EFFECTS OF TREMATODE PARASITES ON HABITAT USE AND DISTURBANCE RESPONSE OF FRESHWATER SNAILS IN THE GENUS *ELIMIA*

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EFFECTS OF TREMATODE PARASITES ON HABITAT USE AND DISTURBANCE RESPONSE OF FRESHWATER SNAILS IN THE GENUS *ELIMIA*

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DISSERTATION ABSTRACT

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Freshwater snails in the genus *Elimia* are abundant and important grazers in many southeastern USA streams; in addition, *Elimia* serves as intermediate hosts to many species of parasitic trematodes and is frequently subject to disturbance from floods. Previous work has shown that parasites can increase *Elimia* feeding and reduce reproduction; however, the prevalence of trematode infections within *Elimia* as well as the relationships among parasitism, snail habitat use, and response to disturbance are unknown. I quantified *Elimia* density, trematode prevalence, and physicochemical habitat conditions, both in wet and dry years, within 7 stream sites in Alabama. All snail populations sampled were parasitized by trematodes to some degree (1–26%). Patterns among streams showed that adult snail density was negatively related to prevalence and percent shade, and was positively related to mean current velocity.

Surveys demonstrated that within-streams, parasite prevalence was positively correlated with snail density and stream substrate size; however, it is unknown whether these patterns resulted from parasites altering habitat use by snails, or because infection rates were higher in these habitats for other reasons. I used in situ enclosures with 2 snail density and 2 substrate size treatments to test if substrate size and snail density affected parasite prevalence and growth of *Elimia flava*. Compared with their contrasting treatment, the higher snail density and larger substrate treatments both showed significantly higher prevalence. In addition, snail growth was significantly lower in the high- (vs. low-) density treatment. There was no effect of parasite prevalence on snail growth.

Last, I used artificial streams to investigate how parasitism, snail size, substrate type, and snail behavior influenced the likelihood of snail dislodgment from substrates during high flows. I placed *Elimia flava* in artificial streams containing tile or gravel substrates, and then exposed snails to progressively increasing flow velocities. Earlier survey results indicated lower snail density and trematode prevalence in high- (vs. low-) flow years. Artificial stream experiments indicated that snails with high parasite loads dislodged faster than snails with low parasite loads or lacking parasites.

Given the widespread distribution of trematodes within *Elimia* populations and the potential for trematodes to influence snail density both within and between stream sites, trematodes appear to have an important influence on population dynamics of their host snails in many southeastern streams. Parasitic trematodes do not appear to alter host behavior as suggested by others, but, because risk of infection is related to substrate type, snails may potentially alter their behavior to minimize risk of parasitism.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The harsh-benign hypothesis (Peckarsky 1983) predicts that a given system's disturbance regime will drive which forces influence community structure. Systems with relatively low or infrequent disturbance will be structured by biotic factors such as predation and competition, whereas systems with more frequent or more severe disturbance will be structured by abiotic factors such as flow and scour. In streams, disturbances such as floods can vary greatly in frequency and severity from year to year. Because of this variation, both abiotic and biotic forces may alternate in their importance structuring stream communities over time. Therefore, it is necessary to quantify the interplay between biotic and abiotic factors to understand separate and interactive influences of these factors on communities.

In many communities, predation is a biotic factor that greatly affects prey habitat use, foraging (Turner et al. 2000), and reproductive success (Fraser and Gilliam 1992). Parasites occupy the same position in a functional food web as predators, and thus can exact similar impacts on their hosts as predators on prey. Digenean trematodes are a widespread group of parasitic flatworms that use snails almost exclusively as first intermediate hosts (Schmidt and Roberts 1996). Trematodes have a complex life cycle involving several host species, with a vertebrate usually serving as the definitive host. Snails become infected when they ingest eggs from the adult worm or by miracidia, which hatch from eggs and then penetrate the snail. Several larval stages generally

develop within snails including sporocysts, redia, and cercaria, with the later stage exiting the snail and infecting the next host (Schmidt and Roberts 1996).

Freshwater snails are ubiquitous throughout the southeastern United States. The Family Pleuroceridae contains >150 species, many of which are in the genus *Elimia* and are subject to low predation because of hard noncrushable shells. Snails in this genus can reach densities of 500 snails /m^2 (Dazo 1965) and are the primary grazers in many streams, exerting strong effects on algal biomass and composition (Tuchman and Stevenson 1991, see review Feminella and Hawkins 1995). *Elimia* also may affect benthic communities by limiting food resources in streams, and thus negatively affecting other grazers (Hill 1992). Large infections of trematodes have been documented in 2 populations of *Elimia* in the southeastern US (Richardson and Scheiring 1994, Lenertz 2002).

To understand and ultimately predict the potential impacts of trematodes on their hosts, and thus stream communities, it is important to 1) quantify the prevalence of trematode infection in their hosts, and 2) identify which environmental factors influence trematode infection within consumers. Several studies have documented the presence of larval trematodes in *Elima* (Richardson and Scheiring 1994, Lenertz 2002), although the prevalence of infection among several populations and species, and the degree to which infection relates to habitat conditions in streams, are unknown. In marine systems, trematode infection is often highly spatially variable (Lafferty et al. 1994, Smith 2001). For example, in mangrove systems infection rate is strongly related to the number of definitive hosts depositing feces (i.e., parasite source) in an area (Smith 2001). However, in streams, the input of infective material may not be as important as physical properties

such as stream current velocity and substrate composition, which may affect the dispersal of infective stages (e.g., miracidium) and thus influence their ability to infect potential hosts. Richardson and Scheiring (1994) found that the riffle-dwelling snail *Elimia clara* showed lower parasite loads than *E. cahawbensis,* a species that occurred equally in riffles and pools. Richardson and Scheiring (1994) hypothesized that, because of high flow rates, stream riffles may serve as a physical refuge from trematode infection.

High spatial heterogeneity of trematode infection within host snails also may result from direct alteration of host snail habitat use by parasites. For example, trematodes may cause host behavioral modifications, inducing hosts to move to habitats that increase the likelihood of parasite transmission to the next host (reviewed by Poulin 1995, see also Levri 1999). Jokela and Lively (1995) found that snails infected with the trematode *Microphallus* spp. were more prevalent in shallow shore-bank habitat where snails were more accessible to waterfowl, the definitive host. Conversely, Shinagawa et al. (1999) found that snails infected with several species of trematodes selected deep-water habitats where emerging cercaria could more easily swim to the next host, usually a fish. Shinagawa et al. (1999) hypothesized that habitat shifts by snails may not be adaptive for the parasite, but rather represent either side effects of infection or shifts in resource use. An alternative explanation for the spatial heterogeneity of trematode infection is that differences in trematode prevalence result from differential mortality of infected snails. Parasitized marine snails had a higher mortality rate under low dissolved oxygen concentrations than uninfected snails (Sousa and Gleason 1989). Thus, it is possible that in stressful environments parasitized snails will decrease in abundance because they have a higher mortality rate than unparasitized snails.

Stream populations, including pleurocerid snails, or communities also can be influenced by abiotic disturbance, often in the form of floods. Scour and substrate disturbance during floods can result in decreased density and species abundance of benthic organisms (McCabe and Gotelli 2000). Holomuzki and Biggs (1999) demonstrated that frequency of floods affects snail distribution and can greatly reduce snail density. Whereas disturbance may release an individual organism from the pressures of competition, predation, and future parasitic infection, it does not release it from the effects of existing parasitic infections. Parasites deplete host resources for their own development, which can lead to reduced growth (Gerard and Theron 1996) and castration (Krist and Lively 1998, Lenertz 2002) of the host snail. Parasitic infection also can increase host mortality in stressful conditions (anoxia, Sousa and Gleason 1989). Therefore, it is possible that parasitism affects vulnerability of snails to flow disturbances. Last, if trematodes exert strong direct or indirect effects on *Elimia* habitat use and response to disturbance, then trematodes may play a key role in structuring stream communities under a variety of disturbance regimes.

CHAPTER ONE:

ENVIRONMENTAL FACTORS INFLUENCING ABUNDANCE OF SNAILS IN THE GENUS *ELIMIA* (PLEUROCERIDAE) AND THEIR TREMATODE PARASITES IN ALABAMA STREAMS, USA

INTRODUCTION

Freshwater snails are ubiquitous throughout the southeastern United States. In particular, the family Pleuroceridae contains >150 species, most of which are in the genus *Elimia*. Pleurocerid snails can reach densities of 500 snails m^{-2} (Dazo1965) and are the primary grazers in many streams, exerting strong effects on algal biomass, productivity, and species composition (Steinman et al. 1987a, b, Tuchman and Stevenson 1991, Feminella and Hawkins 1995). In this context, *Elimia* and other pleurocerids may influence benthic communities by limiting food resources and thus negatively affecting other stream grazers (Furnish and Hawkins 1987, Hill 1992). Given the abundance and role of pleurocerid snails in stream communities, it is important to understand the degree to which environmental factors, both biotic and abiotic, contribute to variation in snail abundance and distribution, both within and among streams.

Predation is an important biotic factor that may affect the abundance and distribution of prey directly by consumption (Flecker 1984, Eggleston et al. 1997) or indirectly by altering prey behavior (i.e., habitat use, foraging; Gotceitas and Colgan

1990, McIntosh and Townsend 1994, Turner et al. 2000), and reproductive success (Fraser and Gilliam 1992, Dorn and Mittelbach 2004). Parasitism also may have pronounced effects on host organisms. Unlike predators, parasites typically do not kill their host (Jokela et al. 1999), although, like predators, parasites occupy the same trophic level and consume valuable prey resources (host tissues). Parasites can alter host behavior and distribution (Bakker et al. 1997, Damsgaard et al. 2005, Gourbal et al. 2001) and limit host reproductive success (Brockerhoff 2004, Newey and Thirgood 2004) in a manner similar to the way predators affect prey.

Production of trematode larval stages within their snail hosts represents a significant drain on snail resources, with infection impacting both host reproduction and behavior. Trematodes can affect host reproductive success by destroying host gonadal tissue (castration) chemically, where trematodes interfere metabolically with gonadogenesis (Cheng et al. 1973) or mechanically, when trematodes directly consume gonadal tissue (Probst and Kube 1999). Castration has been demonstrated both in *Elimia flava,* where infected snails showed decreased gonadal development (Lenertz 2002), and in *E. livescens*, where infected snails were no longer able to reproduce (Krist 2001). Parsite-induced modification of host behavior may occur when trematodes cause host movement to habitats that increase the likelihood of parasite transmission to the next host (Levri 1999, also see review Poulin 1995). Jokela and Lively (1995) found that *Potamopyrgus antipodarum* infected with *Microphallus* spp. were more prevalent in shallow shore-bank habitat than uninfected snails, and thus were more accessible to waterfowl, the definitive host. Conversely, Shinagawa et al. (1999) reported that *Semiulcospira libertina* infected with several trematode species, were more likely than

uninfected snails to select deep-water habitats, where emerging cercaria could more easily access their next host, usually a fish. Given their ability to alter both snail reproduction and habitat use, trematodes may have the potential to influence the abundance and microdistribution of *Elimia.*

Like other organisms, pleurocerid snails persist only in environments for which they are physiologically adapted. Thus, physicochemical habitat variables such as substrate type and dissolved oxygen (DO), may be the ultimate abiotic factors influencing snail abundance and microdistribution, whereas biotic factors, such as parasitism, only modify snail distributions within the abiotic templet (Southwood 1977, Townsend et al. 1997). Therefore, studies of the influence of parasitism on snail distribution also must include measurement of abiotic habitat variables that underlie the basic environmental conditions necessary to parasites and their host snails.

Large infections of digenetic trematodes have been documented in 2 populations of *Elimia* in the southeastern US (Richardson and Scheiring 1994, Lenertz 2002), although the prevalence of infection among different populations and species and the degree to which infection is associated with host habitat conditions, are unknown. The objectives of my study were to 1) determine the extent of trematode infection within several populations of *Elimia*, 2), quantify the relationships between snail and parasite abundance both within and among streams and stream microhabitats, and 3) quantify the degree to which key abiotic factors are associated with snail abundance and trematode parasitism among snail populations.

MATERIALS AND METHODS

Study Sites

I quantified snail density, their trematode parasites, and several physicochemical variables in 7 stream reaches within 6 streams and 3 river drainages within Alabama, USA (Table 1-1). I selected these sites because preliminary data showed a wide range of parasite prevalence, as indicated by percentage of snails parasitized among sites. All sites were in predominantly forested catchments composed mostly of pine and mixed hardwoods, with many sites either in or adjacent to National Forests (NF). Stream parent geology, which can influence *Elimia* abundance (Huryn et al. 1995), also varied across sites, ranging from primarily alluvium to primarily limestone, phyllite, or schist (Szabo 1988).

Flannagin and Hagood creeks (FL and HAG, respectively; Black Warrior Catchment) are limestone streams within the Bankhead NF, with substrates consisting of exposed bedrock as well as sand and cobble. Geologies at Choccolocco and Hatchet creeks (CL and HAT, respectively; Coosa Drainage) are predominantly phyllite, and chert, phyllite, and schist (Szabo 1988), respectively, with substrate composed mostly of exposed bedrock and cobble. Both streams border the Talladega NF. Opintlocco Creek (OPT, Tallapoosa River Catchment) is primarily alluvial sand and gravel. I selected 2 stream sites on Choctafaula Creek (CFa and CFb, near and within Tuskegee NF, respectively; Tallapoosa River Catchment) because of their contrasting geology (schist and greenstone bedrock vs. alluvial respectively; Szabo1988) and differences in prevalence of snail parasitism (26 and 1%, for CFa and CFb, respectively). CFa was

Table 1-1. Stream names, geographic location, species of the snail *Elimia*, catchment parent geology, and selected stream physicochemical parameters of for the 7 study sites.

Site (abbreviation)	Ecoregion	Coordinates	Catchment	Species	Mean stream gradient (%)	Parent geology	NO_3-N (mg L^{-1})	PO_4 (mg L^{-1})	pH	Specific conduc-tance (µS cm^{-1})
Flannagin Creek (FL)	Southwestern Appalachians	34°, 20', 19.6" N 87°, 23', 17.1" W	Black Warrior	*E. cahawbensis*	0.58	Limestone	0.243	0.824	7.71	156
Hagood Creek (HAG)	Southwestern Appalachians	34°, 20', 38.6" N 87°, 24', 8.5" W	Black Warrior	*E. cahawbensis*	0.03	Limestone	0.167	0.867	7.78	81
Hatchet Creek (HAT)	Piedmont	33°, 7', 47.0" N 86°, 3', 15.8" W	Coosa	*E. modesta*	0.56	Schist/ Greenstone	0.142	0.467	7.82	38
Choccolocco Creek (CL)	Piedmont	33°, 49', 39.1" N 85°, 34', 56.4" W	Coosa	*E. modesta*	1.34	Phyllite	0.086	0	8.34	60
Choctafaula Creek, site a (CFa)	Southeastern Plains	32°, 31', 44.7" N 85°, 33', 19.2" W	Tallapoosa	*E. flava*	1.29	Schist/ Greenstone	0.498	0.283	7.39	60
Choctafaula Creek, site b (CFb)	Southeastern Plains	32°, 28', 3.7" N 85°, 38', 22.6" W	Tallapoosa	*E. flava*	0.50	Alluvial	0.374	0.24	6.05	97
Opintlocco Creek (OPT)	Southeastern Plains	32°, 24', 49.1" N 85°, 37', 0.1" W	Tallapoosa	*E. flava*	0.26	Alluvial	0.396	0.555	7.13	55

located on the fall line, a sharp geologic boundary separating upland piedmont from lowland coastal plains streams (Mount 1975), had substrate consisting mostly of exposed bedrock, sand, and cobble; in contrast, CFb was below the fall line and had mostly sand and gravel substrate.

Study species

Three species of *Elimia* occurred in the study area (Table 1-1). *Elimia cahawbensis* occurred in the Black Warrior River sites (FL and HAG), *E. modesta* in the Coosa River sites (HAT, CL), and *E. flava* in the Tallapoosa River sites (CFa, CFb, OPT). Snails with in this genus are dioecious (Dazo 1965, Lenertz 2002), have an estimated life span of 3–4 to 10–11 y (Dazo 1965, Huryen et al. 1994, Richardson and Scheiring 1994), and reach sexual maturity at ~1 y or at 6 mm shell width (Lenertz 2002).

Digenean trematodes are a widespread group of parasitic flatworms that use snails almost exclusively as their 1st intermediate hosts (Schmidt and Roberts 1996). They have a complex life cycle involving several host species, with a vertebrate usually as the definitive host. Snails become infected after ingesting eggs from adult worms or by miracidia, which hatch from eggs and then penetrate the snail. Several larval stages generally develop within snails including sporocysts, redia, and cercaria, with the latter stage exiting the snail and infecting the next host (Schmidt and Roberts 1996).

Three cercaria types occurred in the sampled *Elimia* populations including cotylomicrocercous cercaria, virgulate cercaria, and monostome cercaria (Schell 1970, 1985). Cotylomicrocercous cercaria (Family Opecoelidae) typically use fishes as definitive hosts (Schell 1970, 1985).

Sampling

Snails and parasites.—I sampled each site in Summer 2002, the season when prevalence of trematode parasites (hereafter "parasites") is highest for *Elimia* (Lenertz 2002). 2002 was a relatively dry year, with January to August rainfall across sites averaging ~82 cm (6^{th} driest period between Jan.-Aug. in 50 y; National Weather Service, Birmingham, AL 2002). I conducted surveys during the baseflow period at least 1 wk after a large rainfall event, as high flows after heavy rain reduce snail densities (AMT, personal observations). I quantified snail density from 18 to 20 quadrats per site over a 200- to 300-m reach per site using a Surber sampler (quadrat area = 0.093 m^2). However, I sampled only 9 quadrats at HAG because of extremely low-flow conditions. I stratified quadrats evenly between fast-flow (riffle or run) and slow-flow (pool) microhabitats (Hawkins et al. 1993), and sampled each microhabitat randomly.

I preserved snails in 95% ethanol in the field, transported them to the laboratory, and then measured them for maximum shell width (nearest 0.01 mm). I classified snails 6≥ and ≥6mm in width as juveniles and adults, respectively (Lenertz 2002), and sexed and dissected adults for trematodes. I removed snails from their shells by fracturing the shell with pliers and using forceps to remove shell fragments. I then used a watchmakers' forceps tip to slice through the body wall along the body length, and probed all tissues under a dissecting microscope (25–50×) for the presence of larval parasites. I did not dissect juveniles (<6mm width) because they are rarely parasitized by trematodes (Lenertz 2002).

Microhabitat variables.—I quantified several abiotic microhabitat variables previously shown as correlates of abundances of both *Elimia* (Huryn et al. 1995, Johnson and Brown 1997, Stewart and Garcia 2002) and their parasites (Jokela and Lively 1995, Shinagawa et al. 1999), including dissolved oxygen (DO, YSI Model 55), streamwater pH (Oakton pHtester3, Model 35624-30), electrical conductivity (YSI Model 33 S-C-T meter), NO_3-N (N, DX-120 Ion chromatography, Dionex, Sunnyvale, California), and total P concentration (P, molybdate blue method; Murphy and Riley 1962, Watanabe and Olsen 1965), and current velocity (Marsh-McBirney Flo-mate, Model 2000), substrate size/composition, and % riparian shade (spherical densiometer, Lemmon 1957). I measured current velocity, depth, substrate composition, DO and percent shade at each Surber quadrat. I quantified current velocity in the center of each quadrat, ~1 cm from stream bottom, substrate size by measuring the longest axis of 10 randomly selected particles, and mean % shade by positioning the densiometer immediately above the quadrat and recording measurements from 4 aspects (upstream, downstream, right and left bank directions).

Statistical analysis

I used a combination of regression approaches to quantify snail–parasite relationships with abiotic variables, at 2 spatial scales: 1) among sites within different streams (large spatial scale); and 2) between runs/riffles and pool microhabitats within individual streams (small spatial scale). For the large-scale analysis, I used simple linear regressions to examine relationships between snail density, prevalence of parasitism (as

% of adult snails with parasites, hereafter "prevalence") and mean microhabitat variables among the 7 sites. For the small-scale analysis, I used multiple regression (maximum adjusted *R*-square for model selection, Neter et al. 1996) to determine which abiotic and biotic factors (i.e. current, % shade, substrate size, depth, DO, N, P, prevalence) best predicted adult and juvenile snail abundance within each site. Proportional prevalence and shade data were arcsine-transformed before analysis (Zar 1996). Last, I used Student's *t*-test to assess differences in snail densities between runs/riffles and pools within streams, and χ^2 to assess differences in prevalence between runs/riffles and pools within streams. I used $\alpha = 0.05$ for all analyses except where adjusted for multiple comparisons.

RESULTS

Microhabitat variables

Amount of riparian shading varied greatly among sites, ranging from open sunlit reaches (mean = 17% shade in OPT) to almost entirely shaded reaches (91% in FL; Table 2). Within sites, % shade did not differ between pools and runs. Across sites, mean current velocity ranged from to 0.01 m s^{-1} at HAG to 0.21 m s^{-1} at CFb. At the time of sampling (7 August 2002), HAG had flow only in shallow pools, so I sampled snails only from this microhabitat. Mean current was higher in runs than in pools for all other sites except FL (Table 1-2). Mean DO concentration ranged from 5.43 mg L^{-1} (65% saturation) at HAG to 7.29 mg L^{-1} (85%) at CL. No measurements were taken at CFa because of a meter malfunction, although subsequent measurements revealed that

Table 1-2. Mean (±1 SE) microhabitat variables quantified for the snail *Elimia* and their trematode parasites. Asterisks indicate significant difference between pools and runs of a given study site (1-way ANOVA, *$p<0.01$). Site abbreviations as in Table 1. ND = data not available. Only pools were sampled in HAG.

Site	Micro-habitat	Shade (% canopy cover)	Current velocity ($m\ s^{-1}$)	Dissolved O_2 ($mg\ L^{-1}$)	Depth (m)	Substrate (*phi*)
FL	All	91 ± 1	0.06 ± 0.02	5.93 ± 0.12	0.10 ± 0.02	-2.73 ± 0.51
	Pool	91 ± 1	0.01 ± 0.01	5.91 ± 0.15	0.14 ±0.02	-2.09 ± 0.62
	Run	91 ± 1	0.14 ± 0.02	5.95 ± 0.19	0.04 ±0.03	-3.76 ± 0.78
HAG						
	Pool	89 ± 5	0	5.43 ± 0.46	0.12 ± 0.07	-1.43 ±1.70
HAT	All	54 ± 4	0.12 ± 0.03	6.15 ± 0.1	0.16 ± 0.02	-5.92 ± 0.46
	Pool	47 ± 6	0.00 ± 0.04*	5.88 ± 0.14	0.18 ± 0.03	-4.81 ± 0.67
	Run	59 ± 5	0.18 ± 0.03*	6.32 ± 0.11	0.14 ± 0.02	-6.62 ± 0.53
CL	All	82 ± 1	0.07 ± 0.01	7.29 ± 0.16	0.14 ± 0.02	-5.27 ± 0.33
	Pool	80 ± 1	0.01 ± 0.01*	6.89 ± 0.15	0.16 ± 0.02	-5.14 ± 0.45
	Run	84 ± 1	0.15 ± 0.01*	7.79 ±0.17	0.10 ± 0.02	-5.43 ± 0.51
CFa	All	80 ± 11	0.06 ± 0.02	ND	0.23 ± 0.04	-4.65 ± 0.53
	Pool	80 ± 4	0.01 ± 0.01*	ND	0.25 ± 0.03	-4.21 ± 0.70
	Run	79 ± 4	0.14 ± 0.02*	ND	0.12 ± 0.04	-5.25 ± 0.25
CFb	All	34 ± 3	0.21 ± 0.05	5.72 ± 0.12	0.15 ± 0.02	-2.97 ± 0.92
	Pool	35 ± 5	0.00 ± 0.06*	5.22 ± 0.17 *	0.17 ± .03	0.091 ± 1.13*
	Run	34 ± 4	0.32 ± 0.05*	5.99 ± 0.13*	0.13 ± .02	-5.06 ± 0.83*
OPT	All	17 ± 2	0.10 ± .03	6.82 ± 0.19	0.14 ± 0.02	-2.58 ± 0.66
	Pool	19 ± 2	0.00 ± 0.02*	6.18 ± 0.19*	0.18 ± 0.03	-0.08 ± 0.05*
	Run	17 ± 2	0.18 ± 0.02*	7.40 ± 0.17*	0.11 ± 0.03	-4.83 ± 0.51*

differences in DO between CFa and CFb were negligible (AMT, unpublished data). Mean DO was higher in runs than pools for 2 sites (CFb, OPT; Table 1-2). Mean depth ranged from 0.10 m at FL to 0.23 m at CFa (Table 1-2). Mean depth taken within sampling quadrats did not differ significantly between pools and runs (Table 1-2). Mean substrate size ranged from –2.73 *Phi* (fine gravel) at HAG to –5.92 *Phi* (very coarse gravel) at HAT (Table 1-2). Mean substrate size did not differ between pools and runs except for 2 of the Coastal Plains streams (CFb and OPT), where substrate sizes in runs were higher than pools (Table 1-2).

Snail density and parasite prevalence

Overall, mean *Elimia* density among sites varied by 4-fold, ranging from ~100 snails m^{-2} (in HAG) to 434 (in HAT) (Table 1-3). Juvenile and adult densities were positively correlated at 3 of the 7 sites (CFa $r^2 = 0.72$, $p < 0.001$; CFb $r^2 = 0.69$, $p < 0.001$; OPT $r^2 = 0.25$, $p < 0.039$). All sites and microhabitats had snails that were parasitized by trematodes, although prevalence varied greatly among sites (Table 1-3). Prevalence ranged from 1 to 26%, with CFa and CFb showing the lowest and highest infections, respectively. Prevalence did not differ between runs and pools for any site (Table 1-3). Cotylomicrocercous cercaria were the most common trematode encountered and composed nearly 60% of infections overall; in addition, they were the most common trematode in CFa and HAT, accounting for 95 and 57% of infections, respectively. The virgulate cercaria (Family Lecithodendriidae) use mammals, birds and amphibians as definitive hosts (Schell 1970, 1985). These cercaria composed 23% of infections overall

Table 1-3. Mean (±1 SE) densities of total, adult, and juvenile snails in the genus *Elimia* and % of adult snails parasitized by trematodes (% Prevalence), by microhabitat at each study site. Asterisks indicate significant difference between pools and runs (*t*-test, *p<0.01). Site abbreviations as in Table 1-1. ND = data not available

Site	Microhabitat	Snail density (no. m^{-2})			% Prevalence
		Total	Adults	Juveniles	
FL	All	282 ± 68	56 ± 11	226 ± 69	23.0
	Pool	281 ± 90	49 ± 14	232 ± 92	28.0
	Run	284 ± 112	66 ± 18	218 ± 115	20.4
HAG	All				
	Pool	100 ±12	37 ± 10	63 ± 13	18.0
	Run	ND	ND	ND	ND
HAT	All	434 ± 56	249 ± 29	185 ± 43	5.0
	Pool	376 ± 91	238 ± 48	138 ± 70	4.5
	Run	470 ± 72	256 ± 38	214 ± 56	5.0
CL	All	319 ± 49	192 ± 30	127 ± 34	10.0
	Pool	277 ± 66	201 ± 41	76 ± 43	10.2
	Run	371 ± 74	181 ± 46	190 ± 48	10.6
CFa	All	264 ± 50	133 ± 26	131 ± 26	26.0
	Pool	262 ± 68	132 ± 35	130 ± 36	28.8
	Run	266 ± 80	134 ± 42	132 ± 42	20.8
CFb	All	409 ± 86	232 ± 48	173 ± 43	1.0
	Pool	700 ± 124*	376 ± 72	324 ± 59*	0.1
	Run	246 ± 91*	155 ± 53	91 ± 44*	1.1
OPT	All	268 ± 52	235 ± 46	33 ± 12	5.0
	Pool	205 ± 76	173 ± 66	33 ± 18	6.9
	Run	324 ± 72	291 ± 62	32 ± 17	3.7

and were the most common trematode in CL and FL, accounting for 53% and 69% of infections, respectively. Last, monostome cercaria (Family Pronocephalidae) use birds and turtles are primary definitive hosts (Schell 1970, 1985); these cercaria composed only 18% of infections overall, although they were the most common trematode in CFb, HAG, and OPT (50, 60, and 72% of infections, respectively).

Relationships among snail density, prevalence, and microhabitat variables

Across the 7 sites, % shade, mean current, and prevalence were the best predictors of snail density. Adult density was negatively related to prevalence ($r^2 = 0.68$, $p < 0.02$; Fig. 1-1A) % shade ($r^2 = 0.87$, $p < 0.01$; Fig. 1-1B) and was positively related to current velocity ($r^2 = 0.66$, $p < 0.027$, Fig. 1-1C). In turn, prevalence was positively related to % shade ($r^2 = 0.65$, $p < 0.03$).

Snail size-class distributions varied significantly between high-prevalence sites (FL, HAG, CFa, prevalence ≥18%; Table 1-3) and low-prevalence sites (CL, HAT, OPT, CFb, prevalence ≤10%; Table 1-3; $\chi^2 = 448$, $p > 0.0001$), with higher frequency of small snails (<3 mm width) in high-prevalence sites and higher frequency of large snails (≥5 mm width) in low-prevalence sites (Fig. 1-2). Mean snail width at high-prevalence sites (4.61 ± 0.06) was significantly smaller ($t = -25.74$, $p < 0.0001$) than was mean snail width at low-prevalence sites (6.5 ± 0.04).

Within individual sites, prevalence was a significant factor in explaining snail density (either juvenile or adult) in 4 of the 5 (80%) significant multiple regression models (Table 1-4); however, unlike among-stream patterns, prevalence and snail density were positively related at the scale of individual sites. Mean depth (for HAT, CFb) was

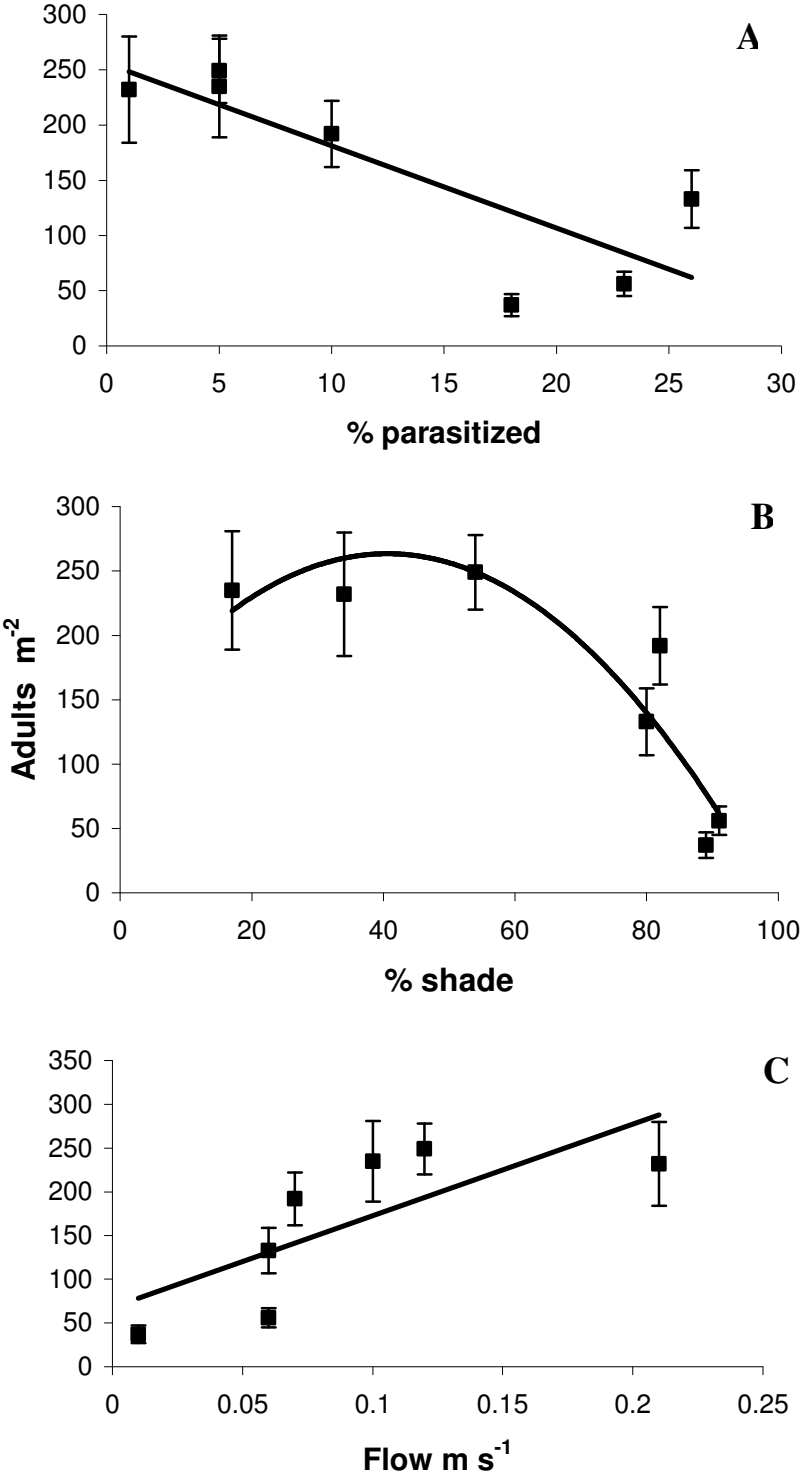

Figure 1-1. Mean (±1 SE) density of adult *Elimia* snails plotted against percent of snails parasitized by trematodes (A), % shade, polynomial model (B) and current velocity (C) at the sampling point across the 7 study sites. All 3 relationships were significant.

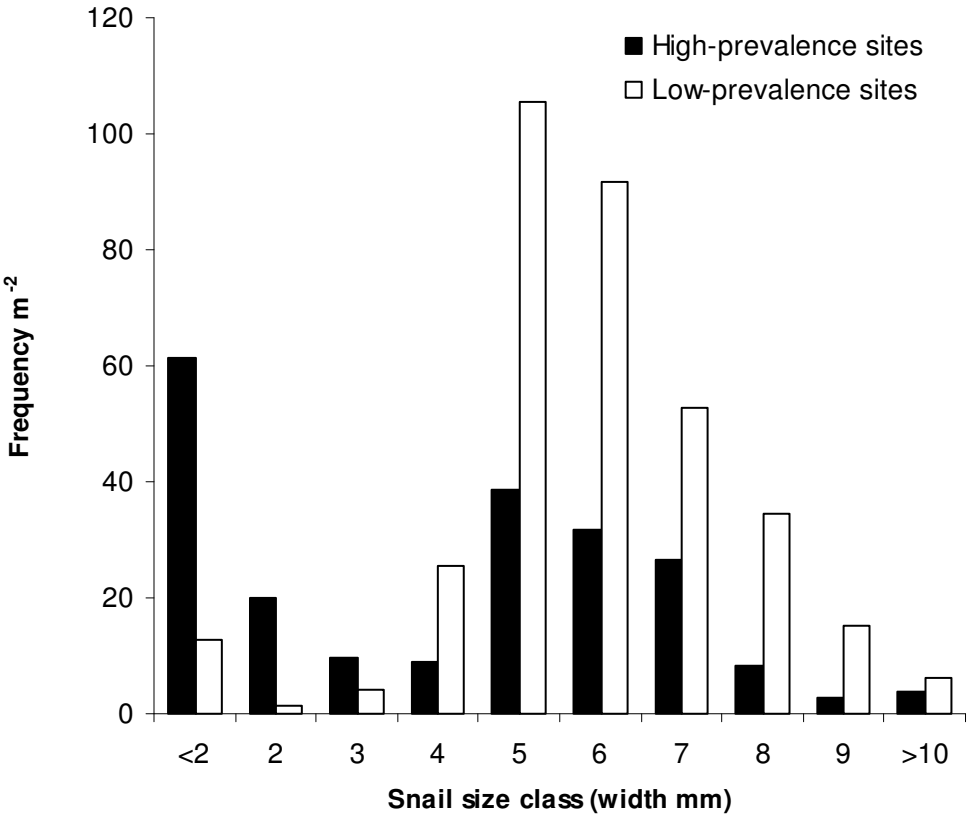

Figure 1-2. Size class frequency distribution in high-parasitism sites (Choctafaula site a, Hagood, Flannagin) and low-parasitism sites (Choccolocco, Hatchet, Opintlocco, Choctafaula site b)

Table 1-4. Results of multiple regression analyses indicating environmental variables best predicting adult and juvenile density of snails in the genus *Elimia,* and % of total *Elimia* parasitized by trematodes (Prevalence) within each study site. Site abbreviations as in Table 1. DO = dissolved oxygen. Adj. R^2 = adjusted R^2.

		Snail density (no. m^{-2})		
Site		Adult	Juvenile	Prevalence
CL				
	Adj R^2			
	p			
FL		Prevalence		
	Adj R^2	0.426		
	p	0.002		
HAG		Current, DO, prevalence		
	Adj R^2	0.8469		
	p	0.0056		
HAT			Depth, prevalence	
	Adj R^2		0.4673	
	p		0.0035	
OPT				% shade
	Adj R^2			0.6848
	p			0.0001
CFb			Depth, substrate	
	Adj R^2		0.4227	
	p		0.0036	
Cfa		% shade, prevalence		Current, substrate
	Adj R^2	0.4964		0.6709
	p	0.0016		0.0001

also a significant factor in explaining juvenile densities in 2 of the 5 regression models (40%, Table 1-4). Abiotic factors significantly predicted prevalence within only 2 sites (CFa, OPT). For CFa, mean current, and substrate size were significant ($R^2 = 0.7294$, $p < 0.0001$), whereas for OPT only percent shade was a significant predictor of prevalence ($R^2 = 0.6848$, $p < 0.0001$; Table 1-4). Environmental variables pH, N, P, and conductivity showed no relationship to snail densities or parasite prevalence within or across streams.

DISCUSSION

Hill (1992) hypothesized that *Elimia* populations are not vulnerable to control by predators because of their hard shells, and are instead subject to bottom-up control through food (periphyton) limitation (see also Hill et al. 1995). My correlative data showing a negative relationship between adult *Elimia* density and % shade support this hypothesis, as more shaded sites, likely with lower periphyton accrual than less-shaded sites(Kelly et al. 2003), also support lower snail densities than less-shaded systems. In laboratory experiments designed to simulate conditions in Oregon streams, high shade levels both decreased algal biomass and reduced growth of the pleurocerid snail *Juga* relative to low and intermediate shading (Lamberti et al. 1989). However, in forested streamside channels in Tennessee, increased light levels alone were not sufficient to increases algal biomass (Rosemond 1993), possibly because of nutrient limitation (see also Hill and Knight 1987). High levels of streamwater nutrients such as N and P have also been shown to increase both snail and algal growth (Stelzer and Lamberti 2001,

2002), although I found no relationships between streamwater N or P concentrations and *Elimia* density in my study.

My data showing an inverse relationship between adult snail density and prevalence of trematode parasites among streams suggest that, similar to predation, parasitism is an important top-down control on snails (Lafferty 1993). Trematodes may decrease host snail populations by increasing mortality (Sorensen and Minchella 1998, de Montaudouin et al. 2003) or by limiting reproduction by castration of both male and female snails (Krist 2001, Lenertz 2002). In my study, snails in sites with high prevalence of parasitism (18 to 26% of adults parasitized in HAG, FL, and CFa) may have been subject to higher mortality and/or lower reproduction than snails in sites with lower prevalence. Parasitized snails have been shown to consume more periphyton and have lower stored glycogen levels than unparasitized snails, thus implying starvation and potentially increased mortality of parasitized snails (Lenertz 2002). This mechanism would explain lower snail density in high-prevalence sites, although it has not been tested experimentally.

Parasitism also may influence snail life history. Differences in size-frequency distributions between high- and low-prevalence sites, with lower proportions of large size classes in high-prevalence sites, also support the hypothesis that parasitism increases snail mortality. Parasitism is more common in large-bodied snails (Lenertz 2002), so increased mortality of large snails from trematode parasitism could explain the lower abundance of large snails in high- vs. low-prevalence streams. Parasitism has also been shown to increase snail growth rate, often resulting in gigantism (Minchilla 1985, Probst and Kube 1999). My results did not support this idea because I found a lower percentage

of large snails in high- vs. low-prevalence sites. However, Gorbushin and Levakin (1999) suggest that effects of parasitism on snail growth depend on snail life history, with gigantism being more common in relatively short-lived snails. However, there is little effect of growth on longer-lived snails such as *Elimia*.

Parasite prevalence also was an important factor in predicting juvenile and adult snail densities within individual streams, with 4 of the 5 significant within-stream models indicating a positive relationship between prevalence and density. This pattern seems counterintuitive given that trematodes may increase host mortality (Sorensen and Minchella 1998, de Montaudouin et al. 2003) or decrease host reproduction (Krist 2001, Lenertz 2002); however, several potential explanations exist for this pattern. The positive relationship between snail density and prevalence cannot be explained by classic density-dependant population models common in many infectious diseases (Gilbert et al 1994), as such models predict that increased host density increases transmission because of high parasite-host contact. In most trematode life cycles, however, snails can only become infected from eggs or miracidia, and they cannot transmit parasites to each other (Schmidt and Roberts 1996). The positive relationship between snail density and prevalence could be explained for *Elimia* if infected snails aggregated more than unparasitized snails. Using y-maze choice tests, Boissier et al. (2003) reported that parasitized *Biomphalaria glabrata* snails aggregated with other parasitized snails about twice as frequently as with uninfected snails, suggesting that aggregations of infected snails may increase parasite transmission to the next host because cercaria release may be more concentrated from a single host source rather than from isolated individuals. If both infected and uninfected *Elimia* are attracted to infected *Elimia*, this pattern would result

in high-density patches of snails with high prevalence, and thus could explain why positive relationships between density, both for adult and juveniles, and parasite prevalence occurred.

There may be other explanations for the high variation in prevalence among sites. For example, in CFa, the site with the highest prevalence, prevalence was only one of several factors that related to snail density (Table 4). In this site, high prevalence was associated with slow-flowing environments with large substrate. One potential explanation for differential parasite prevalence across microhabitats is that parasites manipulate snail behavior to increase the likelihood of transmission to the next host. Previous studies have shown that trematode infections can alter host snail habitat use and cause hosts to aggregate in specific microhabitats such as at greater depths (Shinagawa et al. 1995) or near shore banks (Jokela and Lively 1995). Parasite alteration of host snail behavior to use slow-flowing habitats may increase parasite transmission success either by 1) causing cercarial release in areas where they are more likely to encounter the next host, which is likely a fish, or 2) reducing the likelihood cercaria are swept downstream by the current.

An alternative explanation for differential prevalence across microhabitats may reflect differences in the likelihood of infection between microhabitats. In marine systems, trematode infection is often highly spatially variable (Lafferty et al. 1994). Smith (2001) reported for the snail *Cerithidea scalariformis* that prevalence in mangrove swamps was highest where birds, the definitive host, commonly perched and deposited feces containing trematode eggs. In streams, however, the input of infective material may not be as important as physical properties such as current, that may affect the

dispersal and transmission of snail infective stages (i.e. miracidia) and, thus, their ability to contact potential hosts. Richardson and Scheiring (1994) found that the riffle-dwelling *Elimia clara* showed lower parasite loads than *E. cahawbensis,* which occurred equally in riffles and pools. They hypothesized that the high flow rate within riffles serves as a physical refuge from trematode infection (Richardson and Scheiring 1994). In my study, it is possible that infection was more prevalent in areas of slower flows with larger substrates in CFa because the infective stages (eggs or miricidia) are more likely to settle and not be displaced downstream. In addition, large substrates may facilitate trematode contact with snails more readily than that of smaller substrate, if infective stages might become buried within interstitial spaces of the stream bed.

All populations of *Elimia* I sampled were parasitized by trematodes, albeit to different degrees. These results, while compelling in documenting the prevalence of host-parasite interactions are only correlative; further experimental work needs to be done to 1) assess the degree to which trematodes can directly influence snail populations within or among streams in a top-down fashion, and 2) determine the specific mechanisms accounting for strong associations between prevalence and environmental conditions. Given the abundance of trematodes within snail populations and the potential for trematodes to influence snail abundance and distribution on small and large spatial scales, it appears likely that trematodes play an important role in population dynamics of their snail hosts in many streams.

CHAPTER TWO:

INFLUENCE OF TREMATODE PARASITES ON THE DISTURBANCE RESPONSE OF STREAM-DWELLING *ELIMIA* SNAILS.

INTRODUCTION

The harsh-benign hypothesis (sensu Peckarsky 1983) predicts that stream communities with little or infrequent disturbance are structured by biotic factors such as predation and competition, whereas communities with frequent or severe disturbance regimes are structured by abiotic factors such as flow and scour. Research in New Zealand streams supported this hypothesis where greater influences of biotic factors in stable vs. more disturbed streams (Death 2000). However, Thompson et al. (2002) found in Australian streams that disturbance did not alter the affect a predatory stonefly had on mayfly prey, which was contrary to the harsh-benign model. . In streams, floods are a common form of abiotic disturbance (see Lake 2000) which vary greatly in frequency and severity over time (Holomuzki and Biggs 1999) and can influence benthic organisms in several ways. High flows alone and coupled with destabilized substrate can result in both displacement and mortality of stream invertebrates (Holomuzki and Biggs 2000), while scour and substrate disturbance can result in reductions in total abundance and density of species (McCabe and Gotelli 2000). Because of the variation in disturbance frequency and severity, both abiotic and biotic forces may be important in structuring

stream communities at different times. Therefore, it is necessary to understand how both biotic and abiotic factors interact to affects stream communities

Holomuzki and Biggs (1999) demonstrated that high-frequency flooding can alter snail distribution and reduce density. There are both abiotic and biotic factors that may influence how snails respond to disturbance. Substrate size has been shown to influence the effects of disturbance on snails with gravel substrates providing a refuge from dislodgment over larger substrates (Holomuzki and Biggs 1999) and finer substrates (Moore 1964). Moore (1964) also demonstrated that snail size influenced likelihood of dislodgment in high-flow conditions with small snails being the least susceptible to dislodgment. And finally, aquatic snails have been shown to serve as hosts to trematode parasites (Schmidt and Roberts 1996, Lenertz 2002). While disturbance may release an organism from the pressures of competition, predation, and future parasitic infection, it does not release it from the effects of current parasites. Parasites deplete host resources for their own development, which can lead to reduced growth (Gerard and Theron 1996) and/or castration (Krist and Lively 1998, Lenertz 2002) of the host snail. Parasitic infection can also increase host mortality in stressful environmental conditions (Sousa and Gleason 1989). In this context, it is possible that parasitism may affect the magnitude of snail response to high-flow disturbances, such that parasites may increase host snail vulnerability to flow disturbance.

In this study, I compared snail and parasite abundance over two years with strongly contrasting levels of disturbance. My objectives were to 1) determine what biotic and abiotic factors influenced stream snail abundance at different levels of flow disturbance, 2) quantify the influence of increases in flow disturbance on trematode and

snail abundance and their interactions and 3) experimentally determine the effects of selected biotic factors (snail size and parasitism), and abiotic factors (substrate size) on snail dislodgment during high flow events.

MATERIALS AND METHODS

Study Sites

I quantified snails, their trematode parasites, and several abiotic physicochemical variables in 7 study stream reaches. The reaches were within 6 streams in 3 ecoregions and 3 river drainages within Alabama (Table 1-1). I selected these sites because preliminary data showed a wide range of parasite prevalence among sites. All sites were in predominantly forested catchments composed mostly of pine and mixed hardwoods, with many sites either in or adjacent to National Forests (NF). Stream geology has been shown to be an important determinant of *Elimia* abundance (Huryn et al. 1995), and it varied among the study sites from alluvial channels to those composed primarily of limestone, phyllite, or schist (Szabo 1988).

Flannagin and Hagood creeks (FL and HAG, respectively; Black Warrior River Drainage) are limestone streams within the Bankhead NF, with substrates consisting of exposed bedrock as well as sand and cobble. Geologies at Choccolocco and Hatchet creeks (CL and HAT, respectively; Coosa River Drainage) border the Talladega NF and are predominantly phyllite, and chert, phyllite, and schist (Szabo1988), respectively, with substrate composed mostly of exposed bedrock and cobble. Opintlocco Creek (OPT, Tallapoosa River Drainage) is primarily alluvial sand and gravel. I studied 2

stream sites on Choctafaula Creek (CFa and CFb, near and within Tuskegee NF, respectively; Tallapoosa River Drainage) because of their contrasting geomorphology (schist and greenstone bedrock vs. alluvial, respectively; Szabo 1988) and differences in prevalence of snail parasitism (26 and 1%, respectively). CFa was located on the geologic fall line (Mount 1975), and had substrate consisting of exposed bedrock, sand and cobble, whereas CFb, located below the fall line, had substrate composed largely of sand and gravel.

Study species

Three species of *Elimia* occurred in the study area (Table 1-1). *Elimia cahawbensis* occurred in the Black Warrior River sites (FL and HAG), *E. modesta* in the Coosa River sites (HAT, CL, and *E. flava* in the Tallapoosa River sites (CFa, CFb, OPT). Snails within this genus are dioecious (Dazo 1965, Lenertz 2002), have an estimated life span of 3-4 to 10-11 y (Dazo 1965, Huryen et al., Richardson and Scheiring 1994), and reach sexual maturity at ~1 y or ~6 mm shell width (Lenertz 2002).

Digenean trematodes are a widespread group of parasitic flatworms that use snails almost exclusively as 1st intermediate hosts (Schmidt and Roberts 1996). They have a complex life cycle involving several host species, with a vertebrate usually serving as the definitive host. Snails become infected after they ingest eggs or from larval miracidia, which hatch from eggs and penetrate snail tissue. Several larval stages generally develop within snails including sporocysts, redia, and cercaria, with the later stage exiting the snail and infecting the next host (Schmidt and Roberts 1996).

Field sampling

I surveyed each site for *Elimia* (hereafter "snails") and their trematode parasites (hereafter "parasites") once in summer 2002 and in 2003, the season when prevalence of trematode parasites is highest (Lenertz 2002). 2002 was a relatively dry year, with January to August rainfall across sites averaging ~82 cm (6^{th} driest Jan.-Aug. period in 50 y, Table 2-1, National Weather Service, Birmingham, AL 2002). In contrast, rainfall for the same period in 2003 was ~130 cm (6^{th} wettest Jan.-Aug. in 50 years, National Weather Service Birmingham, AL 2003).

I conducted surveys during the base flow period at least 1 wk after a large rainfall event, as high flows resulting from heavy rain temporarily reduce snail densities (personal observations). I quantified snail density from 18 to 20 quadrats over a 200- to 300-m reach per site using a Surber sample quadrat (area = 0.093 m^2). Only 9 quadrats were taken at HAG during 2002 because of extremely low flow conditions and limited microhabitat. I stratified Surber quadrats evenly between fast-flow (riffle or run) and slow-flow (pool) microhabitats (Hawkins et al. 1993), and I sampled each microhabitat randomly.

I preserved snails in 95% ethanol in the field, transported them to the laboratory, and then measured them for maximum shell width. Snails ≥6 mm in width were classified as adults (Lenertz 2002) and sexed and dissected for trematodes. To assay for trematodes, I removed snails from their shells, made an incision through the body wall, and then microscopically (25 – 50X) probed all tissues with watchmake's forceps for presence of

Table 2-1. Total rainfall (cm) from January to August for 2002, 2003 and 30-y mean. Rainfall data was taken from the rainfall station nearest each study site. Site abbreviations: Hagood (HAG), Flannagin (FL), Choccolocco (CL), Hatchet (HAT), Choctafaula site a, (CFa), Choctafaula site b (CFb) and, Opintlocco (OPT).

Study sites	Rainfall station	30-y mean	2002	2003
HAG, FL	Addison, AL	146.56	118.80	153.64
CL	Heflin, AL	102.97	80.09	134.26
HAT	Sylacauga, AL	98.60	83.70	139.57
CFa, CFb, OPT	Auburn, AL	94.99	75.39	131.27

larval parasite stages. I considered snails with shell widths <6mm as juveniles and enumerated but did not dissect them, as *Elimia* juveniles are rarely parasitized by trematodes (Lenertz 2002).

Abiotic factors.—I quantified several abiotic variables shown in previous studies to relate to both *Elimia* (Huryn et al. 1995, Johnson and Brown 1997, Stewart and Garcia 2002) and parasite abundances (Jokela and Lively 1995, Shinagawa et al. 1999) at each study site, including dissolved oxygen concentration (DO, YSI Model 55), streamwater pH (Oakton pHtester3, Model 35624-30), electrical conductivity (YSI Model 33 S-C-T meter), NO_3-N (N, DX-120 Ion chromatography (Dionex, Sunnyvale, California), total P (P, molybdate blue method, Murphy and Riley 1962, Watanabe and Olsen 1965) concentration, current velocity (Marsh-McBirney Flo-mate, Model 2000), substrate size, and % shade (spherical densiometer, Lemmon 1957). I measured current velocity, depth, substrate composition, DO and % shade at each Surber quadrat. I quantified current velocity in the center of each quadrat, ~1 cm above stream bottom, mean substrate size by measuring the longest axis of 10 randomly selected particles, and mean riparian shade using the densiometer positioned immediately above the quadrat and read from 4 different aspects (upstream, downstream, right and left bank directions). In addition, I calculated link magnitude and used this measure as an index of stream size and catchment area (Osborne and Wiley 1992).

Substrate size – flow experiment

I conducted an experiment to quantify the effects of parasite load and snail size, current velocity, and substrate size on the time necessary for snails to become dislodged and carried downstream by the current. At the Auburn University, North Auburn Upper Fisheries Research Station, I constructed 2 artificial stream channels (1.5 m x 10 cm) from PVC pipe cut in half longitudinally and placed within metal stream channels. The outflow ends of the PVC channels were covered with mesh screen to catch dislodged snails. Water was fed by gravity from a 208-L head tank. In each of the channels a 35-cm length of channel 25 cm from the outflow was covered with 1 of 2 substrate treatments. One treatment (unglazed ceramic tile) simulated bedrock, whereas the other treatment consisted of gravel (2–6 cm diameter). I chose these 2 substrate sizes because previous work had shown that the rate of dislodgment varied greatly between them (Holomuzki and Biggs 1999).

I used *Elimia flava* collected from CFa as the focal species for this experiment, a snail and site that showed a relatively high level of parasitism (26%). At the start of each trial, I randomly selected 10 adult snails, individually marked them using a paint pen, and then placed them in one of the 2 channels, allowing snails to acclimate in a low-flow environment for 5 min. After the acclimation period, I ran a 15-min trial where at time 0 flow velocity was increased to ~10 cm/s, ~40 cm/s at 5 min and ~90 cm/s at 10 min. These flow velocities are well within the range snails experience during a high-flow event (unpublished data). I recorded snail movement patterns (i.e., moving upstream, downstream or to the waterline) during the trial and also recorded the time (s) each snail

became dislodged. After the experiment, I preserved snails in 95% ethanol and brought them into the laboratory, and I measured their width, dissected them for parasites, and quantified parasite volume and parasite abundance (n = 204 snails assayed).

For analysis I used 2 snail size categories, small (6-8 mm in width) and large (≥ 8 mm) and 3 categories of parasite load: no parasites, low (< 150 sporocysts/snail), and high (≥ 150 sporocysts/snail). Categories were established by separating the groups at median snail width and parasite load, respectively. I quantified parasite abundance by counting number of larval parasites present in each snail. I estimated parasite volume by first measuring the length and width of 10 randomly selected parasites in each snail. I used the mean width and length to calculate the volume of a cylinder and multiplied this volume by parasite abundance.

Statistical analysis

I used multiple regression to determine what environmental factors best predicted snail density and/or parasite prevalence across the 7 sites. Proportional parasite prevalence and shade data were both arcsine-transformed before analysis (Zar 1996). I used Chi-square to compare snail size frequency distributions between years (Zar 1996). Snail density between years was compared using the Wilcoxon test (Zar 1996), and one-way ANOVA was used to compare mean snail width between years. Data from the substrate size – flow experiment were analyzed with a 2-way ANOVA

.

RESULTS

Snail density significantly decreased from 2002 (dry year) to 2003 (wet year) in HAG, CFa, CL, CFb and HAT, but not in FL or OPT (Wilcoxon test, $p > 0.05$; Fig. 2-1). Using multiple regression, the best single predictor of adult snail density in 2002 was parasite prevalence ($R^2 = 0.685$ $p = 0.022$) whereas in 2003 link magnitude ($R^2 = 0.661$ $p = 0.022$) was the pest predictor. In 2003, snail density not only declined from 2002 in most sites, but there also was a significant shift in snail size distribution at 6 of the 7 sites (CFa, CFb, HAT, HAG, FL, and OPT; $\chi^2 > 57$, $p > 0.001$, Fig. 2-2) compared with 2002, and mean snail size also increased at 6 of 7 sites (CFa, CFb, HAT, HAG, FL, and CL; 1-way ANOVA, $p > 0.01$, Fig. 2-3). Environmental variables pH, N, P, and conductivity showed no relationship to snail densities or parasite prevalence within or across streams.

Three cercaria types occurred in the sampled *Elimia* populations including cotylomicrocercous cercaria, virgulate cercaria, and monostome cercaria (Schell 1970, 1985). Cotylomicrocercous cercaria are found in the Family Opecoelidae (Lenertz 2002), and opecoelids typically use fishes as definitive hosts (Schell 1970, 1985). Cotylomicrocercous cercaria were the most common trematode encountered and comprised nearly 60% of infections overall, and they were the most common trematode in CFa and HAT (95 and 53% of infections, respectively). The virgulate cercaria were identified as belonging to the Family Lecithodendriiadae and use mammals, birds and amphibians as definitive hosts (Schell 1970, 1985). Virgulate cercaria comprised 27% of infections overall, and they were the most common trematode in CL, HAG and FL (50, 50 and 70% of infections, respectively). Finally, Monostome cercaria belong to the Family Pronocephalidae and birds and turtles are the most common definitive hosts

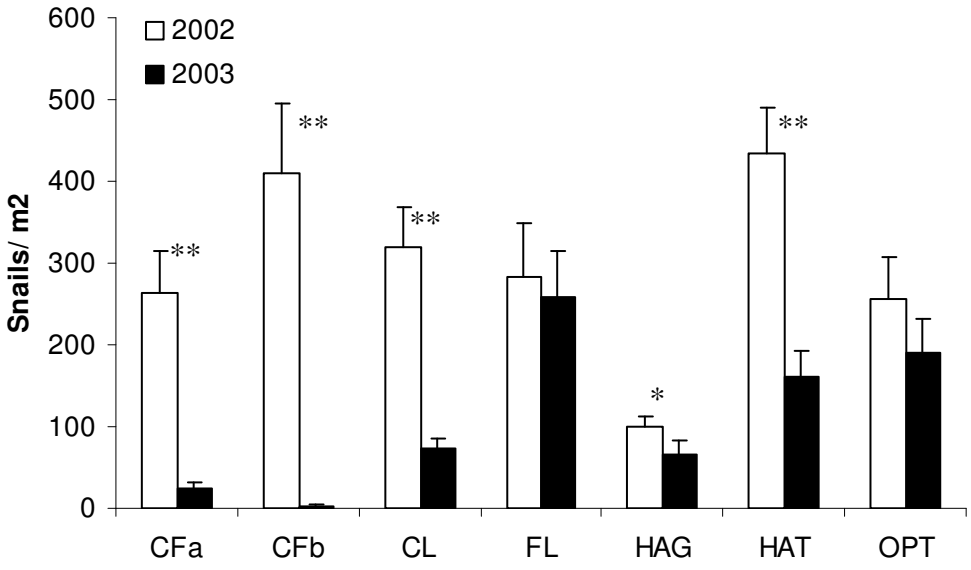

Figure 2-1. Mean (+1 SE) snail density at the 7 study sites during 2002 (dry year) and 2003 (wet year). Asterisks indicate significant differences between years. * p>0.05, ** p>0.001. Site abbreviations: Choctafaula site a (CFa), Choctafaula site b (CFb), Choccolocco (CL), Flannagin (FL), Hagood (HAG), Hatchet (HAT) and, Opintlocco (OPT).

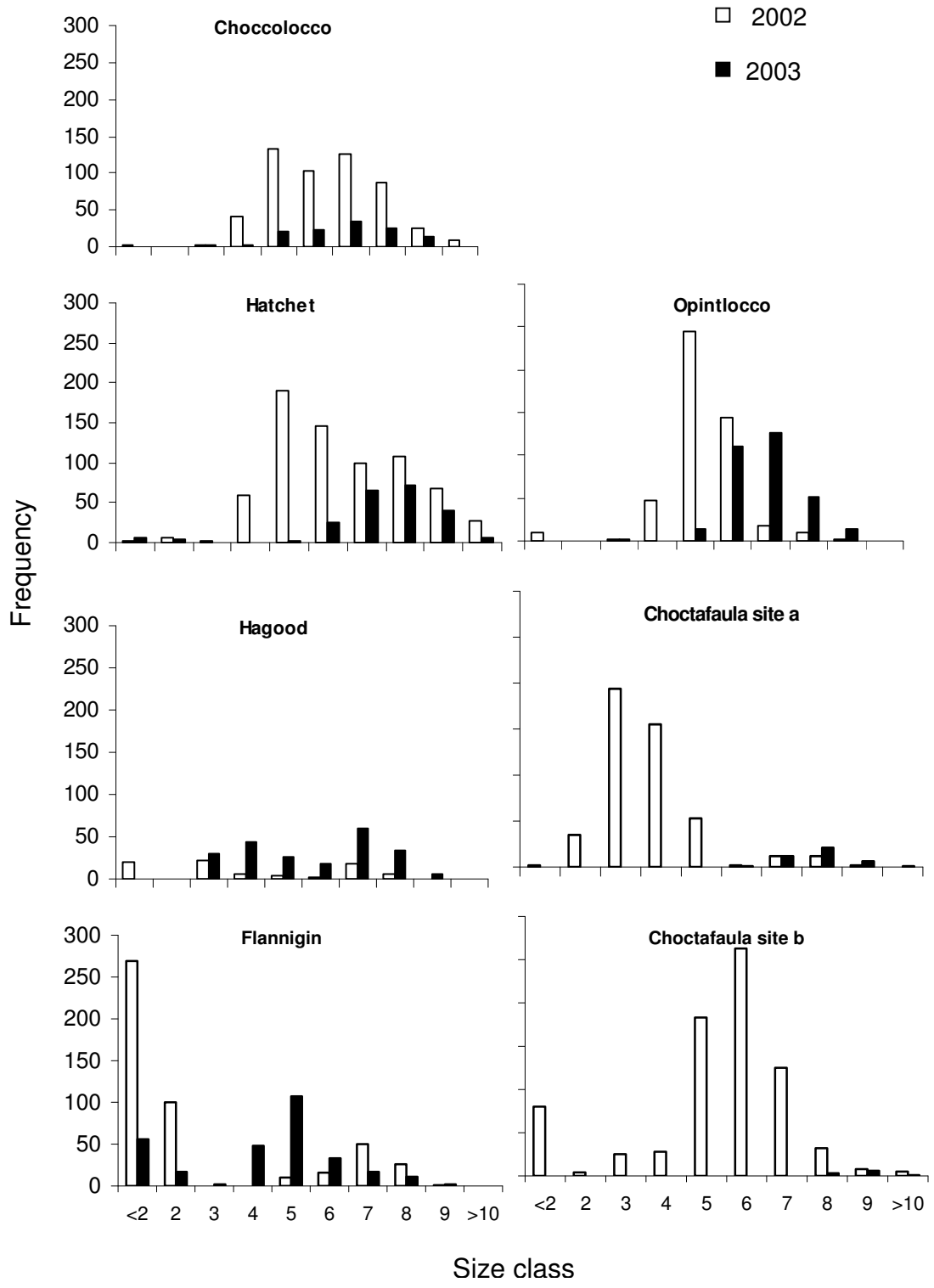

Figure 2-2. Size frequency distributions of *Elimia* snails for 2002 (dry year) and 2003 (wet year) for the 7 study sites.

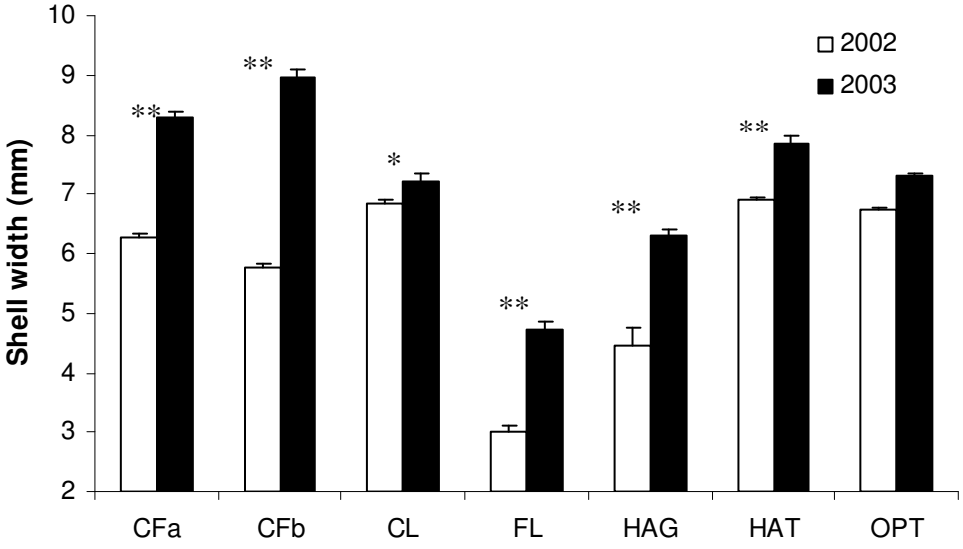

Figure 2-3. Mean (+1 SE) snail width at each site in 2002 (dry year) and 2003 (wet year). Site abbreviations as in Figure 2-1. Asterisks indicate significant differences between years * $p > 0.01$, ** $p > 0.001$

(Schell 1970, 1985). Monostome cercaria composed only 16% of infections overall; however, they were the most common trematode in CFb and OPT (43 and 65% of infections, respectively).

Similar to snail density, the percentage of snails parasitized (hereafter "prevalence") also decreased from 2002 to 2003 ($\chi^2 = 5.05$, $p > 0.025$). However, there was no difference in the percentage of large snails (>8 mm) parasitized between years ($\chi^2 = 0.665$, $p = 0.415$), whereas parasitism in small snails (6-8 mm in width) decreased significantly in 2003 relative to 2002 ($\chi^2 = 14.829$, $p > 0.001$, Fig. 2-4). Last, increases in rainfall were positively correlated with changes in the density of small snails ($r^2 = 0.600$, $p > 0.04$) but were not related to changes in large snail density ($r^2 = 0.116$, $p < 0.45$). This led to the hypothesis that that small parasitized snails were more susceptible to dislodgment than large parasitized snails.

Substrate size – flow experiment

There was a significant effect of parasitism on time to dislodgment, with snails with high parasite loads dislodging faster than snails with low or no parasites (Fig. 2-5). There was no effect of snail size (Table 2-3) or interaction between snail size and parasitism (Table 2-3). There also was no effect of substrate size on dislodgment (Table 2-3); however, there was a significant interaction between substrate size and parasite load (Table 2-3), as snails with low parasite loads dislodged faster on tiles than on gravel substrates (Fig. 2-5).

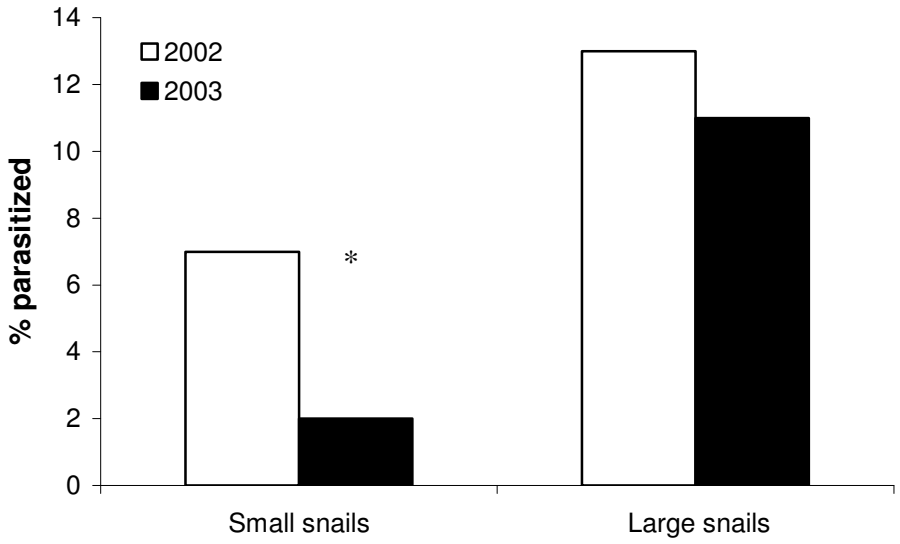

Figure 2-4. The percentage of small (6-8mm shell width) and large *Elimia* snails (>8mm) parasitized by larval trematodes in 2002 and 2003. * indicates p>0.001 (n= 2840)

Table 2-3. ANOVA table showing effects of substrate size, snail size and parasite load on time to dislodgment for the snail *Elimia flava*. * indicates significant model at 0.05 level

	DF	SS	MS	F	p
Model	8	1841666	230208	2.9681	0.0035*
Substrate	1	134345		1.7321	0.1894
Snail size	1	110304		1.4222	0.2343
Parasite load	2	556350		3.5866	0.0293*
Substrate x Parasite load	2	551998		3.5585	0.0301*
Size x Parasite load	2	279661		1.8029	0.1671
Error	230	17838773	77560		
Total		19680439			

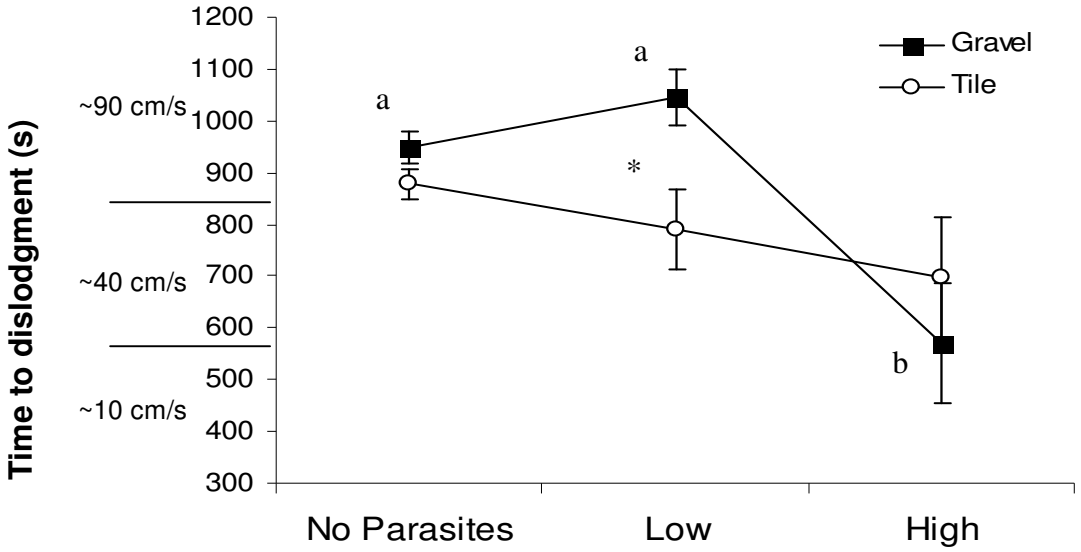

Figure 2-5. Mean time (±1 SE) to dislodgment of *Elimia flava* on two substrate treatments (gravel and tiles) across three levels of parasitism (no parasites, low [<150 sporocysts], and high [>150 sporocysts]). The Y- axis also shows the 3 current velocities. Pair wise comparisons: between tile and gravel * $p > 0.05$, across gravel different letters (i.e. a, b) indicate $p > 0.05$.

Parasitism also was associated with snail movement patterns within the artificial streams: snails showing net downstream movement over the trial had higher parasite loads than those that moved upstream or showed no net movement (t = -2.594, $p < 0.017$, Fig. 2-6). Snail behavior also affected dislodgment probability, as individuals moving upstream (t = -4.534, $p< 0.0001$, Fig. 2-7) or to the waterline (t = -3.642, $p< 0.0003$) resisted dislodgment by ramping flows longer than snails not showing those behaviors.

DISCUSSION

In the relatively dry year (2002) with low levels of flood disturbance, prevalence, a biotic factor, was the best predictor of snail abundance. Whereas, in the wet year (2003), link magnitude, an abiotic factor, best predicted snail density. This result supports the predictions of the harsh-benign hypothesis (Peckarsky 1983), which states biotic factors are more influential during periods of low intensity or infrequent disturbance while abiotic factors are more influential in more frequently disturbed systems. The effects of abiotic factors such as geology (Huryn et al. 1995), light and current (Johnson and Brown 1997), and disturbance (Holomuzki and Biggs 1999) on snail populations have been well studied. Effects of biotic factors such as predation (Turner et al. 2000) and competition (Race 1982) are also well known. However, much less is known about how disturbance mediates the influence on biotic and abiotic factors in stream snail populations.

Results from my experiment showed that snails with high parasite loads were more susceptible to dislodgment from high current conditions than snails with low

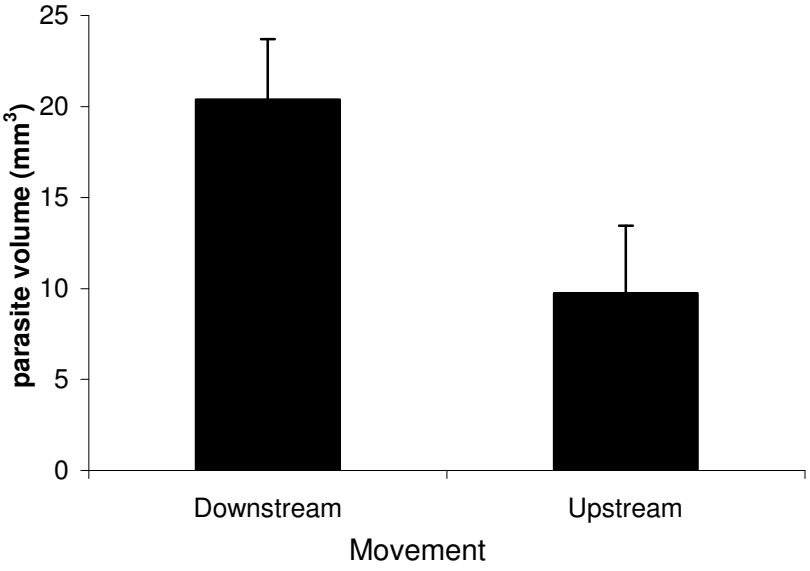

Figure 2-6. Mean (+1 SE) parasite volume of *Elimia flava* snails that showed downstream and upstream movement ($p < 0.017$).

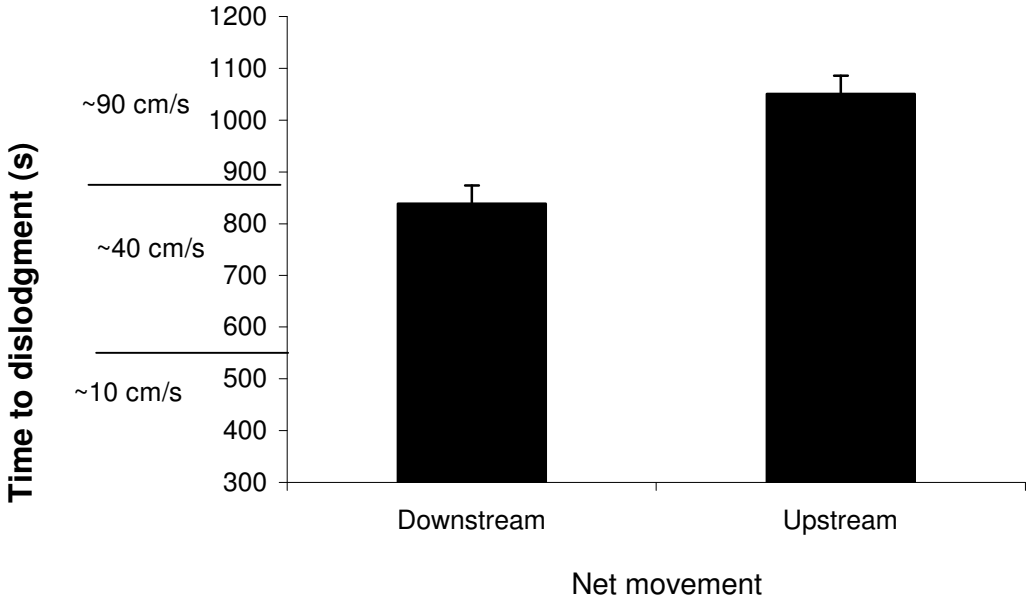

Figure 2-7. Mean (+1 SE) time to dislodgment for snails of *Elimia flava* that showed net downstream and net upstream movement ($p < 0.0001$)

parasite loads or no parasites. Parasitized snails may be more susceptible to dislodgment by current for several reasons. The most likely explanation for this pattern is that consumption of host energy by parasites causes hosts to have less ability to withstand the shear force of water at high velocity. Previous work has shown numerous examples of trematode parasites consuming significant host resources, including all reproductive tissue (castration) (Krist et al.1998, Lenertz 2002,). Parasites do not only limit snail reproduction, but they also may reduce levels of stored glycogen in the body tissue (Lenertz 2002). In my study, individuals with high parasite loads may have less stored energy to withstand the current experienced at high flows than snails with low or no parasites.

Another explanation is that parasites may alter snail behavior to increase the likelihood of transmission to the next host, a pattern commonly seen in other snail-parasite interactions (Levri 1999, Shinagawa et al. 1999). Data from the ramped flow experiment showed that snails that moved downstream had higher parasite loads than snails that moved upstream, and that snails that moved downstream were more likely to be dislodged than upstream-moving snails. *Elimia* has been shown both to orient and display net movement upstream over time (Huryn at al.1994). Parasite-induced movement downstream would cause snails to orient with the spire of their shell facing into the current. This position is much less hydrodynamically efficient than an upstream-facing snail, and would generate lift from water underneath the shell, thus increasing the likelihood of dislodgment (Vogel 1994). Movement or displacement of snails downstream could benefit snail parasites by dispersing the parasitic larval stages to areas where fish, the most likely next host, may be more prevalent. However, it is also possible

that higher downstream movement shown by parasitized snails is simply a byproduct of infection, with downstream movement being a result of depleted energy reserves and the tendency for snails to orient themselves in the least energetically expensive position, irrespective of the consequence of higher risk of dislodgment.

Contrary to Holomuzki and Biggs (1999), I did not find a significant effect of substrate size alone on dislodgment. I did find a significant interaction between parasitism and substrate size, with snails with low parasite loads being dislodged faster on tile than on gravel; however, there was no difference in time to dislodgment in snails with high parasite loads. The roughness of gravel substrate causes eddies and areas of slower flow to form between substrate particles (Davis and Barmuta 1989). Thus, it appears that gravel substrates act as a refuge against dislodgment for snails with low levels of parasitism. In contrast, it appears that snails with high parasite loads are either still subject to dislodgment even in reduced flow spaces in gravel interstices or they may be less mobile because of low energy stores, being dislodged before they can take refuge between substrate particles than less parasitized snails.

My artificial stream experiment did show that level of parasitism affects likelihood of snail dislodgment; however, it did not support the hypothesis that small parasitized snails were more susceptible to dislodgment than large parasitized snails, as there was no interaction between size and parasite load in my analysis. I used naturally infected snails in this experiment, so I had a low sample of small highly parasitized snails ($n = 4$); this limitation may have reduced statistical power to detect a difference in dislodgment time between highly parasitized large vs. small snails. Other factors,

however, may explain why small snails were more susceptible to flow disturbance than larger snails.

My field surveys showed that frequencies of small snails declined more than those of larger snails from 2002 (wet year) to 2003; this result was surprising because 1) there was no effect of size on dislodgment probability in the artificial stream experiment, and 2) previous work using *E. semicarinata* has shown that small snails are actually more common in high-flow habitats than larger snails (Johnson and Brown 1997). `Furthermore, Moore (1964) demonstrated in an artificial stream, small snails of two species (*Stagnicola palustris* and *Physa propinqua*) withstand higher current velocities than larger snails. In that study, small snails were dislodged at a similar rate as large on sand and clay but larger snails dislodged faster on coarser substrates (basalt rock and pea gravel). These studies examined the effect of current velocity on snail dislodgment, but they did not take into account the effects of sediment movement and scour during a flood, which may influence snail's susceptibility to dislodgment. Holomuzki and Biggs (1999) found using artificial stream channels, that dislodgment of snails on loose substrates was positively correlated to the amount of substrate that eroded. However, their study species, *Potamopyrgus antipodarum*, is much smaller and lighter than *Elimia.* Further, their results from experiments with artificially stabilized vs. natural substrate suggest that dislodgment, especially on smaller gravel substrates, was much higher on loose substrate vs. stabilized substrate. It is unknown how scour and substrate movement influence snails of different sizes. In addition, if snails are entrained in the water column during a disturbance, small snails should be entrained longer and thus be carried greater distances than larger snails, which may also explain why I found reduced numbers of smaller snails

in 2003. Holomuzki and Biggs (1999) also demonstrated that once snails were entrained entrained while mortality was low (~ 6%) it varied with substrate size and was highest amongst coarser substrate (cobble).

In addition to scour, substrate deposition may also influence a snail's response to disturbance. Matthaei et al. (1999) found that flood events could deposit as much as 24 cm of substrate in stream patches. It is possible that the differential response to disturbance between size classes could depend on how snails respond to burial. Burial could lead to conditions such as anoxia or starvation, and parasitism has been shown to increase mortality in both conditions (Sousa and Gleason 1989, Jokela et al. 1999). Further work is needed to determine if size or parasitism influences snail survival during burial or the ability to return to the surface.

In conclusion, this study supports the harsh-benign hypothesis by demonstrating that biotic factors such as parasitism best predicted snail abundance in normal water years and abiotic factors such as link magnitude best predicted snail abundance in high-flow years. However, this study also showed that biotic factors such as parasitism can mediate the influences of disturbance, with high levels of parasitism making snails more susceptible to dislodgment during floods.

CHAPTER THREE:

INFLUENCE OF SUBSTRATE SIZE AND SNAIL DENSITY ON THE PREVALENCE OF TREMATODE PARASITISM AND GROWTH IN THE STREAM SNAIL *ELIMIA FLAVA*

INTRODUCTION

Parasitic trematodes commonly use snails as intermediate hosts, and production of trematode larval stages within host snails represents a significant drain on snail resources, with infection potentially affecting host behavior (Levri 1999, also see review by Poulin 1995), growth (Ibrahim 2006), and reproduction (Lafferty 1993). Changes in host behavior can result in a shift in microhabitat use with parasitized snails altering both where and when they are active (Levri and Fisher 2000, Jokela and Lively 1995).

Prevalence of trematodes in snail populations varies spatially in both lotic and lentic habitats (Richardson and Scheiring 1994, Jokela and Lively 1995, Smith 2001). Disparate prevalence of parasites within snail populations among habitats can be explained by at least 3 different mechanisms. First, snails may have a higher likelihood of infection in certain habitats because of higher parasite abundance in those habitats. For example, in mangrove systems parasite prevalence in *Cerithidea scalariformis* is strongly related to the number of wading birds (the definitive hosts) depositing feces (i.e., parasite source) in an area (Smith 2001). Second, parasites may cause behavioral shifts

to new habitats by host snails, possibly to maximize transmission to the next host, or as a byproduct of infection. For example, Jokela and Lively (1995) found that infections of *Microphallus* spp. in *Potamopyrgus antipodarum* were more prevalent in shallow, shoreline habitats, where snails were more accessible to waterfowl, the definitive host, than were uninfected snails. Third, in habitats with high environmental stress, infected snails may show a higher mortality rate than uninfected snails, thus lowering the proportion of infected snails in stressful habitats. For example, Sousa and Gleason (1989) found that parasitized marine snails of *Cerithidea californica* had a higher mortality rate under low dissolved oxygen conditions than uninfected snails.

In addition to altering habitat use, trematodes also may alter growth rates of host snails. The effects of parasitism on growth are highly variable and taxon specific both for hosts and parasites (Gorbushin 1997, Sousa 1983). Parasites may increase snail growth rates, which may cause gigantism in some species (Probst and Kube 1999, Ibrahim 2006), whereas in others they may simply stunt growth or have no effect (Sousa 1983, Gorbushin and Levakin 1999).

Few studies have investigated the mechanisms responsible for the spatial distribution of parasites in natural communities (Shinagawa et al. 1999), or the effects parasites have on growth in pleurocerid snails (Krist 2000). Shinagawa et al. (1999) reported that infected *Semiulcospira libertine* were more likely to select deepwater habitats, where emerging cercaria could more easily access their next host, usually a fish. Previous work on the stream snail *Elimia flava* has shown that parasite prevalence is highest in areas with larger substrate (i.e., bedrock) and high snail densities (Tomba and Feminella, Chapter 1). However, it is unknown if these differences in distribution

occurred from alteration of host snail movement into such habitats, or from other factors such as higher likelihood of infection or increased host mortality in areas where parasites are prevalent. The objectives of my study were to determine if 1) snail density and substrate size influence parasite prevalence, 2) parasite load influences snail growth, and 3) snail density and substrate type influence snail growth. Taken together these objectives allowed determination of whether the association among high parasite prevalence, large substrate, and high snail density results from the parasite altering snail habitat use or an increasing likelihood of infection in this habitat.

MATERIALS AND METHODS

Study species

Elimia flava (Gastropoda: Pleuroceridae) is a stream snail common throughout the Tallapoosa River Drainage in eastern Alabama. Snails within this genus are dioecious (Dazo 1965, Lenertz 2002), have an estimated life span of 3–4 to 10–11 y (Dazo 1965, Huryen et al., Richardson and Scheiring 1994) and reach sexual maturity at ~1 y (Lenertz 2002). Digenean trematodes are a widespread group of parasitic flatworms that use snails almost exclusively as 1st intermediate hosts (Schmidt and Roberts 1996). They have a complex life cycle involving several host species, with a vertebrate usually as the definitive host. Snails become infected when they ingest eggs from adult worms or by miracidia, which hatch from eggs and then penetrate the snail. Several larval stages generally develop within snails including sporocysts, redia, and cercaria, with the latter stage exiting the snail and infecting the next host (Schmidt and Roberts 1996). *Elimia*

flava commonly serves as the 1st intermediate host for parasitic trematodes in the Family Opecoelidae (Lenertz 2002), with opecoelids typically using a fish as the definitive host (Schell 1970, 1985).

Experimental design

I conducted an *in situ* caging experiment with 2 snail density treatments (high and low) and 2 substrate treatments (unglazed ceramic tile and sand) to investigate the degree to which snail density and substrate influence snail infection by parasites (hereafter "prevalence"). *Elimia flava* (hereafter "snails") was collected from a site on Choctafaula Creek within the Tuskegee National Forrest where the prevalence of parasitism was low (~1% of snails infected, Tomba Chapter 1). I measured snails (width, nearest 0.01 mm) and then uniquely labeled them either by affixing a Bee tag® to their shell or by marking them with a paint pen. I used snails that ranged in shell width from 6.5 to 8.5 mm as these snails represent mature adults that were more likely to become parasitized than juvenile snails (<6 mm), but not so large (i.e. <9 mm) that they were already likely to be infected (i.e. large adults are often parasitized; Lenertz 2000). I randomly assigned snails to cages within 4 treatment groups (sand substrate, high snail density; sand substrate, low snail density; bedrock (tile) substrate, high snail density; bedrock substrate, low snail density; 8 cages per group n =32). Cages (20l x 19w x 8d cm) were constructed out of 10-mm-mesh plastic hardware cloth and lined with 2-mm-mesh fiberglass window screening to contain the snails. High-density cages were stocked with 20 snails = 526/m^2 and low-density cages received 10 snails (= 263/m^2); these densities were within the

range of ambient snail densities in Choctafaula Creek (unpubl. data). For the large size substrate treatments, I used unglazed ceramic tiles (15 x 15 cm) to simulate bedrock and placed a tile in the bottom of each cage, which was then anchored in primarily cobble and boulder habitats of the stream. For the small size substrate treatment, I placed cages in sandy habitats installed such that the lower 2 cm of the cage bottom was buried within the stream bed. I installed cages in Choctafaula Creek several km upstream of the collection site where parasitism is considerably higher (26%, Tomba Chapter 1). I arranged cages in a stratified block design over a 200-m reach of stream and secured them with rebar. Each block consisted of 1 replicate from each of the 4 treatments.

I measured current velocity (Marsh-McBirney Flo-mate, Model 2000), % shade (spherical densiometer, Lemmon 1957) and depth at each cage, and attempted to minimize variation in habitat variable across treatments. I removed accumulated debris every 2 to 3 d to maintain flowing conditions for a 9-wk period (23 June 2003 – 25 August 2005). At the end of the experiment, I removed cages, preserved snails in 95% ethanol, and then measured them for maximum shell width (nearest 0.01 mm). To dissect snails for trematodes, I removed snails from their shells, used a watchmaker's forceps tip to slice through the body wall along the snail's length, and then probed all tissues under a dissecting microscope (25–50×) for the presence of larval parasites. I quantified parasite load by simply counting the number of larval parasites per snail. I used ANOVA to test if substrate size and/or snail density influenced parasite prevalence and/or snail growth. I also used linear regression to examine the relationship between parasite load and snail growth.

RESULTS

Mean current velocity, % shade and depth did not differ among treatments (Table 3-1). Ten cages out of 32 were excluded from the analysis because >75% of the snails had escaped during the experiment. Losses were roughly similar across treatments with 3 cages each excluded from the low- and high- density tile treatment and the high-density sand treatment, and 1 cage excluded from the low-density sand treatment.

There was a significant effect of both snail density and substrate size on parasite prevalence (Table 3-2). Prevalence was higher in cages containing tile substrates (vs. sand) and higher in cages with high (vs. low) snail densities (Fig. 3-1). However, there was no interaction between substrate size and snail density (Table 3-2). Prevalence of snails at the source population remained at 1.25 % of snails infected (n = 80) after the experiment.

The ANOVA model describing the effect of snail density and substrate size on snail growth was significant ($F = 10.049$, $p < 0.0004$, Table 3-3). Snails in the high-density treatment grew an average of 0.26 mm over the 9-wk experiment whereas snails in the low-density treatment grew and average of 0.50mm ($p < 0.001$, Fig. 3-2A). Substrate size did not significantly affect growth (Table 3-3, Fig. 3-2B) nor was there an interaction between snail density and substrate size ($p = 0.350$). Last, there was no relationship between snail growth and parasite load ($r^2 = 0.0003$, $p = 0.706$), or between growth and parasite prevalence ($r^2 = 0.072$, p = 0.227).

Table 3-1. 1-way ANOVA and means (±1 SE) for habitat variables across treatment groups. Treatment abbreviations: Sand-Low Snail Density (Sand-Low), Sand-High Snail Density (Sand-High), Tile-Low Snail Density (Tile-Low), Tile-High Snail Density (Tile-High).

	Treatments				*F*	*p*
	Sand-Low	Sand-High	Tile-Low	Tile-High		
n	7	5	5	5		
Current velocity (m/s)	0.065 ± 0.026	0.046 ± 0.023	0.082 ± 0.027	0.086 ± 0.033	0.40	0.75
Depth (m)	0.259 ± 0.018	0.270 ± 0.04	0.269 ± 0.031	0.227 ± 0.283	0.44	0.73
% shade	89.2 ± 1.1	89.4 ± 1.5	89.9 ± 1.3	86.3 ± 1.6	1.28	0.31

Table 3-2. ANOVA table showing effects of snail density and substrate type on parasite load.

Source of variation	df	SS	MS	*F*	*p*
Model	3	0.011	0.0037	3.614	0.033
Snail density	1	0.005		5.0224	0.038
Substrate size	1	0.005		4.8524	0.041
Snail density x Substrate	1	0.001		0.5618	0.463
Error	18	0.018	0.0011		
Total	21	0.029			

Table 3-3. ANOVA table showing effects of snail density and substrate size on snail growth.

Source of variation	df	SS	MS	*F*	*p*
Model	3	0.348	0.1159	10.0486	0.0004
Snail density	1	0.307		26.6425	0.0001
Substrate size	1	0.023		2.0214	0.1722
Snail density x Substrate	1	0.011		0.9307	0.3475
Error	18	0.208	0.0115		
Total	21	0.555			

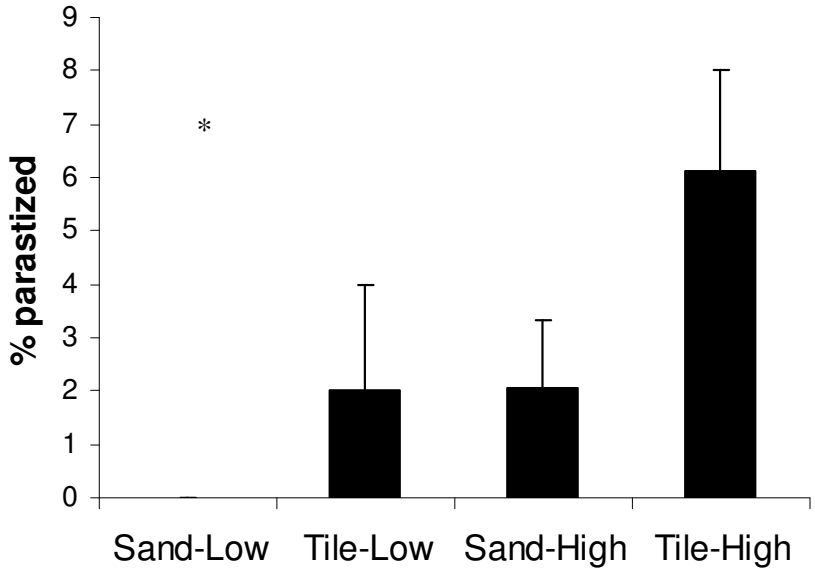

Figure 3-1. Mean (+ 1SE) parasite prevalence across 4 treatment groups. * $p < 0.04$ for sand vs. tile treatments and low snail density vs. high snail density treatments. Treatment abbreviations: same as Table 3-1.

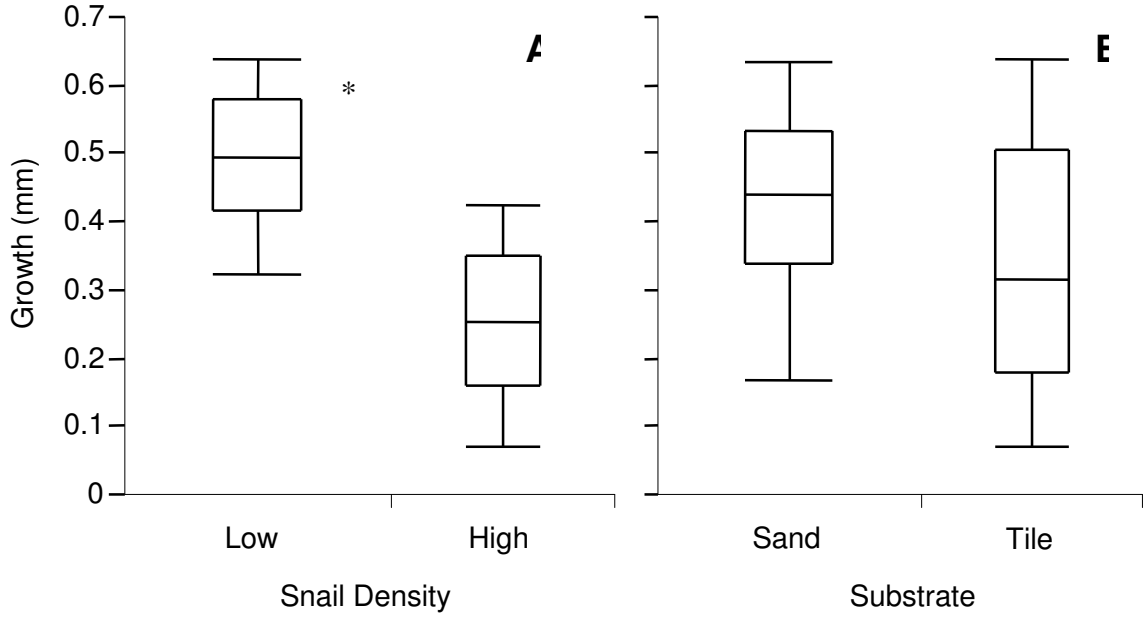

Figure 3-2. A.— Mean growth in the low- vs. the high-density snail treatment. B – Mean growth in the sand vs. tile substrate treatment. Box ends represent 25th and 75th quartiles, middle horizontal lines indicate the mean, and the whiskers indicate the outermost data point within 1.5 x interquartile range. . * $p < 0.0.001$

DISCUSSION

Results from my experiment support the hypothesis that an increased likelihood of infection in certain habitats is a mechanism responsible for the spatial variation in trematode prevalence in *Elimia flava*. As such, it refutes the hypothesis that parasites alter snail habitat use in this population, as caged snails in areas of large substrate had a higher prevalence of parasitism than snails caged in sandy areas. If parasites did in fact alter snail behavior as shown by others, then there would be no difference in parasite prevalence between the substrate or snail density treatments. Additionally, there was no interaction between density and substrate on prevalence; thus it appears that host density and substrate operate independently on likelihood of infection, with the highest prevalence occurring in areas with high snail density and coarse substrate, and the lowest prevalence of parasitism occurring in sandy substrates with low snail density.

Details of the trematode life cycle provide an explanation why snails may be more likely to become parasitized on large (vs. small) sized substrates. In opecoelid trematodes, the definitive host is usually a fish, and the fish host passes trematode eggs into the water with its feces (Schell 1970, 1985). There, the eggs develop and hatch into ciliated miracidia. Snails then may become infected either by penetration of the miracidia or by ingesting trematode eggs (Schell 1975). The flowing nature of streams, which frequently cause shifting of small substrates such as sand, increases the probability that eggs/larvae landing in sandy environments become buried vs. eggs entrained on coarse substrate. Such burial in sand may, in turn, reduce the likelihood that miracidia or eggs come into contact with the host snail. In contrast, eggs on larger substrates could

remain be less subject to burial, and more likely to be ingested by the host, or the hatching miracidia could more easily contact the host.

Previous work on other parasitized snail, such as *Biomphalaria glabrata* has demonstrated that parasitized snails are attracted to each other and thus aggregate together, potentially explaining why parasite prevalence is higher in areas of high (vs. low) snail density (Boissier et al. 2003). This explanation is not supported by my results; instead, my data suggest that snails in high-density areas within a stream have an increase likelihood of infection, as snails in the high-density treatments had higher prevalence than snails in low-density treatments.

In most trematode life cycles, parasites are not transmitted from snail to snail (Schmidt and Roberts 1996); therefore, the higher prevalence at higher snail density response I observed cannot be explained by classic density-dependent population models common in infectious diseases (Gilbert et al. 1994). Such models predict that increased host density increases transmission because of high parasite-host contact. It is important to note, however, that larval sporocysts of some opecoelid trematodes can contain adult trematodes, which can then produce eggs and infective miracidia while still inside the snail (e.g., *Plagioporus sinitsini*, Barger and Esch 2000). Such a life cycle could potentially explain the higher prevalence of parasitism at higher snail densities; however, the abundance of this species in my study area is unknown, although none of the snails I dissected showed adult trematodes within sporocysts (pers. observ.). Thus, the most probable explanation for high parasite prevalence in areas of high snail density may be that large densities of snails are more easily detected by the infective stage (miricidia). Miracidia exposed to snail-conditioned water have been shown to alter their swimming

behavior and aggregate at the source of the conditioned water (Nollen 1994, Chernin 1970). In addition, miracidia appear to show a stronger aggregative response when exposed to water from large (vs. small) snails (Chernin 1970). Finelli et al (2000) found the blue crab *Callinectes sapidus* was more successful and efficient at locating a food odor source when the odor was delivered at a higher rate. They also found that the odor release rate of prey items (clams) was exponentially related to prey size. A high-density patch of snails could have a higher release rate of odor cues and thus would be more likely to attract miracidia and become infected, than a low-density patch.

Parasite induced changes in growth have been documented for many snail species (Mohamed and Ishack 1979, Gerard and Theron 1997, Krist and Lively 1998, Mouritsen et al. 1999, Krist 2000), although, the overall result differs widely. Some studies reporting gigantism from parasitism (Mohamed and Ishack 1981, Probst, and Kube 1999, Gorbushin and Levakin 1999), where as others reported a decreased growth in infected snails (Gerard and Theron 1997, Gorbushin and Levakin 1999, Mouritsen et al. 1999) or no difference in growth between infected and uninfected snails (Gorbushin and Levakin 1999, Mouritsen et al. 1999). One explanation for these disparate growth responses to parasitism is that increased growth is more common in shorter-lived semelparous, species and there is less of an effect on growth in longer-lived, iteroparous species (Sousa 1983). This idea was supported by Gourbushin and Levakin's (1999) study that measured the effect of parasitism on growth of three snail species with different life histories. *Elimia flava* is a relatively long-lived (4-11 y) iteroparous species, so my results that indicating no relationship between parasite load and growth further support this idea. However, at

least one study of a congener *E. livecens* showed increased growth when parasitized (Krist 2000), so this explanation does not appear universal.

Parasitism did not affect growth, but at low snail densities growth nearly doubled relative to high snail densities. This indicates that these snails are food limited at ambient densities, a common pattern in small shady streams (Hill et al. 1995). Being food limited, especially in the high density treatment, may provide another explanation for increased parasite prevalence in the high density treatment. Calow (1974) reported that both *Ancylus fluviatilis* and *Planorbis contortus* showed an increase in movement when deprived of food. For *E. flava* increased movement from low food resources could cause snails to encounter infective eggs or larvae more frequently and thus explain why parasite prevalence is higher in high snail density treatments.

In conclusion, it appears that parasite alteration of behavior is not responsible for the spatial variation in parasitism within this snail population and that certain habitats, namely high snail density and coarse substrate habitats, represent a greater risk of infection than do areas of low snail density and finer substrates. It is not known if snails can select habitats to minimize their risk of infection. Additionally, my results further support the idea that parasitism has limited effects on the growth of these iteroparous, long-lived snails.

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