasitology*, *89*(3), 458-463.

Harper, M. P., & Peckarsky, B. L. (2006). Emergence cues of a mayfly in a high-altitude stream ecosystem: potential response to climate change. *Ecological Applications*, *16*(2), 612-621.

Hartig, F., & Hartig, M. F. (2017). Package 'DHARMa'. *R package*.

Higuera, P. E., & Abatzoglou, J. T. (2020). Record-setting climate enabled the extraordinary 2020 fire season in the western United States. *Global change biology*, *27*(1).

Hominick, W. M., and H. E. Welch. "Mermithids (Nematoda) and mayflies (Ephemeroptera)." In *Advances in Ephemeroptera Biology*, pp. 491-502. Boston, MA: Springer US, 1980.

Hossack, B. R., Lowe, W. H., Honeycutt, R. K., Parks, S. A., & Corn, P. S. (2013). Interactive effects of wildfire, forest management, and isolation on amphibian and parasite abundance. *Ecological Applications*, *23*(2), 479-492.

Johnson, P. T., Dobson, A., Lafferty, K. D., Marcogliese, D. J., Memmott, J., Orlofske, S. A., Poulin, R., & Thieltges, D. W. (2010). When parasites become prey: ecological and epidemiological significance of eating parasites. *Trends in ecology & evolution*, *25*(6), 362-371.

Kaiser, S. W., Greenlees, M. J., & Shine, R. (2021). Wildfires modify the parasite loads of invasive cane toads. *Biology Letters*, *17*(12), 20210470.

Knudsen, R., Amundsen, P. A., & Klemetsen, A. (2002). Parasite-induced host mortality: indirect evidence from a long-term study. *Environmental Biology of Fishes*, *64*, 257-265.

Kohler, S. L. (2008). The ecology of host-parasite interactions in aquatic insects. In *Aquatic insects: challenges to populations* (pp. 55-80). Wallingford UK: CABI.

Kohler, S. L., & Wiley, M. J. (1992). Parasite-induced collapse of populations of a dominant grazer in Michigan streams. *Oikos*, 443-449.

Kumar, D., Nagal, P., Sidhu, H. S., Ranga, P., & Kumari, P. (2023). Role of Mermithid Nematodes in the Management of Agricultural and Household Insect Pests: A review. *Environment and Ecology*, *41*(4C), 2890-2899.

Kuris, A. M., Hechinger, R. F., Shaw, J. C., Whitney, K. L., Aguirre-Macedo, L., Boch, C. A., ... & Lafferty, K. D. (2008). Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature*, *454*(7203), 515-518.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). lmerTest package: tests in linear mixed effects models. *Journal of statistical software*, *82*, 1-26.

Lafferty, K. D., & Morris, A. K. (1996). Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology*, *77*(5), 1390-1397.

Lafferty, K. D., Dobson, A. P., & Kuris, A. M. (2006). Parasites dominate food web links. *Proceedings of the National Academy of Sciences*, *103*(30), 11211-11216.

Lagrue, C., Besson, A. A., & Lecerf, A. (2018). Small alteration–big impacts: effects of small-scale riparian forest management on host–parasite dynamics in streams. *Journal of helminthology*, *92*(1), 64-73.

Lagrue, C., & Poulin, R. (2015). Bottom–up regulation of parasite population densities in freshwater ecosystems. *Oikos*, *124*(12), 1639-1647.

Liu, Y., Stanturf, J., & Goodrick, S. (2010). Trends in global wildfire potential in a changing climate. *Forest ecology and management*, *259*(4), 685-697.

Lively, C. M., & Dybdahl, M. F. (2000). Parasite adaptation to locally common host genotypes. *Nature*, *405*(6787), 679-681.

Łopieńska-Biernat, E., Żółtowska, K., & Rokicki, J. (2006). The content of carbohydrates in larval stages of Anisakis simplex (Nematoda, Anisakidae). *Helminthologia*, *43*, 125-129.

MacDonald, H., Akçay, E., & Brisson, D. (2021). The role of host phenology for parasite transmission. *Theoretical Ecology*, *14*, 123-143.

MacDonald, H., & Brisson, D. (2022). Host phenology regulates parasite–host demographic cycles and eco-evolutionary feedbacks. *Ecology and evolution*, *12*(3), e8658.

Malison, R. L., & Baxter, C. V. (2010). Effects of wildfire of varying severity on benthic stream insect assemblages and emergence. *Journal of the North American Benthological Society*, *29*(4), 1324-1338.

Marcogliese, D. J., & Cone, D. K. (1997). Food webs: a plea for parasites. *Trends in ecology & evolution*, *12*(8), 320-325.

Martens, A. M., Silins, U., Proctor, H. C., Williams, C. H., Wagner, M. J., Emelko, M. B., & Stone, M. (2019). Long-term impact of severe wildfire and post-wildfire salvage logging on macroinvertebrate assemblage structure in Alberta's Rocky Mountains. *International Journal of Wildland Fire*, *28*(10), 738-749.

May, R. M. (1973). Stability and complexity in model ecosystems (Vol. 1). Princeton University Press.

Mellon, C. D., Wipfli, M. S., & Li, J. L. (2008). Effects of forest fire on headwater stream macroinvertebrate communities in eastern Washington, USA. *Freshwater biology*, *53*(11), 2331-2343.

Minshall, G. W., Robinson, C. T., & Lawrence, D. E. (1997). Postfire responses of lotic ecosystems in Yellowstone National Park, USA. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(11), 2509-2525.

Minshall, G. W., Brock, J. T., Andrews, D. A., & Robinson, C. T. (2001). Water quality, substratum and biotic responses of five central Idaho (USA) streams during the first year following the Mortar Creek fire. *International Journal of Wildland Fire*, *10*(2), 185-199.

Minshall, G. W. (2003). Responses of stream benthic macroinvertebrates to fire. *Forest Ecology and Management*, *178*(1-2), 155-161.

Musetta-Lambert, J., Kreutzweiser, D., & Sibley, P. (2019). Influence of wildfire and harvesting on aquatic and terrestrial invertebrate drift patterns in boreal headwater streams. *Hydrobiologia*, *834*, 27-45.

Nickle, W. R. (1972). A contribution to our knowledge of the Mermithidae (Nematoda). *Journal of Nematology*, *4*(2), 113.

Ortega, N. (2018). *Flames and Frogs–The Impact of Environmental Disturbances on Host-Parasite Dynamics* (Doctoral dissertation, University of South Florida).

Petersen, J. J. (1975). Penetration and development of the mermithid nematode *Reesimermis nielseni* in eighteen species of mosquitoes. *Journal of Nematology*, *7*(3), 207.

Phelps, R. J., & DeFoliart, G. R. (1964). Nematode parasitism of Simuliidae. *Research Bulletin*, (245).

Poinar Jr, G. O., & Benton Jr, C. L. (1986). Aranimermis aptispicula ng, n. sp.(Mermithidae: Nematoda), a parasite of spiders (Arachnida: Araneida). *Systematic Parasitology*, *8*(1), 33-38.

Ponton, F., Biron, D. G., Joly, C., Helluy, S., Duneau, D., & Thomas, F. (2005). Ecology of parasitically modified populations: a case study from a gammarid–trematode system. *Marine Ecology Progress Series*, *299*, 205-215.

Preston, D. L., Mischler, J. A., Townsend, A. R., & Johnson, P. T. (2016). Disease ecology meets ecosystem science. *Ecosystems, 19*, 737-748.

Preston, D. L., Layden, T. J., Segui, L. M., Falke, L. P., Brant, S. V., & Novak, M. (2021). Trematode parasites exceed aquatic insect biomass in Oregon stream food webs. *Journal of Animal Ecology*, *90*(3), 766-775.

Preston, D. L., Trujillo, J. L., Fairchild, M. P., Morrison, R. R., Fausch, K. D., & Kanno, Y. (2023). Short-term effects of wildfire on high elevation stream-riparian food webs. *Oikos*, e09828.

Pritchard, G., & Zloty, J. (1994). Life histories of two Ameletus mayflies (Ephemeroptera) in two mountain streams: the influence of temperature, body size, and parasitism. Journal of the North American Benthological Society, 13(4), 557-568.

R Core Team, R. (2023). R: A language and environment for statistical computing (v. 4.2.2). R Foundation for Statistical Computing. https://www.R-project.org/

Sato, T., Egusa, T., Fukushima, K., Oda, T., Ohte, N., Tokuchi, N., ... & Lafferty, K. D. (2012a). Nematomorph parasites indirectly alter the food web and ecosystem function of streams through behavioural manipulation of their cricket hosts. *Ecology Letters*, *15*(8), 786-793.

Sato, T., Watanabe, K., Tamotsu, S., Ichikawa, A., & Schmidt-Rhaesa, A. (2012b). Diversity of nematomorph and cohabiting nematode parasites in riparian ecosystems around the Kii Peninsula, Japan. *Canadian Journal of Zoology*, *90*(7), 829-838.

Shaw, J. C., Korzan, W. J., Carpenter, R. E., Kuris, A. M., Lafferty, K. D., Summers, C. H., &

Øverli, Ø. (2009). Parasite manipulation of brain monoamines in California killifish (Fundulus parvipinnis) by the trematode Euhaplorchis californiensis. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1659), 1137-1146.

Silins, U., Bladon, K. D., Kelly, E. N., Esch, E., Spence, J. R., Stone, M., Emelko, M. B., Boon, S., Wagner, M. J., Williams, C. H. S., & Tichkowsky, I. (2014). Five-year legacy of wildfire and salvage logging impacts on nutrient runoff and aquatic plant, invertebrate, and fish productivity. *Ecohydrology*, *7*(6), 1508-1523.

Statzner, B., & Bittner, A. (1983). Nature and causes of migrations of Gammarus fossarum Koch (Amphipoda): a field study using a light intensifier for the detection of nocturnal activities. *Crustaceana*, 271-291.

Svatos, E. C., Falke, L. P., & Preston, D. L. (2023). Disturbance and disease: host–parasite interactions in freshwater streams remain stable following wildfire. *Oecologia*, 1-11.

Swartz, A., & Warren, D. (2022). Wildfire in western Oregon increases stream temperatures, benthic biofilms, and juvenile Coastal Cutthroat Trout size and densities with mixed effects on adult trout and Coastal Giant Salamanders. *Canadian Journal of Fisheries and Aquatic Sciences*, *80*(3), 503-516.

Thieltges, D. W., Amundsen, P. A., Hechinger, R. F., Johnson, P. T., Lafferty, K. D., Mouritsen, K. N., ... & Poulin, R. (2013). Parasites as prey in aquatic food webs: implications for predator infection and parasite transmission. *Oikos*, *122*(10), 1473-1482.

Thomas, F., Renaud, F., Rousset, F., Cézilly, F., & Meeuûs, T. D. (1995). Differential mortality of two closely related host species induced by one parasite. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *260*(1359), 349-352.

Vance, S. A. (1996). Morphological and behavioural sex reversal in mermithid-infected mayflies. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *263*(1372), 907-912.

Vance, S., & Peckarsky, B. (1996). The infection of nymphal Baetisbicaudatus by the mermithid nematode Gasteromermis sp. *Ecological Entomology*, *21*(4), 377-381.

Vance, S. A., & Peckarsky, B. L. (1997). The effect of mermithid parasitism on predation of nymphal Baetis bicaudatus (Ephemeroptera) by invertebrates. *Oecologia*, *110*, 147-152.

Vieira, N. K., Clements, W. H., Guevara, L. S., & Jacobs, B. F. (2004). Resistance and resilience of stream insect communities to repeated hydrologic disturbances after a wildfire. *Freshwater Biology*, *49*(10), 1243-1259.

Westerling, A. L. (2016). Increasing western US forest wildfire activity: sensitivity to changes in the timing of spring. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1696), 20150178.

Wilzbach, M. A., Cummins, K. W., & Hall, J. D. (1986). Influence of habitat manipulations on interactions between cutthroat trout and invertebrate drift. *Ecology*, *67*(4), 898-911.

Wood, S., & Wood, M. S. (2015). Package 'mgcv'. *R package version, 1(29), 729.*

APPENDICIES

Supplemental Material 1

Supplemental Methods and Results

Species accumulation curves – We used species accumulation curves to examine the expected number of observed trematode taxa as a function of sampling effort (i.e., snails dissected per site). We used data from a previous study, in which trematode infections were identified from 3,255 *Juga* snails sampled from second-order streams (Soap, Oak, and Plunkett Creeks) in the Willamette River Basin (Falke and Preston 2022). *Juga* snails were sampled from the three streams in August, 2020, using a 50-m transect-quadrat design, and all snails > 8 mm were dissected to quantify trematode infections. We included only snails > 10 mm in the species accumulation curves because this matched the sampling approach used in the current study. This resulted in sample sizes of dissected snails of 456 (Soap Creek), 1630 (Oak Creek), and 1183 (Plunkett); differences in samples size were a result of differences in densities of snails >10 mm at each site. Notably, the sites also differed in overall infection prevalence, which was highest in Plunkett (48.3%), intermediate in Soap Creek (38.6%), and lowest in Oak Creek (22.7%). We used the 'vegan' package and 'poolaccum' function in R to estimate trematode species richness for random ordering of sampling units (i.e., individual snails) with 1,000 permutations. We then plotted the resulting richness estimates and standard error to visually assess the expected efficiency of sampling effort in detecting new taxa (Figure S1.1).

Table S1.1. Examples of definitive hosts and host home ranges for trematode taxa in the Willamette River Basin, Oregon. Potential hosts and trematode taxa were compiled from Pratt and McCauley 1961

Definitive Host	Trematode	Host Home Range
Bat (Myotis)	*Acanthatrium oregonensis*	65 ha (Owen et al. 2003)
Racoon	*Metagonimoides oregonensis* *Nanophyetus salmincola*	204-681 ha (Gehrt and Fritzell 1997)
Cutthroat Trout	*Nanophyetus salmincola*	101 m^2 (Minns 1995)
Rainbow Trout	*Nanophyetus salmincola*	174 m^2 (Minns 1995)
Red-legged Frog	*Metagonimoides oregonensis*	150 m (Fellers and Kleeman 2007)
Mute Swan	*Notocotylus imbricatus*	8 km (Sousa et al. 2008)

Table S1.2. Summary of burn area percentage and severity within 500-m buffer of all survey sites (n=17) in 2021, one year after fire, grouped by sub-basin (McKenzie or Santiam). Data were sourced from the Monitoring Trends in Burn Severity (USGS, USFS) program and analyzed using ArcGIS Pro 3 (ESRI)

Location	Total Burn	Low	Med	High
All sites				
BURN	93.2%	17.1%	47.6%	28.6%
CONTROL	1.9%	0.9%	1.0%	0%
McKenzie only				
BURN	87.3%	22.3%	48.4%	16.6%
CONTROL	3.8%	1.8%	2.0%	0%
Santiam only				
BURN	99.1%	11.9%	46.7%	40.5%
CONTROL	0%	0%	0%	0%

Table S1.3. Summary of Generalized Linear Models (GLM) analyses for trematode, snail, and environmental responses before and two years after fire (BACI; n=10 sites). Models tested for the significance of the interaction between burn history ("Burn status") and time since fire ("Year") as well as spatial differences ("Sub-basin") of survey sites. Test statistic ("TS") represents Z-values for Gamma and Negative Binomial models and t-values for Quasi-Poisson models

Variable	Family	Fixed Effect	TS	p-value
Trematode				
Richness	Quasipoisson	Burn*Year	-0.02	0.98
		Sub-basin	-0.88	0.39
Shannon Diversity	Gamma	Burn*Year	0.68	0.50
		Sub-basin	-1.35	1.90
Evenness	Gamma	Burn*Year	1.08	0.29
		Sub-basin	**-3.13**	**<0.01**
Snail				
Biomass	Gamma	Burn*Year	-0.65	0.52
		Sub-basin	-1.03	0.31
Density	Gamma	Burn*Year	-0.07	0.95
		Sub-basin	-0.28	0.78
Environment				
Canopy Cover	Negative	Burn*Year	**-2.01**	**0.04**
	Binomal	Sub-basin	**-2.41**	**0.02**
Riparian Vegetation	Quasipoisson	Burn*Year	**-2.65**	**0.01**
		Sub-basin	0.0	1.00

Table S1.4. Summary of Generalized Linear Models (GLM) analyses for trematode, snail, and environmental responses one year post-fire. Models tested for the significance of burn history (“Burn status”) and spatial differences (“Sub-basin”) of survey sites. Data were incorporated from all survey sites (n=17) in 2021 only. Test statistic (“TS”) represents Z-values for Gamma models and t-values for Quasi-Poisson models

Variable	Family	Fixed Effect	TS	p-value
Trematode				
Richness	Quasipoisson	Burn status	1.13	0.28
		Sub-basin	-1.07	0.30
Shannon Diversity	Gamma	Burn status	**2.68**	**0.02**
		Sub-basin	0.41	0.69
Evenness	Gamma	Burn status	**2.90**	**0.01**
		Sub-basin	**-2.10**	**<0.05**
Snail				
Biomass	Gamma	Burn status	-0.83	0.42
		Sub-basin	**-2.94**	**0.01**
Density	Gamma	Burn status	-1.32	0.21
		Sub-basin	**-2.18**	**<0.05**
Environment				
Canopy Cover	Negative Binomial	Burn status	*-1.73*	*0.08*
		Sub-basin	**-2.34**	**0.02**
Riparian Vegetation	Quasipoisson	Burn status	**-6.07**	**<0.001**
		Sub-basin	**-2.22**	**0.04**

Table S1.5. Summary of Generalized Linear Mixed Models (GLMM) analyses for infection status before and two years after fire (BACI; n=10 sites). Models were specified with a binomial distribution (0=uninfected; 1=infected) and included the random effect of site. Models tested for the fixed effects of the interaction between burn history ("Burn status") and time since fire ("Year"), individual snail size ("Total length"), and spatial differences ("Sub-basin") of survey sites

Trematode	Fixed Effect	Z-value	p-value
All taxa	Burn*Year	-1.183	0.24
	Total length	**19.36**	**<0.001**
	Sub-basin	0.19	0.85
***Acanthatrium* sp. 1**	Burn*Year	**-2.22**	**0.03**
	Total length	**16.05**	**<0.001**
	Sub-basin	**-2.27**	**0.02**
***Acanthatrium* sp. 2**	Burn*Year	-0.87	0.38
	Total length	**4.42**	**<0.001**
	Sub-basin	0.135	0.89
Metagonimoides oregonensis	Burn*Year	-1.04	0.30
	Total length	**10.23**	**<0.001**
	Sub-basin	**2.94**	**<0.01**
***Metagonimus* sp.**	Burn*Year	*1.71*	*0.09*
	Total length	**3.88**	**<0.001**
	Sub-basin	-0.55	0.58
Cardicola alseae	Burn*Year	-0.28	0.78
	Total length	-0.94	0.35
	Sub-basin	0.31	0.75
Deropegus aspina	Burn*Year	0.37	0.71
	Total length	-0.63	0.53
	Sub-basin	-1.35	0.18
Nanophyetus salmincola	Burn*Year	-0.52	0.60
	Total length	**8.99**	**<0.001**
	Sub-basin	*1.90*	*0.06*

Table S1.6. Summary of Generalized Linear Mixed Models (GLMM) analyses for snail infection status one year post-fire. Models were specified with a binomial distribution (0=uninfected; 1=infected) and included the random effect of site. Models tested for the fixed effects of individual snail size ("Total length"), burn history ("Burn status"), and spatial differences ("Sub-basin") of survey sites. Data were incorporated from all survey sites (n=17) in 2021 only

Trematode	Fixed Effect	Z-value	p-value
All taxa	Burn status	*-1.74*	*0.08*
	Total length	**13.39**	**<0.001**
	Sub-basin	1.07	0.28
***Acanthatrium* sp. 1**	Burn status	-0.89	0.38
	Total length	**11.43**	**<0.001**
	Sub-basin	-1.12	0.27
***Acanthatrium* sp. 2**	Burn status	1.00	0.32
	Total length	**4.04**	**<0.001**
	Sub-basin	*1.85*	*0.06*
Metagonimoides oregonensis	Burn status	0.60	0.55
	Total length	**7.34**	**<0.001**
	Sub-basin	**2.83**	**<0.01**
***Metagonimus* sp.**	Burn status	**2.57**	**0.01**
	Total length	**3.87**	**<0.001**
	Sub-basin	-0.08	0.94
Cardicola alseae	Burn status	*1.72*	*0.08*
	Total length	1.44	0.15
	Sub-basin	0.81	0.42
Deropegus aspina	Burn status	**-2.10**	**0.04**
	Total length	-0.64	0.52
	Sub-basin	-1.49	0.14
Nanophyetus salmincola	Burn status	-1.34	0.18
	Total length	**4.98**	**<0.001**
	Sub-basin	**2.30**	**0.02**

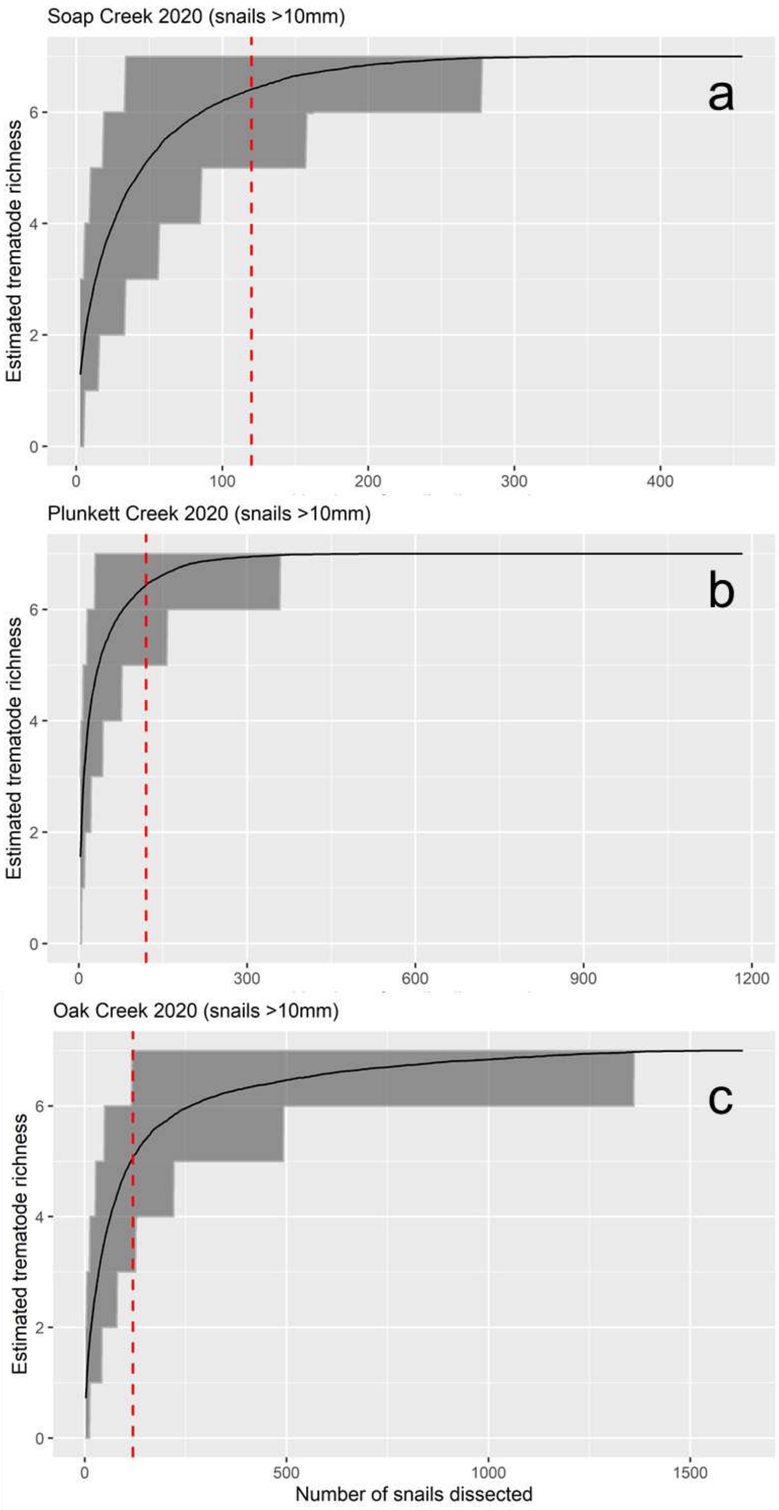

Figure S1.1 Species accumulation curve showing estimated trematode species richness by sampling effort in (a) Soap Creek, (b) Plunkett Creek and (c) Oak Creek, 2020. The grey-shaded area around the curves shows the 95% confidence interval based on standard deviation among permutations. The vertical-dashed red line represents a sampling effort of 120 dissected snails

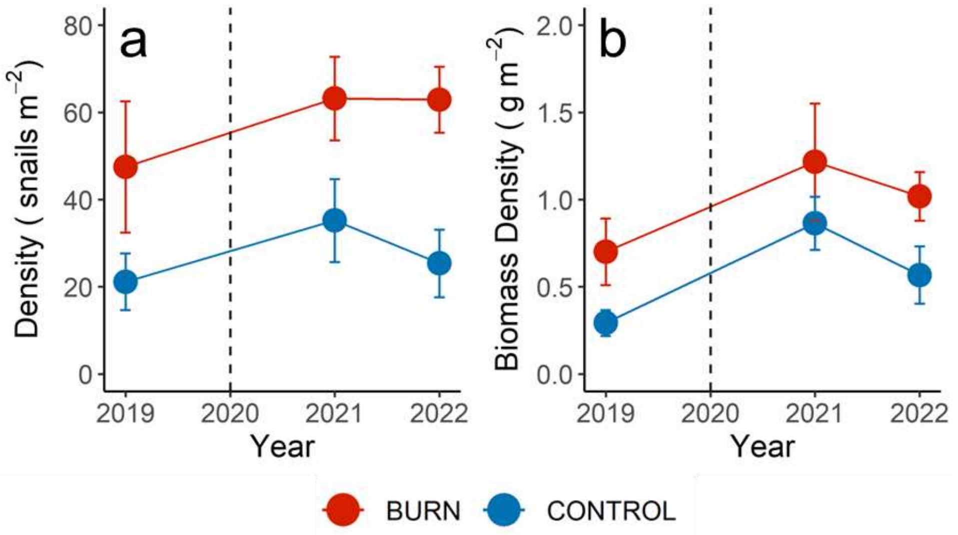

Figure S1.2. Before-After-Control-Impact response of (a) snail density and (b) snail biomass density in relation to wildfire. Points indicate mean density or biomass across burned (red) and unburned (blue) sites, with the vertical error bar indicating ± one SE. Timing of the 2020 wildfires is indicated by the dashed vertical line

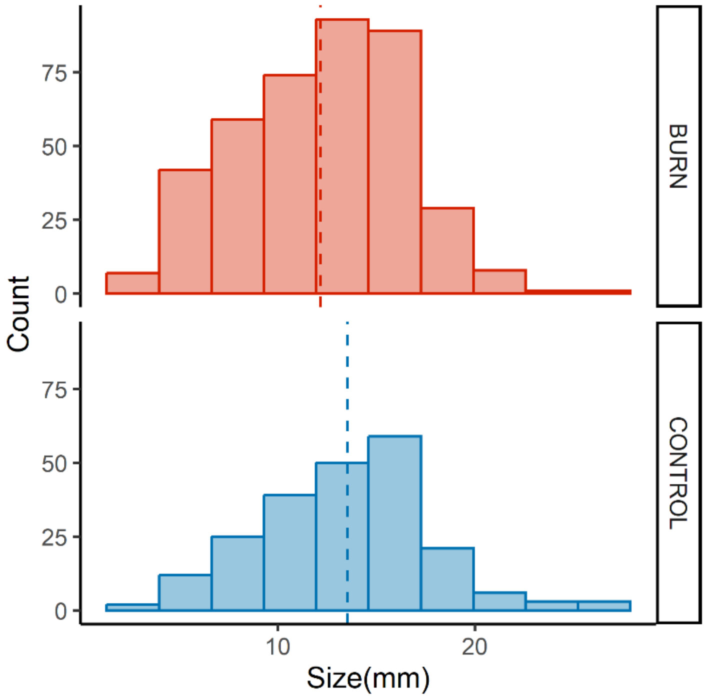

Figure S1.3. Size distribution of *Juga* snails at burned (red) and control (blue) sites one year after wildfires. Mean is indicated by the dashed vertical line

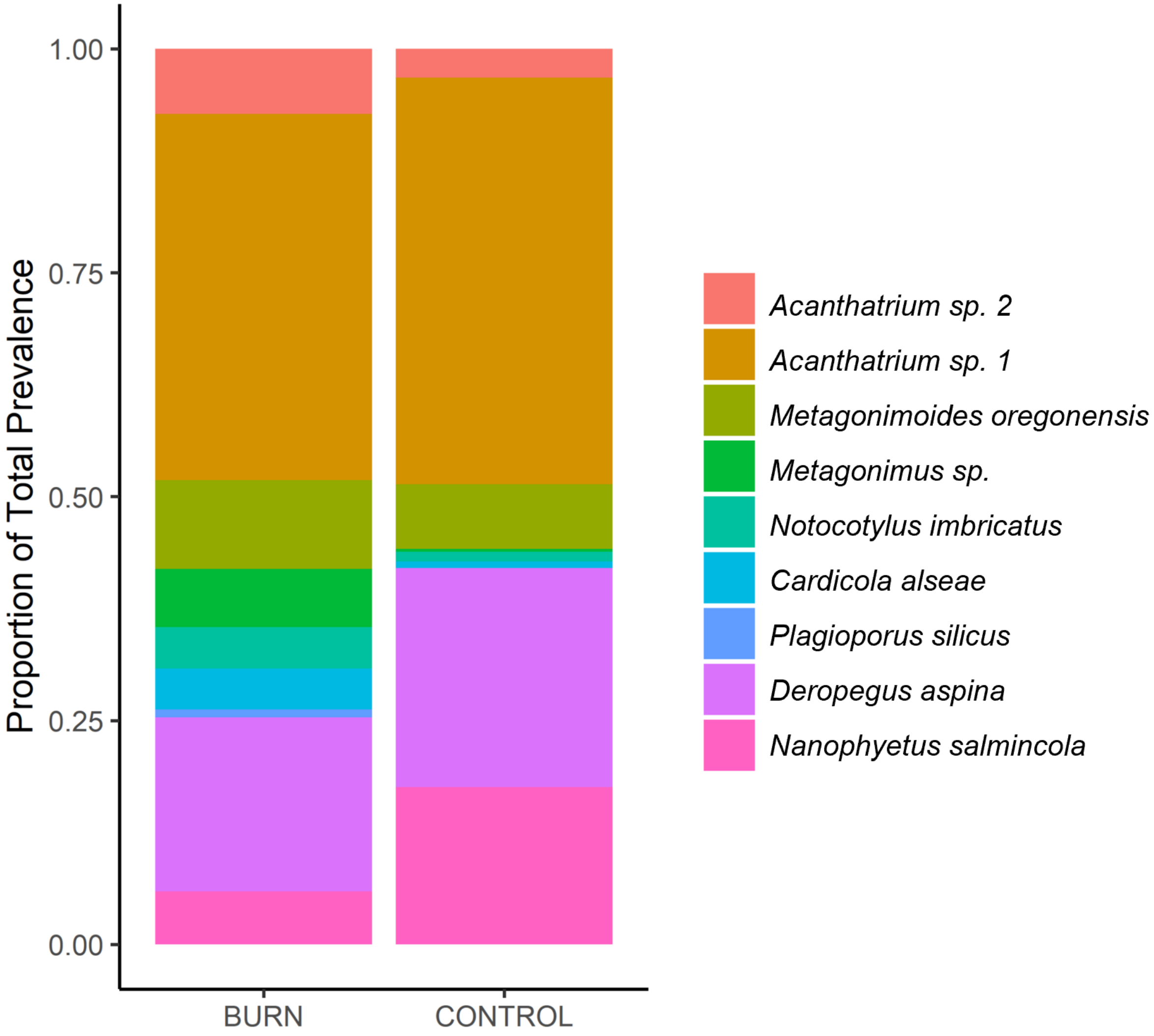

Figure S1.4. Trematode community composition at burned and unburned sites one year post-fire. Each colored bar represents the individual taxon's proportion of total infection prevalence averaged across sites. Data were incorporated from all survey sites (n=17) in 2021 only

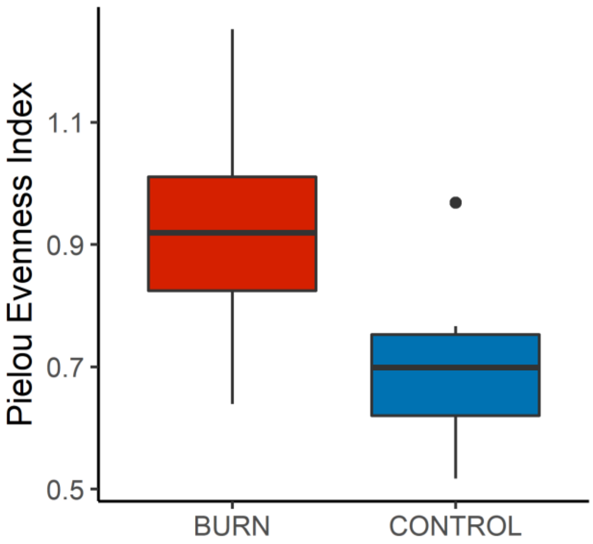

Figure S1.5. Trematode community evenness at burned (red) and unburned (blue) sites one year post-fire. Data were incorporated from all survey sites (n=17) in 2021 only

References

Gehrt SD, Fritzell EK (1997) Sexual differences in home ranges of raccoons. J Mammal, 78(3):921-31.

Falke LP, Preston DL (2022). Freshwater disease hotspots: Drivers of fine-scale spatial heterogeneity in trematode parasitism in streams. Freshw Biol, 67(3):487-497

Fellers GM, Kleeman PM (2007) California red-legged frog (Rana draytonii) movement and habitat use: implications for conservation. J Herpetol, 41(2):276-86

Minns CK (1995) Allometry of home range size in lake and river fishes. Can J Fish Aquat Sci, 52(7):1499-508

Owen SF, Menzel MA, Ford WM, Chapman BR, Miller KV, Edwards JW, Wood PB (2003) Home-range size and habitat used by the northern myotis (Myotis septentrionalis). Am Midl Natl, 150(2):352-9

Sousa CM, Malecki RA, Lembo Jr AJ, Hindman LJ (2008) Monitoring habitat use by male mute swans in the Chesapeake Bay. InProc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies, (62)88-93

Supplemental Material 2

Supplemental Methods and Results

Mayfly size models- Because mayflies <1 mm were rarely infected, we only dissected a subset of mayflies below this size threshold. As a result, we conducted most analyses of infection responses without mayflies under this size threshold and omitted this size class from models including mayfly and infection density estimates. To test whether results were sensitive to this decision, we ran all models that incorporated mayfly density including and omitting small (≤1mm) mayflies to see if they were affecting the analyses and found that all significant model outputs remained the same. In parasite response models, all mayflies <1mm were assumed to be uninfected. Outputs of models including small mayflies are presented in Table S2.3.

Upstream Watershed Burn Area Calculations- Upstream watershed fire effects may have contributed to differences in burned stream reaches, therefore we calculated the percentage area burned in the upstream watershed for each survey site using ArcGIS Pro 3.0, with values provided in Table S1. The analysis in ArcGIS involved several steps: first, the *Mosaic to New Raster* tool was used to combine multiple 10-meter digital elevation model (DEM) rasters of elevation in Colorado's Rocky Mountain region. Next, the *Fill* tool was applied to the 10-meter DEMs covering the study region to create a depression-less grid. We then generated a flow direction grid from this depression-less DEM using the *Flow Direction* tool in the ArcGIS Hydrology Toolbox and used this flow direction grid as the input for the *Flow Accumulation* tool. Once the flow accumulation grid was generated for the spatial area of interest, visually identified the pour point nearest to the site and located the nearby raster cell with the highest flow accumulation. We then used the *Watershed* tool to delineate watersheds upstream of all 16 of our study sites. From there, we converted the watershed raster to a polygon using the *Raster to polygon* tool and calculated polygon area by adding a field to the watershed attribute table and calculating geometry. Using burn severity polygons as boundaries, we applied the *Clip* tool to the study watershed polygon. Finally, we determined the total percentage of upstream burned area for each study watershed by dividing the sum of burned area (of any severity) by the total watershed area, multiplied by 100%. Burn severity data was sourced from Burned Area Emergency Response (BAER) soil burn severity maps generated by the US Forest Service (https://burnseverity.cr.usgs.gov/products/baer).

Molecular Sequencing - We sequenced twenty nematodes from diet and Surber samples. Surber worms were extracted by placing a 1-3mm worm section in 9.8 µl of single worm lysis buffer (121 mg Tris, 380 mg KCl, 51 mg MgCl2*6H2O, 460 µL Nonidet P-40, 460 µL Tween-20 per 100 mL ddH2O) and 0.2 µl of Proteinase K. Extractions were frozen at -80°C for at least 30 minutes and then incubated at 65°C for 2 hours followed by 10 minutes at 95°C. Diet samples were extracted using a DNeasy Blood & Tissue Kit (Qiagen), as per the manufacturer's protocol with the following modifications. Prior to each extraction, ethanol was removed by drying the 2-10 mm worm fragment on the side of a clean 1.5ml Eppendorf tube for ~15 minutes. Tissue was then homogenized in 90 µl of phosphate buffered saline using a disposable plastic pestle. After adding 20 µl of Proteinase K and 200 µl of AL buffer, samples were incubated on a Thermomixer set to 56°C and 300RPM. We amplified extracted DNA using the Nem 18s_F and Nem18s_R primers (Floyd et al 2005). PCR reactions contained 12.5 µL OneTaq 2X Master Mix

(New England BioLabs), 0.5 µL of each primer (10 µM), 1-3 µL of extracted DNA and ddH2O to a final volume of 25 µL. Thermocycling parameters were 94°C for 4 minutes, followed by 30 cycles of 94°C for 30 seconds, 54°C for 30 seconds, 68°C for 1 minute and a final extension of 68°C for 5 minutes. PCR products were visualized on a 1% agarose gel, purified using ExoSAP-IT Express (Applied Biosystems) and then Sanger sequenced at Eton Bioscience using the forward primer.

Individual sequences were examined in SnapGene Viewer and trimmed based on sequence quality. These trimmed sequences were compared to existing sequences in the NCBI nucleotide database using NCBI Blast (Boratyn et al 2013). A sequence from the family Marimermithidae (*Aborjinia* sp., MZ504143.1) was downloaded as an outgroup and then high-quality sequences greater than 350 nucleotides (n=18) were imported into R (v 4.2.2, R Core Team, 2022) and aligned using the "ClustalW" algorithm in the *msa* package (v1.30.1, Bodenhofer et al, 2015). Pairwise distances were computed with the "TN93" model using the dist.dna function in the *ape* package (v 5.7.1, Paradis and Schliep, 2019), and then used to construct a maximum likelihood tree in the *phangorn* package (v2.11.1, Schliep 2011). We observed at least one mermithid species infecting more than one mayfly family (Baetidae and Heptageniidae).

Table S2.1. Site information for the 16 sites surveyed, including the stream name, latitude and longitude, elevation, burn status (burned or reference), reach length, percent upstream watershed burned, riparian canopy cover, canopy mortality, whether trout stomach samples were collected, and number of Surber replicates collected in 2021. Sites were surveyed between 7/19/2021 and 8/27/2021.

Site	Lat	Long	Elevation (m)	Burn Status	Reach Length (m)	Upland Burned Area (%)	Canopy Cover (%)	Canopy Mortality (%)	Trout Diet	Surbers (2021)
Pennock Creek	40.5635	-105.5485	2643	REF	50	3	50	0	Yes	5
S Fork Poudre	40.5754	-105.5856	2721	REF	50	6	58	0	Yes	5
Coral Creek	40.5191	-105.7618	3030	REF	50	23	14	0	Yes	5
Bowen Gulch	40.3286	-105.8673	2696	REF	50	10	40	0	Yes	5
Keyser	39.9098	-105.9906	2858	REF	50	<1	66	0	Yes	5
Cascade	40.5237	-105.7059	3220	REF	100	14	0	0	No	3
Poudre 3	40.4509	-105.7689	3164	REF	100	0	0	0	No	3
Big Thompson 3	40.3598	-105.6806	2704	REF	100	<1	17	20	No	3
Little Beaver Creek	40.6193	-105.5515	2496	BURN	50	100	0	100	Yes	5
Fish Creek	40.6034	-105.5487	2516	BURN	50	100	0	100	Yes	5
N Fork Joe Wright	40.5673	-105.8717	2986	BURN	50	49	0	100	Yes	5
Trail Creek	40.2260	-105.9521	2714	BURN	50	100	0	100	Yes	5
Kinney	39.8395	-106.0430	2778	BURN	50	68	0	100	Yes	5
Fern	40.3435	-105.6705	2830	BURN	100	23	0	100	No	3
Hague 1	40.5169	-105.7299	3016	BURN	100	46	0	0	No	3
Hazeline 1	40.5093	-105.7072	3083	BURN	100	40	0	100	No	3

Table S2.2. Water quality and hydrology information for the 16 sites surveyed including the stream name, burn status, dissolved oxygen, pH, water temperature, conductivity, turbidity, and discharge in 2021. Sites were surveyed between 7/19/2021 and 8/27/2021.

Site	Burn Status	DO (mg/L)	pH	Water Temp (C)	Conductivity (us/cm)	Turbidity (fnu)	Discharge (m^3/s)
Pennock Creek	REF	8.9	7.5	8.5	15.1	1.8	0.32
S Fork Poudre	REF	8.2	8.1	9.1	12.3	1.6	1.13
Coral Creek	REF	6.5	7.7	15.5	18.4	4.1	0.28
Bowen Gulch	REF	9.0	7.7	12.5	49	5.9	1.44
Keyser	REF	7.8	7.9	8.8	65.5	0.9	0.66
Cascade	REF	8.6	7.4	12.2	12.1	1.3	0.16
Poudre 3	REF	9.7	7.3	8.1	42	3.3	0.17
Big Thompson 3	REF	9.5	6.7	9.8	15.1	1.5	1.25
Little Beaver Creek	BURN	8.0	8.2	11.8	38.6	4.7	0.22
Fish Creek	BURN	6.7	7.9	19.6	64.4	5.7	0.02
N Fork Joe Wright	BURN	7.5	7.9	9.4	39.6	1.3	0.25
Trail Creek	BURN	9.5	8.0	10.2	70.2	13.7	0.43
Kinney	BURN	8.3	8.4	12.5	72.1	7.3	0.93
Fern	BURN	9.1	7.2	14.2	16.4	2.4	0.90
Hague 1	BURN	8.2	7.5	12.3	26.2	2.4	0.34
Hazeline 1	BURN	9.8	7.7	8.8	18.8	1.3	0.16

Table S2.3. Linear mixed effects model outputs including all mayflies' sizes. Mayfly density was log-transformed in all models to fit normality assumptions. Model significance was the same including or excluding mayflies ≤1 mm.

Response	**Fixed-Effect**	**t-value**	**p-value**
Host Responses			
Mayfly Density	Burn*Year	2.008	***0.047***
Baetidae Density	Burn*Year	3.196	***0.002***
Ephemerellidae Density	Burn*Year	0.517	0.606
Heptageniidae Density	Burn*Year	1.156	0.250
Parasite Responses			
Mermithid Density	Mayfly Density	7.382	***<0.001***
Infection Prevalence	Mayfly Density	-0.901	0.369

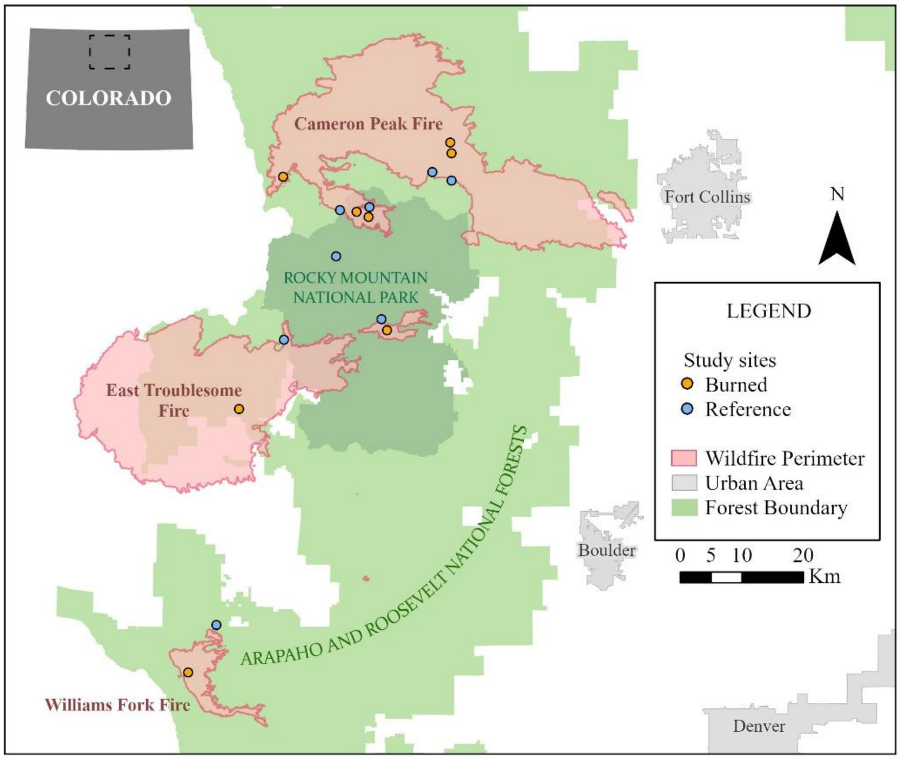

Figure S2.1. Map of northern Colorado's Rocky Mountain region with survey locations (circles) characterized by burn status (burned in orange or unburned in blue). Burned areas of the East Troublesome, Cameron Peak, and Williams Fork wildfires are indicated by the pink colored polygons. Protected forest boundaries are represented in green, with Arapaho & Roosevelt National Forests in light green and Rocky Mountain National Park in dark green. Major urban areas (gray) are shown for scale and spatial orientation.

Figure S2.2. Heptageniidae mayflies parasitized by mermithid nematodes. (A) The silhouette of the mermithid (indicated by black arrow) can be seen through the host prior to dissection, filling the thorax and abdomen. (B) After dissection, the mermithid is removed and the mayfly's abdomen is visually hollow. Mayflies can only be infected by one individual mermithid.

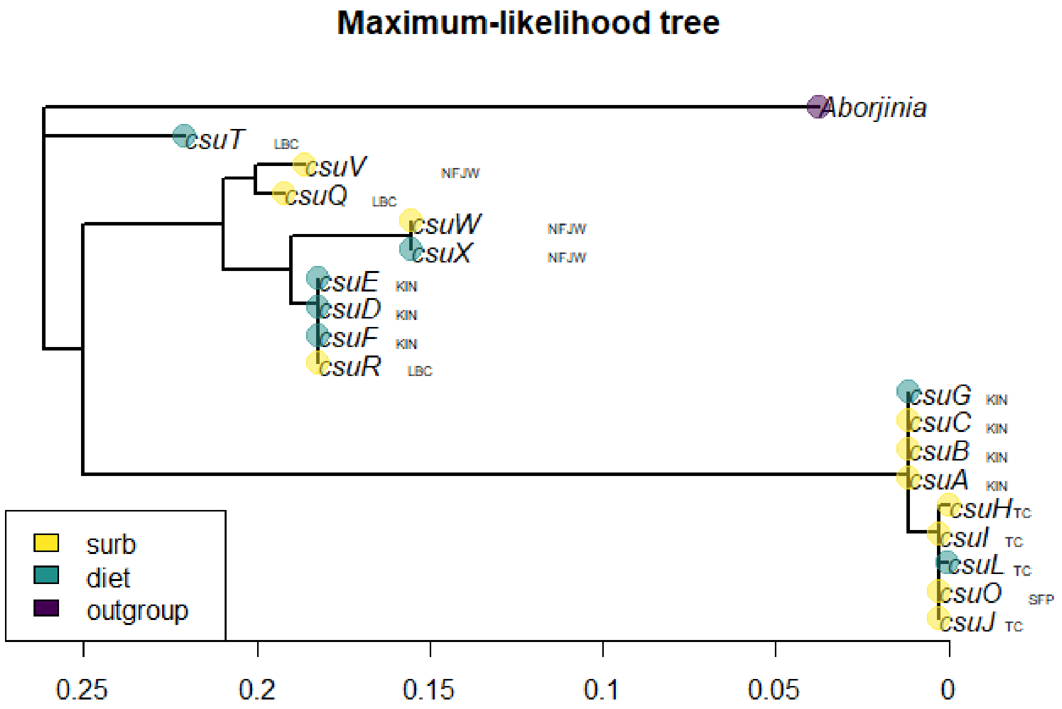

Figure S2.3. Maximum-likelihood tree of 18 mermithid sequences sampled from Surbers (yellow circle) and trout stomach contents (green circle) from five stream sites (Little Beaver Creek [LBC], North Fork Joe Wright Creek [NFJW], Kinney Creek [KIN], Trail Creek [TC], South Fork Poudre [SFP]). Five mermithid groups have sequence similarity <99%, indicating unique species: groupA, groupD, groupQ, groupT, and groupW. A sequence from the family Marimermithidae (Aborjinia sp., MZ504143.1) is shown as an outgroup (purple circle).

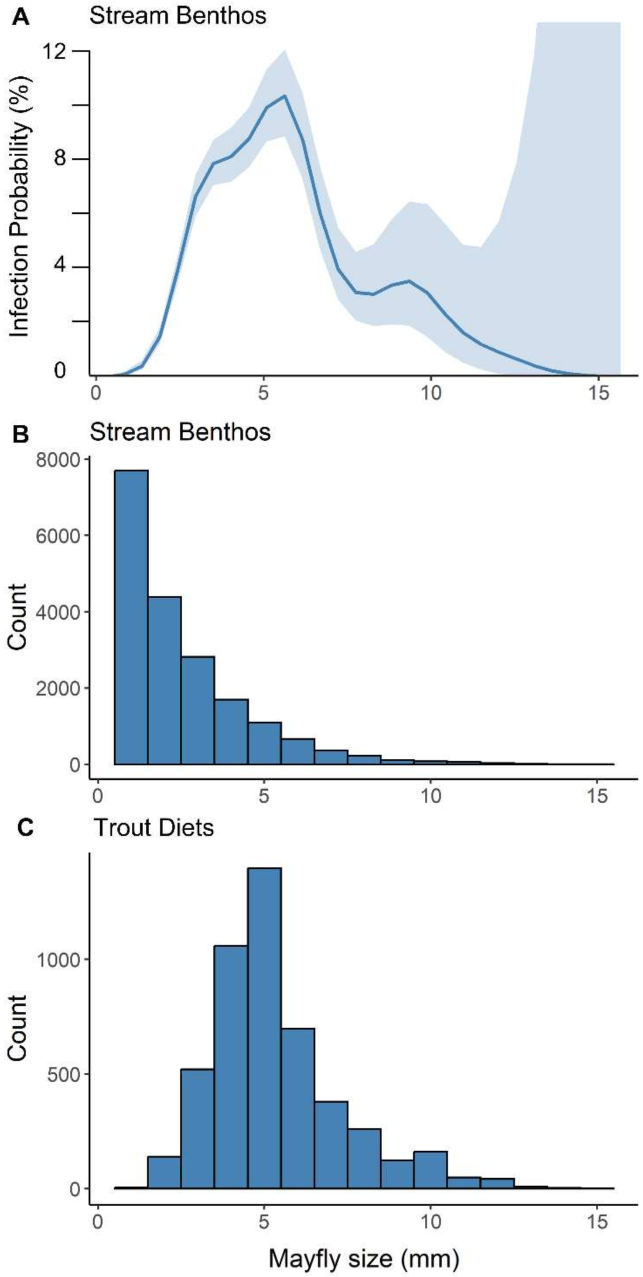

Figure S2.4. (A) Infection probability of mayflies collected from the stream benthos compared to counts of mayflies collected from (B) benthic stream samples and (C) trout stomach contents by size class (rounded to nearest whole millimeter). Only a subset of mayflies 1mm or smaller were dissected (2,358 dissected out of 4,983 total individuals) for infection probability estimates.

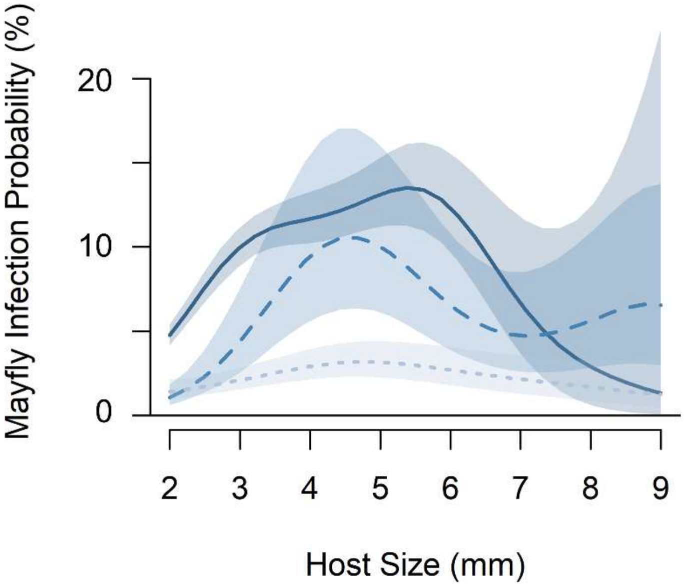

Figure S2.5. Infection probabilities for the three most abundant mayfly families, Baetidae (dark blue, solid line), Ephemerellidae (blue, dashed line), and Heptageniidae (light blue, dotted line), collected from Surber samples by host size (mm). Infection probabilities for mayflies > 9 mm are omitted from the figure due to low sample size. Data do not include mayflies ≤ 1 mm in size.

References

Bodenhofer, U., E. Bonatesta, C. Horejs-Kainrath, and S. Hochreiter (2015) msa: an R package for multiple sequence alignment. Bioinformatics 31(24):3997-9999. DOI: 10.1093/bioinformatics/btv176.

Boratyn GM, Camacho C, Cooper PS, Coulouris G, Fong A, Ma N, Madden TL, Matten WT, McGinnis SD, Merezhuk Y, Raytselis Y, Sayers EW, Tao T, Ye J, Zaretskaya I. (2013) “BLAST: a more efficient report with usability improvements. ”Nucleic Acids Res. 41:W29-W33.

Paradis E, Schliep K (2019). “ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R.” Bioinformatics 35: 526-528. doi:10.1093/bioinformatics/bty633

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Schliep K.P. 2011. phangorn: phylogenetic analysis in R. Bioinformatics, 27(4) 592-593