HOST-PARASITE ECOLOGY OF *BAETIS BICAUDATUS* MAYFLIES AND *GASTEROMERMIS* SP. NEMATODES IN HIGH-ALTITUDE STREAMS

By

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DEDICATED TO

John Smouse

and his great-granddaughters,

Blythe & Monroe Anderson

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Contents

Abstract

HOST-PARASITE ECOLOGY OF *BAETIS BICAUDATUS* MAYFLIES AND *GASTEROMERMIS* SP. NEMATODES IN HIGH-ALTITUDE STREAMS

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Understanding parasite strategies for dispersal, transmission, production and survival within heterogeneous environments is critical to forecasting patterns of disease risk across space. Nevertheless, there is limited insight into how environmental drivers influence most parasites and their ecological interactions. This dissertation addresses that knowledge gap with empirical data collected via observations and experiments at different scales (microcosm, mesocosm and largescale field manipulation) to study the interaction of a common, abundant mayfly host, *Baetis bicaudatus*, and its castrating nematode parasite, *Gasteromermis* sp. in a dynamic high-altitude stream network. Chapters 1 and 2 investigate the role of abiotic factors (habitat and temperature) in driving hotspots of parasite infection, and Chapters 3 and 4 evaluate consequences of host biotic interactions (foraging and predator exposure) for parasite performance and survival. Findings demonstrate that, while this parasite apparently manipulates its host to take advantage of host dispersal behavior, the variability in suitable oviposition habitat that limits host recruitment does not limit its parasite. Therefore, spatial heterogeneity in infection rates may be shaped more strongly by post-colonization conditions within patches than by between-patch differences that influence host density or parasite dispersal. For example, multiyear surveys indicate that temperature has differential impacts on host and parasite development, with the potential to match (in colder sites) or mismatch (in warmer sites)

susceptible hosts and infective parasites, influencing spatiotemporal patterns of parasitism. We also discovered that, when top predators are present, parasites change their trophic strategy within the host so that their growth remains uncompromised but host resources are significantly depleted. Further, parasites decrease the exposure and predation of their hosts to benefit their own survival in risky environments. Those strategies are consistent with parasite adaptations that favor longevity and growth within the host, despite environmental fluctuations, which aligns with the life history requirements of a parasitic castrator. Together these findings illustrate that, despite the intricate connections between parasites and hosts, they nevertheless diverge in responses to key abiotic and biotic gradients. Therefore, predicting what conditions will promote parasitism should account for details of a parasite's natural history and how it interacts with environmental variables to achieve dispersal, transmission, and survival.

INTRODUCTION

Understanding the causes and consequences of disease has been a focus of health sciences and epidemiology for nearly two centuries (Snow 1849), but has relatively recently garnered attention from ecologists. Bringing ecological perspectives to bear on the central questions of pathogen evolution, transmission and virulence has yielded elegant explanations of parasite life history strategies (Poulin 2006), along with ample evidence that parasites not only change behavior and fitness of individual hosts, but also can regulate populations (Kohler and Wiley 1992, Hudson 1998), structure communities, and alter ecosystem function (Thomas et al. 2005, Hudson et al. 2006, Dunn and Hatcher 2011). Spilling over from the accumulation of ecological knowledge, a wealth of stories have made inroads into popular literature and media, presenting parasite life histories as a parade of eco-evolutionary dramas (e.g., Zimmer 2001, Zuk 2008, Drisdelle 2011) and illuminating the connection between ecology and human disease risk (e.g., Quammen 2012).

An interest in the ecology of disease has also coincided with a search for what patterns, predictions and pathways to sustainability ecology can reveal in the context of global change (e.g., Lubchenco et al. 1991, Kareiva and Kingsolver 1992, Vitousek 1994). At the confluence of these efforts, ecologists seek to understand how changing environments interact with disease transmission (Wilson 2001, Lafferty et al. 2005, Dobson 2009, Lafferty 2009), not only for the numerous parasites and pathogens that affect humans (Patz et al. 2004, Wilcox and Gubler 2005, Johnson et al. 2009), but also for diseases of plants and wildlife that potentially threaten both commerce and biodiversity (Knops et al. 1999, Daszak 2000, Bradley and Altizer 2007, Johnson et al. 2008a, Dobson 2009).

The fascinating, often intricate relationships among parasites, hosts and environment emerge from a tendency of parasites to be specialized and deeply embedded within ecological interactions (Price 1980, Lafferty et al. 2006, Hatcher et al. 2012). When transmission is governed by a complex interplay of biotic and abiotic variables, the outcomes for disease risk can be highly context-dependent, with transmission hotspots emerging at some times and places, but not in others (Vale et al. 2011, Duffy et al. 2012). This context-dependency suggests that environmental changes can, in turn, remap the spatiotemporal mosaic of disease by shifting gradients of habitat quality or patch size that influence connectivity, dispersal, or survival of hosts and parasites (Patz et al. 2000, Hall 2010, Rohr et al. 2011). Nevertheless there is little mechanistic understanding of how environmental drivers influence the ecological interactions and transmission of most disease-causing organisms (Lafferty and Kuris 1999, Plowright et al. 2011).

A precursor to forecasting how environmental variability and change could influence disease risk is understanding parasite strategies for dispersal, transmission, production and survival within heterogeneous environments (Thomas et al. 2002). Stream networks stand out as naturally heterogeneous and disturbance-prone systems that are also

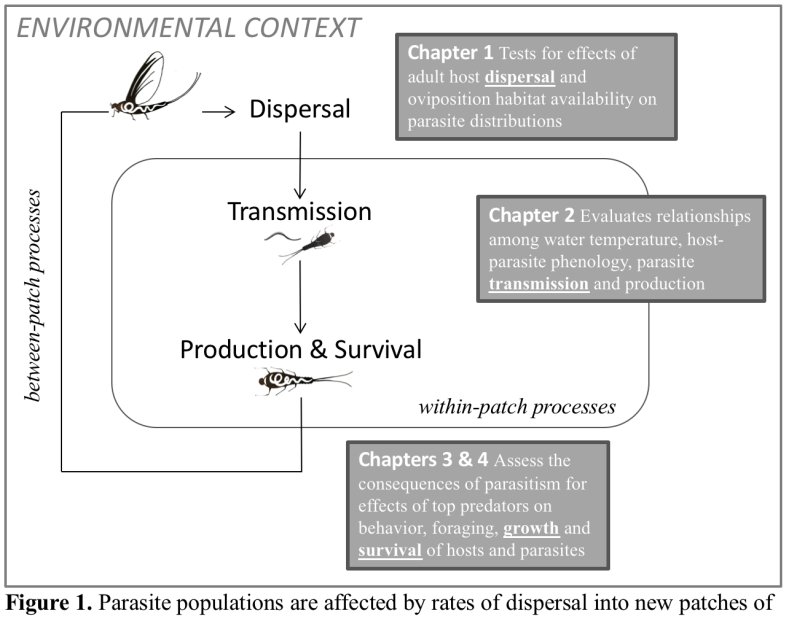

Figure 1. Parasite populations are affected by rates of dispersal into new patches of available hosts, transmission into susceptible hosts within patches, resources available for production, and survival through the parasitic stage, all of which are influenced by environmental context. Focusing on the four elements of this cycle, my dissertation investigates how key abiotic and biotic environmental gradients influence host-parasite interactions between *Baetis bicaudatus* mayflies and parasitic *Gasteromermis spp.* nematodes.

vulnerable to the myriad alterations that face human-dominated landscapes, including changes to climate, land cover, connectivity, chemistry and species distributions (Johnson et al. 2008b, 2010, Johnson and Paull 2011, Okamura and Feist 2011). Nevertheless, we know especially little about disease organisms and processes in streams or other freshwaters, although investigating them could provide useful models of parasite strategies in dynamic, heterogeneous and potentially novel systems (Okamura and Feist 2011).

The aim of this dissertation is to examine key abiotic and biotic influences on and consequences of parasitism of a foundation species in stream ecosystems, the mayfly grazer *Baetis bicaudatus*, in the context of environmental heterogeneity. This goal comprises 3 related objectives: 1) to test for effects of adult host dispersal and oviposition habitat availability on parasite distributions; 2) to evaluate relationships among water temperature, host-parasite phenology, parasite transmission and production; 3) to measure the consequences of parasitism for the effects of top predators on foraging behavior, growth and survival of hosts and parasites (Fig. 1).

Natural History

This dissertation focuses on parasitism in *Baetis bicaudatus* mayflies, an abundant and widespread insect whose larvae live in high elevation streams in the western US. This species is the most efficient insect grazer of attached algae within streams of the region (Alvarez and Peckarsky 2005) and is preferred prey to predatory invertebrates and salmonids (Allan 1983, Peckarsky and Penton 1989).

Baetis has demonstrated resilience to the existing heterogeneity of streams, including patchy distributions of both resources (food, suitable habitat) and risks (disturbance, top

predators and parasites), but changes to this template could generate novel conditions with unpredictable effects (Peckarsky et al. 2011). Recent episodic and directional environmental changes in high elevation ecosystems (Inouye et al. 2000) present complex, multiple, interactive and/or novel stressors to biota (Williams et al. 2007). Although *Baetis* mayflies may be inconspicuous in the public perception of such iconic settings, decades of research on their functional ecology suggests that abundance, commonness, and exceptionally strong food web interactions (Alvarez and Peckarsky 2014) position them as foundation species (Ellison et al. 2005). Therefore responses of *Baetis* to a changing environment could exert strong higher-order effects. Failing to understand impacts of human-accelerated environmental change on *Baetis* could precede the failure to manage regional watersheds for the intrinsic value of biodiversity, the healthy function of ecosystems, and the distinctive cultural services trout streams provide. A key to understanding the status and fate of *Baetis* in altered ecosystems is elucidating the role of the "hand in the puppet" (Hechinger et al. 2008)—a parasite with the potential to infect > 50% of individuals and mediate nearly every ecological interaction in which its host participates.

Gasteromermis sp. mermithids (Poinar 1991) parasitize early instars of *Baetis* larvae by penetrating the integument of the host and eventually growing within its abdomen, until the larva molts to an adult (Hominick and Welch 1980, Vance and Peckarsky 1996). Parasitism ultimately castrates the host and causes morphological and behavioral sex reversal of males, so that both male and female adult hosts join unparasitized females in dispersal and oviposition behavior following metamorphosis (Vance 1996a). *Baetis* is highly selective for oviposition habitat, laying its eggs under large rocks protruding from streams in fast flow, which decreases the probability of eggs desiccating (Encalada and Peckarsky 2006). The oviposition flight of parasitized *Baetis*, however, culminates not with egg-laying but with the mermithid emerging

from its host under water at the landing site. Once free, it overwinters as a pre-reproductive adult, and then matures to adulthood and reproduces the following summer; it's progeny will then pursue new (mostly early instar) *Baetis* hosts (Hominick and Welch 1980). Therefore, adult parasites dispersed by *Baetis* of generation *t* produce offspring that infect the next *Baetis* generation, *t+1* (see Chapter 1, Fig. 1 for lifecycle).

Beyond its immediate effects on larval hosts, there are few certainties about *Gasteromermis*; key details of its life history, dispersal, transmission, and role in community interactions remain enigmatic. Previous work has generated many useful hypotheses, however (Vance 1996b, Vance and Peckarsky 1996, 1997), and the fact that any attention has been paid to its ecology makes it a rarity among parasites of stream invertebrates. In contrast to the relative scarcity of knowledge about *Gasteromermis*, the life history and ecology of its host, *Baetis bicaudatus*, have been the subject of decades of research (e.g., Peckarsky 1980, Allan and Flecker 1989, Kerans et al. 1995, McPeek and Peckarsky 1998, Peckarsky et al. 2001, 2002, 2008, 2011, Encalada and Peckarsky 2011b). This foundational knowledge is indispensable for generating hypotheses to target key aspects of parasite life history, transmission and survival strategies, building a strong case study of the impacts of multiple and novel stressors on disease, with potential higher order effects.

Study Area

My approach combined observational field surveys with experiments at different scales (microcosms, mesocosms and field manipulations) in the East River drainage basin near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA (see Chapter 1, Fig. 2 for map of location). These rocky-bottom streams are located at ≈2,900 m elevation and runoff

is generated primarily by snowmelt, though some streams originate from groundwater or lake outlets. Average peak discharge occurs between late May and early July. Streams throughout the drainage have comparable water chemistry, but vary in size and physical characteristics (Peckarsky et al. 2001, 2002). For decades ecologists have investigated this high-elevation watershed which, like other intensively studied ecosystems near RMBL (e.g., Billick and Price 2011), has potential to serve as a touchstone for pressing questions regarding rapid environmental changes that are occurring (Inouye et al. 2000) and forecast for the region (Painter et al. 2010).

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CHAPTER 1 – Parasites that manipulate insect hosts for dispersal do not benefit from increases in host oviposition sites

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ABSTRACT

Parasite-induced changes to host dispersal potentially influence the distribution of parasites and infection risk for hosts. *Gasteromermis* is a castrating endoparasitic nematode that infects both males and female mayflies (*Baetis bicaudatus*) but feminizes male hosts and manipulates them to disperse parasites to aquatic habitats via a mimicry of female oviposition behavior. In a stream network with high spatial heterogeneity in parasitism we used surveys and a large-scale field manipulation to 1) evaluate parasite effects on host dispersal capacity and site selection, and 2) test whether experimentally increasing host oviposition habitat would increase immigration of parasitized hosts, with subsequent increases in parasite recruitment and prevalence in the next generation. Initially assuming that manipulation benefits the parasite by increasing host encounters around oviposition sites, we predicted the parasite would not affect host dispersal capacity or site selection, and that more parasites would be dispersed to sites with abundant oviposition habitat subsequently increasing parasite transmission. In contrast to predictions, we found that parasites decreased host dispersal capacity (flight muscle ratio) and <2% of adult mayflies captured at oviposition sites were parasitized. We also observed a negative relationship between the flight muscle ratio of hosts and the percent biomass of parasite they contained,

consistent with a tradeoff between parasite growth and host dispersal capacity. Furthermore, experimentally increasing oviposition habitat available for dispersal of hosts in natural streams had no effect on parasite recruitment or host infection risk. We conclude that the variability in suitable habitat that limits host recruitment does not limit its parasite and, therefore, does not contribute to observed spatial patterns of infection. Instead, life history events that occur in the months between when parasites are dispersed and when their offspring infect the next generation of hosts disconnect dispersal dynamics from recruitment and dilute colonization effects. Therefore, heterogeneity in infection rates may be shaped more strongly by post-colonization conditions within patches than by between-patch differences that influence dispersal.

Keywords: parasitism, dispersal, tradeoffs, oviposition, host-parasite interactions, mayflies, manipulation, mermithids, feminization

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INTRODUCTION

Like many organismal interactions, the distribution of parasitism is often patchy (Paine and Mullens 1994, Smith 2001, Poinar and Poinar 2003, Stuart et al. 2006, Paull et al. 2011). Discrete areas of high infection, whether sustained "hotspots" or epidemic outbreaks, can have strong local effects on host populations and communities with potential to become sources of parasite spread (Krkošek et al. 2005, Bradley and Altizer 2007, Paull et al. 2011). Therefore, detecting underlying causes of parasitism is a critical step toward predicting where disease hotspots are likely to arise.

Basic theoretical models predict host-parasite encounters to increase with numbers of hosts or parasites, suggesting that dispersal behavior could locally increase infection risk if it results in parasites aggregating with high densities of hosts in destination patches (Anderson and May 1978, Blower and Roughgarden 1989, Hassell 2000). Because parasite fitness is contingent on successful transmission into hosts, there is considerable scope for parasites to benefit from dispersal strategies that enhance host encounters (Lion et al. 2006). Such strategies can include parasites taking advantage of host dispersal to perpetuate their life cycles and increase transmission rates (Pérez-Tris and Bensch 2005).

Beyond being passively dispersed via host movements, some parasites invest in actively manipulating host dispersal behavior (Lion et al. 2006, Binning et al. 2017). Because those parasites rely on their hosts not only for dispersal but also for nutrition, manipulative parasites balance allocating host resources to their own growth against investing in the manipulation (Poulin 1994). Very little is known about the costs of manipulation, but they are likely to include both the direct "induction costs" of manipulating host physiology (Thomas et al. 2005, 2005, Poulin 2010) and the indirect cost of consuming fewer host resources, because the host should be left with sufficient reserves to complete the manipulated behavior (Maure et al. 2013). In the case of dispersal, which is energetically costly, host manipulation may present a substantial tradeoff (Bonte et al. 2012). Nevertheless, manipulating dispersal should benefit parasites that infect hosts within a spatially structured system, if it allows them to penetrate more clusters of susceptible hosts (Lion et al. 2006).

Habitat suitability can be an important driver of dispersal and spatial structuring of populations; therefore habitat features that affect host distributions and performance also have the potential to affect their parasites (Penczykowski et al. 2016). Understanding interactions at

the nexus of dispersal, habitat, and parasite transmission will enhance our ability to identify conditions that underlie disease heterogeneity and intensify host infection risk. However, understanding the role of dispersal in host-parasite interactions requires considering the life histories and behavior of both organisms along with their experience of relevant environmental gradients. Amid these layers of complexity and covariates, it is a challenge to isolate mechanisms that generate parasitism hotspots, and we are lacking empirical studies of parasite dispersal ability and the role of dispersal in shaping spatial dynamics (Ekholm et al. 2017). Combining field observations with experiments that control for variation can be an effective approach to disentangling potentially important factors (Resetarits and Bernardo 2001). For example, experiments that manipulate habitat variables can be used to test process-based predictions about underlying drivers of parasite dispersal, transmission and host infection risk.

With the goal of understanding drivers of natural variation in parasitism across a landscape, we used observations and a large-scale field experiment to examine relationships among parasitism, dispersal, and habitat selection in a stream-dwelling mayfly, *Baetis bicaudatus* (Ephemeroptera: Baetidae). Prior work in this system has demonstrated that dispersing adult *Baetis* are highly selective for oviposition habitat with traits that increase egg survival (Encalada and Peckarsky 2006), and that experimentally increasing oviposition habitat increases *Baetis* dispersal to and subsequent recruitment within streams (Encalada and Peckarsky 2011b). *Baetis* is infected by an endoparasitic helminth (*Gasteromermis* sp., Nematoda: Mermithidae) that is dispersed by adult hosts, suggesting that oviposition habitat availability could also benefit the parasite and mediate infection risk for *Baetis* (Vance 1996, Vance and Peckarsky 1996).

There is empirical evidence that *Gasteromermis* manipulates *Baetis* to facilitate its own dispersal into appropriate habitats by feminizing male hosts (Vance 1996; Fig. 1). Vance (1996) hypothesized that feminization of *Baetis* could benefit *Gasteromermis* not only because female behavior insures parasite dispersal back into streams (whereas adult males never return to streams), but also because female *Baetis* expend less energy than males during the adult phase (do not swarm). Embedded in that interpretation is the prediction that parasites could pay an energetic cost but also experience a transmission benefit by investing in the dispersal behavior of their hosts.

If parasitized and healthy adult *Baetis* aggregate at oviposition sites, then those behaviors could concentrate infective parasites with susceptible hosts of the next generation, benefitting the parasite by creating localized areas of high transmission. By extension, selective oviposition of *Baetis*, which adaptively promotes egg survival (Encalada and Peckarsky 2006), could also facilitate post-recruitment epidemics if the parasite manipulates that behavior to its advantage. In general, dispersal is expected to play a key role in the distribution and metapopulation dynamics of mermithids of aquatic insects (Stuart et al. 2006, Micieli et al. 2012) but to our knowledge the connection between dispersal and distributions has not be directly assessed in the field.

Key Terms

Parasite Prevalence: Proportion of the host population infected by parasites = no. parasitized hosts / total number of hosts; also an indicator of host infection risk

Parasite Recruitment: Successful establishment within a host, measured as parasite density = no. of parasitized hosts / m^2 (each host is infected by 1 parasite)

Flight Muscle Ratio (FMR): Proportion of an individual insect's biomass composed of flight muscle = flight muscle dry mass / total body dry mass

Our specific objectives were 1) to evaluate dispersal capacity and behavior of infected and uninfected *Baetis* and 2) to determine whether spatial variation in parasitism results from variation in availability of and dispersal to oviposition habitat. We measured flight muscle ratios (proxy for dispersal capacity) in infected and uninfected *Baetis* larvae before emergence to the

adult stage, and used sticky traps to compare dispersal of infected and uninfected adult *Baetis* to oviposition sites. To address the role of oviposition habitat in driving parasitism, we combined surveys with a large-scale habitat manipulation in the field, in which substrates used for oviposition were either added, subtracted, or unmodified in 12 study reaches (N = 4). This experiment enabled us to test the hypothesis that increasing oviposition habitat would increase parasite recruitment (i.e., successful establishment within a host, measured as parasite density) and host infection risk (i.e., parasite prevalence, measured as % hosts infected) relative to conditions before the manipulation.

METHODS

Study area

We studied streams of the upper East River catchment, which drains an area of 45-km^2 near the Rocky Mt. Biological Laboratory (RMBL) in Gothic, Colorado, USA. These rocky-bottom streams are located at approximately 2,900 m elevation with run-off generated primarily by snowmelt, although some streams originate from groundwater or lake outlets. Streams throughout the drainage have comparable water chemistry, but vary in physical attributes such as size, gradient, current velocity, substrate particle sizes and water temperature (Peckarsky et al. 2001, 2002b, Wilcox et al. 2008).

Study organisms

Our research focused on *Baetis bicaudatus*, an abundant and widespread mayfly whose larvae live in high elevation watersheds in the western USA. Following decades of research, many aspects of the organismal, population and community ecology of *Baetis* are well

understood (e.g., Peckarsky et al. 1993, 2000, 2001, 2011). However, a significant knowledge gap remains around the interaction of *Baetis* with the endoparasite *Gasteromermis* sp., including the fundamental question of why parasitism consistently reaches high levels in some streams while remaining low in other sites within the same watershed (Vance and Peckarsky 1996; Fig. 2). Details of the behavior of parasitized adults, including the effect of parasites on dispersal, are also lacking as most studies have focused on the larval stage.

Baetis larvae are the most common grazers of perilithic algae in streams of the East River drainage. Populations in this location are univoltine, with oviposition occurring in the summer (June-August) after dispersal by winged females. *Baetis* mayflies are recruitment limited (Encalada and Peckarsky 2011a) mediated by the availability of optimal oviposition habitat for which they are highly selective, aggregating egg masses under large rocks that protrude from the stream in areas of swift flow, which decreases the probability of eggs desiccating during the 14 d period of embryogenesis (Encalada and Peckarsky 2006). Adults are very short-lived (< 4 days; Vance and Peckarsky 1996, Peckarsky et al. 2002a), with females dying after ovipositing a single egg mass (Peckarsky et al. 2000). Once hatched, larvae overwinter as first instars and advance through later instars beginning in April, completing larval development in the summer (Fig. 1).

Through the spring and summer, *Gasteromermis* infects early instars of *Baetis* larvae, causing significant changes to the development, behavior and fitness of its host. Newly hatched pre-parasitic worms burrow through the cuticle of *Baetis* larvae and reside in the body cavity (one parasite per host), capturing nutrition by absorption (Hominick and Welch 1980, Vance and Peckarsky 1996). Infected hosts are parasitically castrated and the larval period is prolonged so that infected *Baetis* metamorphose to winged subimagos two or more weeks later than uninfected

larvae in the same stream (Vance and Peckarsky 1996, Chapter 2). They enter the adult phase still bearing the parasite. Observations suggest that, like ovipositing females, infected adult *Baetis* disperse to protruding substrates where they mimic oviposition behavior, although they have been castrated by their parasite and consequently have no eggs to deposit (Vance 1996). A remarkable aspect of the *Gasteromermis* parasite is its manipulation of host traits related to dispersal and oviposition. Although both males and females are infected (Vance 1996), when *Baetis* males become infected they experience morphological and behavioral feminization: morphologically they develop secondary sex characteristics resembling females, and behaviorally they exhibit the oviposition behavior of adult females; however, instead of depositing eggs on substrates at the destination patch, they release a single parasitic worm into the stream (Vance 1996). After exiting its host, the parasite overwinters in a free-living stage and reproduces the following spring; its progeny then pursue new (early instar) *Baetis* hosts (Hominick and Welch 1980). Therefore, adult parasites dispersed by infected *Baetis* of generation t produce offspring that infect the next *Baetis* generation, $t+1$, and the parasite lifecycle alternates between a free-living stage and environmental transmission into a single host (Fig. 1).

Surveys

Parasite distribution, prevalence and density. To assess spatiotemporal variation in *Baetis* parasitism in the East River catchment we surveyed parasite prevalence and density in a total of 15 streams during summers 2012-2015, sampling the host population 2-4 times over the summer at dates scheduled to capture the peak in seasonal infection rates. We considered the peak prevalence value to be maximum local prevalence measured before *Baetis* began emerging

from that site, because parasitized *Baetis* typically emerge at a later date resulting in biased prevalence estimates in late summer. At each sampling event ≈100 *Baetis* larvae were captured from the benthos by kick sampling, preserved in 95% ethanol and returned to the lab where the developmental stage, morphologically-determined sex, and infection status were determined by examination and dissection under microscopy at 20-100× magnification. We calculated parasite prevalence (an indicator of host infection risk) as the number of parasitized larvae divided by the total number of larvae dissected. Once per summer, near the date of peak infection, we measured parasite recruitment as the density of infected *Baetis* collected in 3 benthic invertebrate samples in a modified Hess sampler (0.104 m^2 per sample) placed on the streambed at random locations within the stream. *Baetis* larvae were counted and returned to the lab for processing and dissection. If the number of *Baetis* collected by this method was <100, more larvae were collected by kick sampling to obtain 100 individuals and estimate parasite prevalence (as above); subsequently we estimated infection density by dividing the number of larvae by the total area sampled and multiplying by the proportion infected. We defined parasite recruitment as the initial establishment of a parasite within a host. For *Gasteromermis*, in which one parasite infects one host, recruitment is a measure of density of infected hosts. This contrasts to host recruitment, which for *Baetis* mayflies has previously been measured as egg density.

Oviposition habitat availability. To estimate variation in the quantity of substrates available to *Baetis* for oviposition and parasite dispersal we surveyed one 30-m reach in each stream during peak flight season of *Baetis* (mid July-early August) each year from 2012-2015. We measured the density of rocks (no. rocks / m^2 stream) that had 3 traits reported by Encalada & Peckarsky (2006) as minimal criteria for a rock to be used by *Baetis* for oviposition: 1) a dry

surface protruding above the water, which *Baetis* requires as a "landing pad"; 2) a horizontal surface area $\geq$150 cm^2; and 3) surrounded by flowing water on all sides.

Baetis Dispersal Capacity and Behavior

Flight muscle ratio. As an indicator of the ability of adult parasitized and unparasitized *Baetis* to disperse, we measured the flight muscle ratio (flight muscle dry mass [DM] divided by total body DM) of late instar larvae (stage III, developing wing pads are longer than wide; Delucchi and Peckarsky 1989) from each group (parasitized N = 26, unparasitized N = 13). To obtain the flight muscle DM and total DM of an individual, we first removed head, abdomen, legs, and any non-muscular tissue (e.g., esophagus and contents) from the thorax, leaving only the flight muscles intact. Next, all portions were dehydrated at 60 °C for 24 h, and then weighed. Total DM was calculated as the sum of all dried body portions. Then the dry thorax was soaked in 0.35 M NaOH until all flight muscle tissue dissolved. To clear all remnants of tissue, the thorax was rinsed with a stream of water until only the thoracic exoskeleton remained. It was then dried again at 60 °C for 24 h and reweighed. We computed the flight muscle DM as the difference between the original DM of the thorax before tissue removal, minus the DM of the cleared thorax exoskeleton. In infected individuals the parasite was separated then dried and weighed separately. This protocol enabled us to compute the ratio of parasite:host biomass and examine its relationship to FMR.

Baetis *dispersal to oviposition sites.* During the same year as the oviposition habitat manipulation (detailed below) we estimated the immigration of fertile and parasitized adult *Baetis* into 12 sites, by distributing 5 sticky traps through each study reach at the peak of the dispersal period (28 July -13 August 2014). Because the sticky traps were intended to capture

Baetis dispersing into specific oviposition sites, rather than intercept them on a flight path, they were constructed as horizontally-oriented landing platforms protruding from the stream in high flow, mimicking the position and function of oviposition rocks. Each trap platform was an empty compact disc case in the open position (28 x 12.5 cm), painted to blend with stream substrates and with the top surface coated with Tanglefoot® adhesive to trap insects that landed on the platform. Platforms were mounted to a 10 cm square safety caps fitted on the head of rebar stakes anchored in the streambed. We retrieved and replaced traps 3 times during the flight period. After retrieval, we returned traps to the lab, removed all *Baetis* and dissected them to determine whether they contained eggs or parasites.

Field Experiment

Site selection.

In 2014 we began a 2-year field experiment in the upper East River drainage basin near RMBL (further site details in Peckarsky et al. 2014) to test the effect of variation in oviposition habitat availability on subsequent levels of parasitism in *Baetis* mayflies. To accomplish this experiment we increased the density of oviposition substrates ("addition sites"), subtracted oviposition substrates ("subtraction sites"), and monitored unmanipulated control sites in 30 m reaches in 12 sites in 2014 (N = 4 per treatment). We measured the effect of manipulating oviposition sites available to the year *t* host generation (in 2014) by quantifying parasite recruitment and prevalence in host larvae of the year *t*+1 generation in 2015, after hosts had overwintered as first instars.

We positioned the experimental reaches to be bounded by natural barriers that would disconnect manipulated areas from upstream sites, because larval drift from adjacent reaches

could dilute or amplify evidence of a response to the habitat manipulation if immigration and emigration did not occur at equal rates (an assumption that we tested directly after the manipulation). This protocol imposed some constraints on the spatial scale of the experiment such that reach lengths of 30 m were the longest sites available in smaller streams. However, available evidence suggests that manipulating oviposition habitat at this spatial scale was appropriate for measuring the response variables the experiment was designed to test. Previous studies of healthy *Baetis* behavior and demographics have been done at similar scales (Encalada and Peckarsky 2011a, 2011b), and other evidence suggests that aquatic phase of the mermithid-*Baetis* lifecycle may play out at even smaller scales. First, parasites colonize the substrate at the location where they are dispersed; they crawl over small spatial scales as juveniles and do not rove as adults (Ebsary and Bennett 1973, Poinar and Poinar 2003). Once the host is infected by the parasite, its tendency to drift is suppressed, suggesting that infected *Baetis* should occupy a smaller patch size than uninfected *Baetis* larvae (Chapter 4).

We used a stratified random method to allocate sites to addition, subtraction and control treatments, taking into account natural physical and biological variation among streams. Our first criterion was to select streams with initial parasite prevalence (based on prior surveys in 2012-2013) that would best enable us to detect change due to the manipulation. In particular, because we intended to test for a decrease in parasitism following habitat subtraction, we could not expect to detect a negative effect in streams with low initial prevalence. Therefore streams assigned to the subtraction treatment had higher initial prevalence estimates and, conversely, streams assigned to the addition treatment had lower initial prevalence estimates; there was not a sufficient pool from which to select sites with similar initial prevalence for both treatments. The stratified approach presented 2 major risks. First, that initial prevalence was determined by an

unknown driver that varied systematically between sites with high versus low initial prevalence, limiting the potential for our manipulation to affect prevalence and further limiting our ability to generalize effects beyond the manipulated streams. To mitigate that risk, we avoided systematic variation in intrinsic habitat differences that we were aware could plausibly affect the host-parasite interaction. Therefore, each treatment was assigned a representative set of stream types, attempting to balance the distribution of stream size, temperature, and host density (Table A1). A second risk was that interannual variation would disrupt the prevalence patterns we had observed before the experiment (2012-2013), and upon which we based our treatments in 2014; but this change would not be detectable until after the manipulation had been completed. This variation did occur, and ultimately resulted in mean initial prevalence in the year of the manipulation (2014) not differing among treatments, in contrast to the originally intended stratified experimental design.

Manipulation of oviposition habitat. In addition sites we attempted to increase the habitat available for immigration of dispersing parasitized *Baetis* (and thereby the supply of reproductive parasites) into experimental reaches by adding substrates that the infected hosts use for depositing parasites back into the stream. In each site we increased substrates to 150% of the mean density (no. / m^2) that had been observed during peak *Baetis* previous flight seasons. To avoid modifying benthic habitat by transporting new substrates into the stream thereby changing the larval habitat, we accomplished the addition by repositioning existing rocks so that they protruded above the water surface and became available as dispersal sites for *Baetis* (Encalada and Peckarsky 2011b). In the subtraction reaches we submerged protruding rocks to make them unavailable for dispersing *Baetis*. We eliminated dispersal habitat where possible, but some of the largest, embedded boulders could not be submerged. These substrates were assumed to be

relatively unimportant for dispersal because ovipositing female *Baetis* do not prefer embedded rocks (Encalada and Peckarsky 2006). Since stream water levels decline through the summer, we returned to manipulated sites weekly and submerged all newly protruding rocks (Figs. A1 & A2). In control sites we left the rocks unmodified but monitored the natural availability of dispersal habitat through the summer. The initial manipulation began on 7 July 2014, after spring flow had abated enough for streams to be accessible, and continued for 6 weeks until the end of the *Baetis* flight period (13 August 2014).

Baetis *movement through experimental reaches.* To test the assumption that drift movements of larval *Baetis* did not result in a net import or export of parasitized individuals from experimental reaches, which could bias the response to habitat manipulation, we measured the density of *Baetis* and prevalence of parasites in *Baetis* drifting in the water column both into and out of each study reach. Depending on stream width, 1-2 drift nets (opening 25 x 29 cm) were positioned at the upstream and downstream reach boundaries. Because daytime drift of *Baetis* is low in streams with trout (McIntosh et al. 2002), nets were deployed at 2100 h and each was left for 10-60 min, during which we predicted $\geq$ 100 individuals would be captured, based on previous data on *Baetis* drift density within each site (Wilcox et al. 2008). All samples were preserved with 90% EtOH and returned to the lab where the developmental stage, morphologically-determined sex, and infection status of all *Baetis* were determined by examination and dissection. Drift samples were collected in July 2015, to overlap with the dates in which parasitism was measured as a response to the habitat manipulation carried out the previous year. We calculated and compared immigration ratios (drift density in / drift density out) of parasitized and healthy *Baetis* collected between upstream and downstream drift samples;

ratios >1 indicate net gain of *Baetis* while ratios <1 indicate net loss of *Baetis* from experimental reaches.

Estimates of parasitism. To test whether manipulation of oviposition habitat in 2014 affected parasitism in 2015 we compared estimates of parasite prevalence and density in the generation before (2014) and after the manipulation (2015). Samples from experimental sites were collected and processed as components of the multi-year parasitism survey, by the same methods described above. Because infections occur dynamically and continuously over several weeks of *Baetis* larval development, we used peak prevalence as the response variable of interest, because this estimate represents the maximum potential of a site to reduce fitness in the local host population and to source parasite propagules to other sites throughout the drainage.

Analysis

Surveys. To examine spatiotemporal variation in parasitism in the East River catchment we fit a linear model of peak parasite prevalence with site, year, and their interaction as predictors. We examined spatiotemporal variation in oviposition habitat with a similar linear model of oviposition substrate availability (no. substrates/m^2; log[x]-transformed to meet normality assumptions). We used Pearson correlation analysis to evaluate the relationship between oviposition habitat density in year *t* and parasite prevalence in the subsequent generation of *Baetis* (year *t*+1).

Baetis *dispersal ability.* We used Kolmogorov-Smirnov tests to compare the distribution of FMR between parasitized mayflies (which appear to be female and exhibit female dispersal behavior although their genetic sex is unknown), with unparasitized males (which fly in swarms to obtain mates, but do not disperse back to streams) and unparasitized female *Baetis* (which

disperse back to streams for oviposition). We used linear regression to determine if the ratio of parasite:host biomass was a significant predictor of FMR. We expected the proportion of host biomass allocated to flight muscles to decline (a negative relationship) with increasing proportion of host biomass comprised of parasite if there is a tradeoff between energy allocated to the parasite versus flight muscles of the host.

To detect differences in immigration of adult *Baetis* to oviposition sites used in the field experiment we compared the total number (log[x]-transformed) of unparasitized *Baetis* captured in sticky traps among treatment types using a linear model. Capture of parasitized *Baetis* in sticky traps was too low (4 individuals) for statistical analysis.

Field experiment. To test for an effect of habitat manipulation on parasitism we evaluated two outcomes: parasite prevalence and parasite recruitment (no./m^2). We compared the values of each variable estimated before the manipulation in 2014 to the value measured after the manipulation (2015) by fitting a generalized linear mixed-effects models with treatment (substrate addition, subtraction or control) and time (before or after substrate manipulation) as fixed effects and site as a random effect. We were interested in whether the manipulation affected change in parasite prevalence or recruitment dependent on treatment, which would manifest as a significant treatment × time interaction. In 2015 one of the addition sites (Avery) was scoured by a landslide before we could measure the response variables; therefore we could not estimate prevalence after the manipulation, resulting in an unbalanced design.

Baetis *drift.* We compared immigration ratios of parasitized *Baetis* among treatment types using one-way ANOVA. All analyses were done in R 3.2.4 (The R Foundation for Statistical Computing 2016).

RESULTS

Surveys. During the 5-year survey of *Baetis* parasitism average within-stream prevalence ranged from <2% to 80% (median 13%) across sites in the East River drainage. Prevalence varied significantly among sites ($F = 14.4$, $p < 0.001$) and across years ($F = 9.78$, $p < 0.006$), but site differences were consistent across years ($F = 1.09$, $p = 0.423$ for the site × year interaction; Table A3), such that some sites were consistent "hotspots" of parasitism even in years when overall prevalence was lower at the drainage scale (Fig. 2). Similarly, the availability of oviposition habitat also differed among sites ($F = 15.5$, $p < 0.001$) and across years ($F = 12.6$, $p < 0.005$), and it did not vary significantly within sites over time ($F = 1.37$, $p = 0.306$; Table A3).

The relatively stable availability of oviposition habitats and parasite prevalence among years within sites suggested that intrinsic habitat features were related to parasite hotspots. Accordingly, in the first years of surveys (2012 - 2013) we observed that oviposition rock density in year *t* was positively correlated with parasite prevalence in year *t+1*; those early observations motivated the experimental test for a cause of this relationship beginning in summer 2014 ($r^2 = 0.75$, $p = 0.026$). However, as we continued surveys over subsequent years (2014 - 2016), the original pattern diminished and disappeared altogether (Fig. 3).

Baetis *dispersal ability and behavior.* Average FMR was lowest in parasitized *Baetis* (genetic males and females combined; mean ± SE parasitized: 0.108 ± 0.013; unparasitized females: 0.161 ± 0.015; unparasitized males: 0.129 ± 0.031), and the distribution of FMR observed in parasitized *Baetis* was significantly different from that of unparasitized females that they are manipulated to mimic (K-S test, $p = 0.049$; Fig. 4A). In parasitized *Baetis*, FMR decreased with increasing parasite:host biomass ratio ($r^2 = 0.20$, $p = 0.023$; Fig. 4C), consistent

with a tradeoff between energy allocation of biomass to parasites and allocation to host flight muscles. In fact, the biomass of parasites can even exceed that of their hosts (Fig. 4B).

Sticky traps deployed in all experimental sites to detect treatment differences in immigration into sites indicated no differences among treatments in total number of adult unparasitized ($p = 0.411$) *Baetis* females dispersing to sites (Fig. 5). Remarkably, only 4 of 292 (1.4%) captured adult *Baetis* immigrants were parasitized, in contrast to ≈20% average prevalence in late-stage larvae across the drainage in 2014. This observation limits statistical inferences regarding parasitized *Baetis* immigration, but suggests strong negative effects of parasites at some point in the transition between metamorphosis and immigration at oviposition sites.

Field experiment. Before the manipulation there was no difference among treatments in the baseline availability of oviposition habitat ($F = 2.98$, $p = 0.11$). By manipulating oviposition rocks we were able to increase the density of oviposition habitat in addition sites by ≈150% relative to baseline measurements, and sustain this increase through the *Baetis* flight season (Fig. A1). The subtraction treatment resulted in a smaller shift from baseline than the addition treatment, due to the difficulty of submerging the largest, embedded boulders, and because the maximum potential change was capped by the starting density of substrates in these sites.

We found no systematic changes in parasite prevalence as a result of the rock manipulation ($X^2 = 3.92$, $p = 0.14$ for the treatment × time interaction; Table A4, Fig. 6). Similarly, there was no effect of the manipulation on the change in parasite density ($X^2 = 2.63$, $p = 0.268$; Fig. 6). Further, there were no significant continuous relationships of parasite prevalence or density versus oviposition rock density across all manipulated sites ($r^2 = 0.02$, $p = 0.701$; $r^2 = 0.06$, $p = 0.463$, respectively). Together with the overall lack of a relationship

between the availability of host oviposition habitat and parasite prevalence (Fig. 3), the outcome of this experiment is consistent with the hypothesis that the variability in suitable habitat that limits host recruitment does not limit its parasite and, therefore, does not contribute to observed hotspots of infection risk.

Baetis *drift behavior.* We tested the assumption that parasitized *Baetis* moved into and out of experiment reaches at the same rate. While that assumption was confirmed in control and addition treatment sites, in the subtraction treatment significantly more parasitized *Baetis* larvae drifted out of reaches than into them (i.e., immigration ratio < 1; $t = -4.93$, $p = 0.016$; Fig. A3). This outcome could have further reduced the prevalence of parasites in habitat removal reaches; however, there was not a significant reduction in parasitism in those reaches despite the potential for drift dispersal biasing prevalence of parasites in that direction (Figure 6).

DISCUSSION

Host-parasite interactions occur on a spatial mosaic such that in some patches susceptible hosts confront high risk of parasite infections, where other patches offer low-risk shelters from disease. The supply of infective parasites can influence host infection risk, especially if it covaries with host density as a result of similar dispersal, settlement and recruitment patterns (Byers et al. 2014). We found that, rather than mimicking the dispersal trends of healthy hosts, parasitized *Baetis* mayflies had lower dispersal capacity (flight muscle ratio) and immigrated into oviposition sites at lower rates (reflected in sticky trap captures). Consistent with those differences, experimentally increasing oviposition habitat available for host dispersal in natural streams, which was initially predicted also to increase parasite supply, had no effect on parasite recruitment or host infection risk. We also found a negative relationship between a host's flight

muscle mass and the size of its parasite, suggesting that part of this negative response to manipulating oviposition habitat may be explained by physiological constraints on *Gasteromermis* parasites, resolving a tradeoff between allocating host resources to their own growth and leaving the host sufficient reserves to maintain dispersal capacity. In addition, differences between host and parasite lifecycles suggest reasons that selectivity for oviposition sites would benefit the host but not the parasite. Ultimately the variability in suitable habitat that limits host recruitment does not limit its parasite and, therefore, does not contribute to observed hotspots of infection risk.

Negative effects of parasitism on host locomotion are common (Moore 2002) including examples of parasitism decreasing insect flight ability (Jutsum and Goldsworthy 1974, Marden and Cobb 2004, Bradley and Altizer 2005). However, our observation that infected *Baetis* have lower flight muscle mass contradicts the theoretical expectation that parasites manipulating their hosts for dispersal should not deplete dispersal capacity (Maure et al. 2011, 2013). Castrating parasites like *Gasteromermis* sequester host reproductive resources and can exert high energetic costs (Baudoin 1975, Hall et al. 2007, Lafferty and Kuris 2009); but it has not previously been reported that mermithid parasitism affects developing flight muscles. However, all host manipulations are predicted to have cost-benefit tradeoffs for the parasite (Poulin 1994). The negative relationship between FMR and parasite:host biomass suggests that parasites could face a tradeoff between host dispersal capacity and allocating host reserves to their own growth.

Inequalities between host sexes in baseline dispersal capacity may also play a role in the FMR differences we observed. Because infected male hosts are indistinguishable from females, the distribution of FMR measured in infected *Baetis* combined genetic females with males, which tended to have lower FMR when uninfected. Without knowing genetic sex, we cannot

disentangle direct negative effects of parasitism on FMR from natural variation between host sexes. In any case, we suggest that the benefit of feminizing male hosts is not to exactly mimic adult female dispersal, but to provide a vehicle for the parasite to disperse to habitats where hosts are likely to be available the next summer. Further, mortality is high for aquatic insects in the terrestrial phase (Jackson and Fisher 1986). If their hosts disperse over shorter distances parasites may benefit from lower exposure and higher survival.

The failure of parasitized *Baetis* to respond to variation in the availability of oviposition habitat can be explained by contrasting the selection pressures acting on specific life stages of the host and the parasite; i.e., traits of adult female mayflies that benefit *Baetis* may not benefit the parasite enough to balance out their associated costs. First, the characteristics of oviposition habitat that increase *Baetis* egg survival would not be expected to have an equivalent positive effect on parasite egg incubation. Uninfected female *Baetis* experience selection pressure to fly far enough to locate optimal substrates where their eggs will not desiccate before hatching (Encalada and Peckarsky 2006). Those substrates are characterized by being large and located in areas of fast flow with high splash that keeps eggs hydrated during the 14 day embryogenesis period, which significantly increases probability of egg survival (Encalada and Peckarsky 2006).

In contrast to their hosts, parasites are deposited under protruding rocks by infected hosts but quickly move to the streambed to complete their lifecycle (including mating and oviposition months later; Vance & Peckarsky, 1996). Therefore, parasites use rocks merely as portals for delivery to the stream rather than substrates for egg incubation. This contrast also suggests that parasites should not experience any competition for space on the oviposition rock itself, whereas numbers of *Baetis* egg masses accumulating on small rocks could ultimately be limited by space. The natural availability of high quality oviposition sites for healthy *Baetis* is also temporally

variable because the probability of eggs desiccating increases as spring runoff wanes and stream water levels descend through the summer (Encalada and Peckarsky 2006). In contrast, successful dispersal for the parasite depends only on the instantaneous availability of a rock, but not on the fate of the rock over an extended window of time. Deposition of parasites on a rock that optimizes attributes for *Baetis* oviposition (i.e., large size surrounded with high turbulence) could actually disadvantage the worm by increasing the distance it must travel to the streambed and/or by increasing the likelihood of it being dislodged from the rock while it migrates to the streambed.

Because oviposition and embryogenesis occur in a more predictable environment for the parasite (deeper in the stream bed) than for the host (protruding boulders), host habitat selection criteria should not have similar benefits for egg survival in both host and parasite species. Instead, the rock traits parasites require should be much broader and available in higher proportion (Encalada and Peckarsky 2006), requiring less selectivity and lower dispersal capacity to access them. Ultimately the cost of reduced dispersal capability may be relatively low for the parasite compared to benefits of increasing growth, and this thinking is consistent with the outcome of our field experiment, that host oviposition habitat availability did not affect parasite recruitment or host infection risk. Therefore, we argue that targeting the same oviposition sites used by healthy hosts is not likely to decrease mortality of parasites deposited by hosts, nor is it likely to increase the survival of their eggs.

Nevertheless, theory suggests that preparasitic offspring hatching from those eggs should benefit by experiencing the highest number of encounters when host densities are high (Anderson and May 1978, Blower and Roughgarden 1989, Hassell 2000). Therefore, failing to target dispersal to sites where host recruitment is highest (i.e., sites with high densities of

oviposition substrates; Encalada and Peckarsky, 2011a) should come at a cost to parasite transmission via increase host encounters. However, *Gasteromermis* may be constrained from benefiting from increases in host recruitment because its lifecycle is asynchronous with the host, further offsetting any selective advantage of targeting the sites where host dispersal is highest. Although spatially heterogeneous host populations can initially result from variation in oviposition habitat availability, the period when this effect is strongest does not coincide with the hatching of preparasites. The positive influence of oviposition sites and egg densities on *Baetis* larval population sizes has generally dissipated by the time infections appear suggesting that when preparasites are actively seeking hosts, the effects of variation in host recruitment on host density are overridden by density-dependent post-recruitment processes (Encalada and Peckarsky 2011a, 2011b). At a finer spatial scale, the highest quality oviposition substrates accumulate hundreds of egg masses with subsequent local aggregations of *Baetis* hatchlings around individual boulders in late summer (Encalada and Peckarsky 2006, K. Cromwell, *pers. obs.*). However, *Baetis* larvae are mobile enough to have dispersed beyond those hatching sites before parasites are available to infect them. Even if parasites did selectively disperse to preferred rocks, the asynchrony of the host-parasite lifecycles would cost them the opportunity to infect aggregated hosts at either the rock- or the stream-scale. Instead, this pattern should favor the host, which would benefit from a dilution of encounters with parasites when host local density is high but parasites fail to track it spatially or temporally (Mooring and Hart 1992, Côté and Poulin 1995). Indeed, a negative relationship between host density and parasite prevalence is what we have observed in the upper East River catchment (Fig. A4).

Improving our understanding of the natural history of *Gasteromermis* parasites has enabled us to interpret the outcomes of our surveys and experiments, concluding that

heterogeneity in infection rates may be shaped more strongly by post-colonization conditions within patches than by between-patch differences that influence dispersal or host density (Ekholm et al. 2017). Specific to this case, many life history events occur in the months between when parasites are dispersed and when their offspring infect the next generation of hosts (i.e., overwinter survival, mate location, reproduction, egg survival and host seeking; Fig. 1). Those transitions are potentially subject to many constraints that could disconnect dispersal dynamics from recruitment and dilute colonization effects.

Nonetheless, the possibility that *Gasteromermis* alters dispersal of its host on local (if habitat selection differs) or regional (if flight capacity differs) scales has implications not only for parasite recruitment but also source-sink dynamics, persistence and evolution. While transmission hotspots have the potential to generate regional sources of parasites (Paull et al. 2011), this potential is not necessarily realized if recruitment and dispersal are decoupled (Buck et al. 2017). For example, we observed parasite-induced reductions in host flight capacity that could theoretically decrease contact between dispersing parasites and new patches. In this case, rather than being sources from which parasites spread, the hotspots we observe may retain a disproportionate number of parasites, trending toward local host population sinks but not regional parasite sources. This scenario is supported by the observation that high prevalence sites are consistent over multiple years.

Although we have emphasized the average decrease in host dispersal capacity caused by parasitism, we did measure some infected individuals with flight muscle ratios as high or higher than uninfected hosts. Dispersal can be critical to persistence not only by enabling colonization but also by promoting gene flow (Pulliam 1988, Clobert 2012), ultimately influencing local adaptation of parasites and host-parasite coevolutionary dynamics (Price 1980, Davies et al.

1999). However, colonization and persistence can occur if only a small proportion of individuals are more dispersive (Anholt 1995, Higgins and Richardson 1999, Bohrer et al. 2005). Therefore individual variation could be important for parasites facing a tradeoff between fecundity and manipulating host dispersal (Poulin 1994). Plasticity in the degree of manipulation should benefit parasites exploiting heterogeneous habitats where resources are unpredictable (Thomas et al. 2011).

Conclusion

Understanding parasite strategies for dispersal, transmission and survival within heterogeneous environments is necessary to forecast how environmental variability or future change could influence disease emergence (Thomas et al. 2002). To better understand drivers of parasitism hotspots in a dynamic stream network, we investigated the relationship between host-mediated parasite dispersal and infection risk. We found no evidence that host oviposition habitat density drives the dispersal or infection prevalence of *Gasteromermis* parasites of *Baetis*, which suggests that the variability in infection risk observed in this host-parasite system is driven by other variables that act on recruitment or post-recruitment processes. While this negative result can be understood given what we have learned about the natural history of this host-parasite system, the results presented in this study do enable us to rule out dispersal as an explanation of the distribution of hot spots. Further studies are needed to discover factors that influence the recruitment and risk of infection of *Baetis* by mermithid parasites.

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FIGURES

Figure 1. The univoltine lifecycle of healthy mayflies, *Baetis bicaudatus* (left: subsequent stages proceed clockwise in diagram), and parasitized mayflies with their mermithid nematode parasites, *Gasteromermis* sp. (right: subsequent stages proceed counterclockwise in diagram). The parasite infects both male and female hosts, but causes morphological and behavioral feminization of castrated male hosts to insure its transport back to the stream via mimicked oviposition behavior.

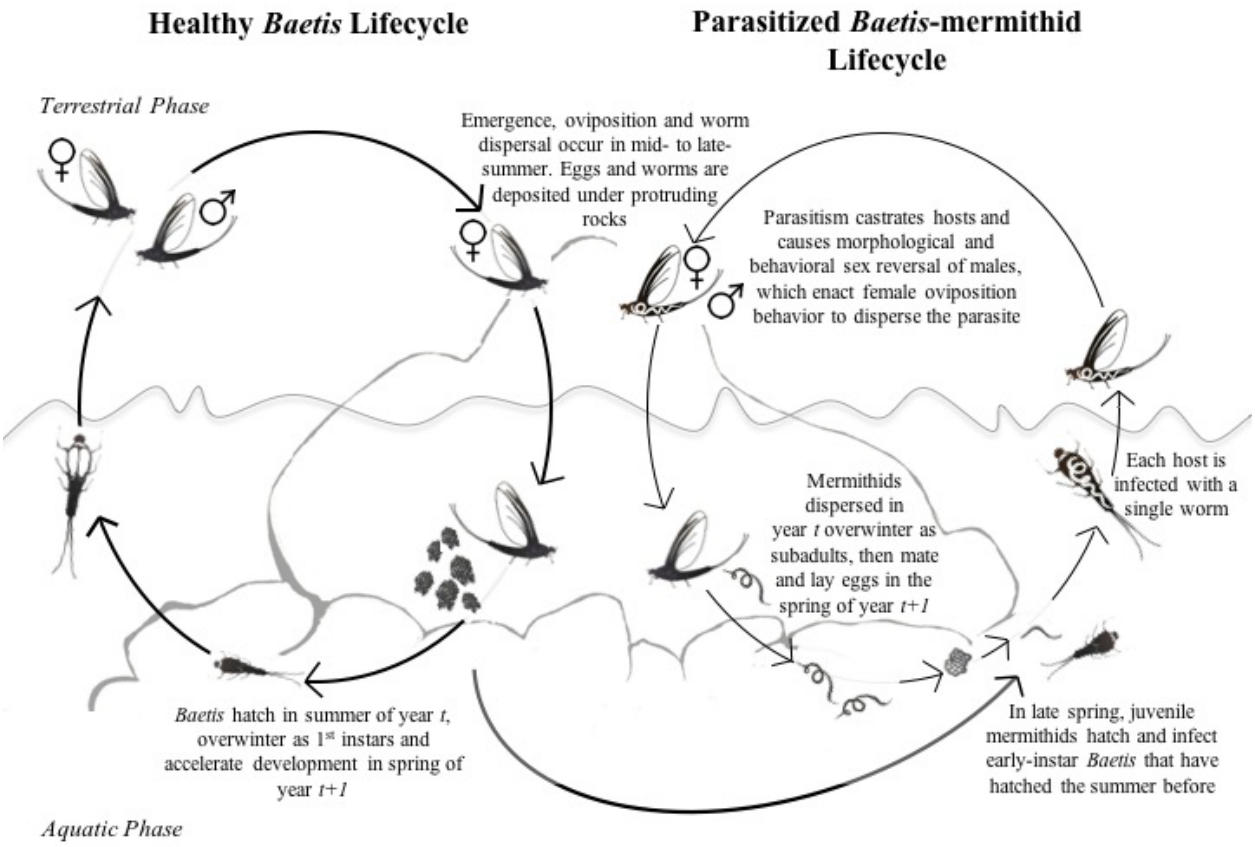


Figure 2. Prevalence (i.e., proportion of hosts infected) of *Gasteromermis* nematode parasites infecting *Baetis bicaudatus* mayfly larvae has high spatial but low temporal variation (see Table A2) in the upper East River drainage near the Rocky Mountain Biological Laboratory (RMBL), Colorado, USA. Sizes of circles are scaled to the average proportion of hosts infected between 2012-2015. Red circles show sites considered "hotspots" and blue circles are "cold spots" for mermithid infections.

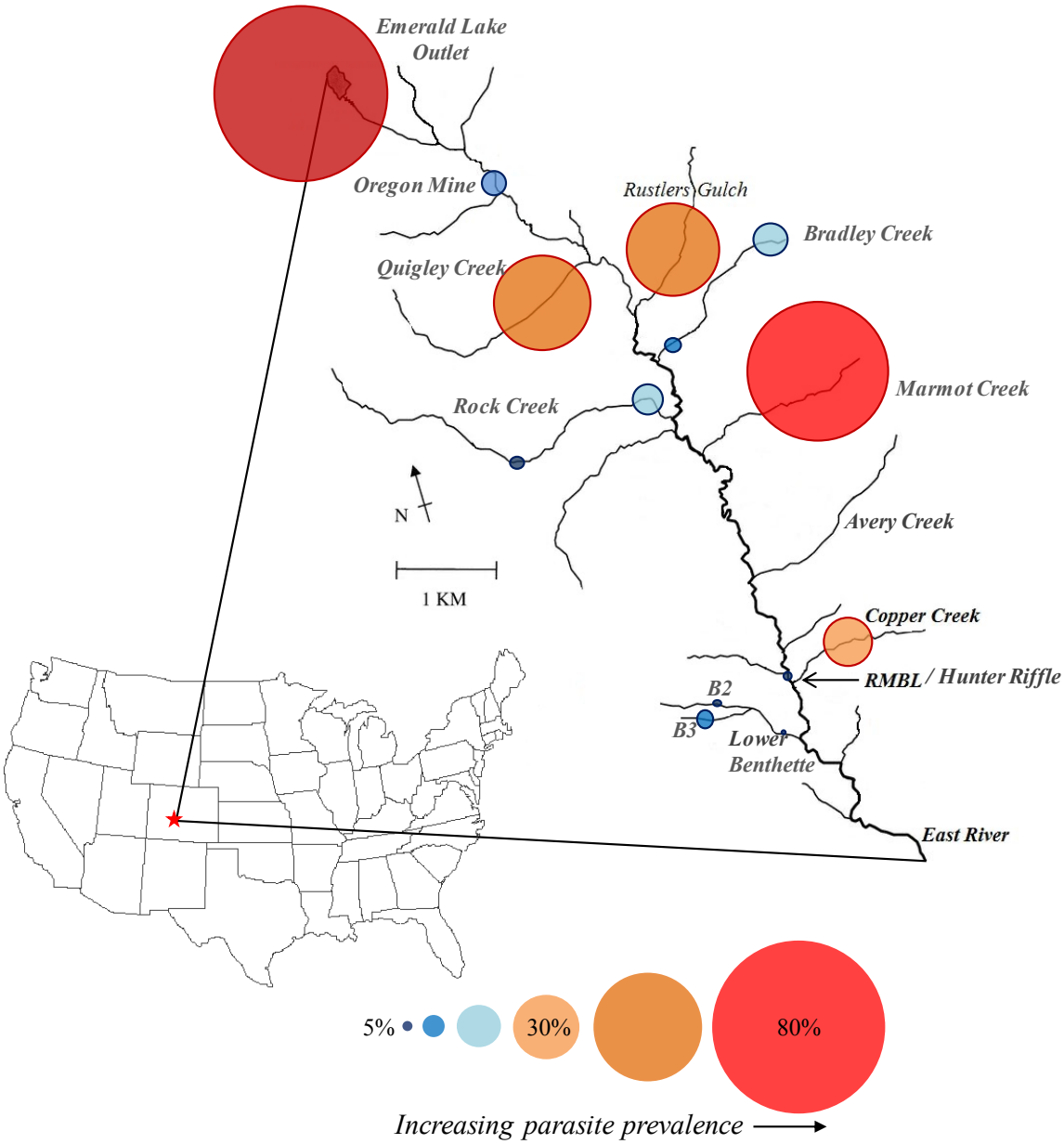


Figure 3. A multiyear field survey of parasite prevalence in *Baetis* mayfly larvae initially (2012-2013) indicated a positive relationship with oviposition habitat that hosts and parasites use for dispersal, but that relationship did not persist through subsequent years. Because hosts and parasites disperse in summer of year *t* but parasites do not reproduce and infect new hosts until spring of year *t*+1 (also see Fig. 1), each point represents measurements made within a site over 2 successive years. Significant regression line presented for 2012 – 2013 data.

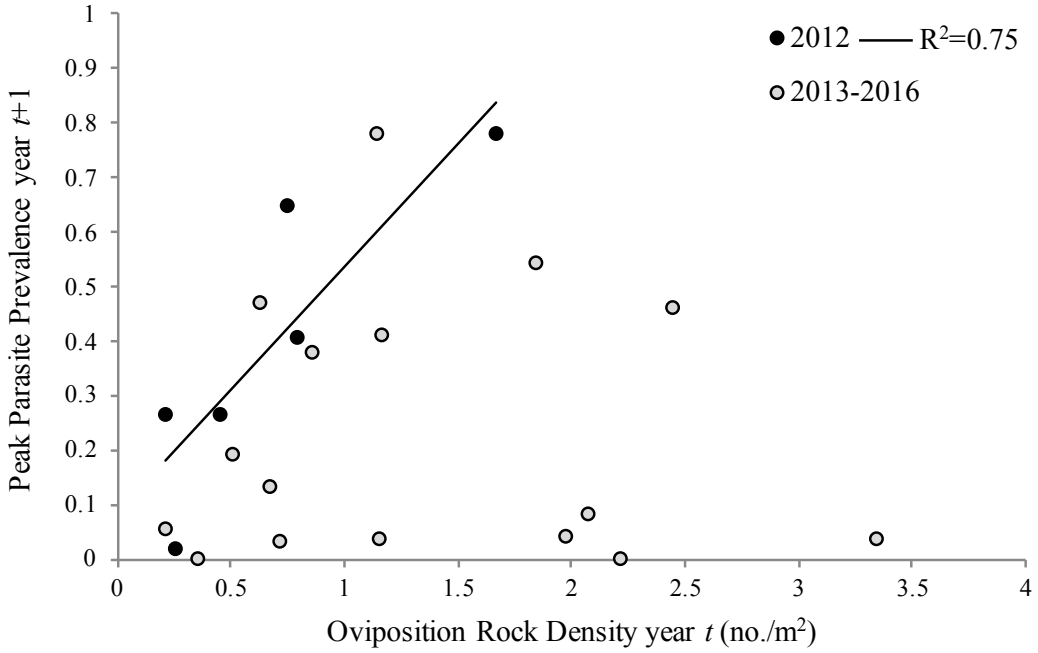


Figure 4. (A) Flight muscle ratios (FMR = biomass of flight muscles:total weight of mayfly host) measured in parasitized *Baetis*, which include genetic males and females (although morphology and adult behavior resembles females), unparasitized females, and unparasitized male *Baetis* collected from a single fishless tributary (Marmot Creek). The distribution of flight muscle ratios differs between parasitized *Baetis* and healthy genetic females that they mimic in oviposition behavior ($p = 0.049$). **(B)** Biomass of *Gasteromermis* parasites can exceed host biomass, and **(C)** the proportion of total host biomass comprising parasite tissue has a negative relationship with FMR.

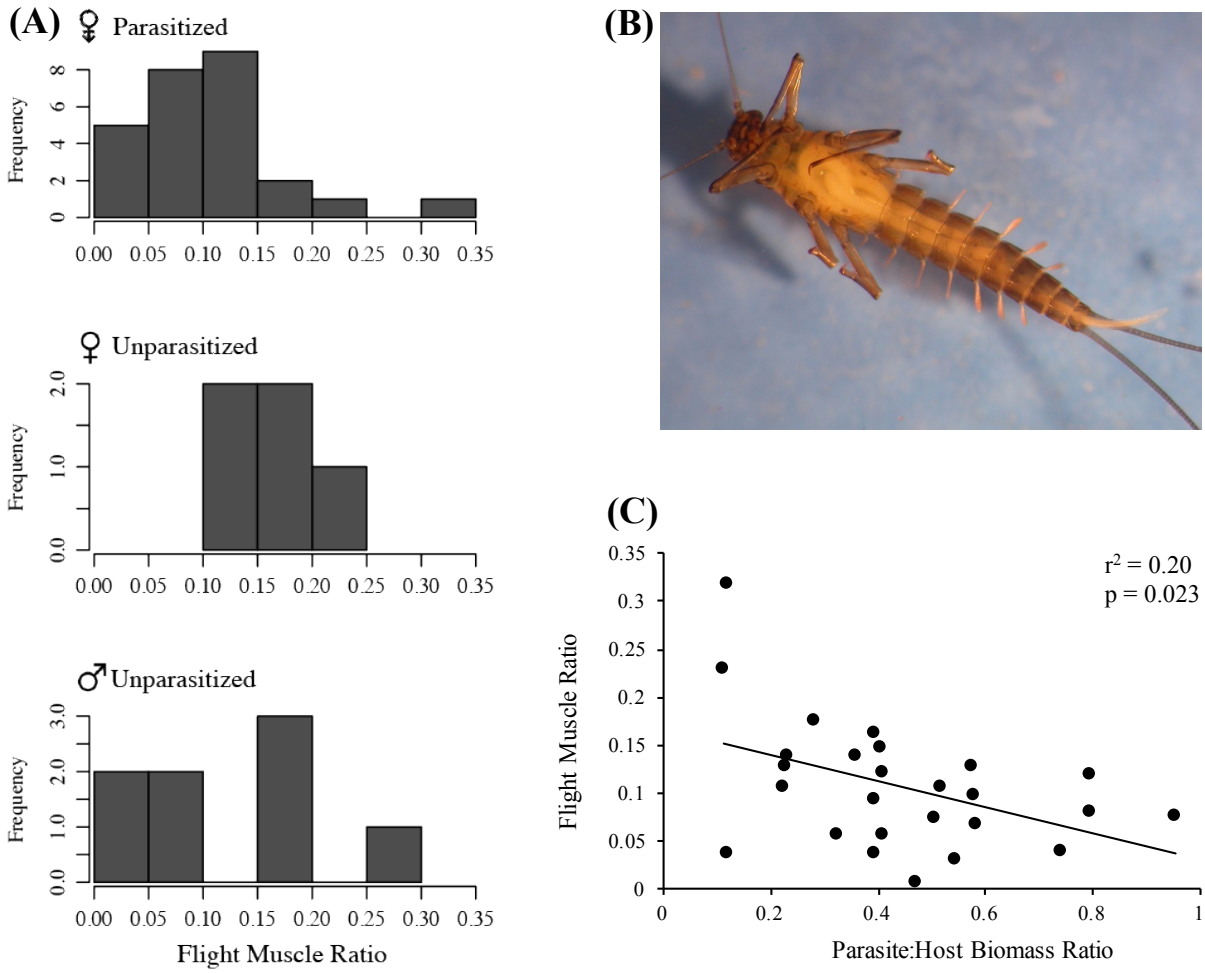


Figure 5. Frequency of parasitized and unparasitized adult *Baetis* immigrating into experimental field sites, collected on sticky platform traps. Less than 2% of captures were parasitized, in contrast to ≈20% of mayflies infected when measured as larvae before they emerged and dispersed (averaged across sites). Dispersing adults were collected at manipulated field sites in 2014 and parasite prevalence at those sites, reflected in the site arrangement on the x-axis, was measured in offspring of the next generation in 2015. Symbols above each bar indicate how oviposition rocks were manipulated in each site during the field experiments: by addition (+), removal (-) or unmodified control (∅). There were no relationships between experimental treatments, host-parasite immigration to a site or subsequent parasite prevalence in the next generation of hosts.

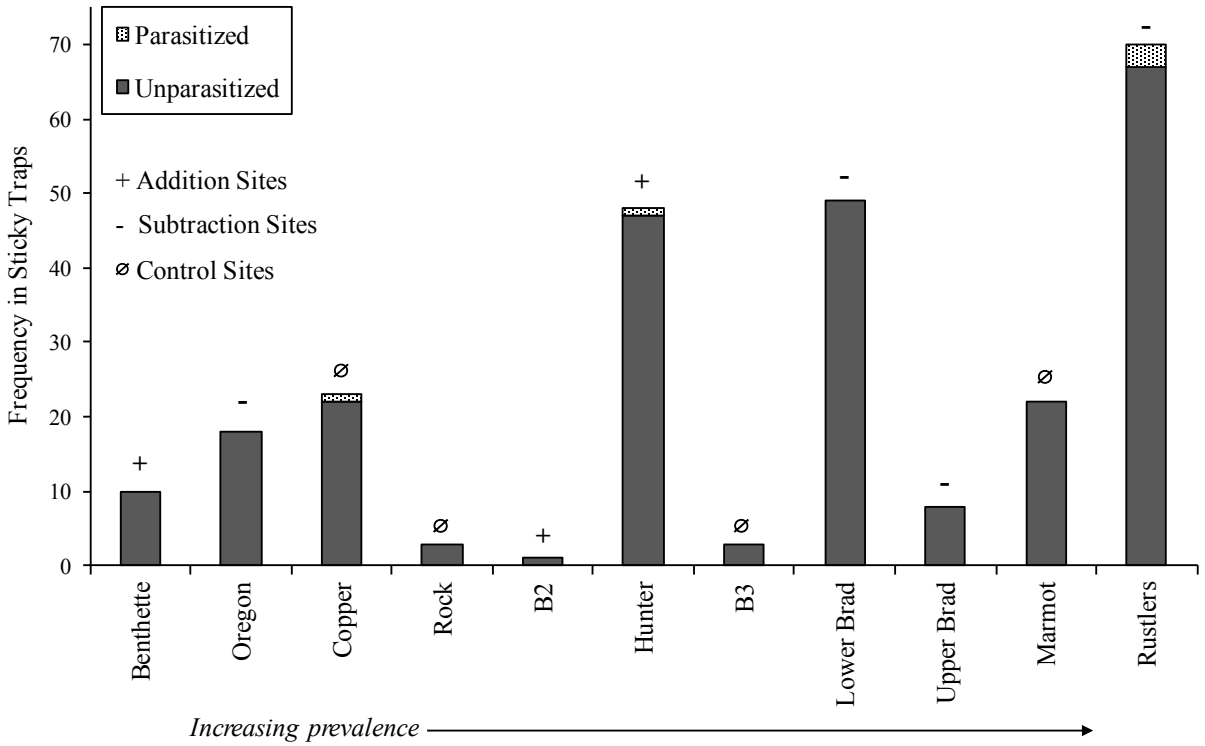
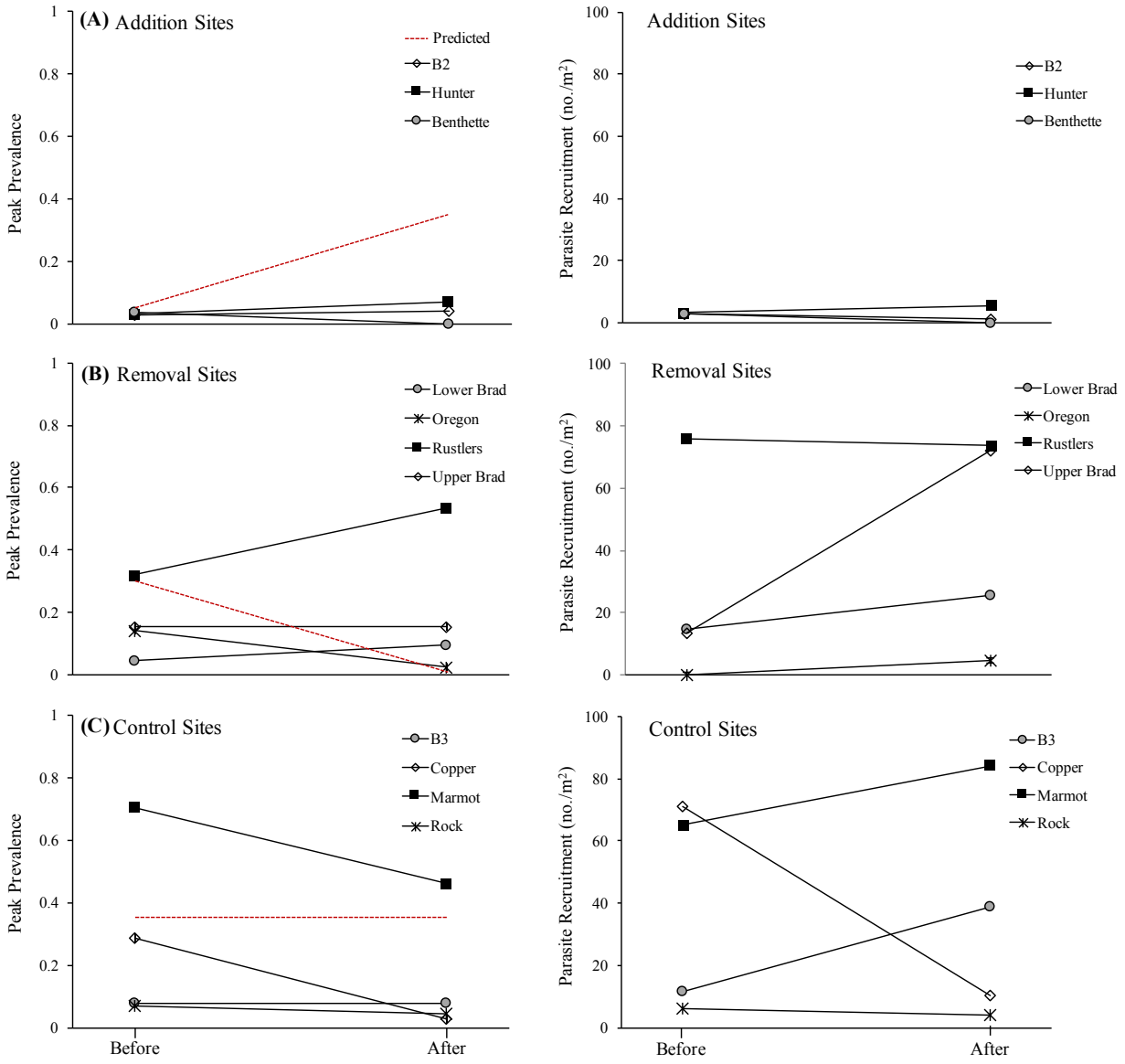


Figure 6. Parasite prevalence (proportion infected; left panels) and parasite recruitment (no./m^2; right panels) in *Baetis* larvae before (2014) and after (2015) the manipulation of *Baetis* oviposition habitat in addition (A), removal (B), and control (C) treatments. Manipulated streams are listed on each panel; red dashed lines on prevalence plots show the direction of change initially predicted to result from each treatment. There were no effects of manipulating habitat available for dispersing hosts (including those dispersing parasites) on subsequent parasite prevalence or parasite recruitment.



APPENDIX

Table A1. Abiotic and biotic environmental variables of 12 stream reaches measured in 2012-2013 in the upper East River drainage, CO, USA.

Streams[a]	Treatment[a]	Drainage basin area (km^2)[b]	Mean stream width (m)	Mean summer temp (°C)	Mean Baetis density ($no./m^2$)
Avery	Add	1.8	1.4	10.9	-
B2	Add	0.05	0.49	5.0	103
East River at Hunter Riffle	Add	45.9	7.8	11.5	83
Lower Benthette	Add	0.34	1.7	8.8	77
	Average±SE	**12.0±11.2**	**2.8±1.7**	**9.1±1.5**	**88±8**
Lower Bradley	Sub	3.8	2.0	5.7	324
East River at Oregon Mine	Sub	7.64	2.8	6.9	79
Rustlers Gulch	Sub	15.1	4.9	6.8	237
Upper Bradley	Sub	3.8	4.0	5.9	192
	Average±SE	**7.6±2.7**	**3.4±0.64**	**6.3±0.31**	**208±51**
B3	Cont	.001	0.7	5.5	248
Copper	Cont	24.3	5.8	8.2	109
Marmot	Cont	.93	.95	6.6	122
Upper Rock	Cont	3.6	3.2	10.1	48
	Average±SE	**7.2±5.7**	**2.7±1.2**	**7.6±1.0**	**132±42**

[a]Streams were assigned to three treatments for host oviposition site manipulation starting in 2014: (Add)ition, (Sub)traction, and (Cont)rol

[b]Data obtained from Wilcox et al. 2008

Table A2. Peak prevalence of *Gasteromermis* sp. parasites in *Baetis bicaudatus* mayflies measured in summers 2012-2016 at sites in the upper East River Drainage, CO. Prevalence varies spatially ($p < 0.01$) but remains stable within sites over time ($p = 0.50$).

	Year				
Site	**2012**	**2013**	**2014**	**2015**	**2016**
B2	-	-	0.03	0.04	-
B3	-	0.12	0.04	0.08	-
Copper	0.22	0.26	0.38	0.03	0.13
Emerald	0.96	0.65	0.78	-	-
East River at Hunter Riffle	0.03	0.02	0.04	0.07	-
Lower Benthette	-	-	0.04	0	-
Lower Bradley	-	-	0.05	0.1	-
Lower Rock	0.14	-	-	-	-
Marmot	0.91	0.78	0.42	0.46	0.54
Easter River at Oregon Mine	0.17	0.26	0	0.03	-
Quigley	0.44	-	-	-	-
Rustlers Gulch	-	-	0.32	0.54	0.47
Upper Bradley	-	0.26	0.05	0.15	-
Upper Rock	-	-	0.07	0.05	-

Table A3. Summary of analyses (linear models) of spatiotemporal patterns of parasite prevalence (proportion of hosts infected) and host oviposition site density (no. rocks/m^2) measured across the upper East River drainage basin, CO between 2012-2016.

	Parasite Prevalence		Oviposition Rock Density	
Effect	***F***	***p***	***F***	***p***
Site	14.4	<0.001	15.5	<0.001
Year	9.78	0.006	12.6	0.005
Site × Year	1.09	0.423	1.37	0.306

Table A4. Analysis of deviance table for the fixed effects from the linear mixed model on parasite prevalence (proportion of hosts infected) and parasite recruitment (no./m^2) in a 2-year before/after field manipulation in which host oviposition habitat was subjected to three treatments: addition, subtraction, unmanipulated control.

	Parasite Prevalence		Parasite Recruitment	
Effect	$\mathbf{X^2}$	***p***	$\mathbf{X^2}$	***p***
Treatment	1.15	0.562	3.28	1.94
Year	0.07	0.794	0.65	0.422
Treatment × Year	3.92	0.141	2.63	0.268

Figure A1. Effects of experimental manipulation of oviposition substrates in natural streams that were modified by rock addition (top row), subtraction (middle row), or control (bottom row) over the summer of the experimental manipulation (7 July – 12 August 2014). The first measurement was taken as a baseline, and the dotted vertical line shows the week of the initial manipulation. In addition sites, rocks were increased at the beginning of the summer. In subtraction sites rocks were submerged weekly as the hydrograph declined, preventing a natural accumulation of protruding rocks over the summer (exemplified by control sites where rocks were not manipulated).

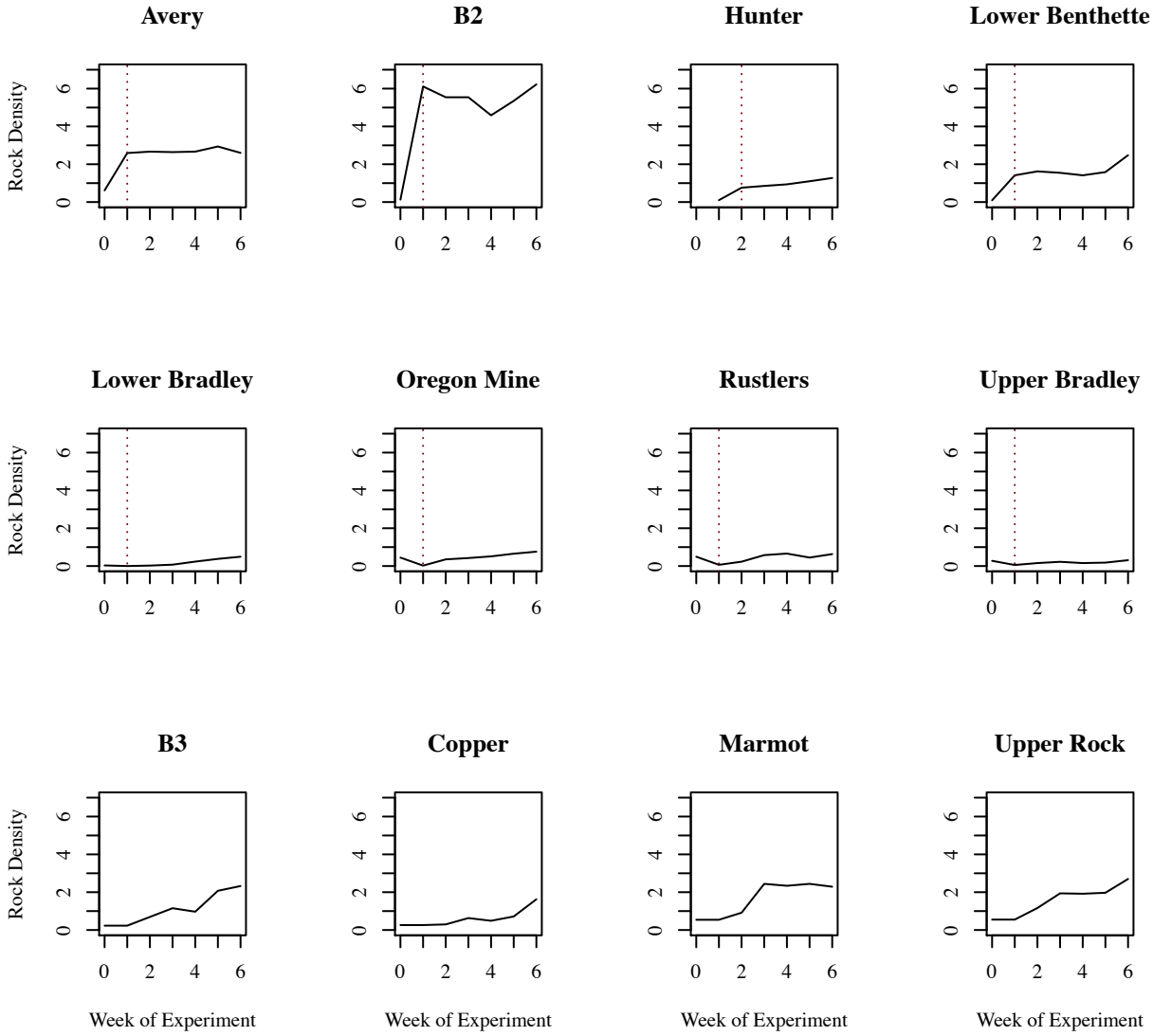


Figure A2. The natural decline of the stream hydrograph over the summer can influence the availability of oviposition sites, so we characterized spatial variability of summer flow regimes by monitoring water level with TruTrack stage height data loggers (model WT-HR1000, Christchurch, New Zealand) in June-August 2014 (year of the experimental manipulation). Logger sensors were protected within cylindrical stainless steel housings and anchored to steel fence posts embedded near the edge of each stream channel.

Each plot shows a time series of mean daily stage (left vertical axis, blue line) and weekly counts of oviposition rock density (right vertical axis, connected black points) across summer 2014 when oviposition substrates were manipulated to test for an effect on *Baetis* parasitism. Site plots are arranged in order of increasing parasite prevalence in the following year (2015), when no effect was detected. The dotted vertical line marks the week of the initial manipulation.

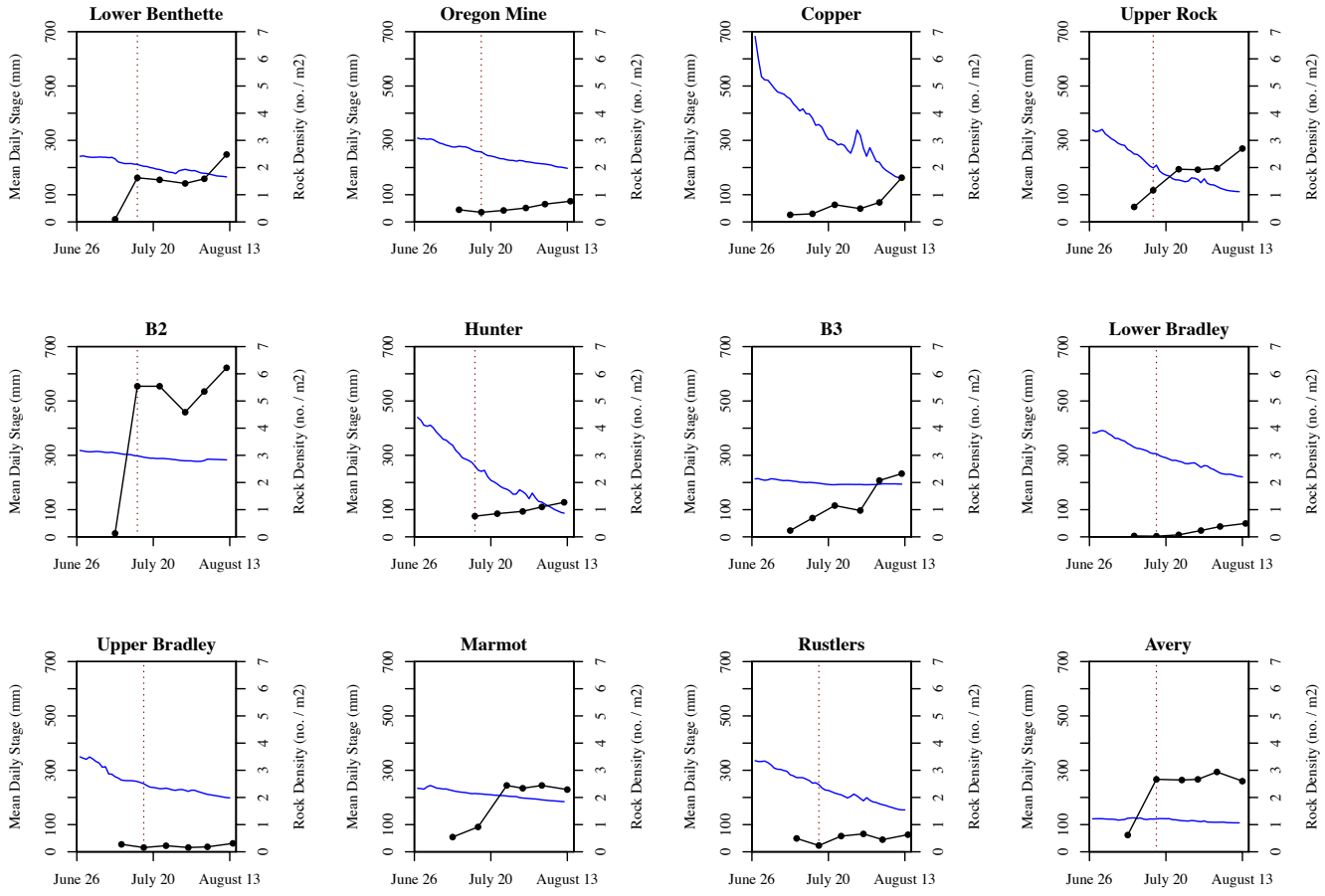


Figure A3. *Baetis bicaudatus* immigration ratio (drift density in:drift density out) in stream reaches for the sites where host oviposition substrates were added (n = 3), removed (n = 4) or unmanipulated (n = 4). Stream reaches with ratios >1 (dotted line) experienced a net gain of individuals, whereas those with ratios <1 experienced net loss. Ratios were significantly <1 in the removal treatment (t = -4.93, p = 0.016). This outcome would have reduced the prevalence of parasites in rock removal reaches; nevertheless there was not a significant reduction of parasitism in that treatment.

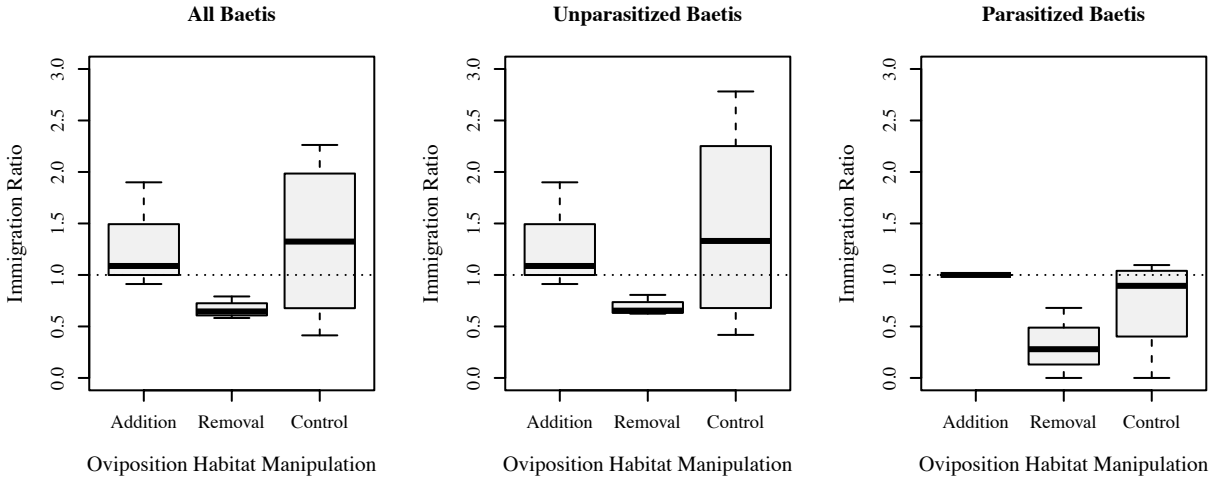


Figure A4. Relationship between host density (no./m^2) and parasite prevalence (proportion infected) in a 3-year survey overlapping the years of the oviposition habitat manipulation (2014-2015).

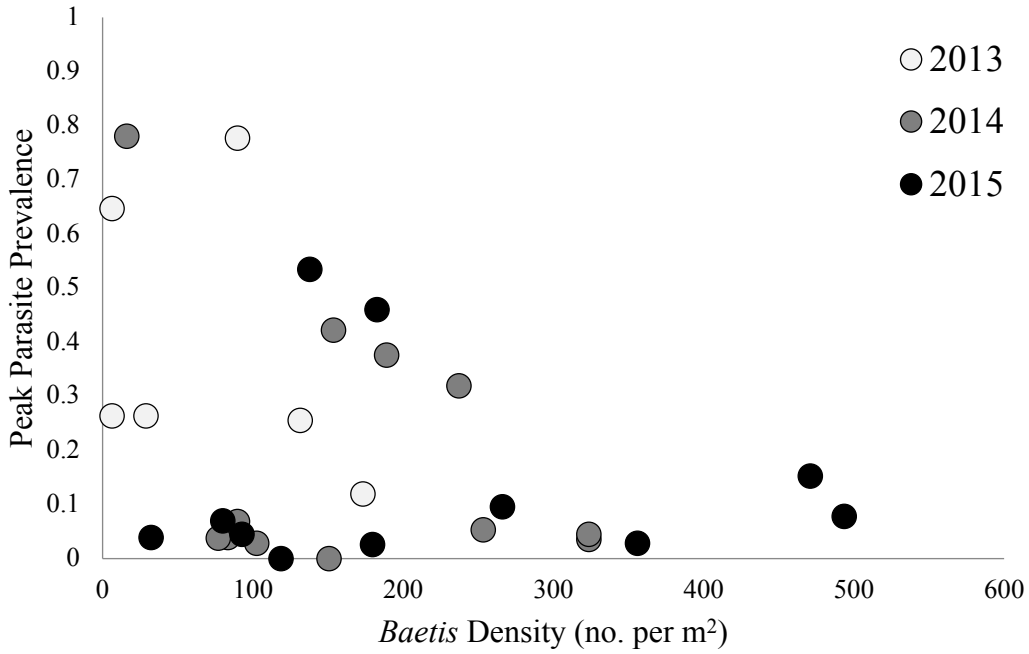
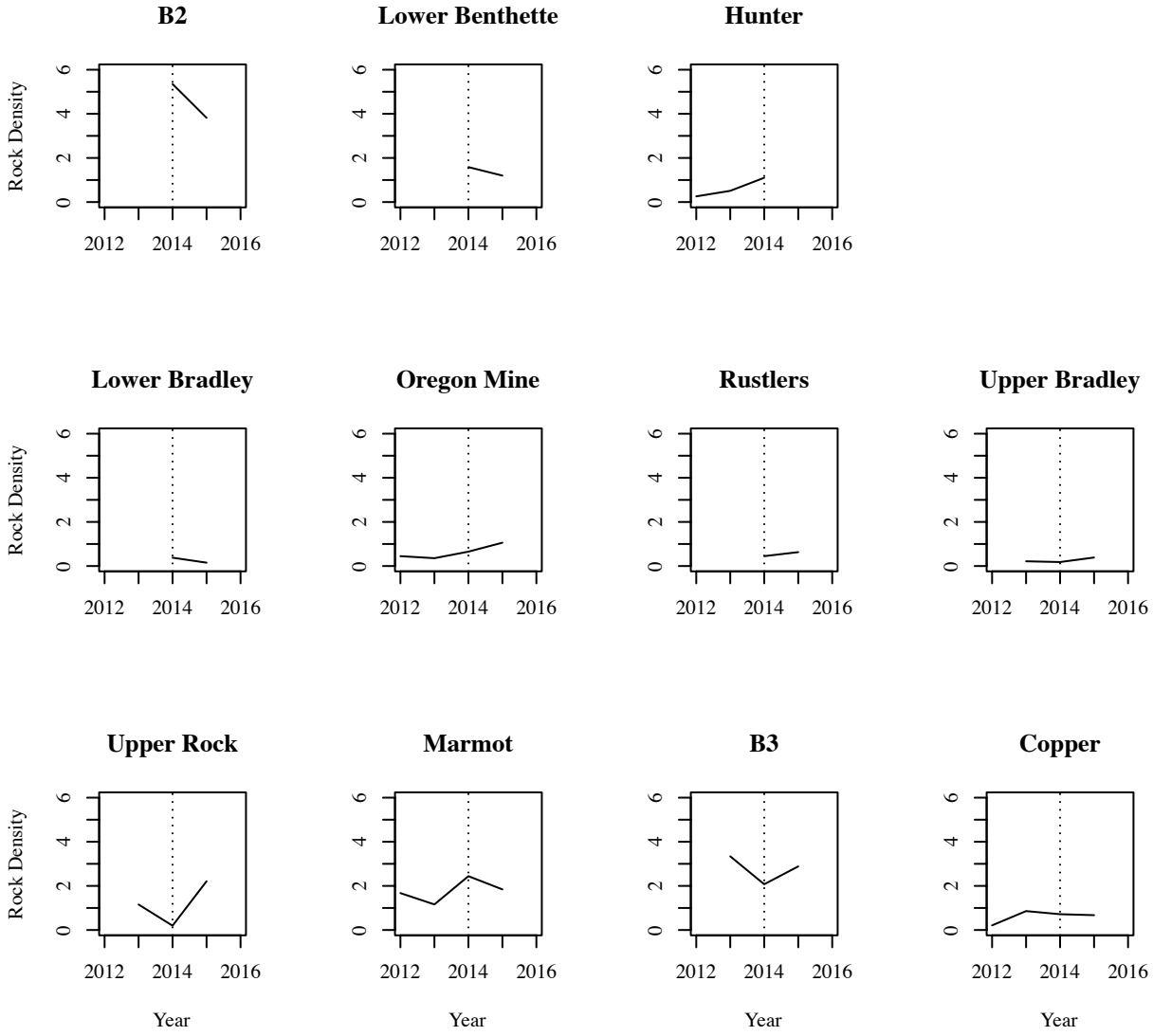
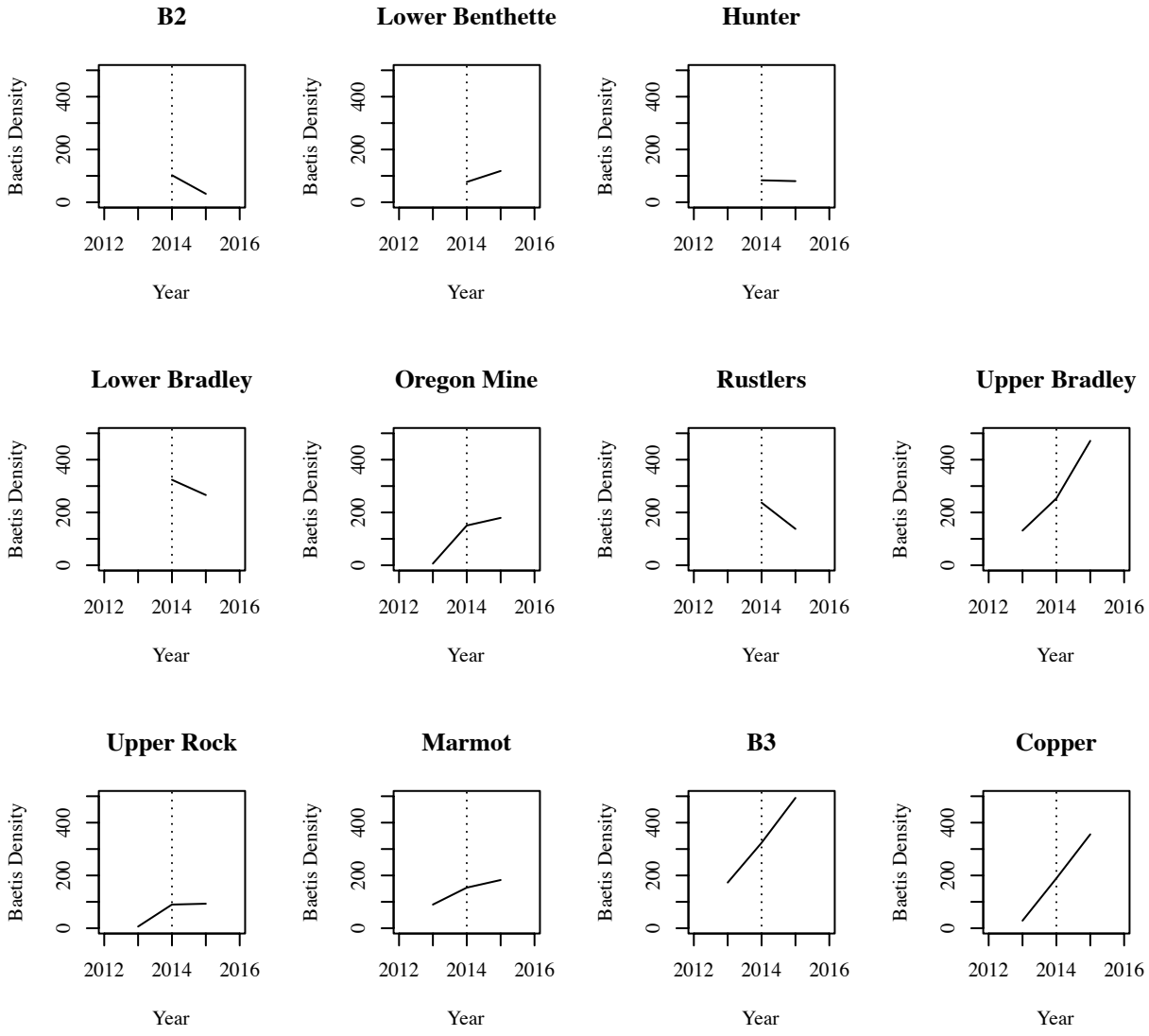


Figure A5. Time series of (A) rock density (no./m^2), (B) *Baetis bicaudatus* larvae density (no./m^2), (C) peak prevalence of *Gasteromermis* sp. parasites (proportion hosts infected), and (D) parasite recruitment (measured as density within hosts, no./m^2) in experimental sites in which host oviposition habitat was added (top row), subtracted (middle row), or unmodified (bottom row) in 2014 to test for an effect on parasitism in 2015. There were no effects as a result of the manipulation.

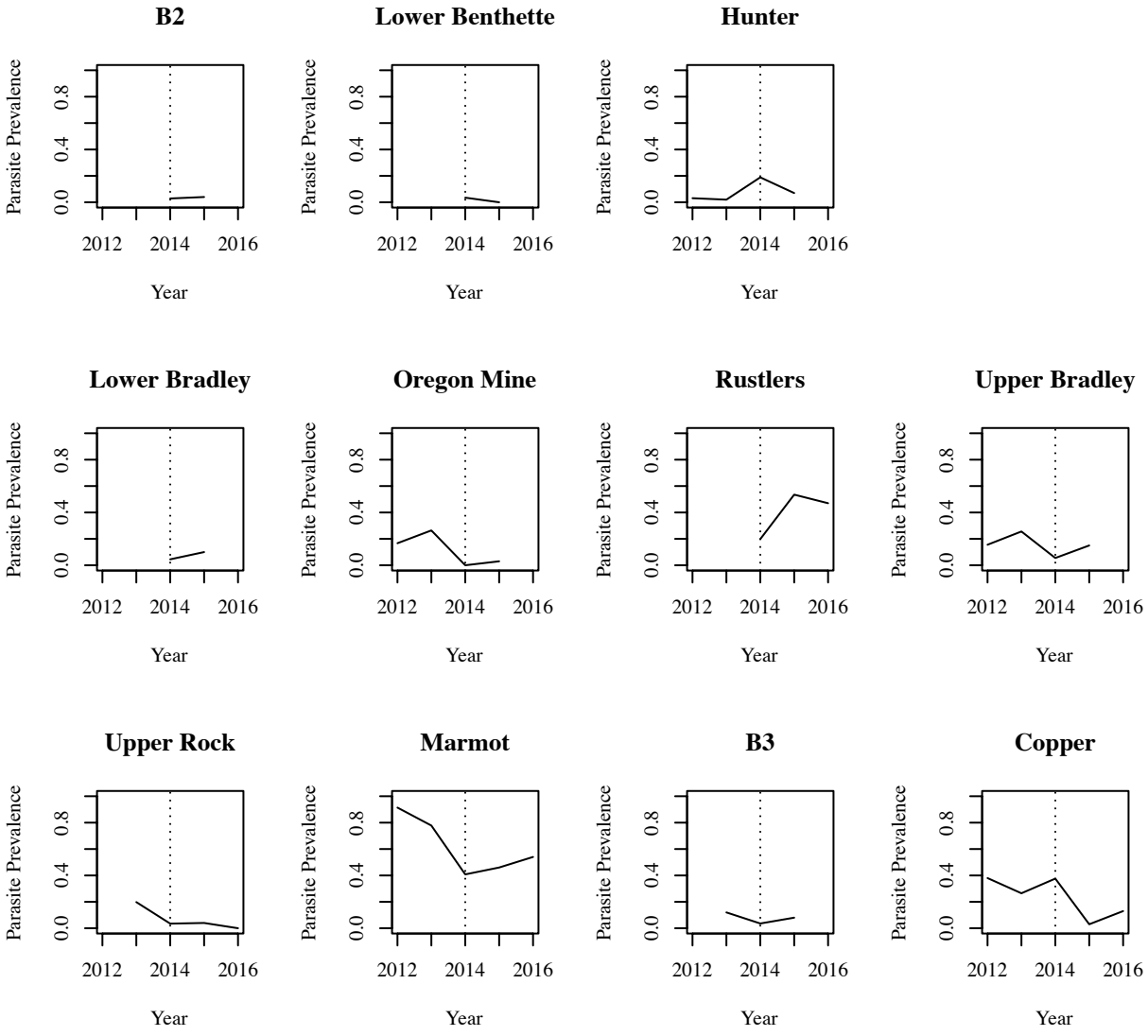
(A)



(B)


B2
Lower Benthette
Hunter
Lower Bradley
Oregon Mine
Rustlers
Upper Bradley
Upper Rock
Marmot
B3
Copper
Baetis Density
0
200
400
2012
2014
2016
Year

(C)


B2
Lower Benthette
Hunter
Lower Bradley
Oregon Mine
Rustlers
Upper Bradley
Upper Rock
Marmot
B3
Copper
Parasite Prevalence
0.0
0.4
0.8
2012
2014
2016
Year

(D)

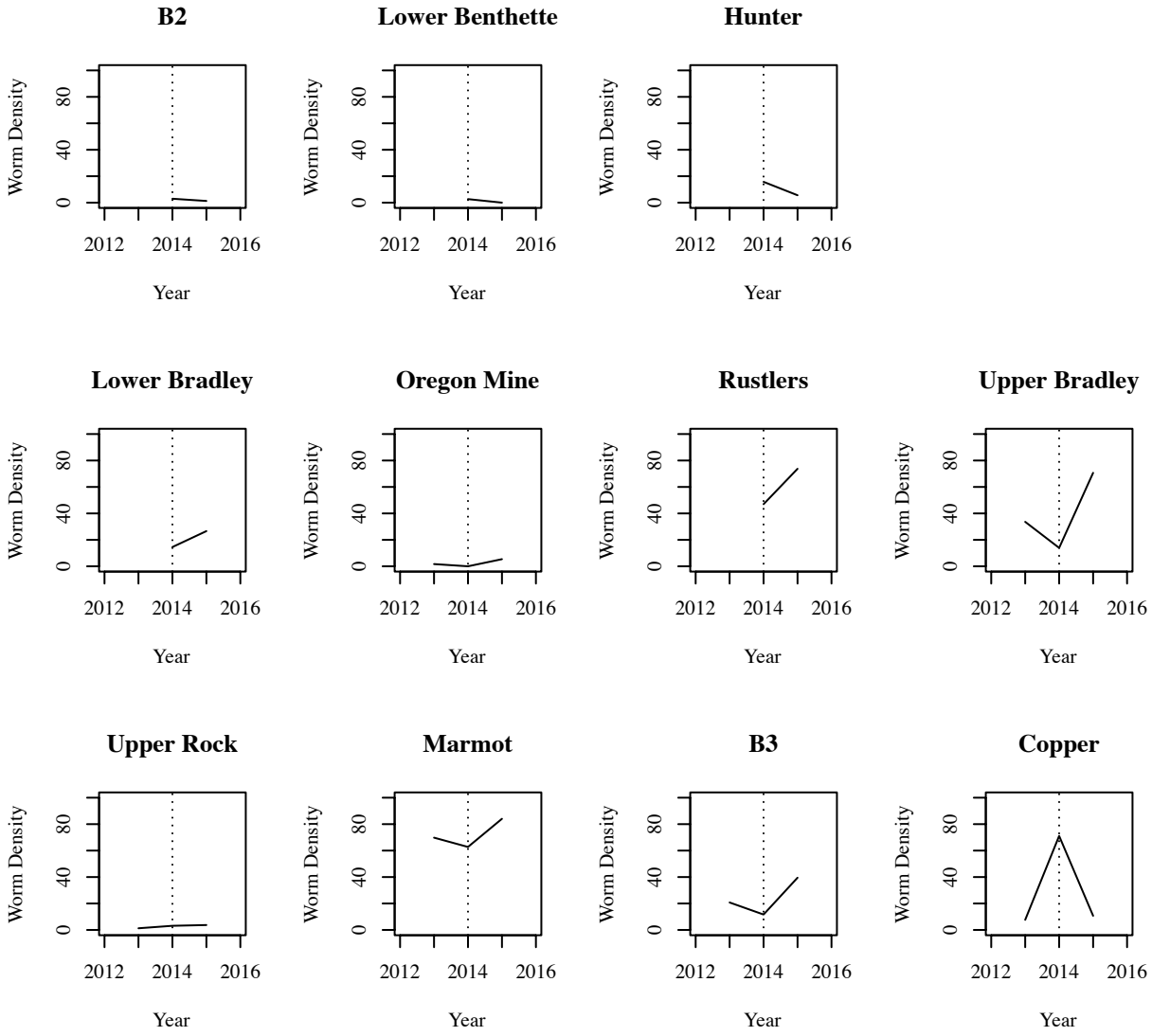
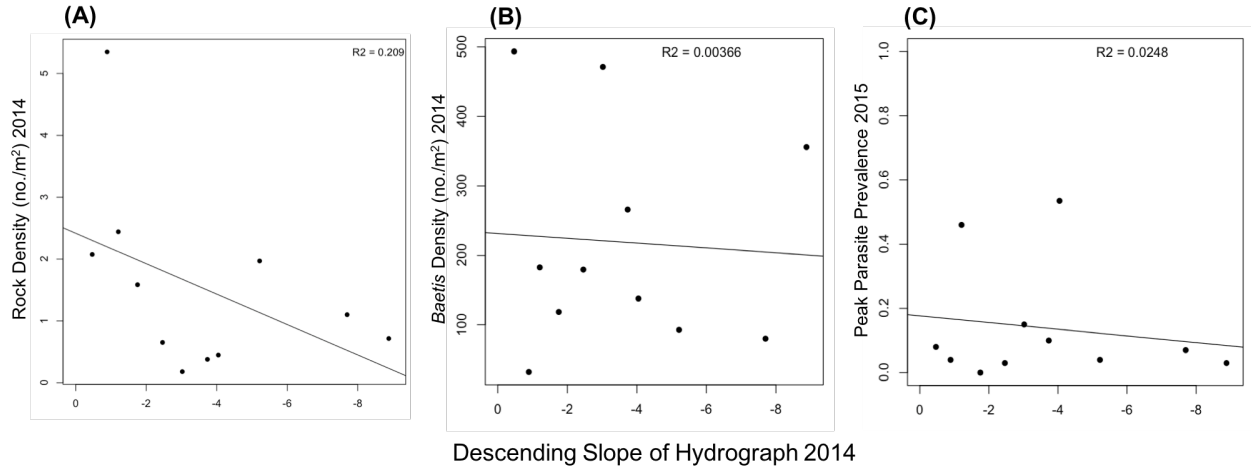
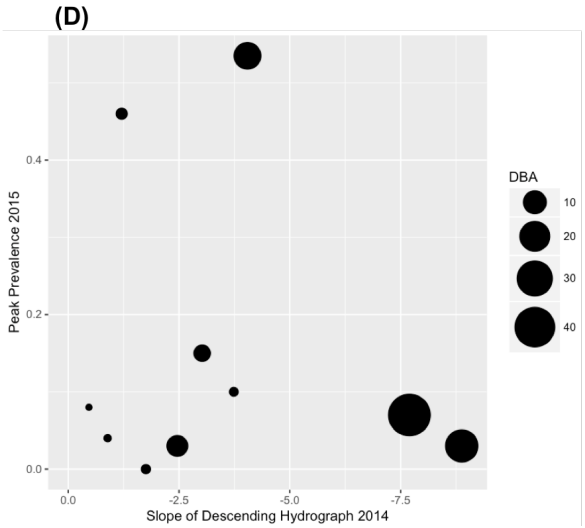

B2
Lower Benthette
Hunter
Lower Bradley
Oregon Mine
Rustlers
Upper Bradley
Upper Rock
Marmot
B3
Copper
Worm Density
0
40
80
2012
2014
2016
Year

Figure A6. These figures display the relationship of the descending slope of the hydrograph (calculated from mean daily stage measured by TruTrack data loggers installed in each site through summer 2014) and the following responses measured in 2015: (A) density of protruding rocks (no./m^2), (B) *Baetis bicaudatus* larvae density (no./m^2), and (C) peak prevalence of *Gasteromermis* sp. parasites (proportion hosts infected). To further examine the prevalence × hydrograph relationship (D) drainage basin area (km^2) and (E) stream gradient (m/km) were added as covariates.





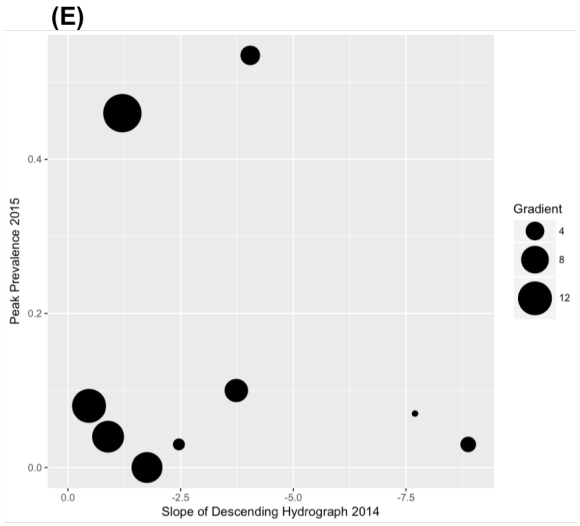


Figure A7. Because the free-living stage of the worm resides in the benthic substrate near the rock where it was originally deposited by the host, we measured between-stream differences in benthic habitat at a fine scale that could relate to parasite dynamics. In summer 2012 we selected 5 rocks in each site that met the criteria for high quality *Baetis* oviposition habitat (detailed in Encalada and Peckarsky 2006), which we predicted would also be frequent sites for parasite dispersal by *Baetis*. At each focal rock we recorded rock composition, rock size dimensions, height it protruded above the water line, water depth around the rock, splash level on a scale from 0-3 (*sensu* Encalada and Peckarsky 2006), flow velocity at four points around the rock perimeter, percent coverage and height of mats of the nuisance diatom *Didymosphenia geminata* on the rock surface (*Didymo* was abundant in some sites but absent from others), and the proportion of sand, gravel, cobble, or *Didymo*-covered substrates within a 0.49-m^2 quadrat centered on the focal rock. We then used Principle Component Analysis (PCA) to describe those microhabitat traits. To determine whether the PCs derived from this analysis enabled us to relate rocks traits with parasitism, we first categorized survey sites by parasite prevalence into low (range), intermediate (range), and high (range) prevalence categories, and then plotted those sites on the 2-dimensional space of PC2 vs. PC1. We extracted two PCs that together explained 47.1% of variance in the dataset. PC1 explained 32.1% variance and was influenced by measurements of the focal rock itself (rock dimensions and water depth around rock), whereas PC2 explained 15% of the variance and was primarily influenced by substrate composition surrounding the rock (e.g., percent cover boulder, cobble, or gravel, percent *Didymo* cover on surrounding substrate) and by the presence of *Baetis* egg masses on the rock. High versus low parasite prevalence sites discriminated along PC1, indicating that low prevalence sites are characterized by large oviposition substrates in deep water, in contrast to high prevalence sites where they tended to be smaller and located in shallower water. There was no separation of sites along PC2, suggesting the relative unimportance of microhabitat surrounding the oviposition substrate compared to inherent traits of the rock itself.

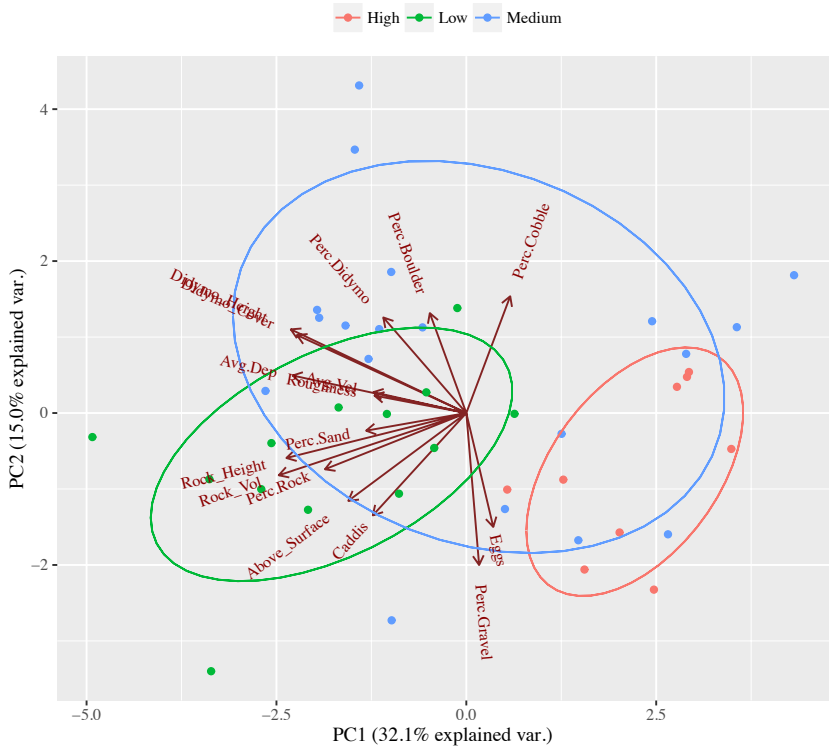
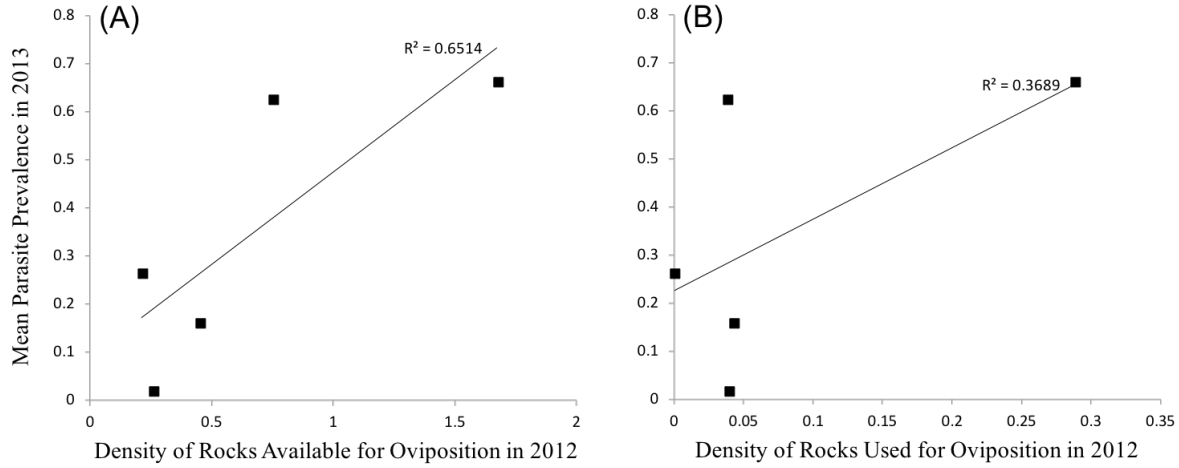


Figure A8. (A-B) Surveys in 2012 provided evidence that parasitized and unparasitized adult *Baetis* showed different preferences for oviposition rock characteristics, with parasitized adults being less selective of traits that unparasitized adult females prefer (e.g., swift flow/high splash). To test this pattern in 2013 we placed 12 pairs of rocks, standardized by size and composition but located in contrasting flow environments (deep/splashy & shallow/not splashy), in the East River near BLPs weatherport at RMBL (Gothic, CO). We coated the protruding dry surfaces of all rocks with Tanglefoot® to trap landing adults, and removed trapped individuals approximately every 2 days for 2 weeks. *Baetis* were preserved in EtOH and returned to the lab for dissection. A total of 133 *Baetis* were captured, but rather than capturing adults as anticipated, 125 (=94%) trapped *Baetis* were newly emerged subimagoes. (C) In total, more subimagos were captured on rocks in slow flow (paired *t*-test, $p = 0.066$) but the proportion of parasitized subimagos was higher on rocks in high flow (paired *t*-test, $p = 0.060$). We do not have a contemporaneous measure of prevalence in this site, but a higher proportion of parasitized *Baetis* were captured on sticky rocks than expected based on prevalence at other timepoints. These patterns could occur if infected individuals have physical changes that cause them to become entrained in swift flow and use rocks to aid their emergence, rather than emerging directly from the water surface.



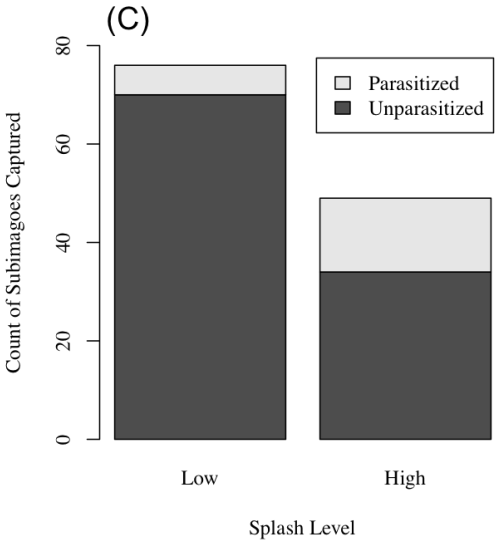


Figure A9. *Didymosphenia geminata* is a single-celled freshwater diatom (A) that can "bloom" by altering its growth form to produce thick, stringy epilithic mats (B). *Didymo* blooms have recently reached nuisance levels in many lotic systems, including oligotrophic streams in the western US (C). Although causes of *Didymo* proliferation are unclear, its consequences include changing composition of non-nuisance algae, decreasing diversity and changing the structure of benthic invertebrate communities. Unlike other mayflies, larval *Baetis* populations have not declined with *Didymo* proliferation, possibly because their mobility and mouthparts enable them to forage on epiphytic diatoms growing on the stalks. However, potential effects of *Didymo* on aerial stages of insects that use substrates (e.g., for oviposition or parasite dispersal) have not been examined. Because female *Baetis* oviposit under rocks, which they select based on key physical characterists (Encalada and Peckarsky 2006), an overgrowth of epilithic diatom mats could affect *Baetis* oviposition and also alter disease dynamics if oviposition rocks are focal areas of parasite dispersal and host-parasite encounters.

We designed a pilot experiment to test for effects of *Didymo* on adult *Baetis* oviposition. We paired suitable oviposition rocks (standardized for size, composition, and surrounding flow conditions) in the East River near BLPs weatherport at RMBL (Gothic, CO) and measured the number of egg masses accumulating on them during peak oviposition (1-20 August 2012). We discovered a tendency for more egg masses to accumulate on rocks with Didymo (paired *t*-test, $p = 0.095$; panel D).

This could occur if more females choose rocks with *Didymo*, or if *Didymo* increases successful oviposition for females that choose those rocks, or both. We suspect that *Didymo* is more important for facilitating survival than attracting females, because they can cling to the structure of the *Didymo* mat and possibly avoid being swept from rocks before laying eggs, which is a high risk of the *Baetis* oviposition strategy (Encalada and Peckarsky 2007). If parasites are dispersed by oviposition behavior of infected mayflies, *Didymo* could influence parasite dispersal by the same mechanisms, potentially resulting in different locations or levels of epidemics.

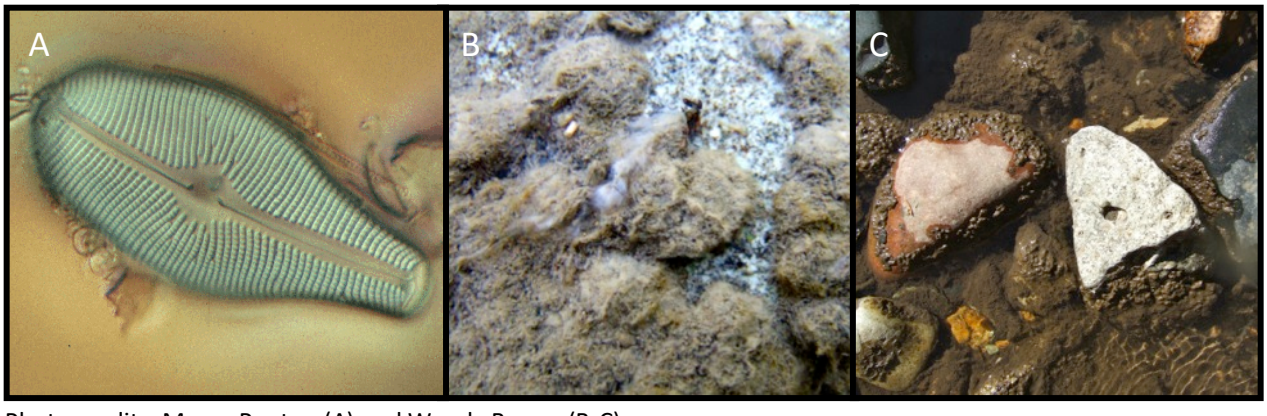
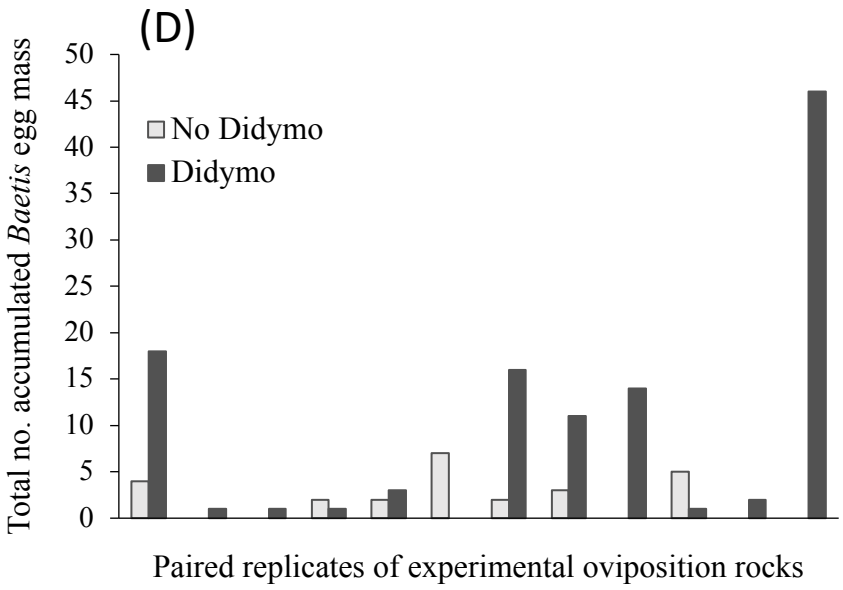


Photo credits: Marge Penton (A) and Wendy Brown (B-C)


(D)
Total no. accumulated *Baetis* egg mass
50
45
40
35
30
25
20
15
10
5
0
No Didymo
Didymo
Paired replicates of experimental oviposition rocks

CHAPTER 2 – The interaction of temperature with parasite phenology, infection prevalence, and growth in a high-altitude stream network

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ABSTRACT

Temperature can affect disease dynamics through multiple pathways, including indirect effects on infectious encounters mediated by host and parasite phenology, and direct effects on organismal biology that influence the outcomes of those encounters. This study explores the relationships among natural variation in water temperature, host-parasite phenology, parasite prevalence, and parasite growth in a high-elevation temperate stream network. In a five-year survey of *Baetis bicaudatus* mayflies infected by *Gasteromermis* sp. mermithid nematodes, we found both temporal and spatial variation in infection phenology associated with water temperatures. While infection prevalence was often higher in warmer years within streams, such inter-annual variation was much smaller than spatial variation of parasite prevalence among streams within years. Across the stream network, warmer stream temperatures, which accelerated host development, were correlated with lower proportions of susceptible hosts (early instar mayfly larvae) present on the date when parasite infections began. Subsequently, those sites with faster developing host populations had lower parasite prevalence over the summer. This observation is consistent with the hypothesis that temperature has differential effects on host and parasite phenology, desynchronizing the temporal "match" of infective parasites with susceptible

hosts in warmer sites. Temperature had a unimodal relationship with parasite growth within the host, such that growth rates were highest at intermediate temperatures. However, parasites in mature larval hosts that are nearing metamorphosis achieved the smallest size in the warmest site. Together, extensive and intensive spatiotemporal observations of host-parasite phenology and performance suggest that warmer temperatures could negatively affect parasitism not only by phenological mismatch due to accelerated host development, but also by declines in parasite production. Controlled experiments are needed to disentangle the effects of temperature from coexisting environmental variables to test hypotheses for effects of temperature on parasitism via multiple pathways.

Keywords: parasitism, temperature, climate change, growth, phenology, host-parasite interactions, phenological mismatch, mermithid

Coauthor: Barbara L. Peckarsky[1,2]

INTRODUCTION

Understanding mechanisms that drive the emergence of disease hotspots has gained urgency as the environment changes along many biotic and abiotic gradients (Wilson 2001, Dobson 2009, Lafferty 2009). As evidence mounts that disease can shape host populations and communities with knock-on effects for ecosystem function (Thomas et al. 2005, Hatcher and Dunn 2011), predicting spatial heterogeneity of disease transmission is a critical task for ecologists and managers (Patz et al. 2004). If parasite transmission depends on environmental context, environmental changes could remap the spatiotemporal mosaic of disease by shifting gradients of habitat quality that influence parasite transmission, production, or survival (Patz et

al. 2000, Hall 2010, Rohr et al. 2011). Understanding the characteristics of infectious patches is a critical first step is building a predictive framework for disease, which can ultimately aid management and conservation strategies (Paull 2012).

Temperature is a key aspect of the environmental mosaic affecting host-parasite interactions, and understanding its effects on disease is compelling in the context of climate change (Marcogliese 2001, Lafferty 2009a, Ostfeld 2009). Evidence suggests that temperature can either amplify or inhibit infections; therefore the net effect of climate change on parasitism has been the subject of ongoing debate (Dobson 2009, Lafferty 2009b, Altizer et al. 2013). Despite a growing body of research on many aspects of parasite ecology, we have very limited ability to predict the dominant effects of warming on parasite transmission and disease distribution.

The effects of temperature on parasitism can occur via direct influences on organismal physiology and indirect influences on interactions among species that lead to infectious encounters (Lafferty 2009b, Rohr et al. 2011, Paull et al. 2012b). One mechanism by which temperature affects physiology and, consequently, biotic interactions, is by regulating the phenology of organisms (Stenseth and Mysterud 2002, Winder and Schindler 2004). Differential responses to temperature can alter interactions between species, including plants and pollinators, predators and prey, and parasites and hosts (Stenseth and Mysterud 2002, Paull and Johnson 2011). Especially in cases where both hosts and parasites are ectothermic, there is the potential for asymmetrical effects of temperature to result in mismatched phenologies with negative consequences for parasite transmission and host disease risk (Paull and Johnson 2014, Gethings et al. 2015).

Temperature also controls many vital processes of ectotherms that can directly affect infection outcomes (Atkinson 1994), including parasite growth and reproduction within the host (Paily and Balaraman 1994, Poulin 2006, Macnab and Barber 2011, Paull et al. 2015). Temperature shifts can cause positive or negative effects on parasite production, depending on where they fall relative to optimal thermal ranges (Paily and Balaraman 1994, Poulin 2006, Paull and Johnson 2011). Under favorable conditions some parasites, such as parasitic castrators, can extend the longevity of their hosts, thereby prolonging their opportunity to consume host resources and allocate energy to growth and reproductive output (Hall et al. 2007, Lafferty and Kuris 2009). Hosts under thermal stress, on the other hand, can have decreased viability with negative consequences for its parasites (Barber et al. 2016). Overall, the net effect of climate change on parasites and disease outcomes will reflect both direct and indirect impacts of temperature across the entire parasite lifecycle, including free-living stages, infective stages, and parasitic stages within the host. Therefore, a thorough understanding of potential climate change impacts requires integration of multiple pathways that are open to temperature effects.

Many parasites of medical and conservation significance occur in freshwater environments (Johnson and Paull 2011), where temperature can have strong effects on organisms and ecological processes (Sweeney and Vannote 1978, Brittain 1983). Aquatic habitats are naturally heterogeneous and disturbance-prone systems at the nexus of climate change and other alterations that face human-dominated landscapes (Johnson et al. 2008, 2010, Johnson and Paull 2011, Okamura and Feist 2011). Therefore, investigating temperature effects on disease processes in aquatic environments could also provide useful models of responses of parasitism to dynamic and changing ecosystems (Okamura and Feist 2011).

To explore temperature effects on parasitism we surveyed summer temperature, host and parasite phenology, and parasite growth across a temperature gradient in streams of a temperate high elevation watershed over five consecutive years. We focused on parasitism of the stream-dwelling mayfly *Baetis bicaudatus* (Ephemeroptera: Baetidae) by the castrating endoparasitic helminth *Gasteromermis* sp. (Nematoda: Mermithidae). We have observed that parasite prevalence (% of host population infected) varies from 2-80% among streams within a catchment but remains relatively stable over time and does not increase with host density (Chapter 1). Those findings suggest that environmental attributes within sites, such as temperature, could drive infection hot spots.

Baetis mayflies and mermithid parasites develop faster at warmer temperatures (Thornton and Brust 1979, Paily and Balaraman 1994, Harper and Peckarsky 2006) but the lifecycles of these species are asynchronous, with parasites hatching from eggs months later than their hosts (Chapter 1). Because mermithids preferentially infect small, early-instar hosts (Vance and Peckarsky 1996, Camino and Reboredo 2000), direct effects of temperature on host and parasite phenology could increase or decrease temporal overlap of infective parasites and susceptible hosts causing indirect effects on infection prevalence. In addition to affecting transmission, temperature could also influence post-recruitment dynamics such as parasite growth within the host via metabolic effects that impact the consumption of its host (Atkinson 1994).

Based on this conceptual framework, our objectives were: 1) to evaluate relationships among temperature, host phenology and parasite prevalence, and 2) to examine effects of natural differences in stream temperature on parasite growth. To accomplish those objectives we conducted extensive (over five summers) and intensive (across multiple streams within years) surveys of spatiotemporal patterns of temperature and parasite prevalence. We tested for effects

of temperature on host development and the stage-specific timing and prevalence of infections and the effects of temperature on host-parasite growth in natural streams (within 1 year). We predicted that warmer temperature, which accelerates development of mayflies, would 1) decrease the overlap of susceptible (early instar) hosts with infective parasites, consequently reducing infection prevalence, and 2) shorten the parasitic phase of *Gasteromermis* due to accelerated growth of the host, thereby decreasing growth opportunity and resulting in smaller parasites at warmer temperatures.

METHODS

Study System

We conducted this research in streams of the upper East River catchment, which drains an area of 45-km^2 near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA. These rocky-bottom streams are located at ≈2,900 m and runoff is generated primarily by snowmelt, although some tributaries are spring-fed. Streams throughout the drainage have comparable water chemistry, but vary in size and physical characteristics (Peckarsky et al. 2001, 2002, Wilcox et al. 2008). For decades ecologists have investigated this high elevation watershed which, like other intensively studied adjacent ecosystems (e.g., Billick and Price 2011), has the potential to serve as a touchstone for pressing questions regarding rapid environmental changes that are occurring (Inouye et al. 2000) and forecast for the region (Painter et al. 2010).

This research focuses on parasitism in *Baetis bicaudatus*, a mayfly whose larvae are abundant in high elevation streams of the western USA. *Baetis* is the most efficient grazer of attached algae within streams of the region (Alvarez and Peckarsky 2005) and is preferred prey to predatory invertebrates and salmonids (Allan 1983, Peckarsky and Penton 1989). *Baetis* is

infected by *Gasteromermis* sp. mermithids (Poinar 1991), which parasitize and castrate early instar hosts. Parasitism causes *Baetis* to consume less algae per capita (Chapter 3) and induces at suite of physical and behavioral changes that decrease consumption of *Baetis* by trout (Chapter 4). Because *Baetis* is a foundation species—both highly abundant and influencing ecosystems disproportionate to its biomass—responses of *Baetis* to parasitism and other interacting stressors could exert higher-order effects (Ellison et al. 2005).

Surveys of Temperature, Phenology and Parasite Prevalence

To assess thermal gradients throughout the drainage, we continuously logged temperature in 30 m study reaches during summers 2012-2016 with Onset HOBO data loggers (Onset Computer, Pocasset, MA, USA). We monitored a total of 14 sites (map of sites presented in Chapter 1), although not all sites were surveyed in every year, resulting in 44 site-year combinations in which temperature logging was paired with surveys of host-parasite dynamics. In the first year we surveyed host populations for temporal resolution within one life cycle, capturing host phenological changes and the onset and progression of parasite infections within sites by making weekly visits to seven sites for 10 w (14 June - 17 August 2012). In four sites we were able to track the entire parasitic phase of *Gasteromermis*, from the date of first infection to the date when all hosts metamorphosed and emerged from streams, which is typically later for parasitized *Baetis* (Vance and Peckarsky 1996). This protocol enabled us to evaluate the impact of temperature not only on the start and progress of infections, but also on the time lag between uninfected and infected host development. In subsequent years (2013-2016) we added sites to increase spatial resolution but decreased frequency, visiting each site every 2-3 weeks for ±7 weeks per summer. At each survey we collected ≈100 *Baetis* by kick sample from the benthos,

preserved them in 95% EtOH, and returned them to the lab. Using a dissecting microscope (20-100× magnification) we assigned individual *Baetis* to developmental stages I-IV based on wing pad development (Delucchi and Peckarsky 1989), and dissected them to estimate parasite prevalence as (no. infected *Baetis* / total no. of *Baetis*).

In Situ Growth Estimates

To determine if temperature affected parasite growth, which is an important determinant of fecundity (Poulin 1996) we quantified host and parasite mass from a subset of survey samples. We collected *Baetis* samples (as above) two weeks apart in seven sites in 2014. We enumerated and dissected individuals, separated hosts from parasites, dried all organisms at 60 °C for 24 h then weighed each to estimate dry mass (DM) of parasite and host tissue, and the ratio of parasite:host DM for infected *Baetis*, which is an indicator of how energy is allocated between parasite and host. We calculated growth rates of parasites as mean final DM of individuals (collected on day 14) dissected from hosts of each developmental stage (I-IV) minus mean initial DM of individuals (collected on day 1) in the previous developmental stage. This would have excluded analysis of any stage IV *Baetis* collected in the initial sample and stage I *Baetis* collected in the final sample, but due to naturally-occurring developmental stages at our sampling dates, those did not occur in our samples. This method produced host stage-specific growth estimates of parasites between stages I-II, II-III and III-IV; measuring parasite growth between discrete stages is valuable because mermithids grow nonlinearly and often accelerate growth near the end of their parasitic phase (i.e., host stage IV; Poinar 1983).

Analysis

We analyzed the effect of temperature on parasite prevalence with a linear mixed-effect model testing for linear and quadratic effects of mean summer temperature, with site and year as a random (blocking) variables. We calculated Pearson correlation coefficients to examine the prevalence-temperature relationship over years within sites. To examine effects of temperature on development rate of hosts and parasites we analyzed correlations between temperature and Julian date of key phenological transitions including first appearance of parasitic infections, first appearance of late-stage *Baetis* instars (i.e., stage III and IV), and date of *Baetis* flight initiation. Because we were also interested in indirect effects of temperature on infection dynamics mediated by host phenology, we calculated the proportion of susceptible hosts (i.e., stage I & II *Baetis*) in each stream on the date when parasite infections were first detected. We fit an exponential function to describe the relationship between that proportion and average parasite prevalence in the same site. We examined temperature effects on parasite size variables using mixed-models with fixed effect of host stage, linear and quadratic effects of temperature and random effect of site. We fit separate models for response variables of parasite size, parasite:host biomass ratio, and parasite growth rate.

RESULTS

Surveys of Temperature, Phenology and Parasite Prevalence

Mean summer temperature varied across study sites and years, with ≈5.5°C difference in mean temperature between the warmest and coldest sites in each year (Fig. 1). The warmest year of the survey was 2012, when the mean temperature reached 13°C in the warmest site (East River at Hunter riffle).

When data for all site-year combinations were analyzed in one model the relationship between mean summer temperature and parasite prevalence was unimodal with the highest prevalence values measured at intermediate temperatures (≈7-10°C; $X^2 = 8.74$, $p = 0.003$; Fig. 2A). Prevalence vs. temperature correlations within sites showed that, of the 12 sites where we had sequential years of data, 9 sites had a positive correlation between temperature and prevalence (Fig. 2A). Two sites had a negative correlation, one of which was the warmest site in every year (East River at Hunter riffle).

In first year of the survey (2012, which was the warmest of the five years) we tracked host phenology and parasite infection dynamics weekly, observing that in warmer streams *Baetis* matured faster ($r^2 = 0.11$, $p = 0.01$); but warmer temperatures did not advance the date of first parasitic infections ($r^2 = 0.27$, $p = 0.134$). Consequently, warmer streams had lower proportions of early instar (i.e., stages 1-2) hosts at the date when infections began ($r^2 = 0.92$, $p < 0.001$; Fig. 2B-D). Furthermore, host populations biased toward later developmental stages when infections began experienced lower mean infection prevalence over the larval period ($r^2 = 0.53$, $p = 0.038$; Fig. 3). Temperature also accelerated the flight initiation date for uninfected *Baetis* ($r^2 = 0.21$, $p = 0.004$), but had no effect on parasitized *Baetis* emergence date ($r^2 = 0.00$, $p = 0.90$).

In Situ Growth Estimates

We observed a positive effect of host developmental stage on parasite size across all temperatures ($X^2 = 34.9$, $p < 0.001$) but overall there was a nonlinear effect of temperature on parasite size such that the scope for growth within maturing hosts appeared to be highest at intermediate temperatures, whereas parasites reached smaller sizes at thermal extremes ($X^2 = 4.85$, $p = 0.028$; Fig. 4A, Table A1). Parasite growth rate increased as hosts matured, with the

highest growth rates measured in hosts developing from stage III to stage IV (the final stage before emergence; $X^2 = 5.36$, $p = 0.068$; Fig. 4C) but without temperature effects ($X^2 = 2.52$, $p = 0.11$; Fig. 4C). Parasite:host biomass ratio also varied across host stages. The highest biomass allocation to parasites occurred in the least developed (and smallest) hosts (mean ± SE stage I: .67 ± .27; stage II: .33 ± .12; stage III: .17 ± .02; stage IV: .33 ± .07; $X^2 = 15.4$, $p = 0.001$; Fig. 4B), and there was not a significant temperature effect ($X^2 = 1.01$, $p = 0.316$).

DISCUSSION

Temperature can affect multiple aspects of the development and interaction of hosts and parasites, potentially contributing to the distribution and emergence of infection hotspots (Poulin 2006, Lafferty 2009b, Altizer et al. 2013). Intensive field surveys within years in multiple streams showed that warmer stream temperatures, which accelerated mayfly development, were correlated with lower proportions of early instar mayflies present on the date when parasite infections first appeared, suggesting that warmer temperature shortens the temporal overlap of susceptible hosts with infective parasites (Fig. 5). Subsequently, those sites with faster host development had lower parasite prevalence over the summer. Temperature did not affect the duration of the parasitic phase (i.e., date of emergence of infected *Baetis*) but did have a nonlinear effect on parasite size, resulting in larger parasites at intermediate temperatures. Parasites in late instar hosts, which are nearing metamorphosis and cessation of feeding, had the smallest mean size at emergence in the warmest site, suggesting that warmer temperatures could alter parasite population dynamics via negative effects on fecundity.

We observed that warmer temperature accelerated host development, but not the date of first parasitic infection, suggesting that temperature has differential effects on host and parasite

phenology that can lead to mismatch of interacting stages (Fig. 5). Other studies have documented accelerated mayfly development at warmer stream temperatures and in experiments (Wright et al. 1982, Harper and Peckarsky 2006). Many parasites including nematodes also have temperature-dependent phenology (Paily and Balaraman 1994, Poulin 2006, Paull and Johnson 2011). Nevertheless, the production of infectious parasite stages could lag behind hosts if temperature accelerates host development to a faster rate than parasite development. Both temperature and temperature-independent constraints may prevent parasites from reaching infectivity earlier in warm sites. For example, while embryogenesis is temperature-dependent, hatching from eggs may be cued by chemical signals from hosts (Wang et al. 2012) or hydrologic cues (Micieli et al. 2012, Wang et al. 2012). Hydrology is an important source of variation in streams that also has the potential to influence parasite free-living stages. In hydrologically dynamic systems such as ours, flow conditions can change drastically from early-season peak to late-season base flow and vary across sites (Peckarsky et al. 2014). It is generally accepted that high flow velocities are often detrimental to parasites at the free-living stage, either through flushing parasites out of their habitats or by decreasing the likelihood that they can successfully infect the hosts they encounter (Marcogliese 2001, Pietrock and Marcogliese 2003). Therefore if flows are high when parasites have achieved a temperature threshold for their own development, their vulnerable preparasitic stages could suffer from those harsh conditions. In general, the net effect of temperature integrates responses of interacting species at all lifecycle stages, some of which will be influenced by other extrinsic or intrinsic factors (Winder and Schindler 2004, Berger et al. 2010).

The consequences of host-parasite interactions are strongly mediated by the developmental stages of the interactors (Johnson et al. 2011). Sites with faster maturing hosts experienced lower

parasite prevalence over the summer, which suggests that warmer temperatures decrease the overlap of infective parasites with the early instar hosts they preferentially infect. Consistent with this pattern, Pritchard and Zloty (1994) also found higher prevalence in mermithid parasites in *Ameletus* mayflies in the colder of two streams although phenological relationships were not assessed. A detailed experimental study involving multiple hosts and different parasite life stages also found that warmed water decoupled the phenology of infective trematode parasites with amphibian hosts, thereby mismatching interacting stages and apparently depressing the rate of infectious encounters (Paull and Johnson 2014). Our findings further illustrate how, in natural streams, constraints on synchronizing with host phenology can be costly for parasites.

Encountering and establishing in hosts is of paramount importance for parasites. Infective stages can be vulnerable and short-lived (mermithid preparasites may survive for only 2-3 days outside a host; Ebsary and Bennett 1973, Platzer 2007, Wang et al. 2012). When the window of opportunity to locate hosts is short, it would be adaptive for infective stages to be released when the highest density of susceptible hosts is available. Infective stages that emerge into an environment dominated by unsusceptible hosts will have lower rates of infectious encounters, and consequently lower recruitment. These trends in our system suggest that warming as a result of climate change could redistribute the pattern of highly infectious patches, potentially decreasing disease risk for hosts in streams at the warmer end of the temperature gradient.

The net effect of warming on parasitism merges the responses of all life stages and physiological processes to temperature, which introduces multiple opportunities for effects of different magnitude or directions to interact (Paull et al. 2012a). Such complexity is one reason generalizable predictions about the effects of climate change on disease remain elusive (Poulin 2006, Lafferty 2009b, Altizer et al. 2013). To evaluate our central question of why *Baetis*

parasitism remains high in some sites, we conducted a spatial comparison and found that the risk of parasitism is higher for hosts developing slowly in colder sites. However, when comparing prevalence over smaller temperature gradients that existed interannually within sites, we observed the opposite pattern: within most streams, parasite prevalence tended to increase in warmer years within the same sites. Although host phenology appears to dominate across-site differences there are other temperature-sensitive phenomena that could influence host-parasite encounters within sites. Below thermal stress thresholds, temperature can have positive effects on survival of reproductive adult parasites (Ebsary and Bennett 1973) and subsequent egg development (Ezenwa 1974), potentially increasing density of parasite propagules. Thermal optima have not been examined for *Gasteromermis*, but warming is known to have variable effects on mermithid infectivity (Kurihara 1976, Camino and Reboredo 2000). Therefore, temperature could directly affect the ability of parasites to infect hosts via changes to host detection capability, mobility, or longevity of the infective stage. Further, the immune response of insect hosts can vary with temperature (Triggs and Knell 2012, Murdock et al. 2012). Importantly, warmer years increased parasitism mainly in the colder sites but not in the warmest, suggesting that at the current range of variation the scope for positive physiological effects of temperature only exists in some sites.

Many attributes of parasite performance follow typical nonlinear response curves as a function of temperature, which could explain why prevalence continued to decline at higher temperatures in the warmest site. There is potential for negative effects on free-living stages by decreasing survival, longevity and infectivity at temperatures above thermal optima (Ebsary and Bennett 1973, Camino and Reboredo 2000). Following initial infections, Paull et al. (2012a) found that successful establishment of parasites in hosts was lower at the highest temperature in

experimental trials. At higher temperatures pathological effects of parasitism can intensify, leading to increased mortality (Paull and Johnson 2011, 2014). Such effects would manifest in lower parasite prevalence at the highest temperatures, which was the general spatial trend we observed, as well as the temporal trend we observed in the warmest site (Hunter). While we cannot disentangle direct and indirect effects of temperature from natural field patterns, our evidence is consistent with the hypothesis that direct effects on the biology of different life stages may also contribute to explaining variation in parasite prevalence, in addition to the effects of warmer temperature on phenological mismatch.

Furthermore, parasites can respond directly to temperature during both free-living and parasitic phases, illustrating the value of understanding temperature effects on each component of the lifecycle and the infection process (Paull et al. 2012a). We discovered that after infections are established, parasites can override subsequent effects of temperature on host development. Although warmer temperatures advanced the date of emergence to the winged adult stage of uninfected *Baetis*, there was no effect of temperature on the duration of the parasitic phase (date of emergence of infected *Baetis*). Even in warmer sites where uninfected hosts emerged fastest, parasitized *Baetis* were found in streams for at least 2 weeks after peak emergence of uninfected *Baetis*. Neither the length of this emergence lag nor the date of final emergence was correlated with temperature. Differing lifecycle requirements between host and parasite suggest reasons that synchronizing with the host at this stage of the lifecycle would not be its optimal strategy. While the timing of mayfly emergence is critical to its ability to locate high quality oviposition sites, which decrease in abundance as the summer progresses (Harper and Peckarsky 2006, Encalada and Peckarsky 2006), parasites have different oviposition requirements that should not have those seasonal constraints (Chapter 1). Instead, prolonging the parasitic phase is expected to

benefit this parasite by extending growth opportunities as it continues to consume the host (Hall et al. 2007). Parasitic nematodes that rely on hosts for all their nutrition experience selection to maximize growth within the host, which can favor prolonging the parasitic phase.

We observed that parasite size was a unimodal function of temperature, reaching higher rates at intermediate temperatures, which is a typical pattern of many performance response curves across parasite taxa (Poulin 2006, Macnab and Barber 2011, Paull et al. 2015). Negative size effects at higher temperatures could be due to direct effects of temperature stressing parasite physiology, or indirect effects on the parasite's ability to consume a host that is experiencing thermal stress. Pathological outcomes of infection can be exacerbated at higher temperatures, potentially decreasing viability of the host as an energy source (Paull and Johnson 2011). While other studies have reported shorter duration of the parasitic phase at higher temperatures leading to smaller parasites at warm temperatures (Craig and Webster 1982), we did not observed a shorter parasitic phase at warmer temperatures. Nevertheless, parasites in stage IV (mature) larvae, for which window of opportunity for growth is closing, had the smallest mean size at emergence in the warmest site. This pattern suggests that warming temperatures could alter parasite population dynamics via negative effects on fecundity in warm sites. Conversely, warming temperatures could benefit parasites in the coldest sites in this catchment, if they are below the thermal optimum for growth, potentially explaining increasing prevalence within the colder sites during warmer years (Fig. 2A).

The complexity of spatial and temporal variation in the relationships between environmental temperatures and parasite prevalence, and the nonlinearity of host and parasite responses to temperature make it difficult to predict disease patterns under climate change scenarios. Temperature effects on parasitism have often been evaluated under controlled

experimental conditions that highlight the discovery of optimal temperature thresholds (e.g., Paily and Balaraman 1994, Macnab and Barber 2011, Gehman et al. 2018). In this survey, we show that unimodal relationships of host disease risk (prevalence) and parasite performance (size) are measurable across the current natural range of temperature variation in one catchment, suggesting that even small temperature increases could have immediate negative or even positive effects on parasitism in wild populations, depending on the temperature regime of streams in question.

Conclusion, Implications and Future Directions

Temperature shifts associated with climate warming are often expected to favor parasites (Patz et al. 2003, Purse et al. 2005, Pounds et al. 2006), but ecologists have also emphasized that a variety of responses could result from warming, including negative effects on parasitism (Lafferty 2009b). Taken together, our results suggest that the dominant effect of warming will likely be to suppress parasitism in this system. At temperatures that are energetically stressful, negative effects on parasitism could be mediated not only by phenological mismatch due to accelerated host development, but also by declines in parasite production. Nonlinear responses appear to be common across the natural range of temperature variation, underscoring that the interval and magnitude of temperature shifts at individual sites will influence the net outcome of climate change on disease distribution.

The effect of temperature cannot be readily disentangled from coexisting environmental variables *in situ*, and controlled experiments could be used to resolve the mechanistic underpinnings of observed relationships between temperature and parasite prevalence and performance. By combining extensive (across sites and years) and intensive (within sites within

seasons) spatiotemporal observations of host-parasite phenology and performance we suggest intriguing hypotheses to explain the complex effects of temperature on parasitism via multiple pathways. These observations provide a foundation for discoveries that will contribute to our limited knowledge of what drives disease in freshwater networks, which are both highly vulnerable to anthropogenic change and crucial to human wellbeing.

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FIGURES

Figure 1. Mean water temperature logged in summers 2012-2016 in 14 sites in the East River drainage, CO, illustrating spatial (Y axis) and temporal (X axis) variation among streams and over years.

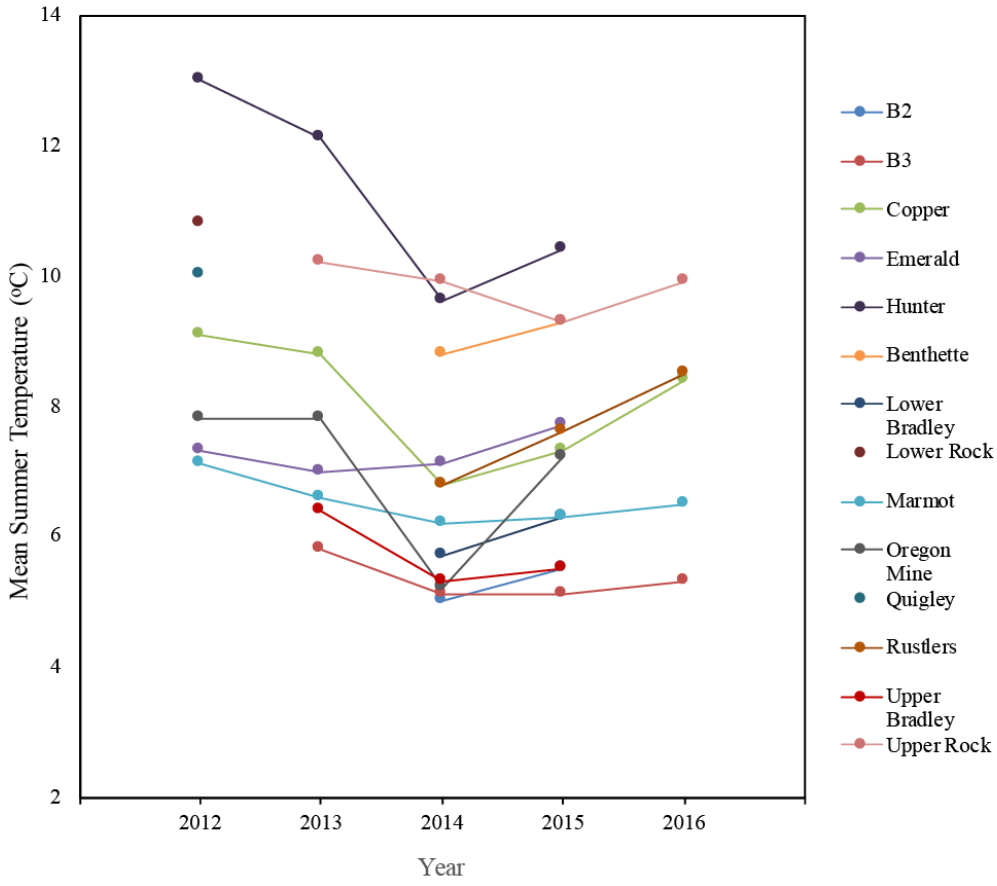


Figure 2. (A) Within 9 of 12 sites surveyed for ≥2 years, parasite prevalence in *Baetis* mayflies increased with mean summer water temperature (connected with lines). Across sites, prevalence peaked at intermediate temperatures and remained low at thermal extremes.
(**B, C, D**) Histograms show the distribution of hosts across the four stages of larval development on the date when parasite infections first appeared, from weekly assessments of host-parasite phenology in 2012, the warmest year of the survey (see Fig. 1) at three sites: **(B)** a cold temperature site with high prevalence (Marmot), **(C)** a cold temperature site with low prevalence (Oregon), and **(D)** a warm temperature site with low prevalence (Hunter). Black bars represent the frequency of parasitized *Baetis* larvae within each stage. Cold streams had higher proportions of susceptible hosts (i.e., stages I-II) on the date when infections began, and the highest prevalence was measured in stage I hosts (**B & C**).

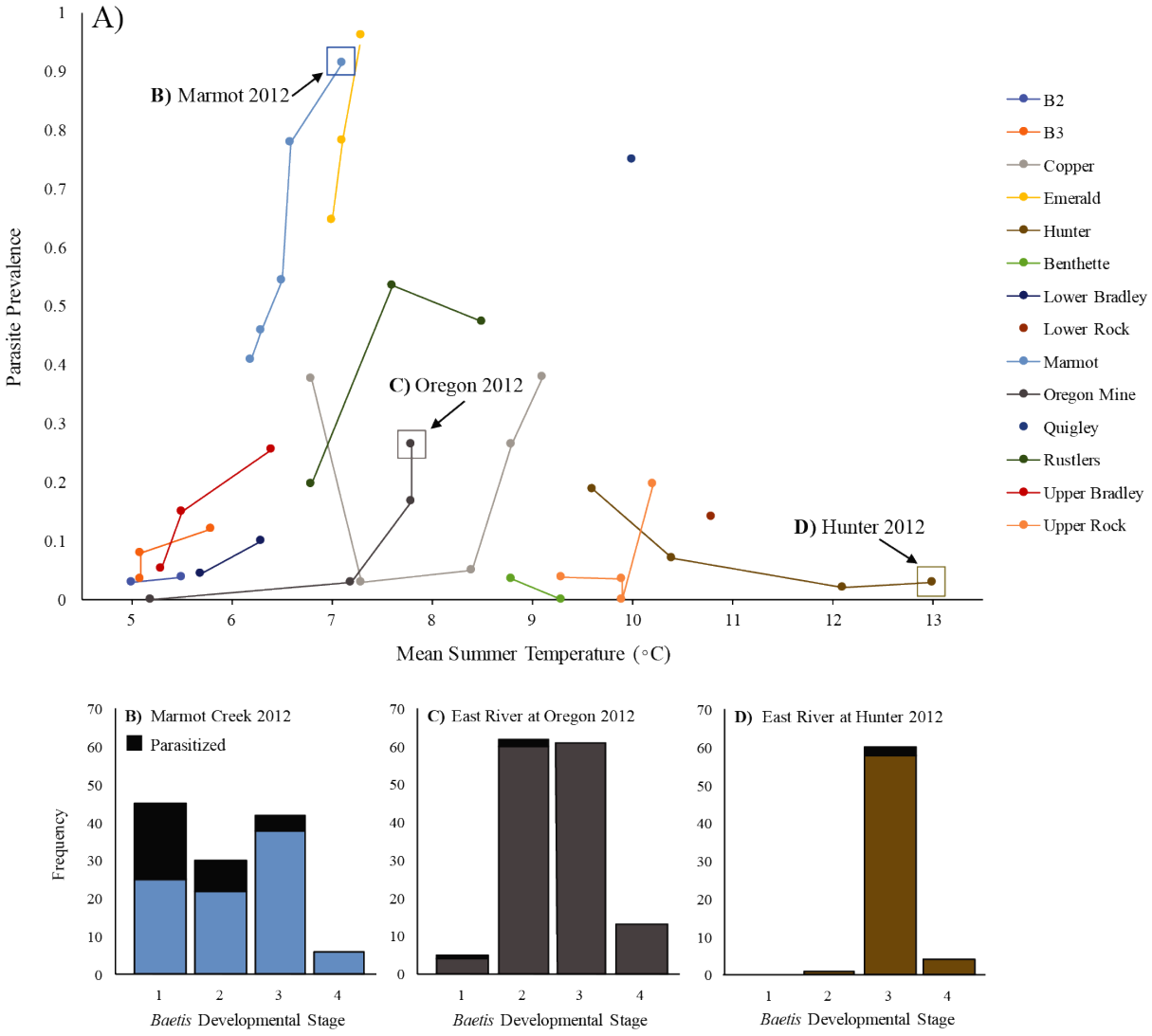


Figure 3. Warmer streams had lower proportions of susceptible hosts (i.e., stages I-II) on the date when infections began in summer 2012 (also see Fig. 2). Sites with faster developing host populations experienced lower mean infection prevalence over the season (p = 0.038).

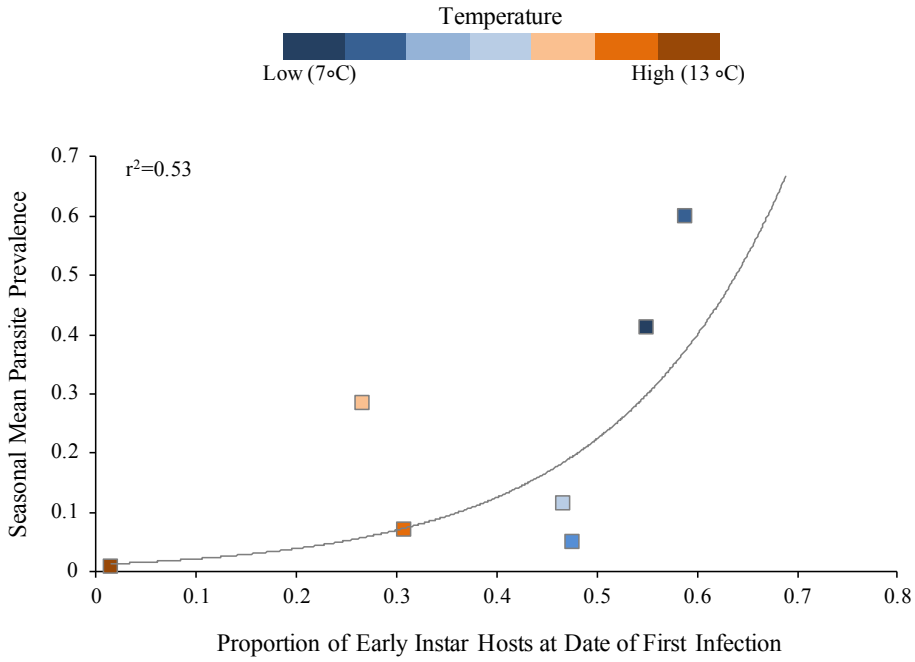
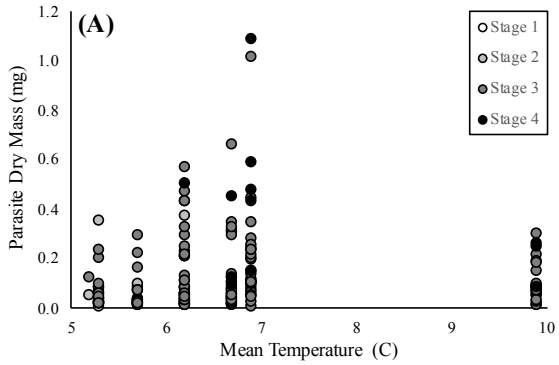
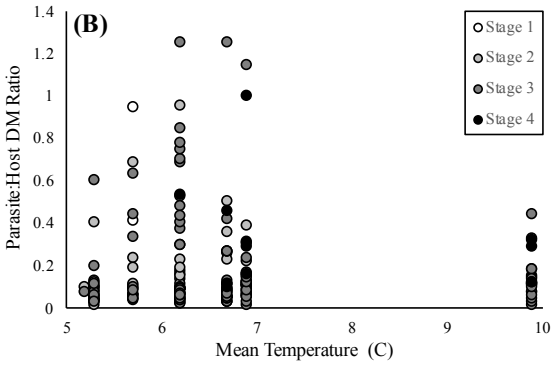


Figure 4. (A) Parasites tended to be larger in more developed hosts ($p < 0.001$), although small parasites occurred across all host stages and temperatures. There was an overall nonlinear effect of temperature such that the scope for growth was highest at intermediate temperatures and parasites reached smaller sizes at thermal extremes ($p = 0.028$). **(B)** Allocation of biomass to parasites relative to their hosts was highest in stage I hosts ($p = 0.001$) but did not vary with temperature after controlling for host stage ($p = 0.316$); **(C)** Parasite growth rate tended to increase as the host matured ($p = 0.068$), reaching the highest mean in hosts developing into the final larval stage (IV); **(D)** Photograph of late instar *Baetis* larvae ventrum showing how large the parasites can grow at the end of the larval life stage.





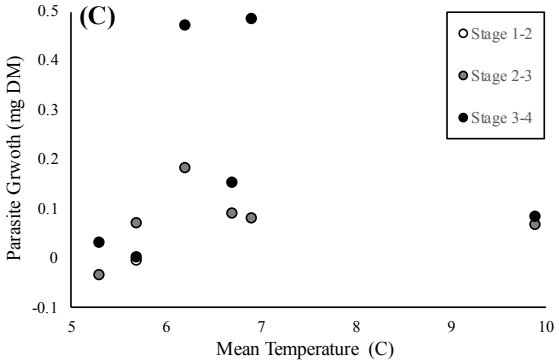
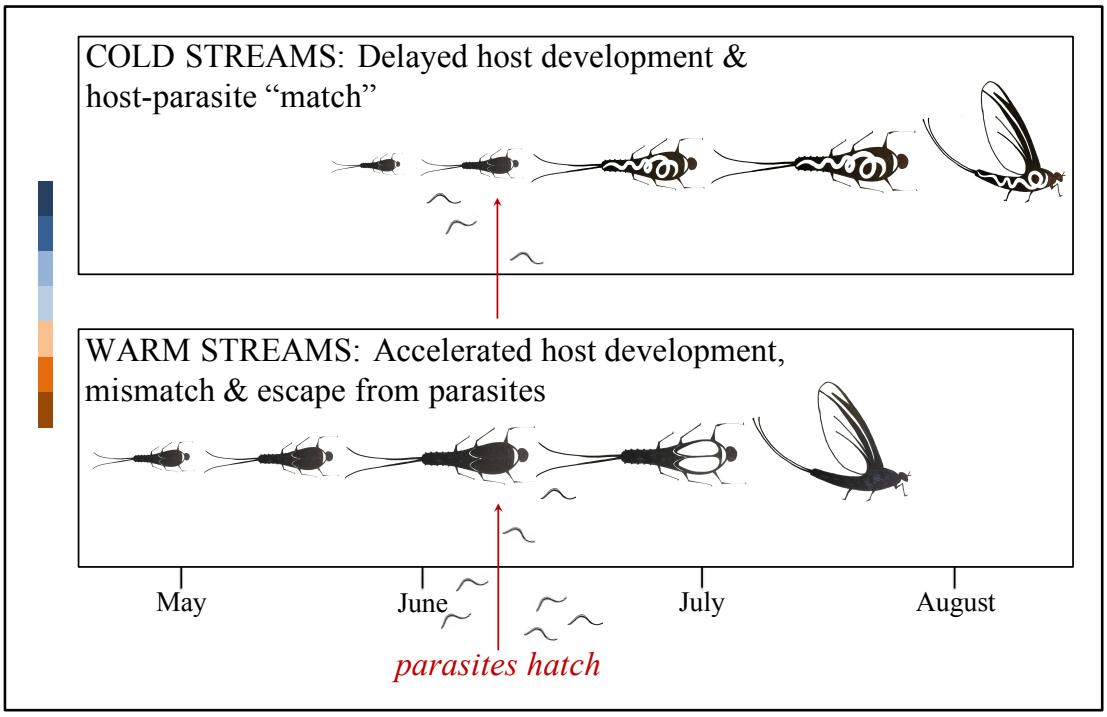


Figure 5. Conceptual model illustrating how temperature-related changes to host phenology could influence parasitism. Accelerated host development is expected to result in fewer infectious encounters due to temporal mismatch of parasites with susceptible hosts, subsequently reducing host infection risk (parasite prevalence) in warmer streams where hosts develop fast enough to "escape" the peak release of infective parasite stages.

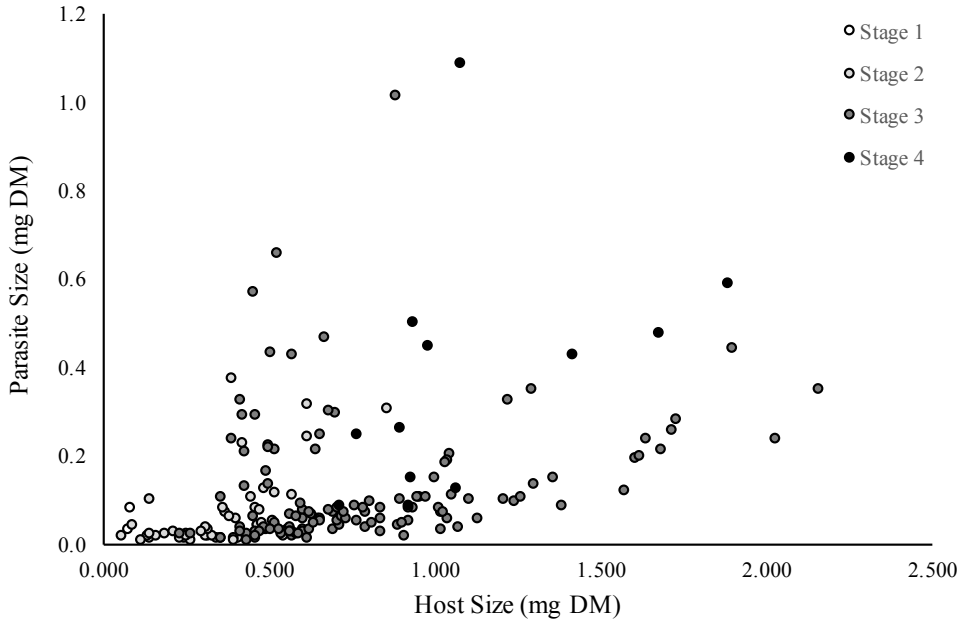


APPENDIX

Table A1. Summary of linear mixed effects models of parasite size (mg DM), parasite growth rate (Δmg DM day^{-1}), and parasite:host biomass ratio as a function of mean summer water temperature (°C) and developmental stage of the host (I-IV) and site included as a random effect.

	Parasite Size		Parasite Growth Rate		Parasite:Host Biomass	
Effect	X^2	*p*	X^2	*p*	X^2	*p*
Temp	4.83	0.028	2.42	0.120	.959	0.327
$Temp^2$	4.69	0.03	2.52	0.112	1.01	0.316
Host Stage	34.9	<0.001	5.36	0.068	15.4	0.001
Temp × Host Stage	.357	0.836	1.21	0.271	.342	0.843
$Temp^2$ × Host Stage	.300	0.861	1.34	0.248	.326	0.850

Figure A1. Relationship between host (*Baetis bicaudatus* mayfly larvae) and parasite (*Gasteromermis* sp. nematode) biomass (mg DM) measured across 7 sites in the upper East River drainage basin, CO in summer 2014.



2016 Warming Experiment Summary

Background. The objective of this experiment was to explore how water temperature alters aquatic host-parasite interactions by testing whether phenological shifts could mismatch the lifecycles of a host and its parasite—a mechanism that is believed to be important in the climate change context, although direct tests of it are uncommon. Because temperature cannot be readily disentangled from other environmental variables in nature, a controlled microcosm experiment was attempted to test the hypothesis that warmer water causes faster development of *Baetis* mayfly larvae, thereby decreasing the overlap of susceptible (early instar) hosts with infective parasites, and ultimately reducing parasite infections.

Methods. We designed a 2×2 factorial experiment in which unparasitized *Baetis* larvae from 2 age categories, stage II (early instar) and stage III (late instar), were reared in microcosms (6 individuals per microcosm; N = 12 microcosms per treatment combination) with stream water at ambient temperature (mean: 6°C, range: 4-9°C) or warmed by 5°C. This temperature regime represents the cold and warm ends of the thermal range of streams in the East River drainage. Microcosms were either supplied with gravel containing parasite propagules collected from the substrate of a stream with known high parasite density (Marmot Creek; methods following Poinar and Poinar 2008) or with boiled gravel (no propagules as control; 3 microcosms from each temperature × stage treatment combination were used as controls, resulting in fewer control than treatment tanks). This approach was intended to reduce parasite mortality, although we could not be confident that the same number of propagules were supplied to all microcosms, and a bias in number of propagules could confound treatment effects. We addressed this challenge by 1) randomly distributing gravel so differences in propagule number are not biased by treatment, and 2) stocking each microcosm with few enough hosts (~12) that parasites were likely to be host-limited.

As responses we measured the number of exuviae produced by molting *Baetis* (proxy for developmental rate), *Baetis* size and growth rate (mg dry mass) over the course of the experiment, and parasite prevalence at the end (after 14 days). The experiment was implemented in July 2016 in a system of 60 circular plexiglass flow-through microcosms housed in a streamside greenhouse and gravity-fed water from a cold fishless stream (detailed in Peckarsky and Cowan 1991). To establish temperature contrasts, natural stream water was warmed by an instantaneous water heater before being dripped through tubing into microcosms, where it continuously mixed with ambient water to maintain natural diel temperature fluctuations (detailed in Harper and Peckarsky 2006). In advance, algae was grown on tile substrates to standardize food for *Baetis* during the experiment.

Predictions. Based on predicted effects of temperature on host development and on our understanding of susceptibility (i.e., early instars are more susceptible, but late instars have periods of vulnerability due to molting) we expected the effect of water temperature to depend on host stage (predictions summarized in Fig. A2). Results contrary to those predictions would be open to other intriguing interpretations. For example, if colder temperatures promote infection in both host stages, then molting *per se* is not a significant source of vulnerability. In general molting can either increase or decrease vulnerability to parasitism however, based on the nature of this interaction, we expected molting to increase vulnerability because it temporarily strips

Baetis of its defensive exoskeleton, which is a barrier the parasite must actively penetrate, and because it temporarily immobilizes *Baetis*, extending exposure time to parasite encounters. If colder temperatures do not promote parasite infection in early instar hosts, then the starting condition of a host population (early or late instar) when first exposed to infective parasites is more important than its developmental rate.

Outcomes. The experiment could not be completed as originally conceived due to equipment failures and challenges capturing and keeping animals in captivity. Ultimately many of these issues were resolved and, although resolution did not come soon enough to capture the narrow window of the host-parasite lifecycle we intended to manipulate, the equipment and methodology developed during this project is a significant contribution to future research on stream host-parasite interactions. First, we were able to streamline and fine-tune a system to provide on-demand warming of natural stream water into flow-through microcosm tanks. This system, now available at the Rocky Mt Biological Laboratory (Gothic, CO), can be used for similar tests on the effects of warming on various aspects of *Baetis* or other macroinvertebrate ecology and host-parasite interactions. Second, for the first time we were able to harvest parasite propagules from streams and introduce infective stages of *Gasteromermis* parasites into microcosms with susceptible hosts. Infections occurred at very low rates, which suggests that these techniques need continued refinement. However, once this is achieved there is great potential to test for the effects of environmental gradients on infection probability, which remains a central question in this and other systems where disease is heterogeneously distributed across space. In general it is difficult to maintain temperature stream organisms in artificial settings due to their sensitivity to environmental conditions, including temperature; therefore it is a significant achievement to have created an experimental system in which animals survive and temperature can be controlled independently from co-occurring variables in the nature.

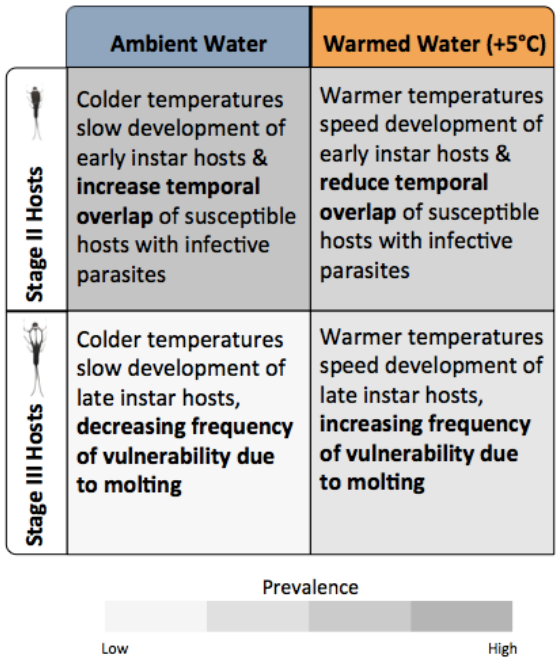


Figure A2. Predicted response of parasite prevalence to treatments in the proposed factorial experiment.

Parasitic infections (the response variable) occurred with very low frequency under all conditions (Table A2). Further, the warming treatment that was central to testing temperature-driven phenological effects on parasitism did not effectively raise mean water temperatures to the extent we desired. Rather, it increased variability in temperature and did so inconsistently. There are indications that the presence of parasites in ambient water conditions may have led to increased host mortality although the mechanism is unclear, which would be consistent with our initial predictions. However, we are treating this outcome conservatively and are unable to report statistical significance or make inferences about the role of temperature with confidence.

Table A2. Counts of *Baetis bicaudatus* larvae in experimental treatments or control tanks (no *Gasteromermis* parasite propagules introduced) that survived or were lost (dead + missing), and that were parasitized at the end of the 2016 warming experiment.

TOTAL NUMBER SURVIVED

TREATMENT				**CONTROL**			
	STAGE 2	STAGE 3	**TOTAL**		STAGE 2	STAGE 3	**TOTAL**
AMBIENT	38	62	**100**	AMBIENT	13	22	**35**
WARM	29	64	**93**	WARM	8	17	**25**
TOTAL	**67**	**126**	**193**		**21**	**39**	**60**

TOTAL NUMBER LOST

TREATMENT				**CONTROL**			
	STAGE 2	STAGE 3	**TOTAL**		STAGE 2	STAGE 3	**TOTAL**
AMBIENT	34	4	**38**	AMBIENT	5	2	**7**
WARM	43	8	**51**	WARM	10	1	**11**
TOTAL	**77**	**12**	**89**		**15**	**3**	**18**

TOTAL NUMBER PARASITIZED

TREATMENT				**CONTROL**			
	STAGE 2	STAGE 3	**TOTAL**		STAGE 2	STAGE 3	**TOTAL**
AMBIENT	8	1	**9**	AMBIENT	2	1	**3**
WARM	1	4	**5**	WARM	1	0	**1**
TOTAL	**9**	**5**	**14**		**3**	**1**	**4**

CHAPTER 3 – Endoparasites deplete host resources to avoid costs of predation risk on growth

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ABSTRACT

Endoparasites consume energy first acquired by their hosts, and therefore could be influenced by host foraging changes made in response to predation risk. Within the constraints of energy available from the host, it should benefit the parasite to sequester nutrition while leaving the host sufficient resources to remain viable and, in some cases, also to perform behaviors that increase parasite transmission. We examined relationships between host foraging and energy allocation to parasites in the context of predation risk, working in a system where mayfly grazers (*Baetis bicaudatus*) are subject to predation by trout and also infected by a castrating, endoparasitic nematode (*Gasteromermis* sp.) for which predation on the host is lethal. We used two experiments to test whether the threat of trout predation, provided by chemical cues 1) affected foraging behavior (grazing on substrate surfaces, and seeking food by drifting in the water column) and, 2) food consumption (algae suppression) of parasitized *Baetis* larvae, with subsequent effects on sequestration of resources by parasites. The first experiment also included a food level treatment (high or low) to explicitly test for responses to the tradeoff between predator avoidance and food acquisition. We found that at low food levels all mayflies increased foraging activity even with predator cues present. Across treatments parasites also tended to

increase foraging activity, albeit at safer (night) times. However, activity increases were not reflected in food consumption. Parasitized *Baetis* removed similar levels of algae per biomass grazer, and removed less per capita than uninfected *Baetis*. Ultimately the growth and size of the parasite, which correlate with fecundity, were not affected by predation risk. Instead, a net loss of host tissue occurred in parasitized *Baetis* exposed to predator cues, suggesting that risk caused the parasite to sequester more available resources, thereby decreasing its host's condition. Although predation risk did not exact a cost on parasite growth, there could be other predator-induced costs to the parasite consequent to depleting its host's energy reserves.

Keywords: herbivory, grazing behavior, resource allocation, growth, predation, parasitism, nonconsumptive effects, food webs, host-parasite interactions, mayflies, mermithids

Coauthor: Barbara L. Peckarsky[1,2]

INTRODUCTION

Organisms optimize fitness by balancing tradeoffs between avoiding risks that threaten their survival or fecundity and obtaining the resources they require for reproduction (Dill 1987, Lima and Dill 1990). For example, most animals cannot remain sheltered from predators while also obtaining food, which generally increases movement, exposure, and predator encounter rates (Sih 1980). In this context, animals can make decisions by integrating information on their own energetic states, quality of available resources, and the distribution of predation risk (Sih 1992). Although a substantial literature on this topic addresses free-living organisms, this framework has not been extended to consider how parasites interact with the mosaic of risks and resources their hosts experience.

Because endoparasites forage within the environment of the host, acquiring energy is a two-phase process. First the host must successfully navigate its external environment to obtain food. Second, the parasite within the host must appropriate nutrition to itself. Tradeoffs exist at both stages: as the host seeks food it risks exposure to predators, which can also be lethal to the parasite, and as the parasite "forages" within the host it must acquire the resources necessary to grow and ultimately achieve transmission, but not cause physiological stress that would weaken the host prematurely (the classic virulence-transmission trade-off; Anderson and May 1982).

The context in which the host forages, including its responses to food and risks, are the first filters of the energy ultimately available to its parasites. For organisms that decrease activity when risk is high, choosing safety can result in fitness decreases due to lost foraging and growth opportunities (Preisser and Bolnick 2008). Although this behavior should decrease total energy available for allocation to the parasite, it is unclear whether parasites pay a fecundity cost for safety. If there is a penalty, then manipulative parasites (which can modify host traits; Poulin 1995, 2010) may have incentive to affect the foraging behaviors of their host when it benefits them to avoid predation, in contrast to trophically-transmitted parasites that famously manipulate their hosts to increase predation as a transmission pathway (Moore 2002, Poulin 2010).

The energy a parasite obtains by direct consumption of its host is constrained by the need to keep the host viable until the parasite is prepared for the next phase of its lifecycle (Anderson and May 1982, Hall et al. 2007). In negotiating this tradeoff it should benefit parasites to refine strategies for the controlled removal of host nutrients, such that the parasite benefits from a stable resource supply while host viability is uncompromised. Host castration is one example of a targeted approach to consumption, in which parasites selectively redirect the reproductive allocation of their host to themselves—this trophic strategy typically extends longevity of the

host and can even increase host somatic growth (Baudoin 1975, Lafferty and Kuris 2009). Because the amount of energy a host consumes can be tightly correlated with energy allocated to reproduction (Schoener 1971), effects on host food consumption can potentially impact castrating parasites that target reproductive resources. Therefore, not only free-living prey but also their parasites could potentially pay a cost for safety from predators.

Because the pathway of energy transfer from the environment to the parasite is lengthy and indirect, it is challenging to predict how foraging, consumption and energy allocation to parasites are affected by variability in the host's environment. Previous studies have investigated the response of parasitized animals to the trade-off between foraging and safety (Lefcort and Blaustein 1995, Williams et al. 2001, Kamiya and Poulin 2012), and at least one has directly measured food consumption by hosts in different risk contexts (Chen et al. 2017). However, the links between host foraging behavior, food consumption and parasite growth in the context of predation risk remain to be resolved.

With the goal of understanding how host responses to environmental variability could ultimately affect parasites, we measured relationships among host foraging movements, food consumption, and host and parasite growth in experiments that controlled for resource availability and predator cues. These experiments were conducted on *Baetis bicaudatus*, a common and abundant mayfly in high-elevation streams of the western US, which are infected by *Gasteromermis* sp. (Nematoda: Mermithidae), a castrating endoparasite with a direct (single host) lifecycle. The parasite does not trophically transmit to another host following its parasitic stage, so consumption of the mayfly host is lethal. The parasite resides in the mayfly through the host's larval period and metamorphosis into adulthood, when it is deposited back into the stream via a mimicry of mayfly oviposition behavior (Vance & Peckarsky 1996, Chapter 1).

Subsequently it overwinters in the stream substrate before sexually maturing, mating and reproducing in the following spring. It cannot feed exogenously as a postparasite, therefore the resources it sequesters from the host are the only nutrition it obtains in its lifetime. Growth achieved within the host not only sustains the postparasite through the prolonged free-living stage, but also predicts its fecundity (Poulin 1996).

Prior research indicates that the threat of trout predation (signaled by chemical cues) exerts strong nonconsumptive effects on *Baetis*, including reduced foraging movements. For *Baetis*, movement by drifting is of paramount importance because it enables location of new food patches (Kohler 1984, 1985); but food-finding comes at the cost of increased visibility and risk of consumption by trout (Allan 1978, 1983, McIntosh et al. 2002). When *Baetis* are exposed to trout chemical cues they respond by suppressing drift during high-risk times (day, when they are most visible to trout) and tend toward nocturnal activity (McIntosh and Peckarsky 1996, Peckarsky and McIntosh 1998). Vance (1996a) and Vance and Peckarsky (1997) reported that parasites changed the drifting behavior of *Baetis* in ways that could alter both vulnerability to predators and grazing effectiveness, but potential knock-on effects of predators on parasite fitness were not tested.

We designed our experiments to answer key questions related to the interaction of parasitism, foraging behavior, food consumption and growth in this host-parasite system: 1) does the threat of trout predation affect grazing behavior and food consumption of parasitized *Baetis*? and 2) does the threat of trout predation affect sequestration of host resources by parasites?

METHODS

Study System

We completed this research in streams of the East River catchment, which drains an area of 45-km^2 near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, USA. These rocky-bottom streams are located at ~2,900-m and run-off is generated primarily by snowmelt. Streams throughout the drainage have comparable water chemistry, but vary in size and other physical characteristics (Peckarsky et al. 2001, 2002, Wilcox et al. 2008). The East River and some of its tributaries contain high numbers of brook trout (*Salvelinus fontinalis*), while other tributaries are fishless due to natural migration barriers. Our work focused on parasitism in *Baetis bicaudatus* mayflies, an abundant and widespread insect whose larvae live in high elevation streams in the western US. In the East River drainage, mermithids infect ≈25% of *Baetis* larvae, although spatial variation is high (Chapter 1). *Baetis* is the most efficient insect grazer of attached algae within streams of the region (Alvarez & Peckarsky 2005) and is a common prey species for predatory invertebrates and salmonid fishes (Allan 1983, Peckarsky and Penton 1989). Because it is a foundation species—both highly abundant and influencing ecosystems disproportionate to its biomass—responses of *Baetis* to parasitism could exert higher-order effects (Ellison et al. 2005).

We conducted two microcosm experiments. First, we evaluated the effects of trout and parasitism on *Baetis* foraging behavior (exposure on substrate surfaces and drift in the water column); second, we measured effects of trout and parasitism on *Baetis* grazing rates (algae removal) and evaluated subsequent energy allocation and growth patterns between the host and parasite. We collected *Baetis* from two fishless streams in the East River drainage near the RMBL, Gunnison County, Colorado, USA. The experiments were conducted in a streamside

system of 60 circular, flow-through Plexiglas® chambers (15 cm diameter, water depth ≈10cm; detailed and illustrated in Peckarsky and Cowan 1991) sheltered in a partially translucent greenhouse (Hansen WeatherPort, Delta, CO). We supplied water continuously to the chambers by gravity from a fishless tributary of the East River. We cleaned chambers daily throughout the experiment to avoid clogging and to account for dead or metamorphosing *Baetis*.

Experiment 1: Foraging behavior

We designed the first experiment to measure the effects of parasitism on the behavioral responses of *Baetis* to variation in resources and risk. We manipulated three variables in a fully factorial design ($2 \times 2 \times 2$); parasitized and unparasitized *Baetis* were offered low- or high-food availability in the presence or absence of trout chemical cues. We randomly assigned each combination of treatments to 7 replicated microcosm chambers containing 6 *Baetis*, which are few enough grazers to have no density effect on behavior (Taylor et al. 2002).

We created low and high food treatments by lining the floor of each chamber with 4 unglazed ceramic tiles (3.5cm × 3.5cm) that had either been left to dry outdoors for 1 week before the experiment so that no algal propagules were introduced (for low food tiles), or incubated in cattle tanks through which water from a natural fishless stream flowed continuously to introduce algae propagules (for high food tiles). Twice during the week we added plant fertilizer containing NH_4 and PO_4 (Continuous Release Bloom Booster, Scotts Miracle-Gro Company, Marysville, OH) to the tanks incubating high food tiles to stimulate algal growth. We placed the tiles in microcosm chambers 4 h before grazers were introduced and 12 h before foraging behavior data were collected. To confirm that algae accumulation was different between the high and low food treatments we extracted chlorophyll *a* from 6 replicates (4 tiles each) from

each food treatment. Extractions were made using cold 90% ethanol for 12-24 h and analyzed by spectrophotometry (Nusch 1980).

We created fish-cue-absent treatments by dripping water that was delivered by gravity from a fishless tributary through Tygon® tubing into half of the chambers. To create fish-cue-present treatments from the same stream, we first delivered water to a 110 L container that housed 2 brook trout caught by angling from the East River. Water from that container was then dripped into the remaining chambers. Chemical cues from trout are readily detected by *Baetis* (McIntosh et al. 1999) but have no fertilization effects on chlorophyll *a* accumulation (Peckarsky and McIntosh 1998). We maintained the same trout for both experiments and fed them every 2 d with mixed macroinvertebrates from the East River.

Because parasitism in *Baetis* could not be experimentally induced, we collected *Baetis* from fishless streams and used microscopic examination of wild-caught, live mayflies to determine whether they were parasitized. To increase our efficiency of detecting parasites within the hosts, we collected infected mayflies from a fishless tributary known to have high prevalence (Marmot Creek) and collected unparasitized mayflies from one known to have low prevalence (B3). We estimated these background levels of parasitism before the experiment by collecting *Baetis* in kick samples, preserving them in 70% ethanol, and dissecting them in the laboratory. Because mayflies disperse across streams within the basin, these streams do not have genetically different populations of *Baetis* (Peckarsky et al. 2005). We used *Baetis* from fishless streams because their foraging behavior is permanently altered after exposure to fish, affecting their responses even in fishless control conditions (Peckarksy and McIntosh 1998).

Before *Baetis* could be examined for parasitism we immobilized them using a nonlethal anesthetic. Water containing dissolved sodium hydrogen carbonate (i.e., original Alka-Seltzer®;

4 oz water per tablet) was dripped by pipette into the viewing dish until the *Baetis* had ceased swimming but respiration continued. Finally, under transmitted light at 40× magnification we used microprobes to flip the *Baetis* on its dorsum. If the *Baetis* was infected by a mermithid parasite the worm could be viewed, coiled within the hemoceol, through the translucent ventral cuticle. We assigned all *Baetis* that appeared to be infected to parasitized treatments and returned those appearing uninfected to the stream. We collected *Baetis* for unparasitized treatments from a fishless stream with such low parasite prevalence (1-2%) that we accepted the small margin of error and allocated them to unparasitized treatments without full microscopic inspection. This significantly reduced time and the number of organisms handled, while insuring that all mayflies had been reared in fishless conditions. To further insure that unparasitized *Baetis* experienced the same pre-experimental conditions as the parasitized group, we transferred them to viewing dishes, anesthetized them, positioned them under the microscope light and flipped them with probes before relocating them to the microcosms. We left all *Baetis* to acclimate to the microcosm environments for 8 h before we began the behavior assay.

To assess behavioral responses to food and predator cues we counted all *Baetis* that were exposed on surfaces of tiles, which serves as a proxy for grazing behavior (Alvarez et al. 2014), and measured drift, a risky food search behavior that makes *Baetis* vulnerable to trout predation. Because predation risk differs between day (high risk) and night (low risk) we were interested not only in total activity in response to treatments, but also how activities were distributed between day and night. Therefore, all observations were made once at night and once during daylight (2100 on 31 July and 0900 on 1 August 2014). We quantified drift by counting the number of drifts past a fixed transect in each channel during 1 min. We could not distinguish whether the same or multiple individuals were drifting, but that problem did not introduce

systematic bias with respect to treatments. We observed drift behavior once at night and once during daylight following exposure counts. We used red lamps at night because red light minimizes disturbance of *Baetis* behavior (Cowan and Peckarsky 1994, McIntosh and Peckarsky 1996, McIntosh et al. 2002).

Analysis. We conducted separate three-way ANOVAs to analyze total exposure counts (sum of day + night) and total drift (log[x]-transformed) with fish (cues present or absent), food level (high or low), parasitism (yes or no) and their interactions as fixed-effect predictor variables. We used the same model structure to investigate how risk, resources and parasitism affected the allocation of these activities to nighttime versus daytime. To do this for exposure, we calculated a nocturnicity index as (no. of *Baetis* exposed at night) / (no. exposed day+night) within each microcosm, and used that ratio as the dependent variable. Similarly, to analyze drift nocturnicity, we calculated (no. nighttime drifts) / (no. day+night drifts), and used this proportion to classify each channel as nocturnal (>50% drifts occurred at night) or diurnal ($\geq$50% occurred during daytime; *sensu* Hernandez and Peckarsky 2014). We analyzed that binomial response using a generalized linear model with predictor variables of fish, parasitism, food level and their interactions.

Experiment 2: Grazing

We transitioned directly from the behavior experiment to the grazing experiment, retaining all *Baetis* in the same chambers and continuing to deliver fish cues. So that we could compare grazing among treatments, we standardized algae availability to "high food" grazing conditions by replacing all tiles in the low food tanks. This resulted in a fully factorial 2×2 design with predator and parasite treatments (N=12). To provide a reference for detecting grazer

impact, we also set up ungrazed control tanks that did not contain *Baetis*, maintaining a balanced design. We allowed *Baetis* to graze for 8 additional days before dismantling the experiment. We then removed the tiles to measure periphyton chorophyll *a*, and preserved the *Baetis* in 70% ethanol before dissecting them and measuring biomass in the laboratory. We estimated per capita growth of *Baetis* and *Gasteromermis* as the difference between per capita dry mass (DM; mg) at the end minus DM at the beginning of the experiment. Starting mass was measured from a random subsample of individuals preserved when *Baetis* was originally collected for the trials. Prior to weighing, we dissected parasites from hosts and dried all organisms for 24 h at 60° C.

Analysis. To analyze *Baetis* grazing and growth rates, we performed two-way ANOVAS with fish, parasitism, and their interaction as predictor variables. We separately analyzed two estimates of grazer impact: chlorophyll *a* removed by *Baetis* per capita and per mg biomass of grazer (sum of host+parasite for infected *Baetis*). We calculated parasitized *Baetis* growth (Δmg) two ways, first using the sum of parasite + host DM, and then using host DM only, after the parasite had been removed. Parasite growth (Δmg) was separately analyzed. All growth metrics were analyzed using one-way ANOVA with fish cues as the predictor. To examine the connection between algae consumption, predation risk, and energy allocation to the parasite we calculated ratios of parasite:host biomass per channel at the conclusion of the experiment and modeled this outcome using ANCOVA with fish cues and total algae grazed over the duration of the experiment (mg chl *a*/cm^2) as predictors. All analyses were done in R 3.2.4 (The R Foundation for Statistical Computing 2016).

RESULTS

Experiment 1: Foraging Behavior

Exposure. Overall, more *Baetis* foraged on exposed substrate surfaces in low than high food conditions ($F = 12.4$, $p < 0.001$; Fig. 1). *Baetis* also showed a bias toward nocturnal foraging when fish cues were present ($F = 3.67$, $p = 0.062$). Within the low food treatment, fish had a negative effect on exposure regardless of whether *Baetis* was parasitized (Fig. 1A). In the high food treatment, however, exposure of parasitized *Baetis* increased with fish cues, whereas fewer unparasitized *Baetis* foraged on exposed substrate surfaces with fish cues (food × fish × parasite interaction, $F = 4.00$, $p = 0.051$, Fig. 1B).

Drift. Like exposure, total *Baetis* drift rate was higher in microcosms with low than high food ($F = 8.05$, $p < 0.001$; Fig. 1C-D). However, in both high and low food conditions, parasitism increased total drift rate ($F = 5.10$, $p = 0.029$). The nocturnicity analysis suggests this outcome is influenced by the tendency of parasitism to increase nocturnal drift, although this trend was not significant ($F = 2.80$, $p = 0.094$). Fish cues did not suppress total drift rate, but instead made both parasitized and unparasitized *Baetis* more nocturnal under high but not low food conditions (fish × food level interaction, $F = 6.30$, $p = 0.012$; Fig.1D).

Experiment 2: Grazing

Grazer Impact. Parasitized *Baetis* removed significantly less chlorophyll *a* per capita and had the lowest grazer impact when fish cues were present ($F = 10.2$, $p = 0.003$, Fig. 2A). In contrast to per capita grazer impact, analysis of chl *a* removal per grazer biomass (sum of host + parasite DM for infected *Baetis*) showed a trend toward higher grazer impact of parasitized *Baetis*, primarily in channels with no fish cues ($F = 3.27$, $p = 0.077$, Fig. 2B).

Growth. Parasitized mayflies (sum of host + parasite) were smaller both before and after the experiment (parasitized *Baetis* DM initial: 0.628 ± 0.079 mg and final: 0.939 ± 0.039 mg; unparasitized *Baetis* DM initial: 0.876 ± 0.054 mg and final: 1.07 ± 0.032 mg). Growth of parasitized *Baetis* (Δmg DM host+parasite) was negatively affected by predator cues ($F = 3.44$, $p = 0.073$). During the experiment, parasitism suppressed growth of *Baetis* tissue (Δmg DM; $F = 6.31$, $p = 0.015$, Fig. 3—parasitized *Baetis* DM measured without parasite). Growth rates tended to be lower for all *Baetis* in the presence of fish cues ($F = 3.66$; $p = 0.061$), resulting in cumulative mean weight loss for parasitized *Baetis* exposed to predator cues (Fig. 3—DM of host only). In contrast to their hosts, the direction and magnitude of parasite growth rate was not affected by predator cues ($F = 0.168$, $p = 0.684$, Fig. 3—DM of parasite only).

Energy allocation. The parasite:host biomass ratio measured at the end of the experiment did not vary with the cumulative amount of algae removed by grazers during the experiment ($F = 0.112$, $p = 0.742$). However, the presence of fish cues increased parasite biomass relative to the host ($F = 4.30$, $p = 0.053$). This effect was also reflected in a significant interaction of fish cues and algae removal driving parasite:host biomass ($F = 9.01$, $p = 0.008$; Fig. 4); higher algae consumption resulted in a higher parasite:host biomass ratio when parasitized *Baetis* were exposed to predator cues (Fig. 4A), but in the "safe" environment, increasing consumption resulted in lower parasite:host biomass (Fig. 4B). These results suggest that the increase in parasite:host biomass in response to fish cues results from host growth being suppressed while the absolute size and growth of parasites remains unaffected by predation risk.

DISCUSSION

To our knowledge ours is the first study to measure relationships between predator exposure, food consumption and growth in a parasitic organism, and we have found evidence that predation risk affects the allocation strategy of parasites. The tradeoff between foraging and predation avoidance has been extensively studied for free-living organisms, with ample empirical evidence documenting the potential for predator-avoidance to alter food consumption with costs for growth and fecundity (Werner and Anholt 1993, Lima 1998, Preisser and Bolnick 2008). We extended this line of investigation to examine how predation risk and resource consumption affect the growth rate of an endoparasitic nematode in its mayfly host. We show that endoparasites experiencing the risk of predation on their host can avoid costs on growth, despite the effect of predators on host foraging behavior and, in some contexts, food consumption. In the presence of cues from predators, parasites sequestered more of the energy that their hosts consumed, resulting in net loss of infected host tissue while parasites maintained growth rates. Because the host is castrated, host growth suppression has no bearing on host fitness and likely represents a modification for parasites to maintain growth under energetic duress.

Effects of resources, predation risk and parasitism on host foraging behavior

Our behavior observations show that both parasitized and unparasitized *Baetis* make compromises between food acquisition and predator avoidance. Food scarcity increased exposure on tile surfaces and motivated *Baetis* to search for better food patches even at risky times (see also Kohler and McPeek 1989, Hernandez and Peckarsky 2014). We extended previous work to consider the role of parasites, and found evidence that parasitism affects the way *Baetis* responds to the foraging-predator avoidance trade-off. Even when predator cues were

present, parasitized *Baetis* continued to graze on exposed surfaces during the day. This result contrasts with the behavior of unparasitized *Baetis* in high food and also with observations of Kohler and McPeek (1989) that *Baetis tricaudatus* (parasitism unknown) in the presence of sculpin predators spent less time grazing on tile surfaces during the day unless they were starved. Parasitism exerts high energetic costs and has been likened to a form of physiologic starvation, which could explain the resemblance between behaviors of parasitized and uninfected but starved animals (Lafferty and Shaw 2013). Although exposed *Baetis* are vulnerable to consumption by sculpin, which are bottom-feeders, sculpin are not present in the East River, where the only fish predator is drift-feeding trout. This context suggests that, although exposure indicates grazing activity, it may not be very risky in terms of trout predation (Allan 1981).

We found that food scarcity and parasitism both increased drifting, which is a risky food search behavior that exposes *Baetis* to trout predation in the water column. Because drifting poses the highest risk of predation during the day (Allan 1981, McIntosh et al. 2002) *Baetis* reduces risk by increasing nocturnicity when trout or their chemical signals are present (Peckarsky and McIntosh 1998, McIntosh et al. 1999, 2002). Consistent with this, we saw that parasite-induced increases in drift occurred mainly at night, resulting in higher nocturnicity in both low- and high food conditions. This could reflect a decision to increase foraging while doing so at the safest times. However, a replicated field study that examined parasite effects on *Baetis* nocturnal drift in natural streams reported that, unlike the microcosm study, parasitism decreased the probability that *Baetis* would enter the drift (Chapter 4). This difference between field and microcosm studies could be attributed to the failure of artificial environments to replicate natural ones, which creates well-acknowledged challenges for extrapolating between experiments and nature (Peckarsky et al. 1997, Power et al. 1998). However, we also suggest that

a plausible mechanistic explanation is provided by observations (also reported in Chapter 4) that parasitized *Baetis* are more buoyant and, although they launch into the drift at a lower rate, they take longer on average to exit the drift. In the present study we did not separately count the number of drift entries and the number of circuits individuals made around the microcosm channel. Therefore, the microcosm data are not inconsistent with the lower "launch rates" known to occur in nature, but we are unable to disentangle behavioral effects on entering the drift from biophysical constraints on exiting it. Importantly, despite variable effects of parasites on *Baetis* drift rates between field and microcosm observations, changes in foraging movements have no apparent consequence for fitness of the host (Hernandez and Peckarsky 2014) or parasite (present study).

Effects of predation risk and parasitism on resource consumption

The main finding from our grazer impact analysis is that parasites decrease per capita grazer impact of their host. There are numerous studies reporting that parasites can either increase or decrease host food consumption, suggesting that effects are both widespread and context-dependent (Moore 2002). In many cases there is evidence that changes to food consumption are mediated by parasite-induced changes in host behavior that can operate in positive or negative directions. For example, *Physa* snails parasitized with trematodes grazed more rapidly and consumed more algae (Bernot and Lamberti 2008), but caterpillars infected with wasp parasitoids had reduced movement, which decreased their ability to locate high quality food patches and resulted in lower consumption (Chen et al. 2017). In other cases, consumption differences may be consequent to size differences because ingestion rate increases with consumer size (Toscano et al. 2014). Although we cannot discount a behavioral component (see

below), we suspect that parasitized *Baetis* consumed less algae per capita mainly due to their smaller size, which also potentially explains why they consumed more algae per unit biomass. Decreased per capita consumption by mermithid-infected mosquito larvae has been reported elsewhere, but underlying causes were unclear (Giblin and Platzer 1985).

Because our behavior observations were made before starting the grazing experiment, we cannot unequivocally connect behavior to food consumption rates measured over a longer interval. However, previous studies suggest that, when food is abundant (as it was in the grazing experiment), lower mobility (drift) may reduce the risk these grazers experience but have little effect on how much food they consume (McIntosh et al. 2004, Alvarez and Peckarsky 2005, Hernandez and Peckarsky 2014). Our observation that unparasitized *Baetis* modified behaviors but did not sacrifice food acquisition or growth for predator avoidance is consistent with other experimental and field studies conducted across multiple scales (McIntosh et al. 2004, Alvarez and Peckarsky 2005, Hernandez and Peckarsky 2014). The disconnect between food search behavior and food acquisition is also consistent with our finding that parasitized *Baetis* had higher search (drift) rates, but not higher consumption. As discussed above, it is possible that the number of drifts reflects not only foraging decisions, but also physical constraints on exiting the drift. In addition, previous observations of food search behavior of individual parasitized *Baetis* have reported that parasitism decreases their ability to locate high quality food patches, which could contribute behaviorally to lower grazer impact (Vance 1996a). Those two explanations are not mutually exclusive as they both suggest that, for infected and uninfected *Baetis* grazers alike, search rate can be decoupled from food intake.

The literature examining effects of predators on food consumption of parasitized prey remains limited, but there is evidence for a variety of effects. In some cases parasitism increases

foraging activity and food consumption, such as in sticklebacks infected by cestode worms (Godin and Sproul 1988) and trematode-infected crayfish (Reisinger and Lodge 2016). However, most of those examples are drawn from case studies of animals infected by trophically-transmitted parasites, which infamously manipulate their hosts to increase activity, thereby attracting predators, because the parasite transmits to the definitive host by becoming its prey (Moore 2002, Poulin 2010). Parasites that do not require transmission via predation have received less attention. However, theory predicts that those organisms can benefit from heightened risk aversion (Parker et al. 2009), and there are reports of parasites suppressing feeding activity and food consumption, such as amphipods in early stages of infection by acanthocephalan parasites (Dianne et al. 2011, 2014) and caterpillars infected by parasitoid wasp larvae (Chen et al. 2017). Another case study reports that predator cues can decrease risky behaviors in parasitized snails without affecting host food consumption (Kamiya and Poulin 2012), which is more similar to our observations of *Baetis*.

Although algae accrual can be influenced not only by the top down processes we emphasize here, but also by nutrient availability, we do not think variation in nutrient availability among treatments explains why more algae accrued in the parasite treatment. Unparasitized *Baetis* excrete more N per unit mass and are larger (A. Sanders unpubl. data), making it likely that more N was recycled in microcosms with unparasitized grazers. Therefore, if there was a positive effect of N on algae accumulation, then that would only serve to dampen the parasite effect rather than providing a proxy for it. Perhaps more importantly, we would not expect differences in N excretion to influence chl *a* measurements because algal biomass in these streams is almost universally P limited (Moslemi 2010).

Effects of predation risk on energy allocation and growth rates of hosts and parasites

We discovered that while parasites do not sacrifice growth in risky environments, they consume a greater proportion of available host resources, shifting the balance of allocation away from the host toward the parasite. Tradeoffs between predator avoidance and growth in aquatic free-living organisms have been reported elsewhere (Nakaoka 2000, Nicieza 2000), although experiments with *Baetis* have shown no such costs despite effects of fish on foraging behavior (Hernandez and Peckarsky 2014). Those studies have considered the tradeoffs organisms make to optimize energy intake and expenditure in the context of predation risk. Because parasitism as a consumer strategy requires foraging in (or on) the environment of the host, tactics that parasites employ for allocation in the context of tradeoffs are cryptic, indirect, and challenging to investigate. In addition to numerous studies of nonconsumptive predator effects on free-living organisms (Werner and Peacor 2003, Preisser et al. 2005, Abrams 2007), there is a small body of studies that examine the nonconsumptive effects of predators on parasitized prey, accounting for interactive effects of predators and parasites on host foraging activity (Benton and Pritchard 1990, Wise de Valdez 2007, Kamiya and Poulin 2012, Soghigian et al. 2017). However, those studies have not conceptually been extended to question the allocation strategy of parasites acting as consumers within a host in a risky environment.

Although predation risk did not suppress growth in uninfected *Baetis*, consistent with previous experiments (Hernandez and Peckarsky 2014), we found a negative effect of fish cues on the growth of infected *Baetis* (host+parasite). This suggests that predation risk decreases total energy available for allocation between the host and its parasite. It is informative to consider this pattern in light of the food web energetics underlying it. Energy ultimately available for the parasite to assimilate should be depleted by inefficiencies at two stages, both in the trophic

transfer of energy from algae to the host, and subsequently from host tissue to the parasite. Therefore, in theory consuming an equal amount of algae would result in more energy available for assimilation into an uninfected *Baetis* alone, than into the combined biomass of infected *Baetis* plus its endoparasitic consumer. We found that predation risk had a stronger negative effect on algae consumed by parasitized *Baetis* than healthy *Baetis*. That initial deficit in energy intake, while small, could ultimately translate into suppressed growth in the two-trophic host-parasite system. Nevertheless, parasites sequestered enough energy to grow comparably in fish and fishless conditions, but this came at the cost of by consuming more of their host when risk was high.

We have shown that the parasite does not pay a fecundity cost in the presence of predators, but it may nevertheless pay other costs related to depletion of host resources resulting in a host with poorer condition that may be less viable or unable to perform functions that benefit transmission. Selection should favor strategies that optimize the exploitation of host resources while maintaining host viability as the parasite matures (Anderson and May 1982). Manipulative castrators (parasitic puppet masters that not only directly re-engineer the energy budgets of their hosts but may also alter their morphological and behavioral traits) may have a particularly complex portfolio of interests. By maximizing growth they face the risk of compromising host viability, but it is also in their interest to leave the host sufficient resources to perform the behavior for which it is manipulated (Maure et al. 2013, 2016). Maure et al. (2013) predict that in a context of limited resources manipulative castrators benefit from accepting reductions in size and fecundity as required to preserve minimum resources required by the manipulated host. In the case of the *Baetis*-*Gasteromermis* interaction, the parasite induces sex reversal of male hosts, causing them to look and behave like females during the adult phase—an extreme manipulation

that ensures dispersal of the parasite to the aquatic habitats it requires to continue its lifecycle (Vance 1996b, Vance and Peckarsky 1996). Because the musculature and physiological processes entailed in insect flight are energetically costly (Marden 2000) *Gasteromermis* should be limited in the extent to which it can consume its host without compromising dispersal ability. Even in fishless conditions, *Gasteromermis* has a negative effect on the flight muscle ratio of its host (Chapter 1), implying that increasing the parasite:host biomass ratio could tax *Baetis* flight systems and dispersal capacity.

Conclusions and Implications

By considering how trophic ecology of a parasite influences its resource assimilation we recognize both top-down and bottom-up perspectives. Other studies suggest that the biomass parasites extract from food webs can be considerable, even dominant (Kuris et al. 2008). Our findings raise the possibility that allocation to parasite biomass partly depends on indirect effects of predators on parasite resource assimilation. In the system we describe, it now seems likely that predators influence the total production energy diverted toward host biomass not only through direct and indirect effects on the uninfected host population (McPeek and Peckarsky 1998, Peckarsky et al. 2001, 2002) but also via predator-induced differences in energy allocation to the parasite population.

Further, it is possible for parasite-induced grazing changes to influence biomass and community composition at lower trophic levels (Wood et al. 2007, Bernot and Lamberti 2008, Hernandez and Sukhdeo 2008). Those changes may also be sensitive to predator presence, although empirical evidence for that effect is sparse (Reisinger and Lodge 2016). Previous studies in this system have found that, despite predator-induced changes in foraging activity,

predator effects do not cascade to differences in grazer impact (Peckarsky et al. 2015). However, results reported here suggest that there could be a top-down effect of parasites, such that increasing prevalence of parasite infections results in decreased algae removal by *Baetis*, and that predators may increase the magnitude of that effect.

Our findings are relevant not only for higher levels of biological organization but also for larger spatial scales. For example, parasitism negatively affects flight musculature of *Baetis* (Chapter 1) and we question whether parasitized hosts reared in high risk environments, and consequently having lower body condition, would have decreased dispersal capacity compared to hosts reared in low risk environments. If parasites trade-off dispersal capacity for growth when predators are present, that could affect not only the local abundance of parasites, but also the metapopulation dynamics and genetic structuring of parasites at a regional scale.

In summary, through investigating the responses of parasites to both risk and resources we have joined salient aspects of predator-prey ecology (the foraging-predator avoidance trade-off and nonconsumptive predator effects) with host-parasite ecology (the virulence-transmission trade-off and parasite life history evolution) to discover that when their hosts experience predation risk endoparasites change energy sequestration strategies and consume more of the host to maintain their own growth. This finding generates further hypotheses related to the food web and dispersal ecology of endoparasites responding to predation risk.

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FIGURES

Figure 1. Total counts (± SE) of *Baetis* visible foraging on exposed substrate surfaces were higher in low **(A)** than high **(B)** food conditions ($p < 0.001$). Within the low food treatment, fish cues decreased exposure counts regardless of whether *Baetis* was parasitized. In the high food treatment fish cues had opposite effects on exposure of unparasitized (negative effect) and parasitized (positive effect) *Baetis* (p = 0.051). Total *Baetis* drift rate (± SE) was higher in microcosms with low **(C)** than high **(D)** food ($p < 0.01$). In both food conditions, parasitism increased total drift rate ($p = 0.03$) and tended to increase nocturnicity ($p = 0.094$). Fish cues did not suppress total drift, but instead, made *Baetis* more nocturnal under high food conditions ($p = 0.012$).

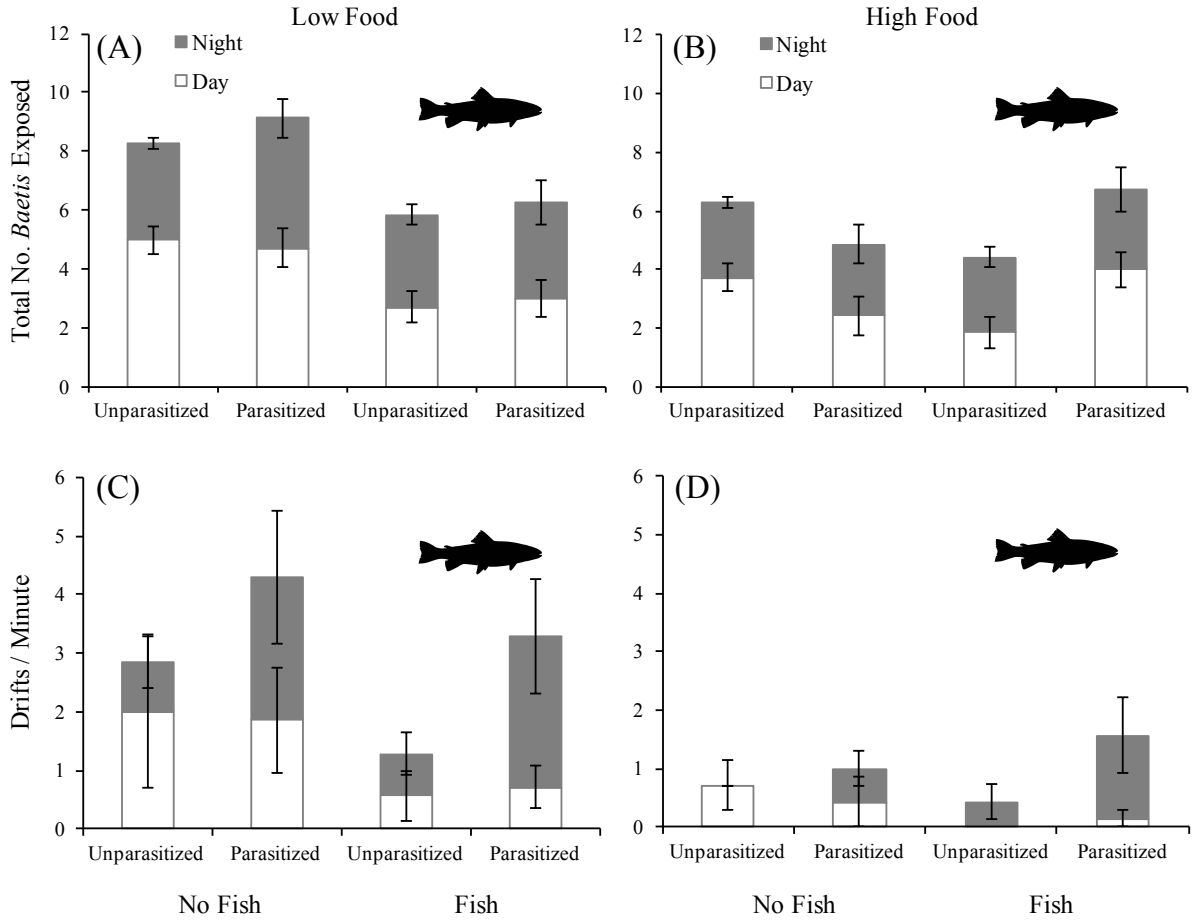


Figure 2. (A) Parasitized *Baetis* removed significantly less chlorophyll *a* per capita independent of fish cues ($p < 0.01$). **(B)** Analysis of algae removed per mg grazer (sum of host + parasite DM for infected *Baetis*) showed a tendency for parasitized *Baetis* to have higher impact per unit biomass, primarily in channels without fish cues ($p = 0.077$), potentially explained by smaller size of parasitized *Baetis*. Labels above bars designate pairwise contrasts significant at $\alpha = 0.05$.

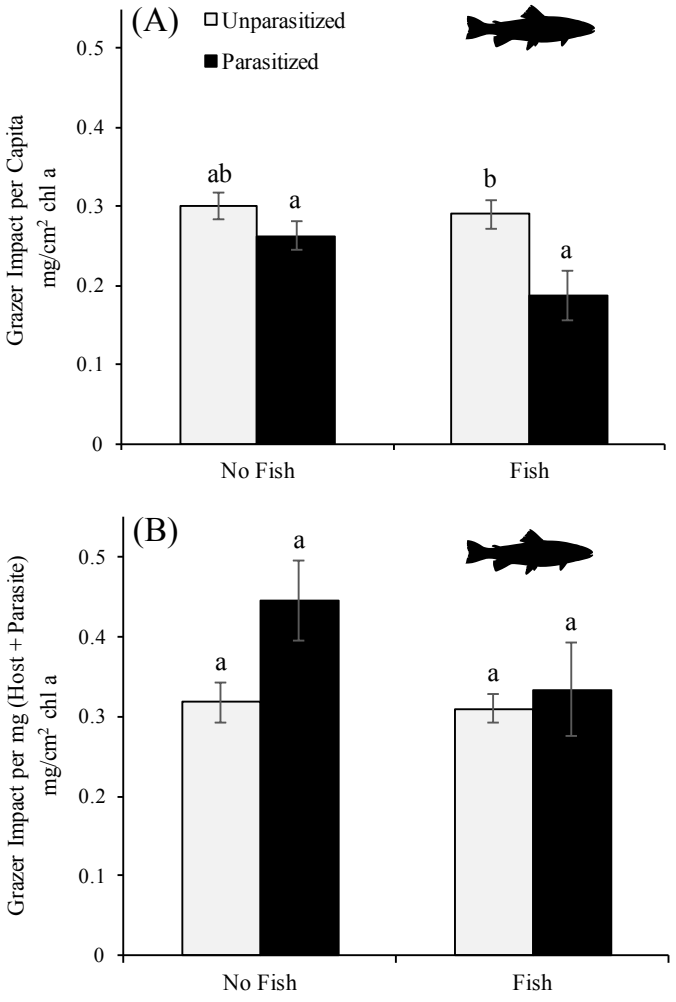


Figure 3. Parasitism suppressed *Baetis* growth rate over the duration of the grazing experiment (final - initial mg DM—measured as host without parasite for parasitized *Baetis*; $p = 0.015$). Fish cues also suppressed *Baetis* growth rate ($p = 0.061$), resulting in cumulative mean weight loss for parasitized hosts exposed to predator cues. In contrast to their hosts, the direction and magnitude of parasite growth was not affected by predator cues ($p = 0.684$).

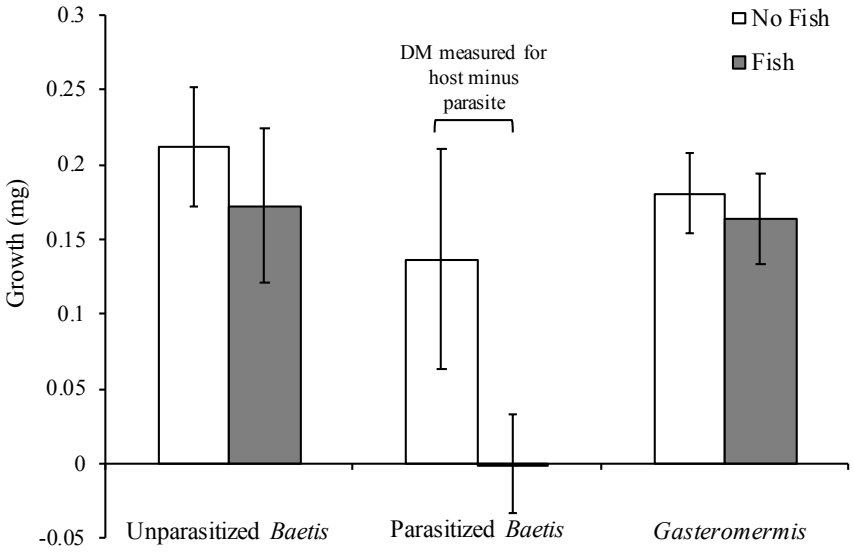
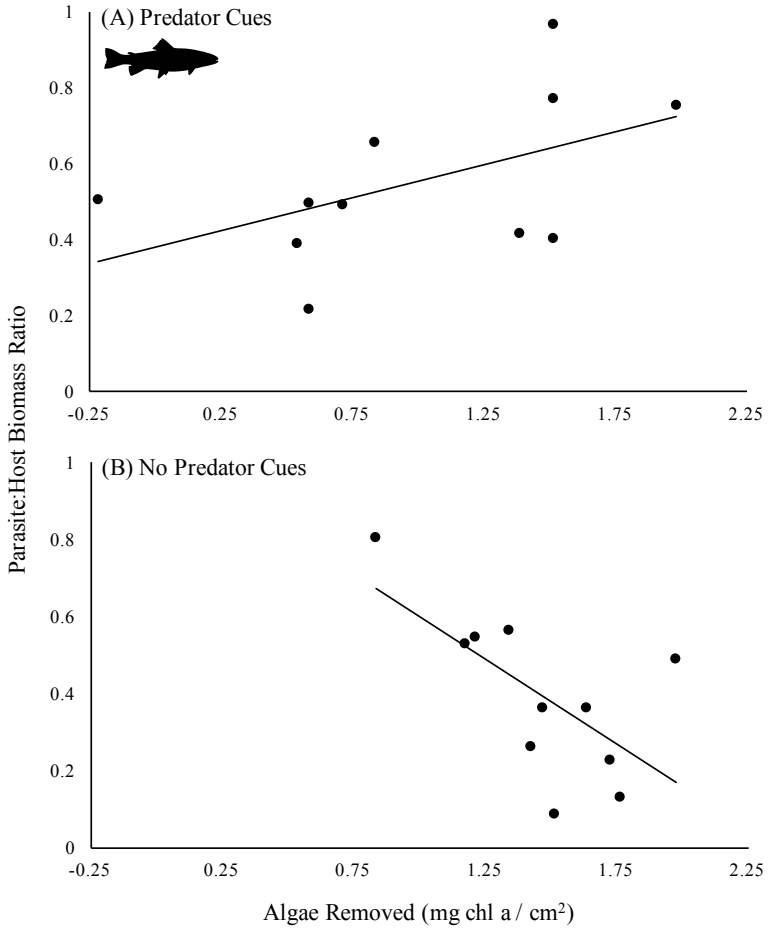


Figure 4. There was a significant interaction of fish cues and algae removal driving the parasite:host biomass ratio ($p = 0.008$), such that higher algae consumption resulted in a higher parasite:host biomass ratio when parasitized *Baetis* were exposed to predator cues (A), but increasing consumption resulted in decreasing parasite:host biomass without predator cues (B). This observed increase in parasite:host biomass in response to fish cues resulted from host growth being suppressed while the absolute size and growth of parasites remained unaffected by predation risk (see Fig. 3).



APPENDIX

Table A1. Summary of analyses of microcosm Experiment 1 testing foraging behavior of *Baetis* in response to presence/absence of fish cues, high or low food levels, and presence/absence of parasite infection**.**

Analysis	***F***	***p***
Number *Baetis* exposed		
Fish cues	6.52	0.014
Food level	12.4	<0.001
Parasitized	1.07	0.306
Fish cues × Food level	6.52	0.014
Fish cues × Parasitized	2.51	0.119
Food level × Parasitized	0.04	0.837
Fish cues × Food level × Parasitized	4.00	0.051
Total Drift		
Fish cues	0.20	0.660
Food level	8.05	<0.001
Parasitized	5.10	0.029
Fish cues × Food level	0.30	0.589
Fish cues × Parasitized	0.45	0.507
Food level × Parasitized	0.36	0.550
Fish cues × Food level × Parasitized	0.03	0.869
Exposure Nocturnicity		
Fish cues	2.42	0.127
Food level	0.01	0.907
Parasitized	5.10	0.029
Fish cues × Food level	0.03	0.866
Fish cues × Parasitized	3.67	0.062
Food level × Parasitized	2.26	0.139
Fish cues × Food level × Parasitized	0.913	0.344
Drift Nocturnicity		
Fish cues	1.86	0.173
Food level	0.55	0.458
Parasitized	2.80	0.094
Fish cues × Food level	6.30	0.012
Fish cues × Parasitized	0.00	1.000
Food level × Parasitized	2.40	0.121
Fish cues × Food level × Parasitized	0.00	1.000

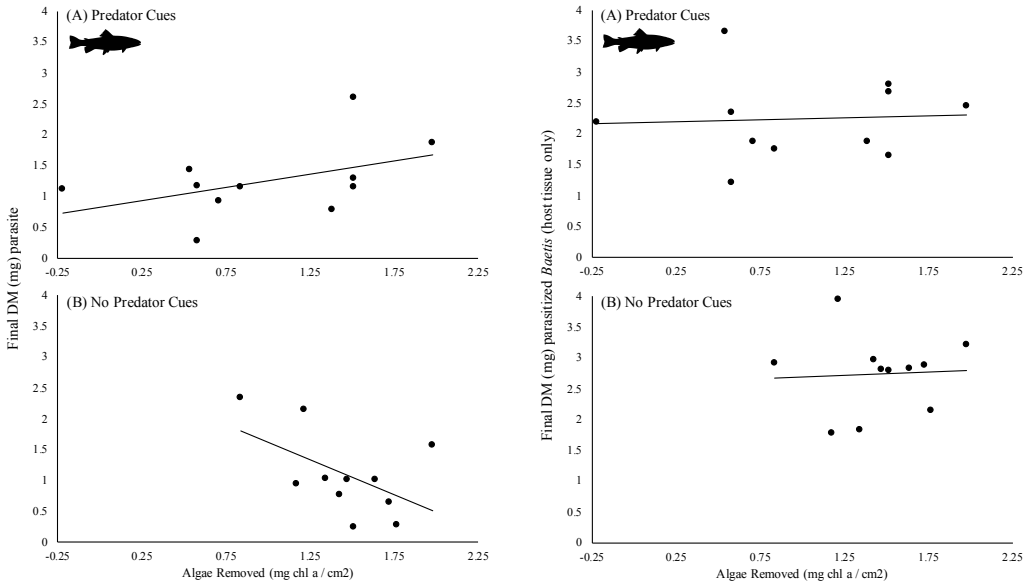
Table A2. Summary of grazer impact analyses from microcosm Experiment 2 testing grazing of *Baetis* in response to presence/absence of fish cues and presence/absence of parasite infection**.**

Analysis	***F***	***p***
Grazer Impact (per Capita)		
Fish cues	3.74	0.060
Parasitized	10.2	0.003
Fish cues × Parasitized	2..27	0.139
Grazer Impact (per mg)		
Fish cues	2.08	0.156
Parasitized	3.27	0.077
Fish cues × Parasitized	1.55	0.219

Table A3. Summary of growth analyses following microcosm Experiments 1&2, testing host and parasite growth variables in response to presence/absence of fish cues, the parasite:host biomass ratio in response to fish cues and the amount of algae consumed by the host.

Analysis	***F***	***p***
Parasitized *Baetis* Growth (host + parasite)		
Fish cues	3.44	0.073
Parasitized *Baetis* Growth (host only)		
Fish cues	6.31	0.015
Parasite Growth		
Fish cues	0.17	0.684
Parasite:Host Biomass		
Fish cues	4.30	0.053
Algae consumed	0.11	0.742
Fish cues × Algae consumed	9.01	0.008

Figure A1. Relationship of food consumption to final parasite biomass and final host biomass (infected *Baetis*, host tissue only) with and without predator cues, at the end of the grazing experiment. These figures clarify the relationships between food consumption and size estimates presented as the numerator and denominator of parasite:host ratio in Figure 4.



2012 Behavior Pilot Experiment Summary

Background. This experiment was designed as an initial test of how parasitism by *Gasteromermis* sp. nematodes influences non-consumptive behavioral effects of top predators (trout) on *Baetis* mayfly larvae.

Methods. Baetis bicaudatus larvae were captured from Quigley Creek, visually assessed for parasitism, and held in the weatherport, with 1 individual per channel. Channels were randomly assigned into 4 treatments in a fully crossed parasite present/absence × fish cue present/absent design, n = 14 per treatment. Replication was limited by the number of parasitized *Baetis* found (= 28 individuals). Drift behavior was observed twice daily (day and night) between 17-19 July 2012 by BLP and KJC. Experiment set-up and behavior observations were completed as detailed in Chapter 3 Methods. All individuals were dissected after the experiment to verify parasite classification and to estimate biomass of parasites and hosts.

Results. Nine *Baetis* were misclassified in a priori visual assessments (8/9 were assigned to unparasitized treatments, but were discovered to be parasitized when dissected) resulting in an unbalanced design (fish cue × parasitized = 17; fish cue × unparasitized = 11; no fish cue × parasitized = 18; no fish cue × unparasitized = 10). We observed that drifting decreased steeply during the day and in the second day of the experiment, so data were only plotted for the 1st nighttime observation (Fig. A2). Unparasitized mayflies tended to drift more in the presence of fish (4/11 drifted) than when fish were absent (2/10 drifted, but 1 of these was very active), whereas parasitized mayflies showed the opposite pattern: they did not drift at all in the presence of fish, but did drift when fish were absent (6/18 drifted). The effect size of fish was greater for parasitized than unparasitized larvae. Parasites in stage III hosts tended to be larger than those in stage IV hosts, and represented a great fraction of host biomass (Fig. A3)

Discussion. We found evidence that parasitized mayflies are strongly averse to predation risk, based on the observation that no parasitized mayflies drifted in the presence of fish. However, a few parasitized mayflies did drift on the second night (results not shown). Overall drift was very low, possibly because Quigley is a fish stream and/or because only 1 individual was observed per channel. To account for possible effects of habituation to fish, the next pilot experiment was completed with *Baetis* captured from a fishless stream (Marmot Creek).

Figure A2. No. of drifts/min observed during the first night of the experiment only (*B. bicaudatus* from Quigley Creek). Parasitism decreased drift in the presence of fish (W = 60, p = 0.01). All other comparisons are n.s.

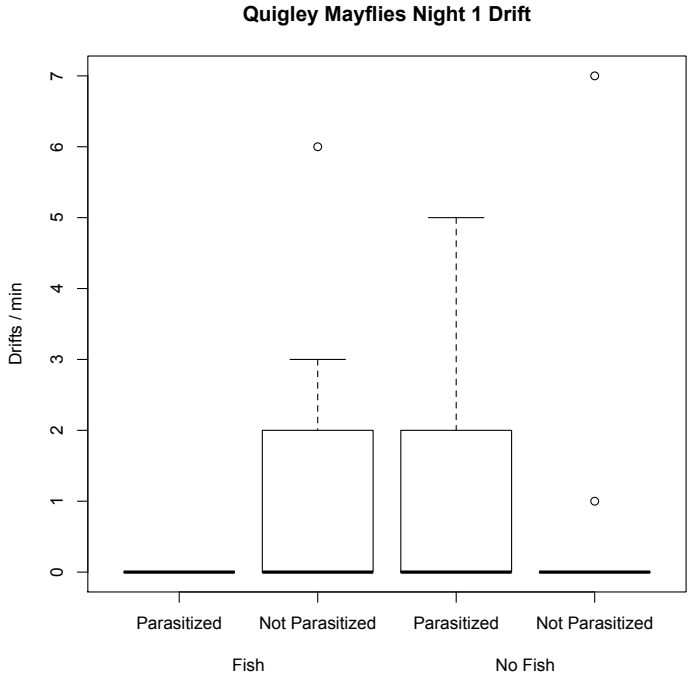
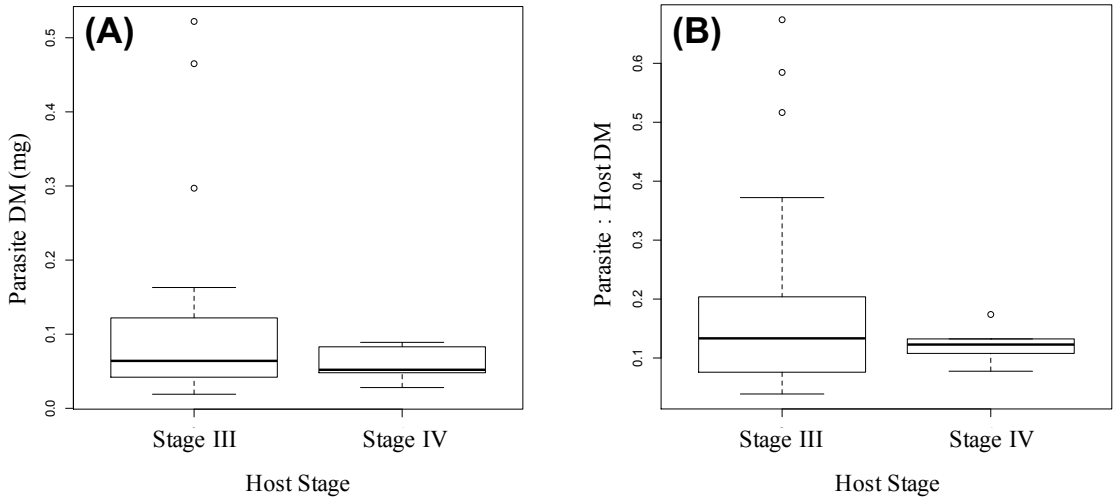


Figure A3. (A) Dry mass of *Gasteromermis* sp. parasites and (B) parasite:host biomass ratio of parasites dissected from stage III and IV *B. bicaudatus* collected in Quigley Creek in July 2012.



2012 Trade-off Pilot Experiment Summary

Background. This experiment was designed as a follow-up test of how parasitism by *Gasteromermis* sp. nematodes influences non-consumptive behavioral effects of top predators (trout) on *Baetis* larvae. In addition, we manipulated food levels to examine how parasitism affects the activity-predator avoidance trade-off of its mayfly host.

Methods. Baetis bicaudatus were captured from Marmot Creek, visually assessed for parasitism and held in microcosms in BLPs weatherport, with 1 individual per channel. Channels were randomly assigned into 8 treatments in a fully crossed food × parasite × fish design, N = 7 per treatment. 1 additional unparasitized replicate was added to compensate for potential misclassifications of "unparasitized" mayflies. High food treatment channels contained 6 tiles that had been incubated in cattle tanks for 1 week with occasional fertilization to grow algae; low food treatments included 6 tiles that had been scrubbed and left to dry for the same length of time. Differences between high and low food treatments were confirmed by estimating chlorophyll content of tiles with spectrophotometry. Drift behavior was observed twice daily (day and night) between 31 July – 2 August 2012 by BLP and KJC. Experiment set-up and behavior observations were completed as detailed in Chapter 3 Methods. All individuals were dissected after the experiment to verify classification and to estimate biomass of parasites and hosts. No statistical analyses were conducted.

Results. All *Baetis* drifted more in the low food treatment. Overall, fish presence depressed drift, more so for parasitized *Baetis*, but parasitism tended to increase drift in safe environments (Fig. A4). The drift behavior of parasitized *Baetis* in low food conditions was not explained by the biomass of the parasite (Fig. A5.A), although parasites that reached the largest size relative to host biomass did not drift (Fig. A5.B).

Figure A4. Total no. of drifts/min recorded during 2 night observations of hungry *Baetis* (= low food treatment) collected from Marmot Creek. Drift in the high food treatment was negligible.

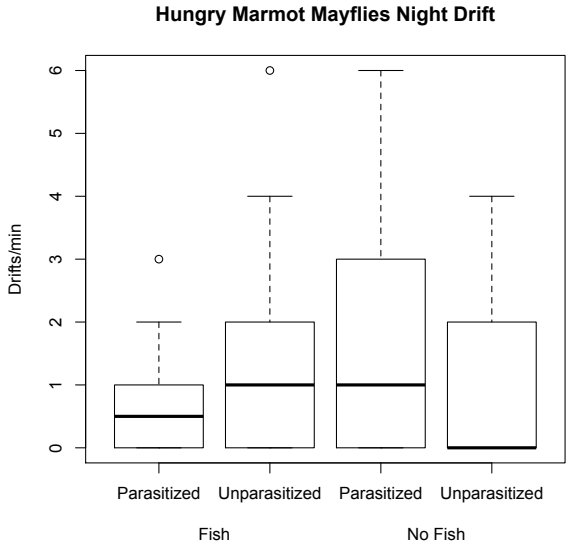
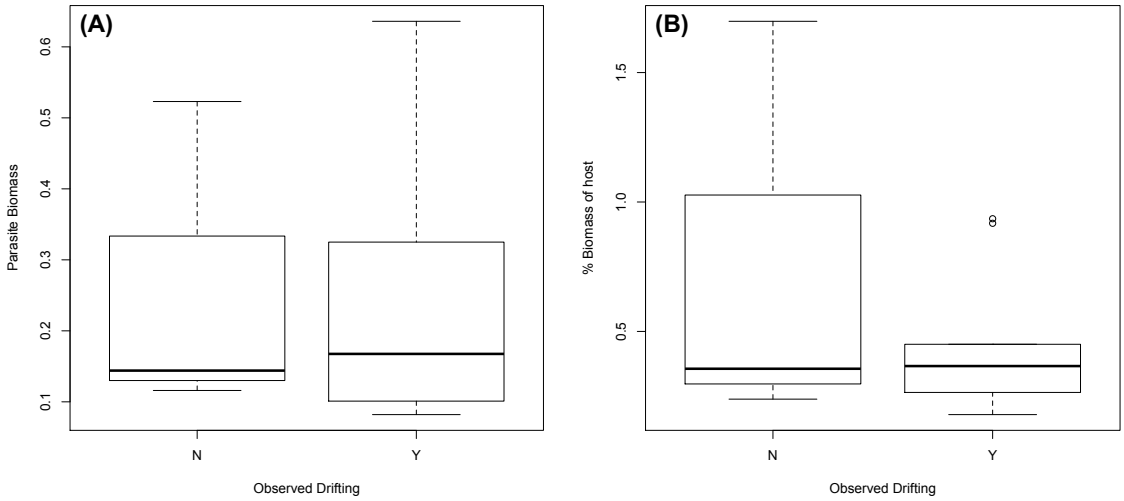


Figure A5. (A) Biomass (mg DM) and (B) parasite:host DM ratio of *Gasteromermis* sp. parasites in *Baetis* larvae that either did (Y) or did not (N) drift in the low food treatment of the trade-off experiment (fish treatments combined in this figure).



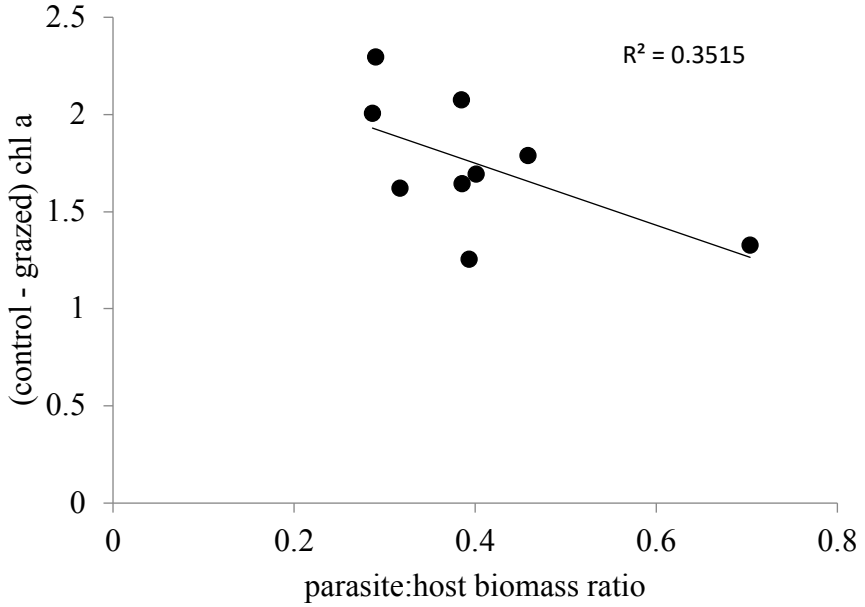
2012 Grazing Pilot Experiment Summary

Background. This experiment was designed to test whether parasitism affects the grazer impact of *Baetis* larvae consuming epilithic algae.

Methods. Baetis bicaudatus were captured from Marmot Creek, visually assessed for parasitism and held in the weatherport, with 6 individuals per channel. Channels were randomly assigned to 3 treatments: no grazers (control); parasitized grazers; unparasitized grazers. Each microcosm was provisioned with 6 tiles that had been incubated in cattle tanks for ~1 week with occasional fertilization to grow algae, and 6 *Baetis* were placed in each channel. No fish cues were introduced. At the end of the experiment (which ran from 13-20 August 2012) differences between grazed and ungrazed tiles were measured with spectrophotometry. Experiment set-up and chlorophyll measurements were completed as detailed in Chapter 3 Methods.

Results. We found that microcosms with higher parasite biomass relative to the host were not grazed as heavily as microcosms with lower parasite:host biomass ratios (Fig. A6). We conducted this analysis as an alterative to the original parasitized vs. unparasitized comparison because when *Baetis* were dissected at the conclusion of the experiment we discovered that most were parasitized, but their parasites had initially been too small to observe by nonlethal inspection under the dissecting microscope. After that realization, the unparasitized *Baetis* used in all subsequent experiments reported in the dissertation were captured from streams known to have low levels of parasitism (B3 or Upper Rock Creek) to reduce the impact of observer error, while we continued to sample parasitized *Baetis* from Marmot Creek.

Figure A6. Chlorophyll a estimated in grazed microcosms compared to ungrazed controls (control-grazed = consumption) versus worm:host biomass in the microcosm (averaged across 6 *Baetis* per channel).



2013 Foraging Behavior and Consumption Experiment Summary

Background. Following pilot experiments in 2012, this experiment was designed to 1) compare risky foraging behavior and algae consumption of parasitized vs. unparasitized *Baetis bicaudatus* in the presence of trout predators, and 2) test whether fear has different effects on grazing of parasitized vs. unparasitized *B. bicaudatus* under low food conditions

Methods. Microcosm channels were randomly assigned to treatments of high or low food × presence or absence of predator cues × parasitized or unparasitized mayflies in a fully-crossed factorial design (n = 7). After preparing channels with food and predator treatments, *Baetis bicaudatus* larvae were collected from Marmot Creek and Benthette 3 on 29 July 2013 and transported in coolers to the lab where they were examined nonlethally for parasitism. Six larvae were placed in each channel. *Baetis* behavior (active grazing and drift) was observed once at night and once during the day between 29-30 July 2013. At the end of the experiment differences between grazed and ungrazed tiles were measured with spectrophotometry. Experiment set-up, behavior observations, and chlorophyll measurements were completed as detailed in Chapter 3 Methods. Data were analyzed with linear models (variables transformed as necessary) including all interaction terms.

Results. High food conditions decreased total drift rate ($p = 0.04$) whereas parasitism increased drift rate ($p < 0.01$; Fig. A7). No daytime drift was observed in high food conditions when fish cues were present. When food was limited, parasitized *B. bicaudatus* grazed significantly more algae than unparasitized ($p < 0.01$; Fig A8). Fish cues did not influence algae consumption ($p = 0.98$), and parasitized and unparasitized *B. bicaudatus* did not respond differently to the presence of fish ($p = 0.74$).

Figure A7. Total *Baetis* drift rate (± SE) was lower in microcosms with high (A) than low (B) food. In both food conditions, parasitism increased total drift rate and tended to increase nocturnicity.

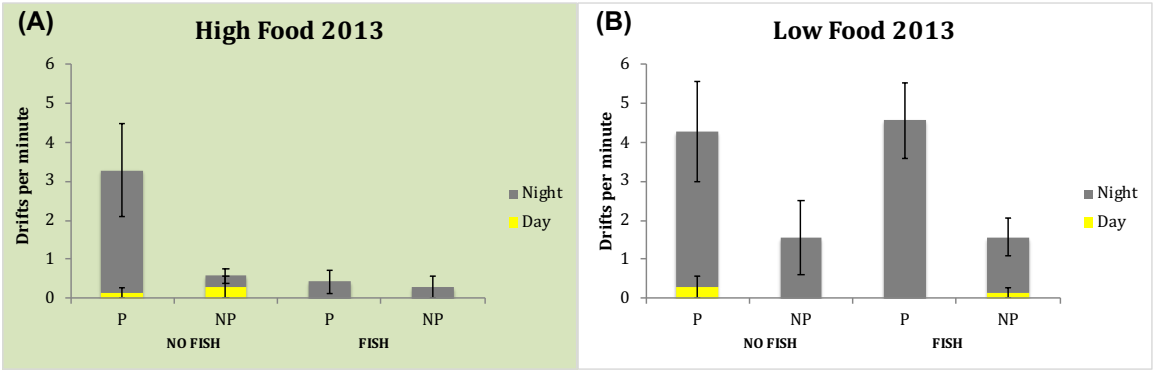
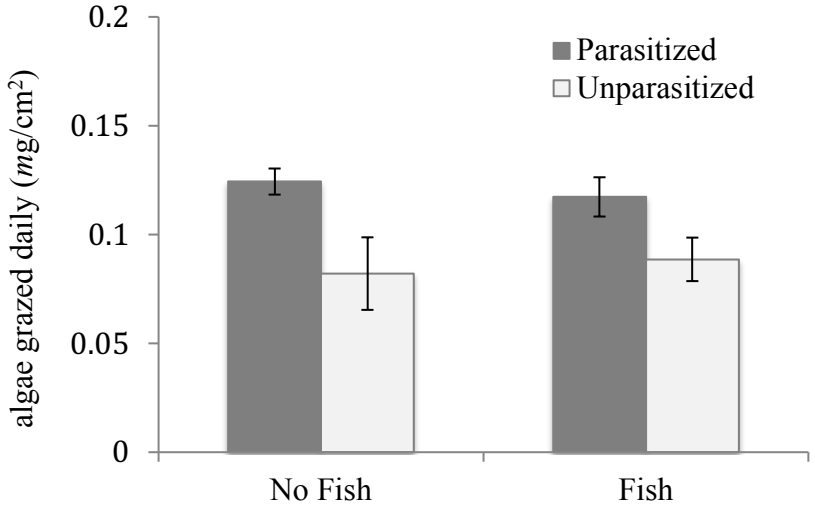


Figure A8. Algae consumption by *Baetis* larvae was increased by parasitism but not influenced by fish cue presence.



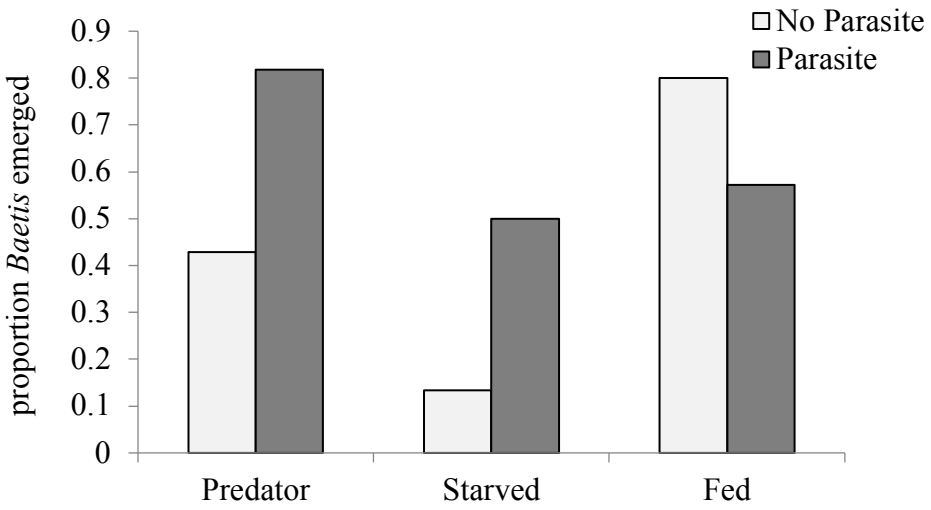
1990 Sublethal Predator Effects Experiment Analysis

Background and Methods. BLP and Cathy Cowan measured emergence date and biomass (mg DM) of *Baetis* species B in the presence of 2 predatory stoneflies (*Kogotus* and *Megarcys*, here grouped in one "Predator" treatment), compared to *Baetis* larvae held without predators but with different food conditions (either ad lib or starvation treatments). Following the experiment they discovered that most of the *Baetis* were infected with *Gasteromermis* sp. nematodes. The proportion of *Baetis* emerged by the end of the experiment was examined with Chi-square analysis with treatment (predators, fed, or starved) and parasitism (yes or no) as predictors. (Analysis completed by KJC).

Results. The treatment × parasite interaction had a significant effect on emergence ($p < 0.01$), with a disproportionately higher fraction of infected *Baetis* emerging in the stressful treatments (predator, starved) but disproportionately fewer emerging in the safe, well fed treatment (Fig. A9.

Discussion. This outcome is consistent with experimental findings reported in Chapter 3 that stressful or risky conditions seem to accelerate the parasite's development within its host.

Figure A9. Emergence of *Baetis* species B larvae reared in different stress conditions (stonefly predators present, or starvation) compared to safe, well fed conditions. Treatments had opposite effects on the proportion of parasitized versus unparasitized *Baetis* larvae emerging, with stressful treatments resulting in higher emergence of infected *Baetis*.



CHAPTER 4 – Single-host parasites alter multiple dimensions of host phenotype to decrease consumption by top predators

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ABSTRACT

Parasites that are killed when their host is consumed should benefit from host predator-avoidance behaviors but few studies have linked their effects on host behaviors with mortality due to predation. Further, parasites can modify multiple dimensions of the host phenotype, suggesting that not only behavioral but also morphological changes could affect predator-prey interactions and parasite fitness. To disentangle multiple effects of parasites on host predation, we measured predation rates in response to behavioral and morphological traits of parasitized prey along a gradient of realistic to controlled conditions. In natural streams we measured trout consumption of parasitized mayfly larvae (*Baetis bicaudatus* infected with *Gasteromermis* sp. nematodes) to determine if parasites decrease their host's mortality due to predation. Because field estimates of predation on *Baetis* reflect both its probability of exposure (i.e., drifting in the water column) and its probability of being consumed when exposed, we separately examined those components. First, we measured behavioral effects of parasites on *Baetis* drift in natural fish and fishless streams. Then we used more controlled conditions in a mesocosm observation channel to measure trout predation rates on *Baetis* in the drift. We also measured effects of parasitism on

behavior of drifting mayflies (distance drifted, maximum drift height, time until escape from drift, and swimming behavior in the drift). Last, we used a highly-controlled microcosm to measure interacting effects of parasitism and host size on buoyancy, which has been predicted to act as a biophysical constraint on drift behavior and could affect conspicuousness to predators. We found that trout consumed fewer parasitized *Baetis* than expected based on their availability. That pattern is partially explained by the finding that parasitism decreased *Baetis* exposure to predators by suppressing drift in both fish and fishless streams. Low predation rates were further explained by mesocosm experiments demonstrating that parasitized *Baetis* were less likely to be consumed even after exposure in the drift, although they drifted higher than healthy *Baetis*, which can increase visibility. *Baetis* that actively swam in the observation arena were more likely to be consumed; nevertheless unparasitized *Baetis* increased swimming when fish were present, potentially explaining why predation tended to be higher on them than parasitized *Baetis*. Differences in drift behavior were consistent with the finding that parasitism increased buoyancy of *Baetis* larvae—primarily an indirect effect of parasites reducing host size. We estimate that combined effects of parasitism cumulatively reduced probability of trout predation by an order of magnitude.

Keywords: predation, parasitism, nonconsumptive effects, host-parasite interactions, multidimensional, manipulation, mayflies, mermithids, drift

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INTRODUCTION

Parasite fitness depends on successful transmission into the appropriate host or habitat required to complete its life cycle, resulting in selection for traits that increase probability of transmission (Price 1980, Moore 2013). Predation on infected hosts can have strong effects on parasite transmission, acting in either positive or negative directions. For example, if a parasite is trophically-transmitted to its definitive host it achieves transmission when it is consumed, and selection favors traits that increase predation by target predators (Poulin 1995, 2010, Moore 2002). Conversely, if a parasite is killed when its host is consumed, predation threatens it with complete failure to achieve transmission. In this case avoiding predators should benefit the parasite, just as it benefits the host (Fritz 1982, Parker et al. 2009).

Far from being passive bystanders in the biotic interactions of their hosts, many parasites have the ability to manipulate host traits and behaviors to their benefit (Moore 2002, Poulin 2010). This ability has famously been exemplified by trophically-transmitted parasites that modify their intermediate host to attract predation by definitive hosts, such as killifish that bait predatory birds by "contorting, shimmying, and jerking" (Lafferty and Morris 1996) or nematodes that cause "fruit mimicry" in their ant host, turning its abdomen berry-red to attract frugivorous birds (Yanoviak et al. 2008). In theory parasites should also manipulate host behavior to avoid predation when it benefits them to do so, such as when the parasite has not matured to the life stage at which it can transmit to the definitive host, or when their hosts risk consumption by predators that are not viable definitive hosts (Parker et al. 2009). However, empirical evidence for behavior modifications that suppress predation risk is uncommon, and comes mainly from systems involving trophically-transmitted parasites (Dianne et al. 2011,

2014, Médoc and Beisel 2011, Weinreich et al. 2013; but see Chen et al. 2017, Soghigian et al. 2017).

From the outset ecologists have cautioned against a taking a heedlessly adaptationist view of parasite-induced behavior changes, which could alternatively be pathological side-effects of infection (Poulin 1995). But recently the usefulness of viewing behavior changes as stemming dichotomously from either an adaptive manipulation or a constraint has been questioned, acknowledging that both those mechanisms could theoretically increase parasite fitness if they decrease mortality due to predation (Poulin 2010, Moore 2013). It is also evident that manipulative parasites do not modify their hosts using a scalpel approach as much as a sledgehammer; manipulated hosts are often subject to comprehensive changes on several dimensions falling along the spectrum of "side effect" to adaptation (Lefèvre et al. 2009b, Poulin 2010, Thomas et al. 2010). The debate surrounding how host modifications affect parasite fitness has remained lively in part because empirical studies that disentangle effects of multiple modified traits are rare. Such studies require deconstructing the components of survival and ultimately linking fine-scale mechanistic drivers with behavior outcomes and consequent mortality rates.

Isolating separate effects of parasitism on predation rates is challenging, and benefits from the capacity to measure multiple integrated components of predator-prey-parasite interactions, grounded in detailed knowledge of the organisms and processes involved. We work in a system involving a single-host parasite (*Gasteromermis* sp. Nematoda:Mermithidae; Poinar 1991) that infects and castrates a freshwater grazer (*Baetis bicaudatus* mayflies) subject to predation by trout (Vance and Peckarsky 1996). Decades of previous research have clarified many aspects of *Baetis* interactions with trout but the effects of parasitism on this interaction

remain unknown (Peckarsky and McIntosh 1998, McIntosh et al. 2002, Peckarsky et al. 2002, Hernandez and Peckarsky 2014). For *Baetis*, voluntary movement by drifting is of paramount importance because it enables location of new food patches (Kohler 1984, 1985); but food-finding comes at the cost of increased exposure and risk of consumption by trout (Allan 1978a, 1983, McIntosh et al. 2002). *Baetis* exposed to trout chemical cues respond by suppressing drift during the day, when they are most visible to trout, and becoming more nocturnal (McIntosh and Peckarsky 1996, Peckarsky and McIntosh 1998). Predator-induced changes to larval *Baetis* behavior are proportional to risk based on prey traits. For example, the smallest *Baetis* are not preferred by trout, which tend to be size selective, and are also less likely to modify drift when trout are present (Allan 1978b, McIntosh et al. 1999). In addition to behavioral induced responses, *Baetis* living in trout streams accelerate their larval development and emerge from streams earlier, decreasing their cumulative risk of mortality due to predation, but paying a fecundity cost because they are smaller at emergence (Peckarsky et al. 2001, 2002).

Because consumption of *Baetis* is lethal to its parasite, both should benefit from balancing risk-avoidance with fecundity gains. In the context of foraging, if *Baetis* and its parasite share the same optimal balance between host food acquisition and predator avoidance, parasites should accept their host's strategy of depressing drift or shifting foraging activity to safer times of day when trout are present. Empirical evidence for the effect of parasites on drift of stream insects has been mixed, but in general does not conform to the null expectation of no behavior difference between parasitized and unparasitized hosts (Wilzbach et al. 1986, Cummins and Wilzbach 1988, Benton and Pritchard 1990). Vance (1996a) observed that *Baetis* parasitized by *Gasteromermis* drifted less frequently in microcosms, which, in nature, would decrease trout predation relative to uninfected hosts, although foraging opportunities could be lost as a result.

However, those initial observations did not control fish chemical cues in the water, which have since been shown to suppress drift, so the effect of parasitism remains unknown (McIntosh and Peckarsky 1996, McIntosh et al. 1999).

In addition to effects on foraging movements (drift), parasites also cause morphological changes that could affect host appearance and performance after it launches into the drift. Specifically, parasitized *Baetis* are smaller than their uninfected counterparts in the same stream (Vance and Peckarsky 1996), and appear to be more buoyant, which has not been tested but could constrain their performance while drifting (Vance 1996a). Those changes seem likely to influence vulnerability to visually-foraging trout, but it is unclear what the net outcome would be. Importantly, trout predation on parasitized *Baetis* has never been measured, so the fitness consequences of any trait changes, behavioral or morphological, remain untested.

Founded on our knowledge of *Baetis*-trout interactions there is considerable potential to use this system to examine the spectrum of endoparasite effects on host predation. Our first goal was to measure consumption of *Baetis* in natural streams to determine if parasites decrease their host's mortality due to predation. Then, because field predation estimates reflect both the probability of exposure (i.e., drifting) and the probability of being consumed when exposed, we examined those components separately, measuring behavioral and morphological traits of parasitized *Baetis* that could influence each. In streams, we measured behavioral effects of parasites on propensity to drift. Then we used more controlled conditions to test for effects of parasitism on behavior of mayflies once they were in the drift, and trout predation rates directly from the drift. Last, we used a highly-controlled microcosm to measure effects of parasitism on host buoyancy, which could act as a biophysical constraint on drift behavior.

METHODS

Study System

We worked in streams of the East River catchment, which drains an area of 45-km^2 near the Rocky Mt. Biological Laboratory (RMBL) in Gothic, Colorado, USA. These rocky-bottom streams are located at ~2,900-m and run-off is generated primarily by snowmelt. Streams throughout the drainage have comparable water chemistry, but vary in size and other physical characteristics (Peckarsky et al. 2001, 2002, Wilcox et al. 2008). The East River and some of its tributaries contain high numbers of brook trout (*Salvelinus fontinalis*), while other tributaries are fishless due to natural migration barriers. Our work focused on parasitism in *Baetis bicaudatus* mayflies, an abundant and widespread insect whose larvae live in high elevation streams in the western US. *Baetis* is the most efficient insect grazer of attached algae within streams of the region (Alvarez & Peckarsky 2005). *Baetis* is a common prey species for predatory invertebrates and salmonids (Allan 1983, Peckarsky and Penton 1989), suggesting that responses of *Baetis* to parasitism could exert strong higher-order effects. *Gasteromermis* parasitize early instars of *Baetis* larvae, eventually castrating the host and causing morphological and behavioral sex reversal of males, so that both male and female adult hosts join unparasitized females in dispersal and oviposition behavior following metamorphosis (Vance 1996a). The oviposition flight of parasitized *Baetis* culminates with the mermithid emerging from its host under water at the oviposition site. Once free, the parasite overwinters in the free-living stage and reproduces the following spring; it's progeny then pursue new (early instar) *Baetis* hosts (Hominick & Welch 1980).

Trout diet analysis

To estimate effects of parasitism on trout predation we captured brook trout by electrofishing and collected their stomach contents by pulsed gastric lavage, a non-lethal method for analyzing fish diets *in situ* (Hartleb and Moring 1995). We removed all *Baetis* from the regurgitate, preserved them in 95% ethanol and later dissected them for parasites. We calculated parasite prevalence for the stomach contents of each fish individually (no. parasitized *Baetis* consumed / total no. *Baetis* consumed). For comparison to stomach contents, we estimated parasite prevalence in *Baetis* available to trout from benthic kick samples collected on the same date and time as trout stomachs. We preserved samples in 95% ethanol and enumerated and dissected all *Baetis* to estimate ambient parasite prevalence for each sampling event. We sampled 2 streams (Rustlers Gulch Creek, Copper Creek) on a total of 9 occasions across 3 years (2014-2016), resulting in 124 trout sampled, of which 54% (N = 67) contained *Baetis* in their stomachs. If trout consume all *Baetis* at random then the proportion of parasitized *Baetis* in stomachs should equal prevalence in the environment. We conducted an ANCOVA on parasite prevalence in trout stomachs to test whether its relationship to ambient parasite prevalence matched the null prediction of a 1:1 relationship.

Larval drift assessment

To test whether parasitism and the presence of trout affected *Baetis* drift propensity we collected *Baetis* in paired drift and benthic samples in fishless (N = 6) and trout (N = 5) streams, preserved all *Baetis* in 95% ethanol, and dissected them for parasites. We collected samples on one date per site in July 2014. We collected drifting organisms in 2-4 drift nets (number based on stream size; 200 μm mesh) divided between two transects (1-2 nets per transect) just after dark

for 10-60 min (at 2100 h MDT, interval sufficient to collect ≅100 *Baetis* based on previous parasite prevalence and drift density estimates). We sampled transects in each stream sequentially to prevent effects of upstream sampling on the downstream transect. Because day drift is low in trout streams (Allan 1987, McIntosh et al. 2002), we collected drift at night to provide a larger sample size for detecting an effect of parasites. Further, methodological studies on best practices for comparing drift across sites have advocated making replicated samples within sites just after dark, as the "first nighttime" sample is highly predictive of drift patterns across the subsequent 24 h (Allan and Russek 1985). We standardized drift estimates by dividing by discharge of water through the net over the sampling interval, resulting in drift density values, which we calculated separately for parasitized and unparasitized *Baetis* (no. drifting per m^3 water; Allan and Russek 1985). We calculated discharge at each sampling transect by multiplying water depth (m), current velocity (m/sec), sampling interval (sec) and net width (0.29 m). The day after taking drift samples, we estimated benthic density by averaging 3 samples collected with a modified Hess sampler (0.104 m^2 per sample) placed on the streambed at random locations within the reach upstream of the drift transects. Finally, we calculated drift propensity by dividing the density of parasitized or unparasitized *Baetis* collected in drift samples by the benthic density measured upstream of the transect to standardize for background abundance (McIntosh et al. 2002, Wilcox et al. 2008). We tested for differences in drift propensity with a linear mixed model with fixed effects of parasitism (Yes/No), fish presence (Yes/No) and their interaction, and a random (blocking) effect of transect nested within site.

Mesocosm Predation Trials: *Baetis* drift and trout feeding behavior

In nature the probability of *Baetis* being consumed by trout integrates the likelihood that it enters the drift with factors that determine its encounters with and selection by trout once it is exposed. Disentangling those mechanistic underpinnings of consumption requires more controlled conditions. We used a streamside flow-through fish observation arena (McIntosh et al. 2002) to release *Baetis* into the drift and then examine relationships among *Baetis* drift behavior, parasitism, trout foraging behavior and probability of predation after *Baetis* were exposed.

We collected *Baetis* for behavior trials from fishless streams and used microscopy to determine visually without dissection whether they were parasitized. We used fishless streams because *Baetis* foraging behavior is permanently altered after exposure to trout (Peckarsky and McIntosh 1998). Because mayflies disperse across streams within the basin, there are no genetic differences in *Baetis* from fish versus fishless streams (Hughes et al. 2003). We collected *Baetis* from the benthos in kick samples, transported them in chilled ambient water to an outdoor shelter and moved individuals by transfer pipette onto opaque circular dishes where they were immobilized using a nonlethal anesthetic. We dripped stream water containing dissolved $NaHCO_3$ (original Alka-Seltzer®; 4 oz water per tablet) into the viewing dish by pipette until the mayfly ceased swimming but respiration continued. Under transmitted light at 40× magnification we used microprobes to flip the *Baetis* on its dorsum. If the *Baetis* was infected by a mermithid parasite the worm could be viewed, coiled within the hemocoel, through the transparent ventral cuticle. We separated *Baetis* that appeared to be infected and uninfected, and held both groups in microcosm tanks continuously fed with natural fishless stream water (tanks are described and illustrated in Peckarsky and Cowan 1991). As a food source, we placed 6 algae-covered tiles in each tank (≈2.25 cm^2 surface area each). The tiles had been left for 2 wk to colonize with

periphyton in adjacent mesocosms through which water was continuously delivered from a natural fishless stream. We left *Baetis* to acclimate in microcosms for ≥8 h before predation assays. When we removed *Baetis* for trials they were visually assessed to choose individuals of comparable size because parasitized *Baetis* tend to be smaller, which decreases trout predation (Allan 1978b, 1981), and we were interested in detecting effects of parasitism independent of size. It was not feasible to measure sizes of live *Baetis* so we assigned them to size categories based on visual inspection (XS, S, M, L, XL). A subsequent test of independence confirmed that the size distribution did not differ between groups ($X^2 = 4.11$, $p = 0.25$).

We captured brook trout (N = 6) by angling and held each one alone without food in the observation arena for 24 h to acclimate prior to the trial. We used each trout in only one trial. The arena held natural stream cobbles arranged to provide areas for the trout to shelter and ambient stream water was continuously pumped through at ≈11 cm sec^{-1} velocity. After acclimation we primed the fish for foraging by pulsing groups of *Baetis* larvae into the current until the fish reacted. We began each trial by alternately introducing individual parasitized and healthy *Baetis* (N = 20 per trial) into the current at the upstream end of the arena by pouring them into a funnel attached to a Tygon® tube that connected to a tube fixed on the floor of the arena. This design simulates the launch of individuals from the substrate, mimicking natural entry into the drift (McIntosh et al. 2002). Observers were positioned behind a blind and watched the *Baetis* until it was consumed, settled on the substrate, or drifted through the outlet of the arena. We measured distance drifted (cm), maximum height reached in the water column (cm), time spent in the drift (sec), and whether the *Baetis* actively swam (Yes/No) as opposed to passively drifting. We simultaneously recorded the reaction distance of the trout (cm) and whether it consumed the *Baetis*. Next we removed the trout from the arena and repeated

observations for the same number of *Baetis* without a predator, which enabled us to analyze both parasite- and trout effects on *Baetis* behavior.

We analyzed drift distance, maximum drift height, and time spent in the drift with linear mixed effects models with fixed effects of parasite (present/absent) and fish (present/absent); date was treated as a random effect to account for background variation in weather conditions that could influence behavior (e.g., temperature, cloud cover), as well as fish foraging differences because each fish was observed at approximately the same time on a different day to minimize effects of diel feeding periodicity. We tested for main and interactive effects of fish and parasitism on whether *Baetis* actively swam (Yes/No) with a generalized linear mixed model with a binomial error structure, also including the random effect of date. To explore how parasitism affected predation in the context of *Baetis* drift behaviors, we used the data from the fish predation trials to construct models predicting fish reaction distance to *Baetis* (log[x]-transformed) and *Baetis* consumption by trout (binomial distributed response) with fixed effects of drift height, drift time, swimming behavior, parasitism, and all interactions between them, with random effect of date. Drift distance was excluded *a priori* due to its correlation with time ($r^2 = 0.60$). However, drift height and time also introduced collinearity (positive correlations among drift time, drift height and active swimming) so we removed them and fit a reduced model with the fixed effects of parasitism, swimming (the most informative variable), and their interaction.

Larval buoyancy test

To refine our mechanistic understanding of parasite effects on *Baetis* behavior in the drift, we measured buoyancy of infected and uninfected *Baetis*. This protocol was intended to

reveal parasite-induced physical changes that could affect involuntary attributes of drifting *Baetis* (i.e., more buoyant organisms may be less able to control, or required to invest more energy in controlling, their voluntary behaviors while drifting in the current). To determine whether the buoyancy of *Baetis* larvae is affected by parasitism we timed the descent of both live and dead *Baetis* larvae through a column of fishless stream water standing in a graduated cylinder. We deposited each live *Baetis* at the water surface with a pipette, timed until it reached the bottom (sec), retrieved and preserved it with a rinse of 2 ml 70% ethanol, then immediately returned it to the water column to obtain a paired settling time for the dead individual. Observers conducted the trials blind to condition. Later we examined each *Baetis* in the laboratory to identify its sex and dissected it to determine if it was parasitized. If we discovered a parasite we separated it from the host and dried (24 h at 60 C°) and weighed (mg) each organism to account for effects of host and parasite mass on settling time. To insure sufficient replicates for this blind trial, we collected *Baetis* from a single fishless site known to have high parasite prevalence (Marmot Creek). We used separate linear models to analyze the effect of parasitism on live settling time (log[x]-transformed), which is a composite of behavioral and biophysical factors, and dead settling time (log[x]-transformed), which reflects the baseline physical traits of the organism without behavioral compensations. Finally, to understand the effect of biomass on buoyancy we used linear models to test the relationship between settling time (live and dead separately) and biomass with parasitism as a covariate. The biomass estimate for parasitized *Baetis* included the combined mass of host + worm. Prior to the analysis, we removed three outlying individuals (2 parasitized, 1 unparasitized) with extremely high settling times (> 40 sec; displayed in Fig. 5B).

Extrapolating from experiments and field data to estimate potential impacts on trout

We combined data from this study (experiments and field survey) with previous studies of trout consumption and *Baetis* behavior to ask how parasite-induced changes to host behavior ultimately affect the probability of parasites being consumed by trout. In addition we extrapolated from existing data to ask how varying parasite prevalence could affect prey available to trout visually feeding during daytime (because nocturnal foraging on *Baetis* low; McIntosh et al. 2002).

Effect of parasitism on probability of consumption by trout. To estimate the effect of parasitism on probability of *Baetis* consumption by trout we combined field drift data with consumption rates measured in the observation arena. From field drift measurements we calculated the instantaneous probability of parasitized and unparasitized *Baetis* drifting in trout streams (*sensu* Elliott 1978). Because drift probability estimates were obtained from nighttime samples whereas consumption estimates were made during the day in the observation arena, we calibrated the drift probabilities to reflect day time drift rates using a night:day drift ratio of 10 for East River *Baetis* (McIntosh et al. 2002), which is also comparable to *Baetis* in similar settings (e.g., Allan 1987). Because drift estimates in microcosm experiments (Chapter 3) suggest that parasitized *Baetis* are at least as nocturnal as unparasitized *Baetis* we used the simplest (and also most conservative) approach of applying the same ratio to both parasitized and unparasitized *Baetis*. We estimated the probability of consumption of parasitized (P) and unparasitized (UP) *Baetis* using the following equations:

[1] $$P(\text{consumption}_P) = (P(\text{drift}_P) / 10) \times P(\text{drift.consumption}_P)$$

[2] $$P(\text{consumption}_{UP}) = (P(\text{drift}_{UP}) / 10) \times P(\text{drift.consumption}_{UP})$$

where P(drift) is the probability of daytime drift and P(drift.consumption) is the probability of being consumed once in the drift (measured in mesocosm assays).

Potential impact of parasitism on prey available to trout. Incorporating experimentally-derived consumption rates with field daytime drift probabilities (as calculated above) and spatially-explicit parameters measured in the field, we extrapolated how parasite-induced decreases in drift could decrease drifting prey density along a gradient of parasite prevalence in a given site, using a back-calculation of Elliott's (1987) equation and allowing drift probability to vary with parasitism:

[3] Drift Density (no./m^3) = [P(parasitism) $\times$ P($drift_P$) + (1-P(parasitism)) $\times$ P($drift_{UP}$)] $\times$ B / D

where the total density of *Baetis* in the daytime drift (no./m^3) at any site is defined by:

P(parasitism)=probability of being parasitized, assumed equal to parasite prevalence and ranging from 0 to 1

P($drift_P$)=instantaneous probability of daytime drift for parasitized *Baetis* calculated from field drift samples as described above

1-P(parasitism)= probability of being unparasitized, assumed equal to (1-parasite prevalence) and ranging from 0 to 1

P($drift_{UP}$)=instantaneous probability of daytime drift for unparasitized *Baetis*, calculated from field drift samples as described above

B=total benthic density of *Baetis* (no./m^2), a spatially-explicit measure made from quantitative benthic samples within each site

D=water depth (m), a spatially-explicit value averaged across 3 measurements in each of 2 transects per site

All analyses were done in R 3.2.4 (The R Foundation for Statistical Computing 2016).

RESULTS

Field Surveys: Trout diets and larval *Baetis* drift

In natural streams brook trout consumed a lower proportion of parasitized *Baetis* than expected if they were consumed in direct proportion to their availability ($F = 34.9$, $p < 0.001$; Fig. 1). Consistent with their infrequency in trout stomachs, our estimates of parasitized *Baetis* in the benthos compared to the drift—which is the source from which trout forage directly—demonstrated that parasitism significantly suppressed nocturnal drift propensity in streams both with and without trout ($X^2 = 32.5$, $p < 0.001$; Fig. 2).

Mesocosm Predation Trials: *Baetis* drift and trout feeding behavior

We found effects of parasites and trout on mechanistic aspects of *Baetis* behavior in the drift. In general trout had a stronger effect on the behavior of parasitized prey but ultimately consumed more unparasitized prey. Trout presence in the channel caused all *Baetis* to shorten their drift distance ($X^2 = 16.9$, $p < 0.001$) and to suppress their drift height ($X^2 = 6.85$, $p = 0.009$; Fig. 3A), but parasitized *Baetis* tended to drift higher in the water column even when trout were present ($X^2 = 3.06$, $p = 0.080$). The time *Baetis* spent drifting before escaping to the substrate also decreased with trout presence ($X^2 = 25.0$, $p < 0.001$; Fig. 3B). Parasitized *Baetis* tended to take longer to settle but that effect was not significant ($X^2 = 1.90$, $p = 0.168$). Our analysis of swimming activity revealed that unparasitized *Baetis* actively swam more than parasitized *Baetis*

in the presence of trout, while unparasitized *Baetis* swam more when trout were absent ($X^2 = 5.48$, $p = 0.019$ for the fish × parasite interaction; Fig. 4A).

We analyzed trout responses to drifting *Baetis*, finding that parasitized *Baetis* only elicited a long-distance reaction from trout (comparable to reactions to unparasitized *Baetis*) if they swam. In contrast, parasitized *Baetis* that did not swim only caused the trout to react at close range ($X^2 = 6.85$, $p = 0.009$). Similarly, we found that swimming, which appears to make *Baetis* more visible to trout, had a positive effect on consumption ($X^2 = 5.30$, $p = 0.021$). Parasitized *Baetis*, which were less likely to swim when trout were present (see above), also tended to be consumed at lower rates ($X^2 = 3.19$, $p = 0.074$; Fig. 4B). These analyses suggest that active swimming is conspicuous and risky, partially explaining why predation rates tended to be lower on parasitized *Baetis*, which were less likely to swim in the presence of trout.

Microcosm Assays: Larval *Baetis* buoyancy tests

Parasitism increased the sinking time of dead *Baetis* larvae (proxy for buoyancy; $F = 14.7$, $p < 0.001$) but had a weaker effect on live *Baetis* ($F = 2.00$, $p = 0.162$), suggesting that voluntary behaviors can at least partially override baseline effects of parasitism on buoyancy. Our analysis of the buoyancy-biomass relationship demonstrated that biomass had a negative effect on buoyancy (i.e., dead sinking time; $F = 12.0$, $p < 0.001$). Parasitized *Baetis* had significantly lower biomass (host + worm combined) than unparasitized *Baetis* ($F = 29.5$, $p < 0.001$) and parasitism did not affect buoyancy when we controlled for biomass (live: $F = .053$, $p = 0.819$, dead: $F = 1.92$, $p = 0.171$; Fig. 5). In addition to decreasing size, parasites may cause some individuals to become aberrantly buoyant even at higher mass. In our full dataset, it appears that slope of negative buoyancy-biomass relationship was shallower for parasitized

Baetis, suggesting that even as they gained mass they remained relatively buoyant (parasite × biomass interaction; $p < 0.001$). This pattern was primarily driven by extreme buoyancy values from parasitized *Baetis* and the slopes of the lines did not differ when these extremes (>40 sec sinking time) were omitted (parasite × biomass interaction; $p = 0.726$). However, those extremes reflect the potential for high variation around the predicted slope of this relationship. Altogether this suggests that parasitism increases buoyancy mainly via indirect effects on mass, although other unknown effects of parasitism are likely to cause deviations from the negative biomass-buoyancy relationship.

Extrapolating from experiments and field data to estimate potential impacts on trout

Effect of parasitism on probability of consumption by trout. From field drift data (N = 11 streams) we estimated the instantaneous probability of daytime drift for parasitized *Baetis* as $P(drift_P) = 1.32 \times 10^{-4} \pm 4.28 \times 10^{-5}$ which is ≈5.5× lower than our estimate for unparasitized *Baetis*, $P(drift_{UP}) = 7.20 \times 10^{-4} \pm 1.22 \times 10^{-4}$. We then used consumption rates from the mesocosm to estimate the probability of parasitized or unparasitized *Baetis* being consumed once in the drift as $P(drift.consumption_P) = 0.238$ and $P(drift.consumption_{UP}) = 0.363$. Using these parameters, we estimated (Equations 1 & 2) that the cumulative probability of a parasitized individual entering the drift and then being consumed by a trout is .0031%, an order of magnitude lower than the estimate of .0261% for uninfected *Baetis*.

Potential impact of parasitism on prey available to trout. Combining experimental and field data to extrapolate effects of parasitism on prey available to trout revealed that drift suppression in parasitized *Baetis* could affect numbers of *Baetis* available to trout in our study area (Fig. A1). Mean parasite prevalence in fish streams of the East River drainage between

2012-2016 was 28% (with minimal interannual variation; Chapter 1). On that basis, we extrapolate that on average parasitism could decrease the density of drifting *Baetis* by 0.2 individuals m^{-3} compared to the low extreme of 0% prevalence. Direct measurements of daytime drift density in East River trout streams estimate average drift density at 0.683 m^{-3} (McIntosh et al. 2002), which aligns well with this extrapolation and suggests that on average trout in this drainage could experience a ≈20% reduction in drifting *Baetis* compared to a scenario without parasites.

DISCUSSION

Studies that connect broad patterns with underlying mechanisms are important for understanding selective forces acting on parasite transmission. Selection should favor parasite traits that increase transmission in the context of predation risk, but the framework for understanding interactions of parasites with predators is limited. By focusing on the predator-prey interaction between trout and *Baetis* mayflies, we developed a detailed understanding of nonconsumptive and consumptive interactions of a top predator with parasitized prey. An integrated view of our findings, observed across multiple scales along a gradient of experimental control and realistic foraging conditions, suggests that: (1) parasitized *Baetis* have a low propensity to engage in risky foraging movements (drift), which corresponds to lower predation rates (trout stomach content analysis); (2) parasitized *Baetis* are capable of perceiving and reacting to predators when exposure is forced (mesocosm behavior assays); however (3) they do not respond to heterogeneity in trout predation risk by increasing exposure in low risk conditions (field drift measurements); (4) low predation rates are also influenced by intrinsic attributes of parasitized *Baetis* that affect conspicuousness (mesocosm behavior assays); (5) via the combined

effect of drift suppression and negative selection by trout, parasitism decreases probability of consumption by an order of magnitude and, in our system, could decrease the drifting density of *Baetis* available to trout by ≈ 20%, creating the potential for bottom-up effects on energy transfer.

Effects of parasitism on risky host behavior

In contrast to the strategy observed in trophically-transmitted parasites (Moore 2002), it should benefit parasites with direct lifecycles to avoid predation because consumption of the host kills the parasite and interrupts its transmission cycle (Fritz 1982). We found parasitized *Baetis* to be highly risk averse, with a lower probability of drifting than healthy *Baetis* even at relatively "safe" times (i.e., night, when predation rates decrease and invertebrate drift is often highest). Our findings were consistent with Vance (1996a), who also reported that parasitism decreased drift captures of *Baetis* in a single trout stream and in microcosms, but this study further advances our understanding by demonstrating that parasites decrease drift propensity of hosts across multiple streams and even in fishless sites.

Tests of behavioral effects of direct lifecycle parasites are rare in the literature, and have shown mixed results. There are reports that infected hosts counterintuitively take more risks, as has been reported for tadpoles, marine mudsnails under certain conditions, and other stream macroinvertebrates (Benton and Pritchard 1990, Lefcort and Blaustein 1995, Williams et al. 2001, Kamiya and Poulin 2012). Those studies have generally interpreted risk-taking either as a disadvantageous side effect (Benton and Pritchard 1990, Williams et al. 2001, Kamiya and Poulin 2012) or as an tradeoff for other potential benefits (Lefcort and Blaustein 1995). Other studies mirror our findings that parasitism decreases risky activity, such as in caterpillars

parasitized by wasp larvae and in mosquitos infected with mermithid or gregarine parasites (Wise de Valdez 2006, 2007, Chen et al. 2017, Soghigian et al. 2017). It has also been acknowledged that predation does not always benefit trophically-transmitted parasites—e.g., if they experience mortality in non-host predators, or if they are consumed before they are ontogenically prepared for that transition. In those contexts, they should benefit from suppressing activity and risk-taking (Parker et al. 2009). For example, *Gammarus* amphipods infected with acanthocephalan parasites decrease activity and increase refuge use early in the infection before the parasite matures to its infective stage, at which point those risk avoidant behaviors reverse (Dianne et al. 2011, 2014). This spectrum of outcomes underscores that selection acts on parasite-induced behaviors in the context of different life history frameworks, which are intricately complex and varied in the parasitic lifestyle (Price 1980). Therefore interpreting behaviors in terms of selective advantage requires understanding their impact on relevant aspects of parasite performance and survival.

Effects of parasitism on predation

Theory suggests that parasite strategies for predation avoidance could be more common than predation facilitation (Parker et al. 2009), but we are not aware of other field studies linking parasite-induced risk avoidance to decreased predation for single-host parasites (but see Chen et al. 2017, Soghigian et al. 2017 for experimental approaches). We show that risk avoidance results in lower predation of parasitized prey in this system and also found trends (in controlled mesocosms observations) that predators are less likely to consume parasitized prey even when they are exposed.

While there are ample reports of parasitism affecting predation, they are dominated by examples of trophically-transmitted parasites manipulating hosts to attract predation (Moore 2002, Lefèvre et al. 2009a, Poulin 2010). Studies of single-host parasites are less common, and their outcomes are less uniform. There are counterintuitive examples in which infected hosts, including mayflies, become less sensitive to risk and increase their exposure to predators, which is hypothesized to be a pathological side-effect of infection (Benton and Pritchard 1990, Lefcort and Blaustein 1995, Williams et al. 2001). In contrast, at least two studies have measured decreased predation as a result risk avoidance, in caterpillars parasitized by wasp larvae and gregarine-infected mosquitoes (Chen et al. 2017, Soghigian et al. 2017). There are also reports of behavioral changes that would appear to benefit parasites by decreasing predation risk, although they could not be linked to any measurable effect on predation rates. For example, mermithid parasites have been shown in some cases to reduce activity of late instar mosquito hosts, but activity changes did not decrease consumption by an invertebrate predator in experiments (Wise de Valdez 2006, 2007). We demonstrate that coherence can be found by linking multiple prey behavior changes to direct predation measures because individual trait changes that would be predicted to have one directional effect on predation may be balanced with other traits for a different net effect.

The disconnects between prey activity and predation underscore that exposure is not the only factor influencing predation rates, and that multiple traits should be considered together. Some parasites change intrinsic attributes that affect their appeal to predators, such as nutritional value (Lefèvre et al. 2009b) or visual conspicuousness (Johnson et al. 2006). We found that parasitized *Baetis* drifted higher in the water column, which alone would be predicted to increase predation by trout (Ware 1972). That fact that we did not observe that outcome could be

explained by other interacting trait changes. Parasitized *Baetis* are smaller in any particular stream (Vance 1996b, Vance and Peckarsky 1996), and smaller size could have contributed to lower predation rates in the field surveys, because trout are highly size selective (Ware 1972, Allan 1981). Closer examination of behavioral interactions in the mesocosm suggests some further mechanistic explanations for decreased consumption of parasitized *Baetis* in the drift. The strongest behavioral trigger for consumption was active swimming by *Baetis*, which caused earlier reactions from trout, suggesting that it made them more visually conspicuous, and ultimately increased the likelihood of being consumed (Ware 1972, Allan 1978b). Although swimming was risky, unparasitized *Baetis* swam more often with trout present; in contrast, parasitized *Baetis* did not modulate their swimming behavior, resulting in similar swimming rates in low- and high-risk environments. Combined with previous reports that mermithids affect mayfly swimming behavior (Benton and Pritchard 1990, Vance 1996b), these observations suggest that parasitism may impose a biomechanical constraint that causes infected *Baetis* to swim rather than drift passively once in the water column. Similar reports that parasitism impairs normal movement of Hymenoptera infected with nematodes (Maeyama et al. 1994). Not every parasite-induced alteration we observed would be expected to reduce predation rates (e.g, drifting higher). Nevertheless that was the net effect likely due to a combination of behavioral changes (swimming) and other morphological changes (size) that contribute to prey selection.

It is increasingly recognized that parasites are commonly preyed upon, and that they could alter food web topology as well as pathways of energy fluxes (Lafferty et al. 2006, Johnson et al. 2010). For example, Sato et al. (2011) found that orthopterans infected with trophically-transmitted nematomorph parasites altered their behavior by entering the stream, generating a significant seasonal prey subsidy for trout. Nevertheless, studies considering the

ecological implications of parasite predation events that do not result in transmission are rare. Although nematode infections are common in aquatic macroinvertebrates there has been no attempt to estimate their direct or indirect contribution to the energy budgets of predators. Our extrapolation suggests that behavior changes induced by parasitism could moderately decrease *Baetis* drift (≈ 20%). Because *Baetis* are common prey items that trout tend to consume in proportion to their drift abundance (Allan 1981), this level of drift suppression could negatively affect energy available to trout and decrease the transfer of *Baetis* biomass to higher trophic levels.

Costs of activity

In the field we found that parasites suppressed *Baetis* drift propensity even in low risk conditions. When risk-taking is suppressed, foraging and growth opportunities may be sacrificed as a result (Peacor and Werner 2000). Therefore decisions about foraging and exposure should be sensitive to the degree of risk in the environment, as increasing foraging during low-risk times can offer significant fitness gains (Lima and Bednekoff 1999). Accordingly, although parasites should manipulate host behavior to reduce their own death rate, theory further predicts that host modification will be proportional to the intensity of predation on the infected host (Fritz 1982). Accordingly, Vance (1996a) reasoned that parasitized *Baetis* drifted less in microcosms (and therefore found fewer high quality food patches) because parasitism increased their vulnerability to predation once they entered the drift. If this were the case we would expect to observe trout selectively consuming parasitized *Baetis* from the drift, and also expect trout presence to induce risk avoidance. In contrast, we found that parasitized *Baetis* were less often consumed while

drifting, and that drift is similarly low for parasitized *Baetis* in fish and fishless streams, indicating that parasitized mayflies express a risk-averse phenotype even when risk is low.

We have demonstrated that parasitized *Baetis* do show behavioral responses to trout while they drift, suggesting that they are able to detect and react to chemical cues of predators. Therefore it is unlikely that drift suppression reflects a pathological constraint or a fixed risk-avoidant trait resulting from an inability to gather information about risk in the environment. Regardless of the mechanistic causes of risk avoidance in infected *Baetis*, it should be favorable to the parasite to decrease mortality due to predation, and could have benefits even when predators are absent.

One or more of the following mechanisms explain why it could be adaptive for parasites to suppress drift in *Baetis* even when predation risk is low: (1) parasite fecundity is not limited by host foraging movements, (2) parasitism increases costs of drift behavior other than predation risk, or (3) parasites and hosts exhibit different risk allocation patterns across the larval period.

First, it is unclear how suppression of host foraging behavior ultimately affects energy acquired by parasites, but seems reasonable that energy gains and costs related to foraging movements are different for the "extended phenotype" of the parasite than for an uninfected host. In the case of *Baetis*, drift is assumed to reflect foraging, but it is not an absolute requirement for food consumption. In fact, some studies have shown a lack of correlation between drift, benthic feeding activity and gut fullness (e.g., Allan et al. 1991), suggesting that *Baetis* may be able to forage continuously without drifting if the quality of food patches is sufficient (Hernandez and Peckarsky 2014). Direct measurements linking the activity of parasitized animals to food consumption are rare and report different outcomes, supporting the hypothesis that parasite fecundity may not be limited by host foraging movements (Strickland 1911, Kamiya and Poulin

2012, Chen et al. 2017). Furthermore, even if infected hosts acquire less food, reduced foraging may not translate into a decline in growth rates for parasites; indeed, we have found no relationship between host size and parasite size in this system (Chapter 2). Studies in mosquitos have also reported that, in some cases, conversion efficiency of their parasites increases even as host foraging decreases, resulting in no decrease in parasite growth (Giblin and Platzer 1985). Understanding the direct effect of altered foraging behavior on food consumption and parasite fitness would require integrated measurements of host behavior, grazing impact and parasite growth under controlled conditions (Chapter 3).

A second, but not mutually exclusive, explanation for risk avoidance in parasitized *Baetis* is that drift movements—including entering, navigating, and exiting flowing water—register excess costs not paid by healthy larvae. Given the high ratio of parasite:host body size typical for parasitic castrators and observed in this system, bio-mechanical or -physical effects due to parasitism would be unsurprising (Lafferty and Kuris 2002; Chapter 3). Detailed observations determined that parasitism significantly increases the buoyancy of *Baetis*, and passively drifting *Baetis* float higher in the water column (Vance 1996b), which is a disadvantageous trait when trout are present. Even without predators, more buoyant individuals would likely invest more energy in escaping the drift. To maintain viable hosts mermithids should minimize such indirect costs, especially since the direct cost of parasitism on host resources is high (Jutsum and Goldsworthy 1974, Lettini and Sukhdeo 2010). There is evidence that parasitized insects decrease movement or tailor their movements for energy-saving. For example, *Ameletus* mayflies infected with mermithids rarely travelled against the current, which was a common behavior for unparasitized mayflies (Benton and Pritchard 1990). Similarly, bumblebees infested with parasitoid larvae visit flowers that are easier to access (Schmid-Hempel and Schmid-

Hempel 1990, Schmid-Hempel and Müller 1991). Behavioral risk avoidance in parasitized *Baetis* may not only benefit parasites by reducing trout predation, but also by reducing costs of parasite-induced morphological changes, further underscoring the multidimensionality of those modifications.

Finally, decreased activity of infected *Baetis* may be viewed as a parasite risk allocation strategy that differs from its host's. *Baetis* optimizes fitness by accepting some proximal risks of foraging at the larval stage but decreasing cumulative time spent in the dangerous environment, emerging earlier and with lower fecundity when trout are present (Peckarsky et al. 2001, 2002). In contrast, mermithid parasitism markedly extends the larval period in both fish and fishless streams (Vance and Peckarsky 1996, Chapter 2). Extending host lifespan is an advantage selecting for host castration, because reallocating energy away from host reproduction toward somatic growth can increase the viability of the host, prolonging the parasite's opportunity to assimilate host resources before reproducing or transmitting (Baudoin 1975, Hall et al. 2007). This may be critical for parasites like *Gasteromermis* whose sole nutrition is obtained from the host because they cannot feed exogenously (Hominick and Welch 1980). As discussed above, parasitized *Baetis* may experience costs of activity even without predators. Therefore the parasite could benefit from accepting short term foraging losses while mitigating movement costs and protecting growth opportunities within the host.

Conclusion and Implications

In summary, through observations of predator-prey-parasite interactions at multiple scales (natural streams, mesocosms and microcosms) we integrated natural patterns of consumption of parasitized prey with controlled predation assays that mechanistically linked

consumption to behavioral and physical prey traits. We conclude that parasitism decreases consumption of *Baetis* by trout through multiple trait changes to the host. First, infected *Baetis* have a lower propensity to enter the drift and become exposed to predators. Further, they are less likely to be consumed by trout once exposed, partly explained by behavior differences due to parasitism (parasitized *Baetis* were less likely to exhibit conspicuous swimming behavior), and in nature also likely due to smaller size. Size differences also contribute to the higher buoyancy of parasitized *Baetis*—a trait that may increase the energetic cost of swimming in or escaping from the water column once drifting. Further understanding of the relationships between host foraging activity and parasite growth or fecundity would reveal how selection shapes parasite effects on host predator avoidance–foraging tradeoffs.

Baetis are locally common and abundant mayflies as well as strong food web interactors, suggesting the potential for its parasites to affect not only individual behavior, but also higher order processes in streams. In theory a predator that has high levels of predation and shows a partial preference for unparasitized hosts should increase parasite-induced suppression of the host population by disproportionately removing healthy, reproductive individuals (Colfer and Rosenheim 2001). Therefore, this trout-mayfly-parasite interaction could partially explain higher mortality of *Baetis* observed in fish streams (Peckarsky et al. 2008). Parasite effects have not been considered in demographic models of *Baetis* (e.g., McPeek and Peckarsky 1998), although this inclusion would enable a more complete view of *Baetis* population dynamics. In addition to the bottom-up effects considered here, the negative effect of parasitism on foraging movements could also affect ecosystem processes by decreasing algae suppression by *Baetis* (Chapter 3), which are the most efficient grazers in the streams they inhabit (Alvarez and Peckarsky 2005).

Further studies could inform this hypothesis by examining quantitative links between host activity and grazing to enhance our understanding of top-down effects of parasites.

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FIGURES

Figure 1. Brook trout consumed fewer parasitized *Baetis* than would be expected if they were eaten in direct proportion to their availability (dashed 1:1 line), estimated from benthic samples ($p < 0.001$). Parasite prevalence in consumed prey (mean ± SE) was estimated as (no. parasitized *Baetis* consumed / total no. *Baetis* consumed) from stomach contents of individual trout (N = 67) sampled from 2 streams over 3 years. Parasite prevalence in available prey (no. parasitized *Baetis* / total no. *Baetis*) was measured in one benthic kick sample collected on the same date and time as trout stomachs.

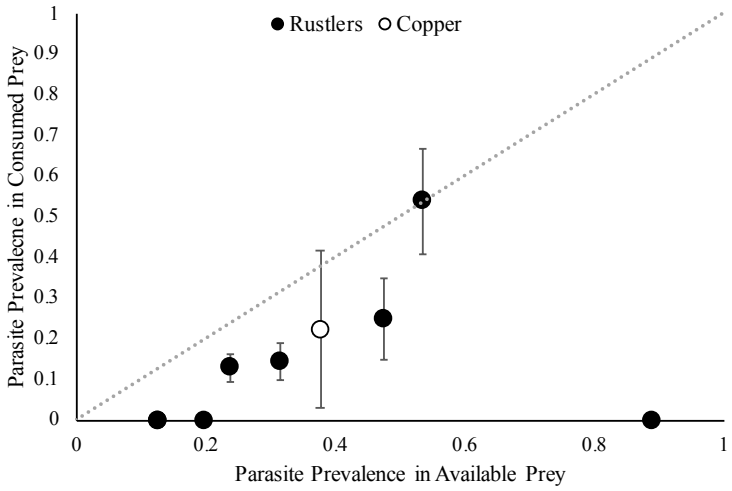


Figure 2. Parasitism suppressed *Baetis* drift propensity (no. *Baetis* drifting m^{-3} / no. *Baetis* m^{-2} benthos; mean ± SE) in streams both with (N = 5) and without (N = 6) trout ($p < 0.001$) in the East River drainage, CO, USA. Drift was measured between 2100-2300h MDT in July 2015.

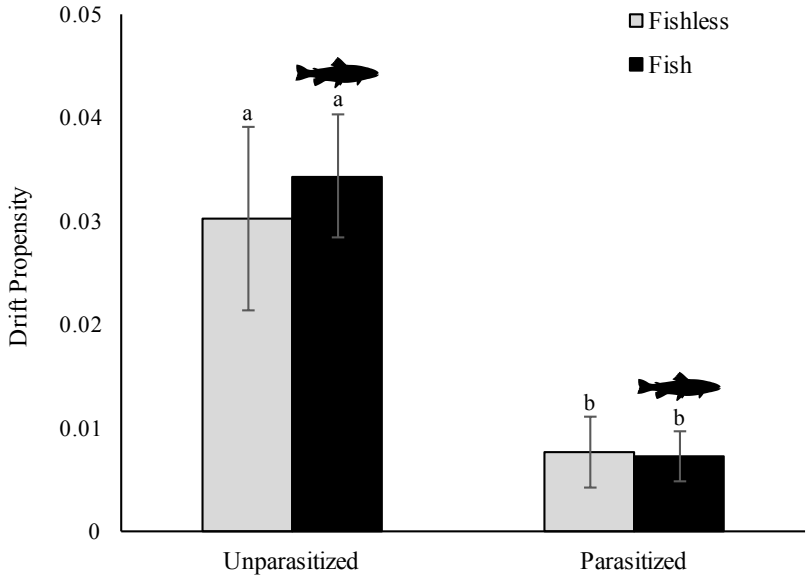


Figure 3. In mesocosm predation trials **(A)** parasitized *Baetis* tended to drift higher in the water column (mean ± SE; $p = .056$), but when trout were present all *Baetis* decreased drift height (mean ± SE; $p = .009$); and **(B)** trout also caused *Baetis* to exit the drift more quickly ($p = .002$). Data were obtained by individually introducing parasitized or unparasitized *Baetis* into the drift of a flow-through observation arena (*sensu* McIntosh et al. 2002) while observers measured behaviors both in the presence and absence of a brook trout in the channel.

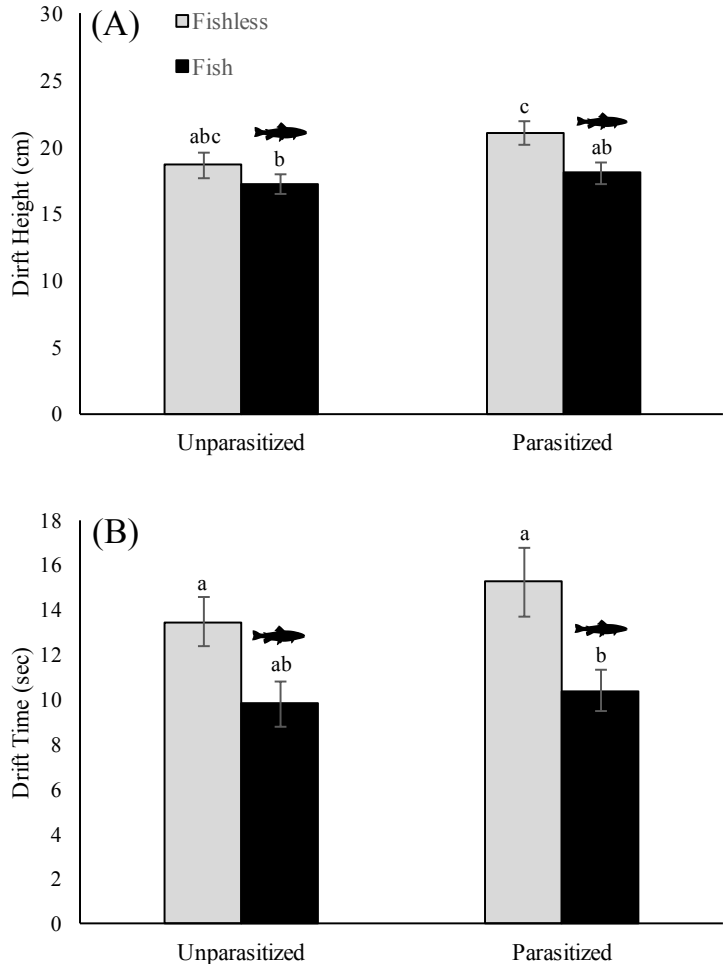


Figure 4. (A) When *Baetis* were introduced directly into the drift of a flow-through mesocosm, more parasitized *Baetis* swam when trout were absent, but more unparasitized *Baetis* swam when trout were present (fish × parasite interaction, $p = .019$). Swimming increased likelihood of being consumed from the drift ($p = .021$), potentially explaining why **(B)** trout consumed a higher proportion of unparasitized *Baetis* ($p = 0.074$). Behavior data were obtained by individually introducing parasitized or unparasitized *Baetis* into an observation channel with and without brook trout (*sensu* McIntosh et al. 2002) while observers measured behaviors and consumption rates.

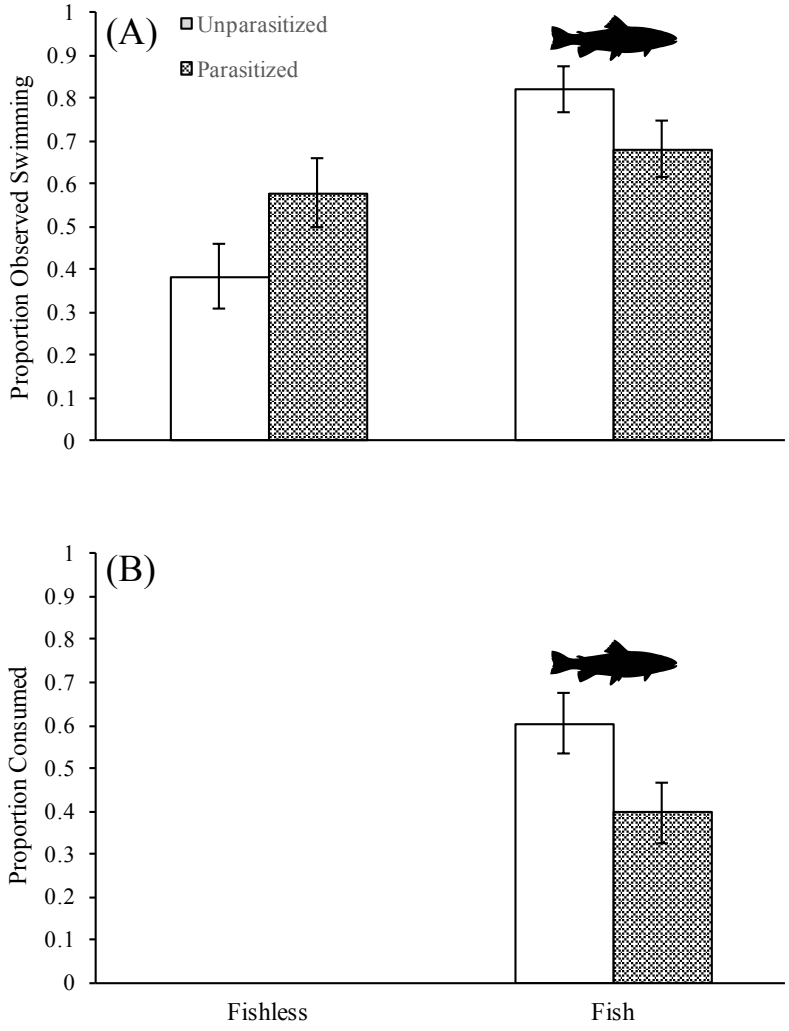
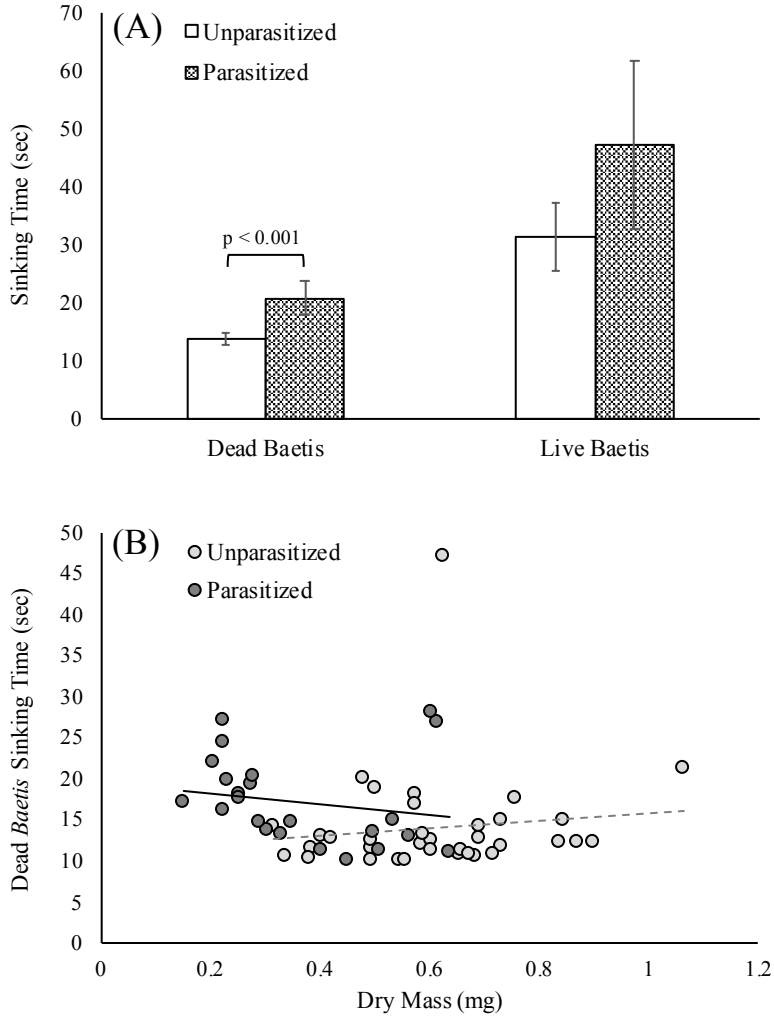


Figure 5. (A) Parasitism increased the sinking time (mean ± SE) of *Baetis* larvae in a still water column. **(B)** Examining relationships between *Baetis* dry mass and sinking time revealed outliers (> 40 sec sinking time) that but were excluded from analysis but remain displayed here. Biomass had a negative effect on sinking time for dead *Baetis* (proxy for buoyancy; $p < 0.001$; solid line), suggesting that the significantly lower biomass of parasitized *Baetis* ($p < 0.001$) contributes to their higher buoyancy.



APPENDIX

Table A1. Summary of linear mixed model analysis of drift propensity (drift density / benthic density) of *Baetis bicaudatus* larvae in streams of the upper East River drainage basin, CO collected across 11 sites in July 2014; all samples were taken at 2100h MDT. Model included fixed effects of fish (present or absent in stream) and *Baetis* parasitic infection status (yes / no), with a random effect of sample transect nested within site.

Analysis	X^2	*p*
Drift Propensity		
Fish	0.07	0.787
Parasitized	32.5	<0.001
Fish × Parasitized	0.19	0.659

Table A2. Summary of linear mixed models used to analyze the behavior of *Baetis* mayfly larvae in an observation arena with or without parasitic infection and in the presence and absence of predatory brook trout and. Responses include distance drifted in the water column (cm), maximum drift height reached in the water column (cm), and exposure time spent in the drift. Date was included as a random effect in all models to account for background variation in weather conditions that could influence behavior (e.g., temperature, cloud cover), as well as fish foraging differences because each fish was observed at approximately the same time on a different day to minimize effects of diel feeding periodicity. We also tested for main and interactive effects of fish and parasitism on whether *Baetis* actively swam (Yes/No) with a generalized linear mixed model with a binomial error structure, also including the random effect of date.

Analysis	X^2	*p*
Drift Distance		
Fish	16.9	<0.001
Parasitized	2.43	0.118
Fish × Parasitized	0.05	0.824
Maximum Drift Height		
Fish	6.85	0.009
Parasitized	3.06	0.080
Fish × Parasitized	0.77	0.382
Drift Exposure Time		
Fish	25.0	<0.001
Parasitized	1.90	0.168
Fish × Parasitized	0.08	0.779
Baetis Swimming (Yes/No)		
Fish	5.36	0.021
Parasitized	0.01	0.928
Fish × Parasitized	5.48	0.019

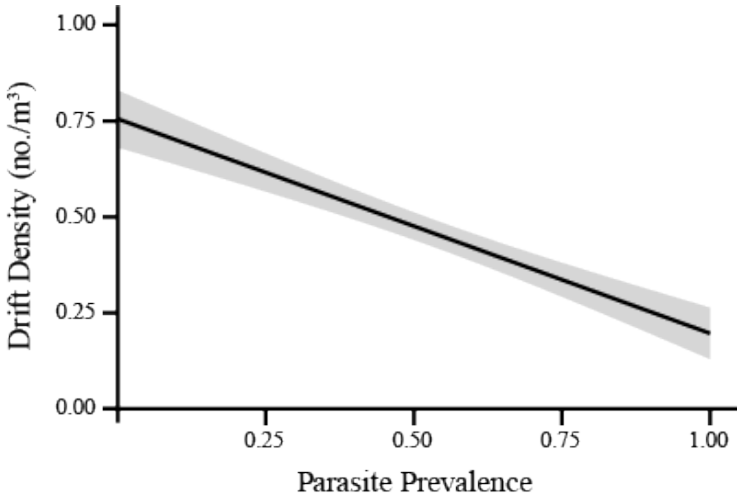
Table A3. Summary of analyses (linear mixed-effects models) of predatory brook trout behavior, recorded from a fish observation arena in which trout were presented with *Baetis* mayfly larvae that either swam or did not swim while exposed in the drift, and did or did have parasitic infection. Responses were fish reaction distance to *Baetis* (cm; log[x]-transformed) and *Baetis* consumption by trout (binomial distributed response) with fixed effects of swimming and parasitism and date included as a random variable.

Analysis	X^2	*p*
Reaction Distance		
Swimming	0.19	0.664
Parasitized	0.06	0.799
Swimming × Parasitized	6.85	0.019
Consumption of *Baetis*		
Swimming	5.30	0.021
Parasitized	3.19	0.074
Swimming × Parasitized	2.15	0.143

Table A4. As a proxy for buoyancy we timed the descent of both live and dead *Baetis* larvae through a column of fishless stream water standing in a graduated cylinder. Sinking times were analyzed with linear models with parasitic infection status (yes/no) and total biomass (host + parasite) as predictors.

Analysis	*F*	*p*
Live Sinking Time		
Parasitized	0.05	.819
Total Biomass	2.70	0.106
Parasitized × Host Biomass	1.56	0.217
Dead Sinking Time		
Parasitized	1.92	.171
Total Biomass	12	<0.001
Parasitized × Host Biomass	0.12	0.726

Figure A1. Hypothesized effects of increasing parasitism on drifting prey available to trout during daytime (mean ± 95% CI), extrapolated from calculations of drift probabilities made from field measurements in the East River, CO, USA. Because parasitism decreases the instantaneous probability of drift, the availability of drifting prey is predicted to decrease as parasitism increases. This outcome implies that average parasite prevalence in our study area (≈ 28%) could reduce the density of drifting *Baetis* by ≈20% compared to a scenario without parasites (see Results).



2013 Pilot Microbial Analysis Summary
Coauthor: Andrea Landiera Debarca

Background. All organisms live associated with bacteria, and symbiotic associations between different organisms are of great importance for evolutionary and ecological processes. Bacteria are particularly valuable symbiotic partners owing to their huge diversity of biochemical pathways that may open entirely new ecological niches for higher organisms. Different types of associations (e.g., ingestion, contribution of exoenzymes, incubation, parasitism) are reported to occur between gut microbes and aquatic invertebrates, and it is clear that gut bacterial communities cannot be treated as single functional entities, but that individual populations require examination. In addition, gut microbes may be either ingested transients or residents, the presence of which have different implications for the invertebrate. Quite a number of authors report the physiological properties of gut microbes (including enzyme activities and attributes such as nitrogen fixation), while less attention has been given to consideration of the colonization sites within the digestive tract, the density and turnover of gut bacteria, and the factors affecting the presence and nature of gut microflora. Furthermore, the indigenous gut bacteria play a role in withstanding the colonization of the gut by non-indigenous species including pathogens. The objectives of this analysis were to 1) document the microorganisms that live associated with *Baetis bicaudatus* larvae by measuring density and community composition of external and gut microorganisms; 2) compare microbial communities of healthy *Baetis* larvae to those infected by *Gasteromermis* sp. endoparasitic nematodes;

Methods. Baetis larvae were collected from Marmot Creek on 12 and 23 July 2013 and acclimatized for 72 h in microcosm chambers supplied with fishless water in BLPs weatherport. *Baetis* were euthanized by dripping in warmed water, then dissected with EtOH-sterilized tools. Males were not dissected because they are not expected to contain parasites. We sampled external microbiota with PBS-washes and plated all host (+ parasite when present) tissue to culture internal microbes. To analyze the density and community of bacteria in *Baetis*, we cultured bacteria in PCA agar and incubated in a chamber at 18-23ºC (because *Baetis* is poikilothermic). Following incubation, we counted the number of CFU, described morphotypes and calculated morph-frequency. We calculated Bray-Curtis similarity percentages (SIMPER) and analyzed similarity (ANOSIM) of microbial communities of infected and uninfected *Baetis*.

Results. Bacterial load tended to be lower in unparasitized *Baetis* ($U = 2877$; $p = 0.067$; Fig. A2). We found significant differences in community frequency ($p = 0.032$; Fig. A3) and marginal differences between groups in the community presence/absence data ($p = 0.08$). Similarity percentages within parasitized *Baetis* were lower than unparasitized (Fig. A4).

Discussion. Higher bacterial load could occur in parasitized *Baetis* if its immune system is depressed, potentially resulting in colonization and establishment of microbial infections. Lower community similarity among parasitized *Baetis* could be caused by different microbes associated with the worm.

Figure A2. Bacterial density is marginally elevated in parasitized *Baetis* larvae.

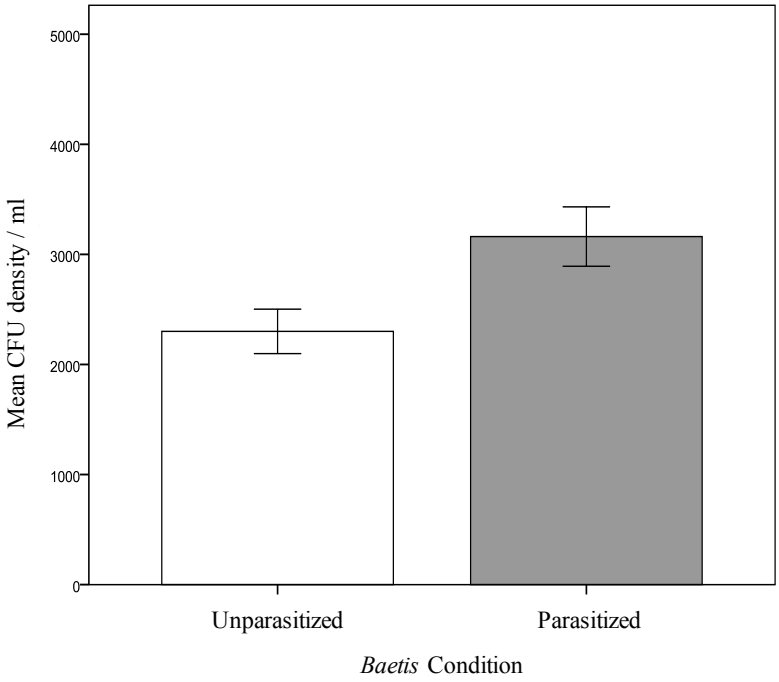


Figure A3. Microbial community frequency differed between (A) unparasitized and (B) parasitized *Baetis* larvae.

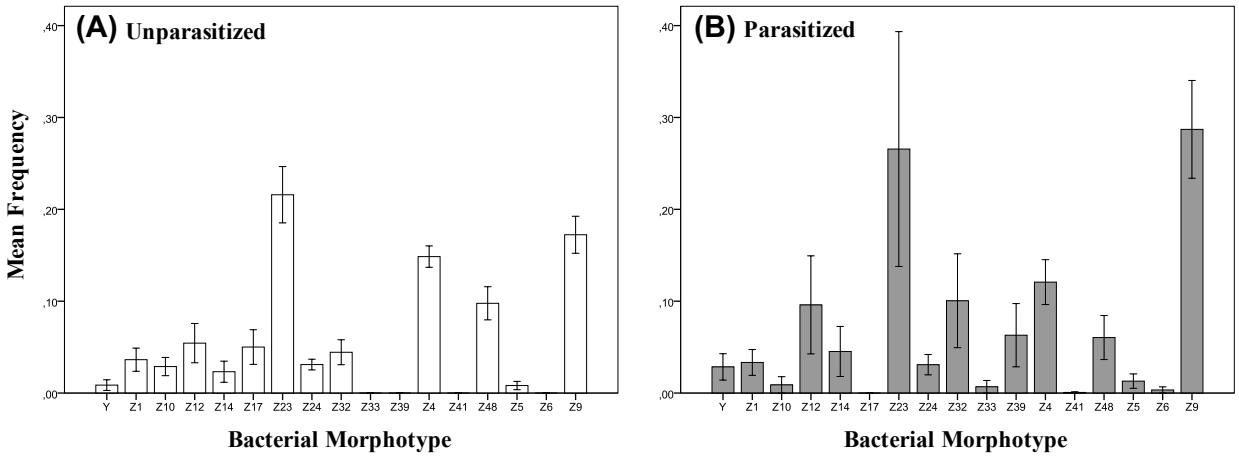


Figure A4. Community similarity among parasitized *Baetis* was lower than among unparasitized *Baetis*.