

THE EFFECTS OF AN INVASIVE CRAYFISH (*FAXONIUS CRISTAVARIUS*) ON NATIVE SYMBIONT
COMMUNITIES IN THE NEW RIVER IN NORTH CAROLINA

A Thesis
by
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Submitted to the School of Graduate Studies
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 2023
Department of Biology

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Abstract

THE EFFECTS OF AN INVASIVE CRAYFISH (*FAXONIUS CRISTAVARIUS*) ON NATIVE SYMBIONT COMMUNITIES IN THE NEW RIVER IN NORTH CAROLINA

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Three dominant, native crayfish species in the genus *Cambarus* that occur in the New River in northwestern North Carolina may host up to seven species of ectosymbiotic worms known as Branchiobdellidans. Branchiobdellidan worms depend on the crayfish for successful reproduction and get a safe habitat to occupy. Most species of branchiobdellidan appear to be commensals, some are obligate parasites and a few species have been shown to engage in a mutualism with their crayfish hosts. *Faxonius cristavarius*, an invasive crayfish, was introduced into the New River drainage in the 1930s. *Faxonius cristavarius* is a noncompetent host for branchiobdellidans and will quickly remove any worms that colonize it. The distributions of all three species of *Cambarus* (*Cambarus chasmodactylus*, *C. robustus*, and *C. bartonii*) now overlap with *F. cristavarius* in the North and South Forks of the New River in northwestern North Carolina. If *F. cristavarius* acts as a sink for these worms, this could reduce the fitness of native *Cambarus* spp. and further, provide a competitive advantage for *F. cristavarius*. I hypothesized that the presence and increasing relative abundance of *F. cristavarius* would have a negative effect on the abundance and richness of the worms on all three native *Cambarus* species. I conducted a survey in various

locations in the watersheds of both the North and South Forks of the New River to determine the diversity and abundance of worms on native *Cambarus* spp. and *F. cristavarius*. Sample sites exhibited varying degrees of invasion by *F. cristavarius* ranging from 0 – 100% invaded. There were significant, negative declines in branchiobdellidan abundance and richness on native *Cambarus* spp. in response to an increasing relative abundance of *F. cristavarius*. I also tested whether *F. cristavarius* caused changes in the abundance of symbiont worms on native *Cambarus robustus* after subjecting individuals to one of two treatments: 1) a control treatment (Cr/Cr) consisting of a single *C. robustus* stocked with 5 worms placed with an unstocked *C. robustus* and 2) an experimental treatment consisting of a single, stocked *C. robustus* placed with an unstocked *F. cristavarius* (Cr/Fc). Significantly fewer worms remained on crayfish in the presence of *F. cristavarius*. This research contributes to a greater understanding of the effects of invasive species on freshwater symbiont communities. It should guide conservation tactics regarding the introduction of non-native hosts and their potential impacts on populations and communities of native symbionts.

Acknowledgments

First and foremost, I would like to thank my Lord and Savior, Jesus Christ, who blessed me with the strength, patience, and courage I needed to fulfill my lifelong dream of becoming a biologist.

I am deeply indebted to my research mentor, Robert Creed, for his patience, invaluable advice, continuous support, and willingness to teach me what a great scientist is. He has laid out the pathway to my success and has given me all the tools needed to succeed by demonstrating his immense passion for ecology and worldly insight.

I could not have undertaken this journey without the love of my precious children, Clair and Daniel. They motivate me to do my best, so I can one day give them the life they deserve.

Words cannot express my gratitude to my sweet mother and father for supporting my two children and me financially and emotionally during my time here at App State; they made it possible for me to go back to school.

Many thanks to my sister, brother-in-law, and nephew for their encouragement; thesis committee members Dr. Michael Gangloff and Dr. Jon Davenport, as well as Dr. Howard Neufeld for excellent advice and accountability; the College of Arts and Sciences; the Appalachian State Department of Biology; and the Cratis D. Williams School for Graduate Studies.

Dedication

I dedicate this thesis to my sweet grandmother, Mary Massie, my guardian angel. I miss you, Yummy.

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Foreword

This thesis will be submitted to the journal *Biological Invasions*, an international peer-reviewed journal. It has been formatted according to the style guide for that journal.

Introduction

Invasive species threaten the diversity of native species in many ways such as through competition, predation, herbivory, and spread of disease (Richardson et al. 2000, Rodriguez 2006, Pejchar and Mooney 2009, Hernández-Brito et al. 2014, Lymbery et al. 2014). Next to habitat loss, invasive species are the second most significant threat to native species diversity (Didham et al. 2005, Pejchar and Mooney 2009). Invasive species are often intentionally and unintentionally introduced to habitats through human activities such as the import and export of plants, animals, and raw materials such as lumber, as well as the introduction of biocontrol agents used against other problematic invasive species (Pimentel et al. 2005, Sakai et al. 2001, Vilcinskis et al. 2013). For example, wild pigs (*Sus scrofa*) are common invasive mammals widely distributed in the United States and many other countries worldwide (Connaly et al. 2021). Wild pigs have been intentionally introduced for food and hunting, and they have adapted to most of these novel habitats easily due to having a generalist lifestyle (Connaly et al. 2021).

In addition to human introductions of invasive species into novel habitats from their native ranges, or other invaded habitats, biotic and abiotic factors can naturally aid in dispersing invasive species. For example, facilitative dispersal by animals such as birds and passive dispersal via wind and water introduce invasive species to new habitats (Sakai et al. 2001). *Physa acuta*, an invasive gastropod, was initially introduced from its native range in North America to other continents via the plant and aquarium trade (Leeuwen et al. 2013). Since the snail's introduction, it has successfully invaded all continents due to facilitative

dispersal between lakes attached to plants attached to the bottom of boats and, further, attached to the feathers of multiple species of water birds (Leeuwen et al. 2013, Velez et al. 2021). However, not all introduced species are able to colonize native habitats.

Non-native species may become problematic and labeled invasive when rapid range expansion and establishment of large populations follow successful colonization of a novel habitat. Once invasive species become abundant, this could lead to the displacement of native species (Sakai et al. 2001). Human-induced disturbances, such as logging and urbanization, increase the likelihood of invasion providing newly opened habitats for colonization (Sakai et al. 2001). Successful invasive species may be dominant competitors especially if they lack natural predators or parasites (Lymbery et al. 2014). Additionally, species with high fecundity, short development time and generalist feeding habits are often successful invaders (Mondor and Addicott 2007).

Adverse effects of invasion have been well studied by investigating effects of invaders, such as competitive exclusion and predation, on individual native species. However, relatively few studies have addressed the indirect effects of invasion including alteration of ecological interactions between mutualist partners (Traveset and Richardson 2006). Invasive species can indirectly affect native species by altering the ecological interactions with their symbiotic partners (Traveset and Richardson 2006). For example, *Boiga irregularis*, commonly known as the brown tree snake, has caused the extinction of 12 bird species in Guam. This loss indirectly threatens native tree species that depend on those bird species for seed dispersal (Rogers et al. 2017). Invasive species may indirectly affect symbiont communities, such as the partnership between mycorrhizal fungi

and many vascular plants. The degradation of fungi prevents movement of water and nutrients into the roots of plants (Stinson et al. 2006). Therefore, disruption of mutualisms, such as with plants, fungi, birds, and trees by invasive species, is yet another way invasive species can exert negative, indirect effects on communities.

Invasive crayfish are well documented in freshwater systems and threaten native freshwater organisms directly and indirectly; alterations in food webs likely disrupt native mutualisms. For example, omnivorous crayfish such as *Faxonius rusticus* consume snails and both snails and crayfishes feed on periphyton in littoral habitats (Lodge et al. 1994). The reduction of snails offsets the direct effects of herbivory on periphyton and indirectly causes a net positive increase in periphyton abundance (Lodge et al. 1994).

Another member of the genus *Faxonius*, *Faxonius cristavarius*, was introduced into the New River drainage in Virginia from Mountain Lake, Virginia in the 1930s (Bell 2018). It has since spread into the headwaters of the New River in northwestern North Carolina (NCWRC, unpublished data, Brown and Creed 2004, Helms and Creed 2005, Fortino and Creed 2007). The distribution of this invasive crayfish now overlaps almost completely with two of the three dominant, native crayfish species (*Cambarus chasmodactylus* and *C. robustus*) that occur in the upper New River in northwestern North Carolina (Fortino and Creed 2007; Helms and Creed 2005). There is some overlap with the distribution of *C. bartonii* and essentially none with a headwater specialist, *C. asperimanus*. Native crayfish, especially *C. chasmodactylus*, have declined at sites now dominated by *Faxonius cristavarius* (Brown and Creed 2004, Helms and Creed 2005, Creed, personal observation).

Crayfish in the Northern Hemisphere often host multiple species of ectosymbiotic annelid worms, known as branchiobdellidans (crayfish worms) (Gelder et al. 1999). Some species of worms have been shown to be dependent upon crayfish for a protective habitat and successful reproduction (Creed et al. 2015). A cleaning symbiosis has been confirmed between *C. chasmodactylus* and *C. bartonii* and two different symbiotic branchiobdellidans (Brown et al. 2002, 2012, Thomas et al. 2016). The worms can benefit the crayfish as they can clean the gills of the crayfish, which increases ammonia excretion by the crayfish; this appears to improve crayfish survival and growth (Brown et al. 2002, 2012, Skelton et al. 2013). Disruption of this mutualism could lower crayfish fitness (Brown et al. 2002, 2012, Lee et al. 2009).

Faxonius cristavarius is a “noncompetent host” for branchiobdellidan worms in the New River in northwestern North Carolina (Creed et al. 2022a, Farrell et al. 2014). *Faxonious cristavarius* displays more grooming when one worm is present than *C. chasmodactylus* displays when ten worms are present (Farrell et al. 2014). In addition to the active removal of the worms during grooming, *F. cristavarius* has been observed consuming the worms (Farrell et al. 2014). The loss of these worms after dispersal to *F. cristavarius* may lower the abundance and richness of the worms on *Cambarus* spp. in the New River, similar to what has been observed in Virginia rivers (Bell 2018, Creed et al. 2022a). Creed et al. (2022a) suggested that a lower survival of worms on noncompetent hosts could result in the extermination of these individual worms and an indirect decrease in the abundance and diversity of native *Cambarus* spp. Consequently, this could lead to a reduction in the fitness

of native *Cambarus* when mutualistic worm species are lost and provide a competitive advantage for *F. cristavarius* (Bell 2018).

The objective of this research was to investigate whether the richness and abundance of branchiobdellidan worms on native *Cambarus* crayfish in the New River and several of its tributaries were influenced by the relative abundance of *F. cristavarius*. In a lab experiment, I tested whether the presence of *F. cristavarius* caused changes in the abundance of symbiont worms on one of the native crayfish, *C. robustus*. I hypothesized that the noncompetent host *F. cristavarius* would have a negative effect on the abundance and richness of worms on native *Cambarus* host crayfish. Therefore, I expected that the presence and increasing relative abundance of *F. cristavarius* would have a negative effect on the abundance and diversity of the worms on native *Cambarus* host crayfish in the survey and the abundance of worms in the experiment.

Methods

Field Survey

A survey of crayfish and their ectosymbiotic, branchiobdellidan worms was conducted in the New River drainage to determine if the relative abundance of *Faxonius cristavarius* affected the abundance and richness of native branchiobdellidans. Surveys were conducted at 19 sites in the watersheds of the North and South Forks of the New River in North Carolina during June and July 2021 (see Table 1 for site locations). Crayfish were collected by two individuals for one hour at each site. Dip nets were placed immediately

downstream of rocks or other appropriate substrate, which was then flipped, and the substrate beneath was disturbed to drive the crayfish into the nets. Crayfish were also caught opportunistically with dip nets if spotted directly on the surface of the streambed.

Upon capture, each crayfish was placed individually into glass containers containing a 70% ethanol solution. After crayfish had been collected, the relative abundance of *F. cristavarius*, hereafter referred to as percent invasion, was determined for each site by dividing the number of *F. cristavarius* collected by the total number of crayfish collected at that site.

In the lab, the sex and total carapace length (TCL, in mm) of all preserved crayfish were determined. In addition, the external surfaces of the crayfish exoskeleton were examined under a dissecting scope for attached worms. The carapace was removed from all crayfish to determine presence of worms in the gill chambers. Any worms found on the exoskeleton and/or gill filaments were transferred to a separate dish for species identification and enumeration. As worms frequently detach from the crayfish when placed in ethanol, the bottom of the sample jar was also checked for worms.

For each crayfish, I determined the total number of worms found, the number of each worm species, and worm species richness. All worms from each crayfish specimen were preserved separately in glass vials containing 70% ethanol. Once all crayfish from all sites had been processed, I determined mean worm number and mean species richness for each site. Finally, the relationship between percent invasion, a measure of the relative abundance of *F. cristavarius*, and mean worm number and mean species richness for each site was analyzed using linear regressions in R package 'GGPLOT2' (version 4.2.2).

Because small crayfish frequently host few if any worms due to their frequent molting and grooming, I also used linear regression to examine the relationship between percent invasion and both mean worm number and mean worm richness for only crayfish greater than or equal to 20 mm TCL. Similar linear regressions were performed using only native crayfish (all individuals regardless of size), just native crayfish that were greater than or equal to 20 mm TCL, and for the three native crayfish species (*C. chasmodactylus*, *C. robustus* and *C. bartonii*).

I also determined the relationship between the abundance of individual worm species on native crayfish at each site as a function of percent invasion of *F. cristavarius*.

Finally, as worm abundance on crayfish can be influenced by the size of host crayfish (Creed et al. 2015, Thomas et al. 2016), mean crayfish TCL was determined for each site and was regressed against percent invasion for all crayfish species to determine whether or not mean crayfish size changed with increased percent invasion of *F. cristavarius*.

Laboratory Experiment

This experiment aimed to test whether the presence of the invasive *F. cristavarius* affected total branchiobdellidan worm abundance on the native crayfish *Cambarus robustus*. In order to determine the effect of *F. cristavarius*, I compared the number of worms remaining on all crayfish between aquaria containing two *C. robustus* crayfish (*Cambarus* treatment, Cr/Cr) and aquaria containing one *C. robustus* and one *F. cristavarius* (*Faxonius* treatment, Cr/Fc). The *Cambarus* treatment consisted of a donor *C. robustus* stocked with 5 *Cambarincola ingens* and a receiver *C. robustus* with no

worms. The *Faxonius* treatment consisted of a donor *C. robustus* stocked with 5 *C. ingens* and a receiver *F. cristavarius* with no worms.

Cambarus robustus and the *C. ingens* were collected from the Middle Fork of the New River. *Faxonius cristavarius* were collected from the South Fork of the New River. Large (> 6 mm in length) and medium-sized (3 – 6 mm) *C. ingens* were removed from the collected *C. robustus* and placed in a dish containing stream water. All crayfish were placed in a 10 % magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) bath for 5 minutes to kill any remaining worms and cocoons. Donor crayfish were stocked with three large and two medium-sized worms. Crayfish used in the experiment ranged in size (TCL) from 22-31 mm. When possible, crayfish of the same size were placed in aquaria. Size differences between the two species were less important in the *Faxonius* treatment given that *Faxonius* in the New River rarely hosts worms regardless of size (Farrell 2012).

Aquaria (37 L) contained water from the South Fork of the New River. A mixture of fine sediments from the New River and aquarium gravel covered the bottom of aquaria. Crayfish refugia were created using stream cobbles. Two refugia were set up on opposite ends of each aquarium to provide shelter for each crayfish. All aquaria were aerated. Lights were on a 14-hr. light:10-hr. dark schedule for the duration of the experiment. I replaced half the water in each aquarium weekly to prevent ammonia accumulation. In addition, water temperature was recorded 2-3x weekly from four randomly selected aquaria. Crayfish were fed two shrimp pellets every other day.

Pairs of aquaria were placed on wire shelves for a total of 6 rows. The experimental design was a randomized complete block design. I randomized the position of each

treatment in each row, which crayfish were placed in which treatment, and, in the *Cambarus* treatment, which crayfish would be the donor and which would be the receiver.

Donor and receiver crayfish were inspected under a dissecting scope on days 7, 14, and 20 for worms and cocoons. Worm and cocoon abundance in the two treatments were compared using a repeated measures ANOVA (R Function lme, version 4.0.2).

Results

Field Survey

There was a significant, negative effect of percent *Faxonius cristavarius* invasion on both mean worm number ($F_{1,17} = 23.448$, $P < 0.0001$, $r^2 = 0.579$, Fig. 1A) and mean worm species richness ($F_{1,17} = 46.495$, $P < 0.0001$, $r^2 = 0.732$, Fig. 1B) on all crayfish, i.e., *Faxonius* and the native crayfish (Table 2a), from all 19 sites.

Faxonius cristavarius was not detected at one site (Appaloosa Trail on Meat Camp Creek) where they had been found previously (Fortino and Creed 2007). This site is also not far from two other sites (Meat Camp Creek at Castleford Road and Roby Greene Road) where *F. cristavarius* were collected during this survey. Further, worm number and richness at this site were lower and similar to values obtained from invaded sites. Because the Appaloosa Trail Site is likely occupied by *Faxonius cristavarius*, I also analyzed worm number and richness with this site excluded. When the Appaloosa Trail Site data were excluded from the regression analyses, the regressions for worm number and species richness

remained significant, and the r^2 values increased: mean worm number ($F_{1,16} = 35.190$, $P < 0.0001$, $r^2 = 0.687$, Fig. 1C); and mean worm species richness ($F_{1,16} = 63.273$, $P < 0.0001$, $r^2 = 0.798$, Fig. 1D).

Branchiobdellidan numbers and richness were highest on crayfish at 0% and 3.6% invaded sites (mean worm number ranged from 45-71 and mean richness ranged from 3.5-4.5 species per crayfish). Worm number and richness declined to values close to or equal to 0 at sites at which 80-100% of the crayfish were *F. cristavarius*.

When I analyzed mean worm number and richness using crayfish (both *Cambarus* and *Faxonius*) with a total carapace length (TCL) ≥ 20 mm, the regressions were significant; mean worm number ($F_{1,16} = 39.723$, $P < 0.0001$, $r^2 = 0.713$, Fig 2A) and mean worm richness ($F_{1,16} = 73.482$, $P < 0.0001$, $r^2 = 0.821$, Fig. 2B). Regressions for mean worm number and mean worm richness on just large (≥ 20 mm TCL), native *Cambarus* crayfish were also significantly affected by percent invasion of *F. cristavarius*: mean worm number ($F_{1,13} = 7.496$, $P = 0.017$, $r^2 = 0.366$, Fig. 2C); mean worm richness ($F_{1,13} = 11.637$, $P = 0.005$, $r^2 = 0.472$, Fig 2D)

Significant, negative effects of *Faxonius* invasion on mean worm number and richness were apparent for large *Cambarus chasmodactylus*: mean worm number ($F_{1,13} = 13.780$, $P = 0.003$, $r^2 = 0.515$, Fig. 3A); mean worm richness ($F_{1,13} = 14.401$, $P = 0.002$, $r^2 = 0.526$, Fig. 3B) but not for large *C. robustus*: mean worm number ($F_{1,8} = 0.264$, $P = 0.621$, $r^2 = 0.032$, Fig. 3C); mean worm richness ($F_{1,8} = 0.736$, $P = 0.416$, $r^2 = 0.084$, Fig. 3D) and *C. bartonii*: mean worm number ($F_{1,6} = 0.545$, $P = 0.260$, $r^2 = 0.205$, Fig. 3E); mean worm richness ($F_{1,6} = 0.0003$, $P = 0.985$, $r^2 = 6.1E^{-05}$, Fig. 3F). In addition, large *F.*

crstavarius crayfish had fewer worms and worm species at sites at which their percent invasion was greater: mean worm number ($F_{1,13} = 11.983$, $P = 0.004$, $r^2 = 0.480$, Fig. 4A); mean worm richness ($F_{1,13} = 15.010$, $P = 0.002$, $r^2 = 0.537$, Fig. 4B).

Seven worm taxa were recovered from the crayfish collected during the survey (Table 2b). Two taxa (*Ankyrodrilus koronaeus* and *Xironogiton instabilus*) were only found at 8 and 3 sites, respectively, and only 191 and 6 individuals were collected, and thus these two taxa were not abundant enough to be included in the regression analyses. There were significant, negative regressions for the abundance of four of the five remaining taxa on the percent invasion of *F. cristavarius*: *Cambaricola ingens*, *Cambarincola fallax*, *Pterodrilis alcicornus* and *Bdellodrilis illuminatus* (*C. ingens*: $F_{1,16} = 29.303$, $P < 0.0001$, $r^2 = 0.647$, Fig. 5A; *C. fallax*: $F_{1,16} = 10.674$, $P = 0.005$, $r^2 = 0.400$, Fig. 5C; *P. alcicornis*: $F_{1,16} = 15.945$, $P = 0.001$, $r^2 = 0.499$, Fig. 5D; *B. illuminatus*: $F_{1,16} = 23.473$, $P < 0.0001$, $r^2 = 0.594$, Fig. 5E). *Camborincola philadelphicus* was not significantly affected by the increasing relative abundance of *F. cristavarius* ($F_{1,16} = 2.475$, $P = 0.135$, $r^2 = 0.134$, Fig. 5B). *Pterodrilis alcicornis* was the most abundant worm species with mean worm numbers per crayfish ranging from 20-34 worms per crayfish at sites with little to no *Faxonius*. *Pterodrilus* were essentially absent when the percent invasion of *Faxonius* reached 80%. Mean worm numbers for *Cambaricola ingens* and *C. fallax* were lower than *P. alcicornis*, ranging from 5-16 worms per crayfish at 0 – 3.6% invasion. *Camborincola philadelphicus* and *B. illuminatus* were the least abundant worm taxa with 1-8 and 4-7 worms per crayfish, respectively, at sites with 0 - 3.6% *Faxonius* invasion. *Cambaricola ingens*, *C. philadelphicus* and *B. illuminatus* were present on crayfish at sites with up to 85% *Faxonius* invasion (Figs.

5A, B & E); *C. fallax* was essentially absent on crayfish at sites with greater than 60% invasion (Fig. 5C).

Crayfish sizes were similar across sites regardless of whether examining all crayfish (Fig. 6A) or just native crayfish (Fig. 6B).

Laboratory Experiment

The repeated measures ANOVA indicated that worm abundance in the Cr/Fc treatment was significantly lower than the control treatment over the course of the experiment ($F_{1,9} = 6.64$, $P = 0.030$, Table 3A, Fig. 7A). There was also a significant day effect ($F_{1,28} = 83.41$, $P < 0.0001$) but no significant treatment x day effect (Table 3A). There was no significant row effect for worm number.

The number of cocoons increased in both treatments but there was no significant difference between the two treatments ($F_{1,9} = 0.503$, $P = 0.496$, Fig. 7B). There was also a significant day effect ($F_{1,28} = 17.382$, $P = 0.0003$) but no significant treatment x day effect (Table 3B). There was a marginally significant row effect for cocoon number (Table 3B).

Discussion

Research investigating the impacts of invading species typically focuses on the responses of native host taxa to invading host species. In contrast, the impacts on the abundance and diversity of native symbionts dwelling on those native hosts have received far less attention (Creed et al. 2022b). Previous surveys and experiments have shown that invasive crayfish in the genus *Faxonius* can affect the abundance and diversity of native

branchiobdellidan symbionts on native *Cambarus* crayfish hosts (Bell 2018, Creed et al. 2022a). In the survey, I observed a decline in branchiobdellidan abundance and richness on the dominant native crayfish, *C. chasmodactylus*, in the headwaters of the New River in North Carolina as the relative abundance of the invasive crayfish *F. cristavarius* increased. Little or no impacts on abundance and richness were observed for the other two native hosts, *C. robustus* and *C. bartonii*. In the lab experiment, I determined that the presence of *F. cristavarius* caused reductions in worm abundance on the native crayfish *Cambarus robustus*.

In the survey, there were significant, negative regressions of total worm number and richness on all crayfish as well as the larger crayfish, i.e., those with carapace lengths greater than or equal to 20 mm. The somewhat stronger relationships for worm number and diversity on larger crayfish were not surprising as small crayfish generally host fewer worms due to frequent molting and more active grooming relative to larger crayfish (Farrell et al. 2014, Skelton et al. 2014, 2016). The relationships between worm abundance and richness and percent invasion of *F. cristavarius* on the three native crayfish differed among the three species. The strongest relationships were for *C. chasmodactylus*, which hosted 78.2% of all worms found in the survey, and this crayfish host was present at all sites except the 100% invaded sites. Far fewer *C. robustus* were collected, and the worm abundance and richness relationships were weaker for this native host; 10.5% of the worms were recovered from *C. robustus*. Only 7.7% of the worms were collected from *C. bartonii* and the vast majority of *C. bartonii* (60%) were from a single, second-order stream (Dog Creek). Dog

Creek contained few *C. chasmodactylus*, no *C. robustus* and 2 *F. cristavarius* (8.7% invaded). Less than 4% of the worms were recovered from *F. cristavarius*.

Some worms were recovered from *F. cristavarius* at sites with a low percent of invasion, i.e., sites that had recently been invaded. The survey results showed that branchiobdellidans colonize *F. cristavarius* and persist long enough to be recovered during surveys at sites with a low percent invasion. Likely, they only persist for a short time, given the intense grooming reported for this species (Farrell et al. 2014). Similarly, in a survey of 171 *F. cristavarius* in the South Fork of the New River, only one crayfish had a worm and none were reported to have any branchiobdellidan cocoons (Farrell, 2012).

The experiment confirmed the survey results since the presence of *F. cristavarius* in experimental aquaria (Cr/Fc treatment) resulted in fewer worms remaining relative to the control treatment (Cr/Cr). Over time, the more significant loss of worms in the Cr/Fc treatment was likely due to the extensive grooming behaviors exhibited by *F. cristavarius* in response to the branchiobdellidans (Farrell et al. 2014). However, worms also declined in the control (Cr/Cr) treatment, although not as fast as in the (Cr/Fc) treatment. The decline in worm numbers in control could indicate other worm regulation mechanisms at play by both the crayfish host and the symbionts themselves (Creed and Brown 2018). First, *C. robustus* collected in the survey hosted an average of 22.37 worms/crayfish compared to an average of 40.35 worms/crayfish for *C. chasmodactylus*. Therefore, this species may initiate grooming at lower worm numbers than *C. chasmodactylus* (Farrell et al. 2014, Creed and Brown 2018). Second, the worms themselves could be regulating their own numbers through cannibalism (Thomas et al. 2016). Third, some of the worms may have naturally

senesced. Branchiobdellidans live for 1-2 months, and we had no way to control the age of the worms.

More cocoons were recorded on crayfish in the control treatment, but the difference was not significant. The pattern of consistently greater cocoon production in the control treatment is different from what was observed in a previous experiment that used *C. chasmodactylus* as the host (Creed et al. 2022a). In that experiment, there was no clear pattern of cocoon production in any of the treatments. However, several of the crayfish molted in the experiment conducted by Creed et al. (2022a), which could have disrupted normal cocoon production by the worms.

Crayfish size has been shown to be a predictor of worm abundance and diversity (Creed et al. 2015, Skelton et al. 2016, Thomas et al. 2016). However, mean crayfish size was similar across all sites sampled during the survey suggesting that the decline in worm number and richness across the gradient of *F. cristavarius* invasion was not due to high percent invasion sites being dominated by smaller crayfish.

Each worm species appeared to respond somewhat differently to the invasion of *F. cristavarius*. *Cambarincola ingens* and *Bdellodrilus illuminatus* showed a similar, linear decrease as the relative abundance of *F. cristavarius* increased. *Pterodrilus alcornis* and *Camborincola fallax* showed pronounced declines in abundance going from sites with <10% invasion to sites with greater levels of invasion. *Cambarincola philadelphicus* was uncommon at most sites and showed the weakest relationship to percent *F. cristavarius* invasion. Further, worm species differed in terms of the percent invasion value at which they were no longer observed on the crayfish. Numbers of the two

smallest worms (*Pterodrilus alcornis* and *Cambarincola fallax*) were essentially zero by 60 percent invasion. *Cambarincola ingens*, *C. philadelphicus* and *B. illuminatus* persisted at sites with up to 85% invasion. It has been suggested that *C. ingens* disperses less often on larger crayfish and when worm densities are lower (Skelton et al. 2015). *Cambarincola ingens* is a large species of worm that typically moves less than other smaller worms that are escaping intraguild predation by *C. ingens* (Skelton et al. 2016, Thomas et al. 2016). Dispersal in the other worms, especially the smaller worms in the presence of *C. ingens*, likely leads to a higher likelihood of being removed by the host, especially by *F. cristavarius*, at highly invaded sites

Invasive species threaten native biodiversity with extinctions of native species at the local and global scale (Aidoo et al. 2022, Cuthbert et al. 2022). In some cases, the invaders become dominant predators and competitors that threaten displacement of native species directly. However, invasive species may also indirectly threaten other native species dependent on directly affected species (Creed et al. 2022b, Rogers et al. 2017). In this case, it appears that *F. cristavarius* is causing an indirect negative effect on native crayfish by removing native symbionts from native crayfish in the New River. While some of these worm taxa are commensals (*C. fallax*, *P. alcornis*) and one is an obligate gill parasite (*B. illuminatus*) two have been demonstrated to be mutualists (*C. ingens* and *C. philadelphicus*) (Brown et al. 2002, 2012, Thomas et al. 2016). Loss of these mutualists could have negative effects on their native crayfish hosts. Specifically, mutualist loss could lead to reduced growth rates as well as affect their ability to compete against *F. cristavarius*.

The dilution effect states that a higher host species diversity will lead to a lower abundance of parasites and pathogens (Civitello et al. 2015, Creed et al. 2022a, Keesing and Otsfeld 2021). This occurs when the addition of noncompetent host species act as “decoys” (Johnson and Thieltges 2010) that eliminate parasites and pathogens from a community and further prevent transmission to other competent hosts (Johnson and Thieltges 2010). Historically, this hypothesis has been used to explain the positive effects of reduced transmission of parasites and infectious diseases. Recent studies have widened the scope of this research to include negative effects on mutualistic symbionts brought on by the invasion of a noncompetent host (Bell 2018, Creed et al. 2022a, Creed et al. 2022b). In this system, *F. cristavarius* is a noncompetent host for all native branchiobdellidans in the New River. *Faxonius cristavarius* do not benefit from the worms because they possess antimicrobial compounds in their hemolymph and groom extensively; this results in a clean exoskeleton and gills (Farrell 2012, Farrell et al. 2014). Worms colonizing *F. cristavarius* would likely shift to parasitism at lower densities due to the lack of resources which could lead to the consumption of gill tissue (Farrell 2012, Farrell et al. 2014, Skelton et al. 2016, Thomas et al. 2016). Thus, the worms have a reduced probability of surviving long enough to disperse onto a native crayfish in heavily invaded sites.

My study confirms the results of previous studies that invading crayfish are causing declines in native, ectosymbiotic branchiobdellidans (Bell 2018, Creed et al. 2022a). Loss of these worms, especially the mutualists, could indirectly affect their crayfish hosts. *Faxonius cristavarius* could be directly and indirectly causing a decline in at least one native crayfish species (*C. chasmodactylus*) as a result of the eradication of their worm symbionts. Many of

these hidden effects in response to invasion likely occur in many communities, and it is fundamental that we understand the full extent of the risks of invasion with regard to community structure and the indirect effects of invasion (Valdovinos 2019, Vitali 2022).

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Tables

Table 1. Crayfish survey sample site locations including GPS coordinates. All watersheds sampled were second, third or fourth order streams within the New River basin in Watauga and Ashe counties of North Carolina.

Site	Watershed	Latitude	Longitude
Intersection of Route 88 and Sutherland Rd	North Fork	36.39437	-81.69141
Intersection of Route 88 and Three Top Creek	North Fork	36.42992	-81.62079
Appaloosa Trail	Meat Camp Creek	36.26365	-81.62783
Three Top Baptist	Three Top Creek	36.39636	-81.61056
Int. How and Stony	Howard Creek	36.24165	-81.66145
Joe Little Rd.	Dog Creek	36.42334	-81.39593
Intersection of Castle Ford and Roby Green	Meat Camp Creek	36.26374	-81.62180
Tweetsie Railroad	Middle Fork	36.16642	-81.64787
Oak Grove Church	Buffalo Creek	36.45223	-81.52210
Meat Camp Creek confluence with South Fork	Meat Camp Creek	36.24925	-81.62302
Howard Creek confluence with South Fork	Howard Creek	36.24570	-81.63320
Intersection of Roby Green and Raven Rock	South Fork	36.24800	-81.62100
Boone Mall	Winkler's Creek	36.20001	-81.66908
Greenway	South Fork	36.21198	-81.65590
First pulloff Above Route 16 Bridge	Helton Creek	36.53435	-81.42072
Second pull off Roby Green	South Fork	36.24485	-81.63254
Brookshire Park	South Fork	36.22707	-81.64312
Wastewater treatment site	South Fork	36.21498	-81.64400
Castle Ford and River	South Fork	36.27100	-81.59000

Table 2. A. Number of crayfish collected by species, distribution by site and total number of worms collected for species. B. The total number of branchiobdellidan species collected from all 19 sites during June and July of 2021 and the number of sites at which they were collected.

A.

Crayfish Species	Total	Worm Number	Number of Sites Present
<i>C. chasmodactylus</i>	188	7811	16
<i>C. robustus</i>	46	1089	11
<i>C. bartonii</i>	33	719	10
<i>F. cristavarius</i>	214	297	15
Total	480	9916	

B.

Branchiobdellidan Species	Total Worm Number	Number of Sites Present
<i>C. ingens</i>	2,144	17
<i>C. philadelphicus</i>	535	15
<i>C. fallax</i>	1,544	14
<i>P. alcornis</i>	4,521	14
<i>B. illuminatus</i>	975	15
<i>A. koronaeus</i>	191	8
<i>X. instabilis</i>	6	3
Total	9916	

Table 3. A. Results of a repeated measures ANOVA examining the effects of treatment, row and day on total worm numbers recovered from all crayfish in each aquarium in the experiment. B. Results of a repeated measures ANOVA examining the effects of treatment, row and day on total cocoon numbers recovered from *Cambarus robustus*.

A.

Factor	DF	F	<i>p</i>
Treatment	1,9	6.638	0.0299
Day	1,28	82.762	< 0.0001
Row	1,9	0.839	0.3836
Treatment x Day	1,28	1.471	0.2353

B.

Factor	DF	F	<i>p</i>
Treatment	1,9	0.503	0.4962
Day	1,28	17.382	0.0003
Row	1,9	4.734	0.0576
Treatment x Day	1,28	0.624	0.4363

Figure Legends

Figure 1. A. Mean number of all worms recovered from all crayfish collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. Data include the collection at the Appaloosa Trail Site on Meat Camp Creek. **B.** Mean richness of all worm species recovered from all crayfish collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. Data include the collection at the Appaloosa Trail Site on Meat Camp Creek. **C.** Mean number of all worms recovered from all crayfish collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. Data from the Appaloosa Trail Site on Meat Camp Creek have been omitted. **D.** Mean richness of all worm species recovered from all crayfish collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. Data from the Appaloosa Trail Site on Meat Camp Creek have been omitted.

Figure 2. A. Mean number of all worms recovered from all large crayfish (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. **B.** Mean richness of all worm species recovered from all large crayfish (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. **C.** Mean number of all worms recovered from all large, native, *Cambarus* crayfish (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. **D.** Mean richness of all worm species recovered from all large native, *Cambarus* crayfish (TCL \geq

20 mm) collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval.

Figure 3. **A.** Mean number of all worms recovered from all large *Cambarus chasmodactylus* (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. **B.** Mean richness of all worm species recovered from all large *Cambarus chasmodactylus* (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. **C.** Mean number of all worms recovered from all large *Cambarus robustus* (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius*. **D.** Mean richness of all worm species recovered from all large *Cambarus robustus* (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius*. **E.** Mean number of all worms recovered from all large *Cambarus bartonii* (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius*. **F.** Mean richness of all worm species recovered from all large *Cambarus bartonii* (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius*.

Figure 4. **A.** Mean number of all worms recovered from all large *Faxonius cristavarius* (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius* with 95% confidence interval. **B.** Mean richness of all worm species recovered from all large *Faxonius cristavarius* (TCL \geq 20 mm) collected at a site versus percent invasion by *F. cristavarius*.

Figure 5. A. Mean number of *Cambarincola ingens* on all crayfish species from all sites except the Appaloosa Trail Site with 95% confidence interval. **B.** Mean number of *Cambarincola philadelphicus* on all crayfish species from all sites except the Appaloosa Trail Site. **C.** Mean number of *Cambarincola fallax* from all sites on all crayfish species except the Appaloosa Trail Site with 95% confidence interval. **D.** Mean number of *Pterodrilus alvicornis* on all crayfish species from all sites except the Appaloosa Trail Site with 95% confidence interval. **E.** Mean number of *Bdellodrilus illuminatus* on all crayfish species from all sites except the Appaloosa Trail Site with 95% confidence interval.

Figure 6. A. Mean carapace length (TCL in mm) for all sizes of all crayfish species from all sites except the Appaloosa Trail Site. **B.** Mean carapace length (TCL in mm) for all sizes of native crayfish species from all sites except the Appaloosa Trail Site.

Figure 7. A. Total worm numbers remaining on both crayfish in an aquarium for both treatments (Control = Cr/Cr and *Faxonius* treatment = Cr/Fc) in the experiment. **B.** Total cocoon numbers on *Cambarus robustus* in the two treatments (Control = Cr/Cr and *Faxonius* treatment = Cr/Fc). No cocoons were observed on *F. cristavarius*.

Figures

Figure 1

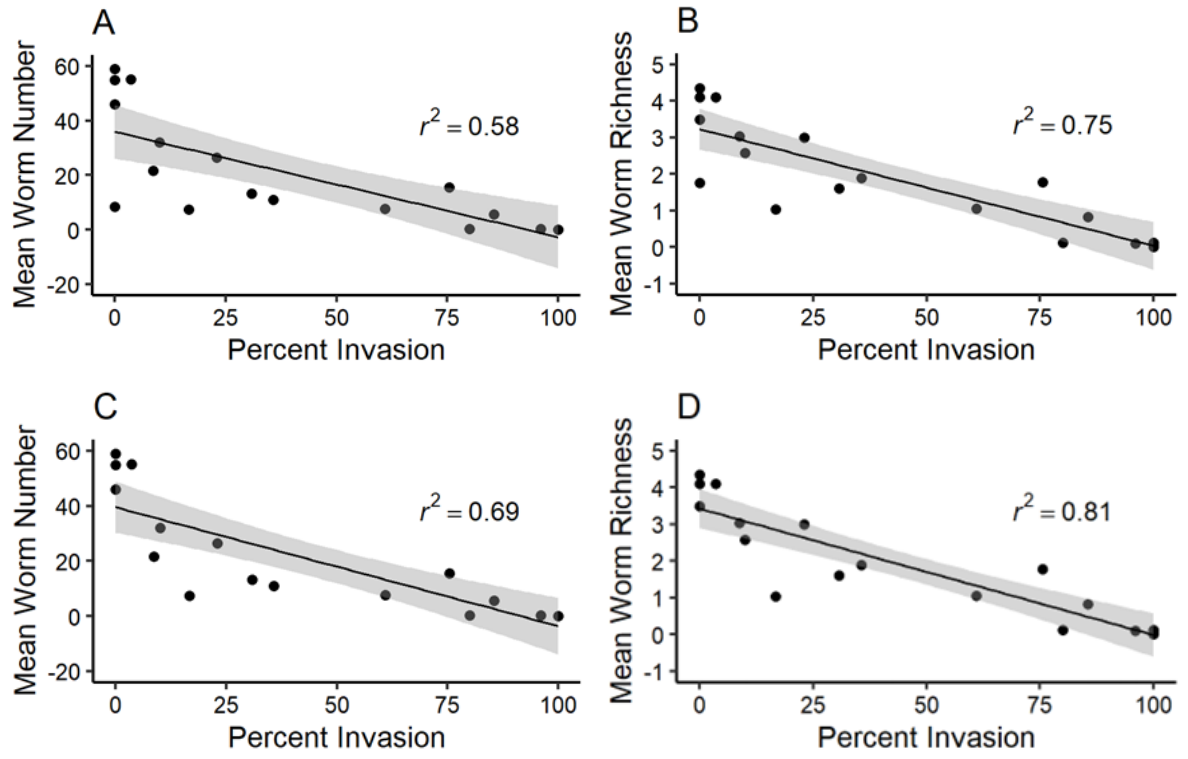


Figure 2

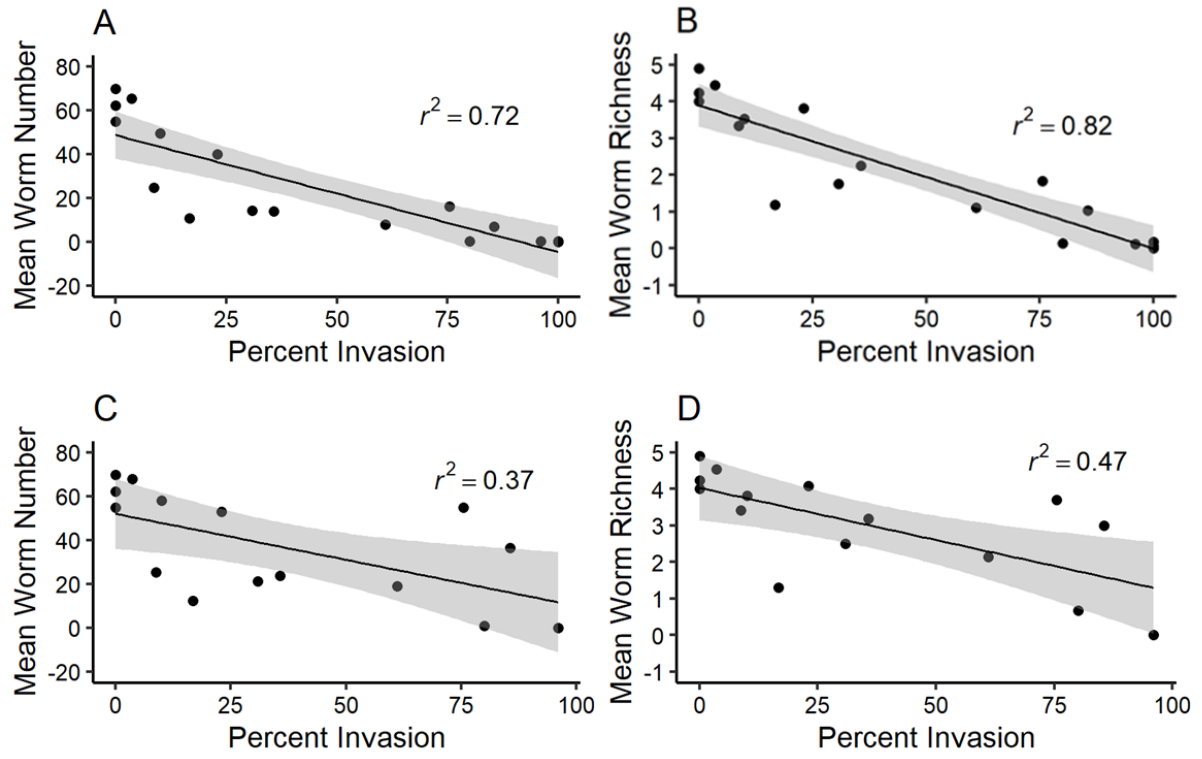


Figure 3

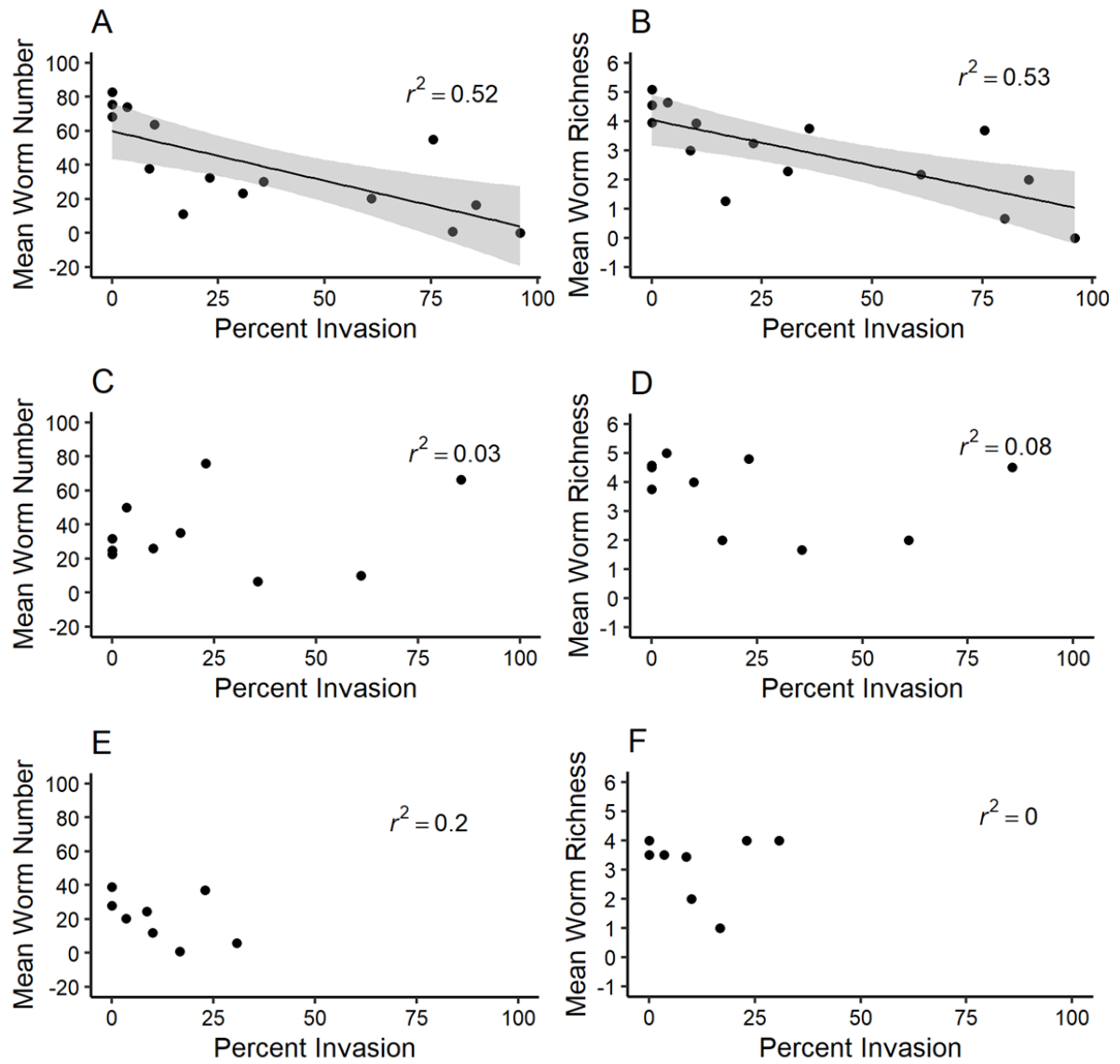


Figure 4

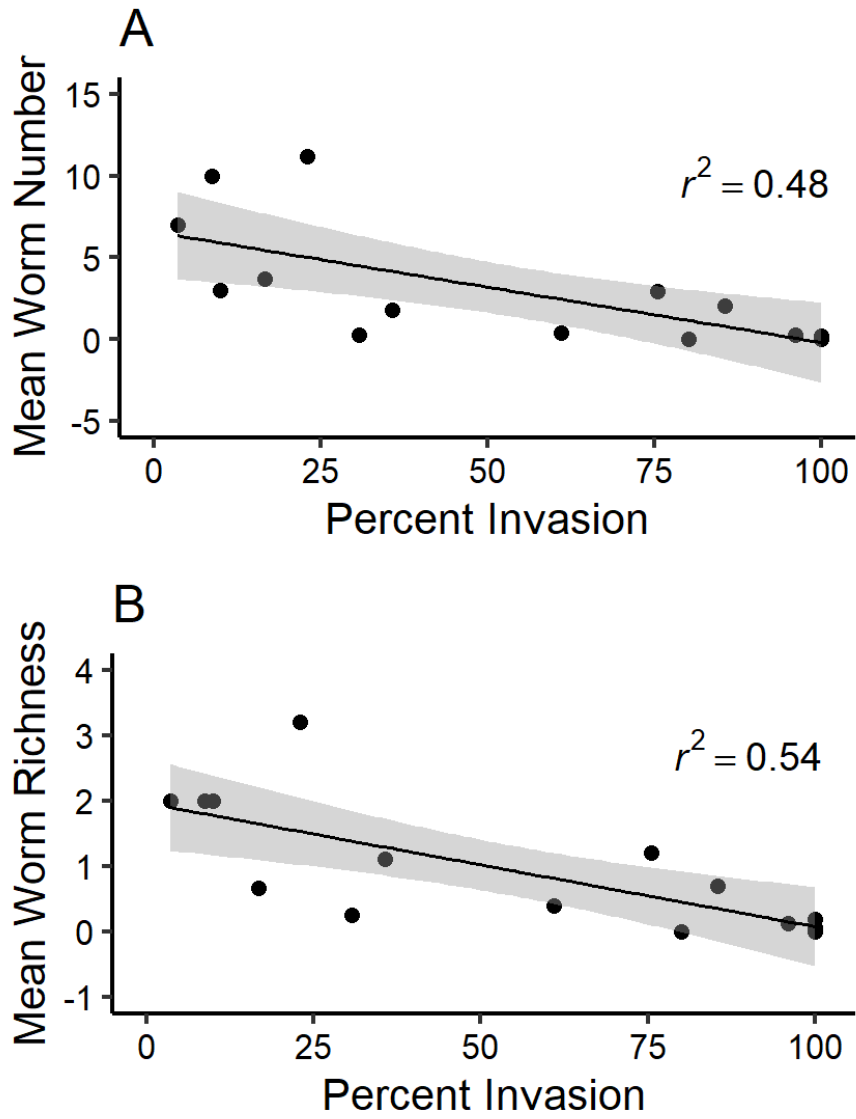


Figure 5

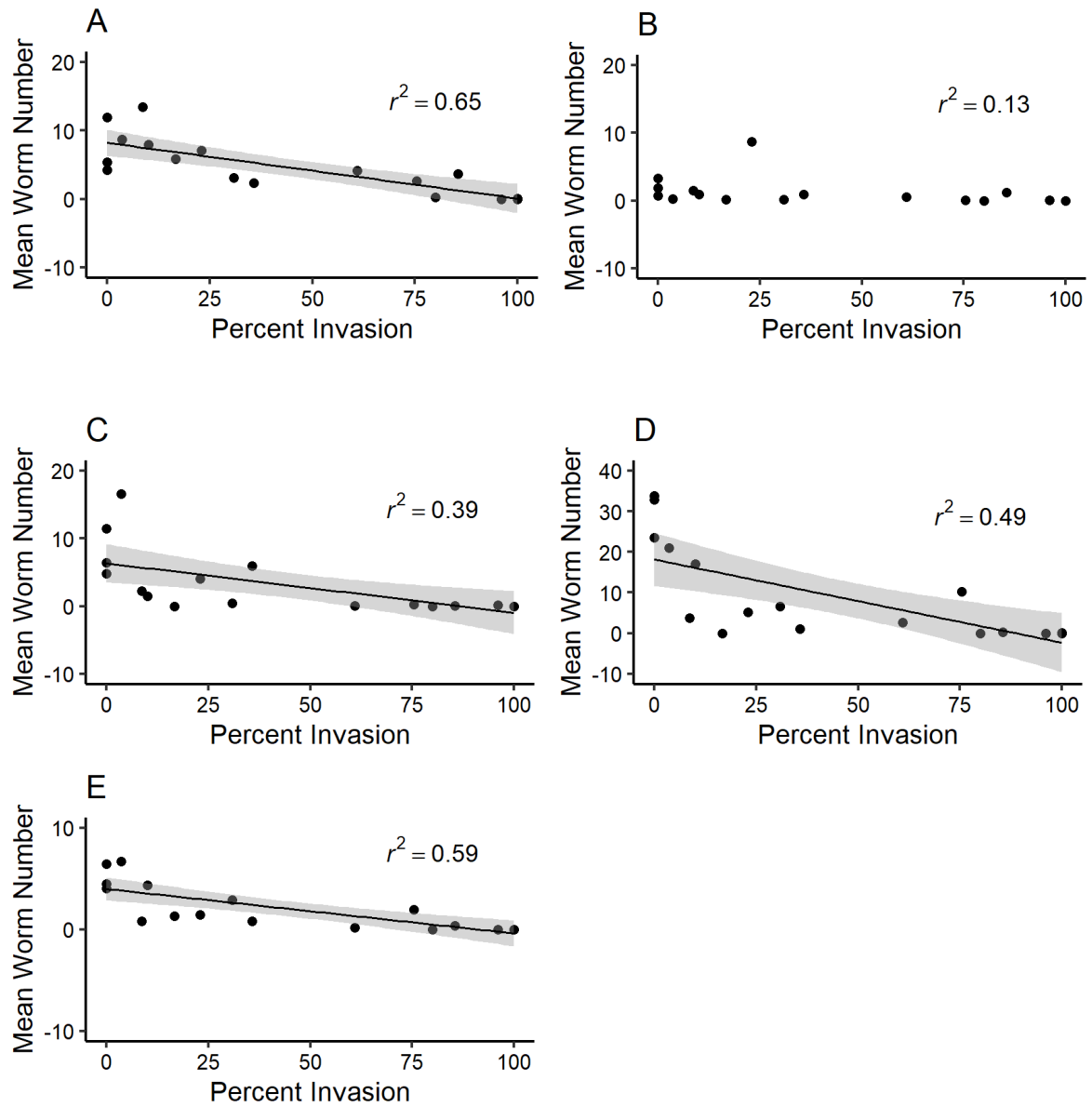


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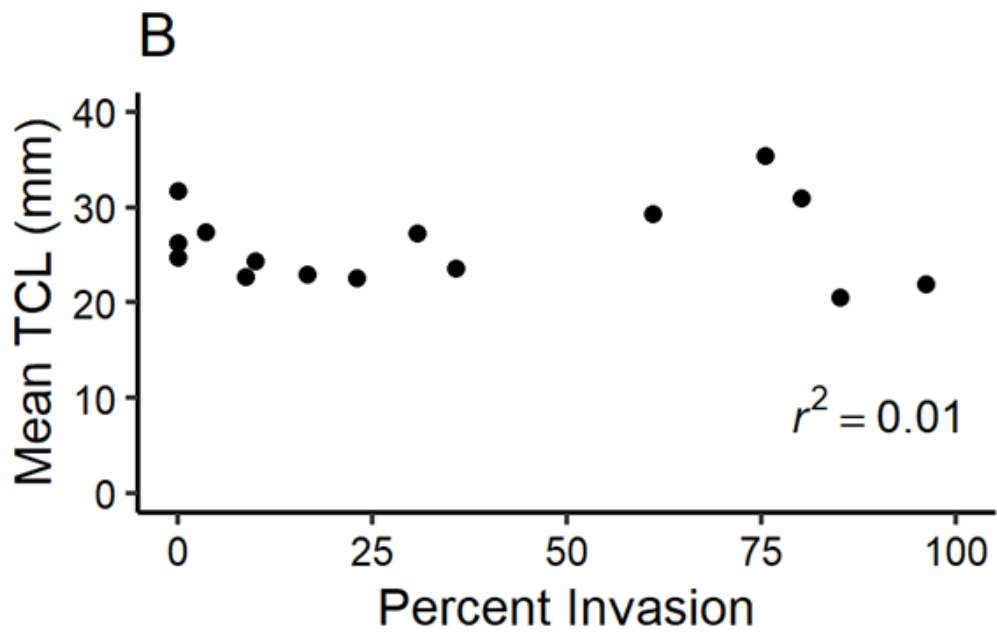
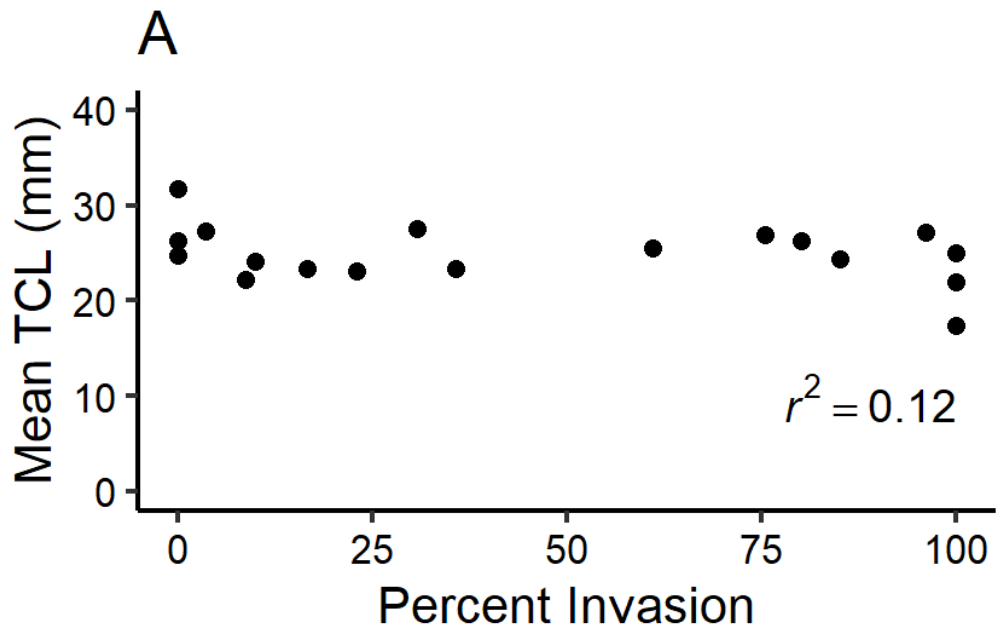
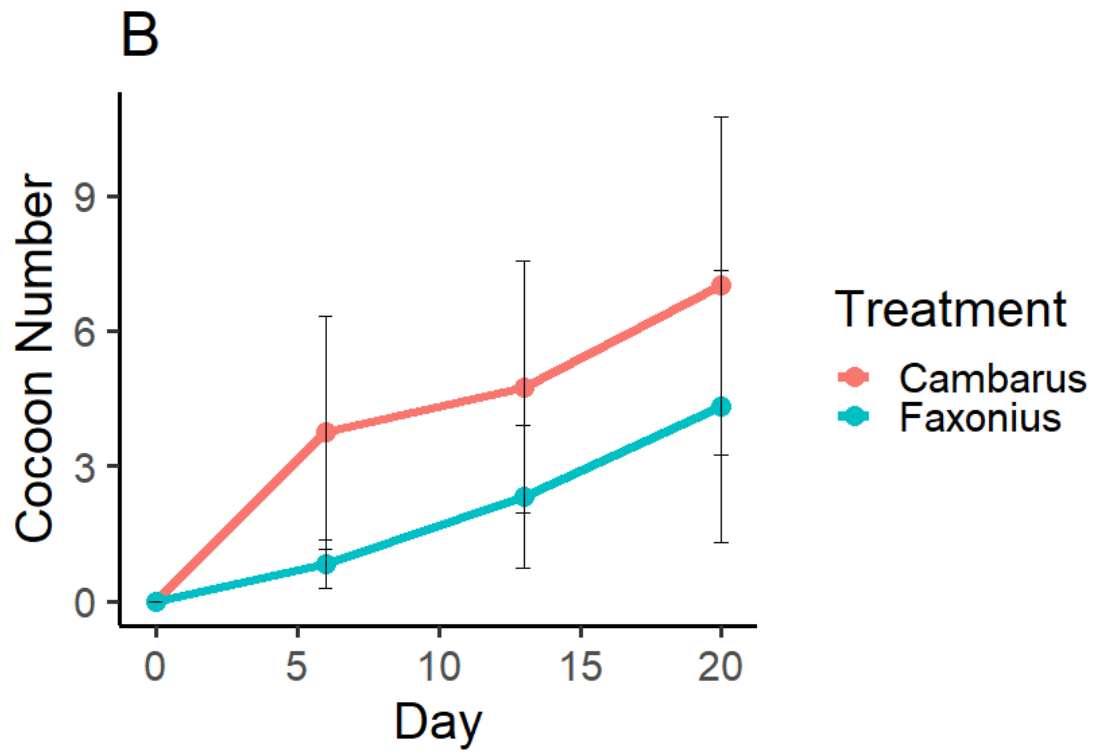
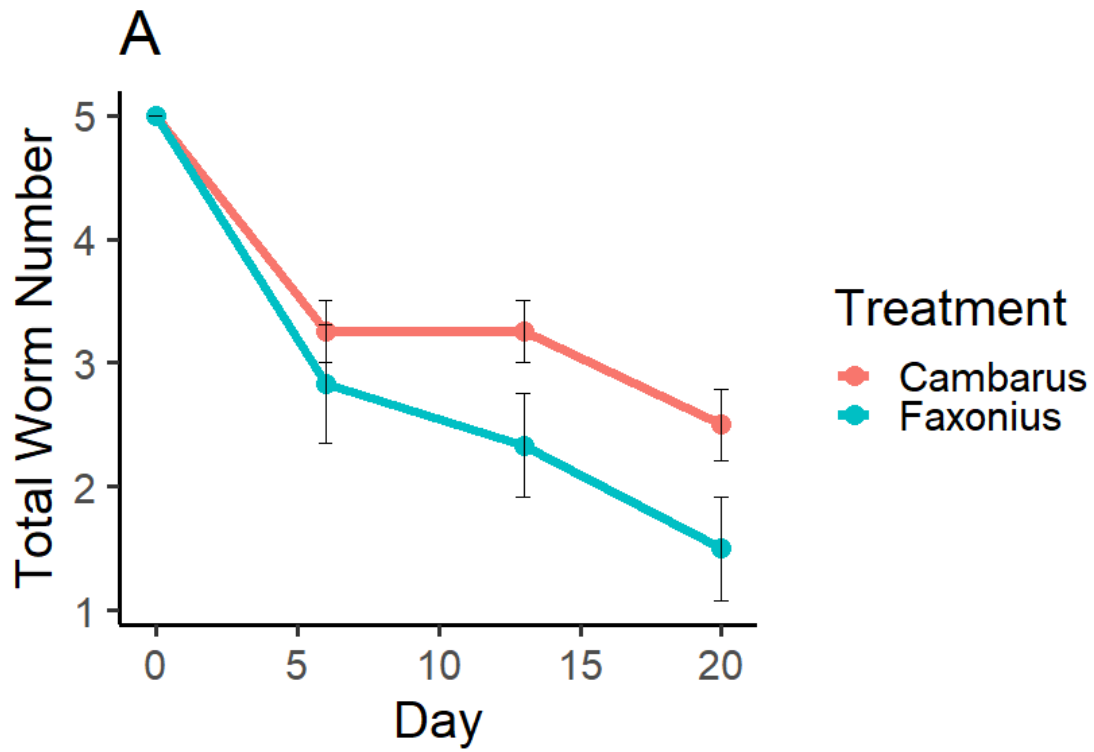


Figure 7



Vita

Mary Caitlin Massie was born in Waynesville, North Carolina, in 1987. She received her GED from Haywood Community College in 2006. Cait married, had two children, Daniel and Clair, and became a stay-at-home parent for several years. In 2008 she returned to school and earned her associate's degree in arts in 2010 from Haywood Community College. She developed a passion for science during her two years at Haywood Community College. One year after graduating, she enrolled at Western Carolina University, where she studied plant ecology and evolution. One year following receiving her bachelor's in science in biology, she was accepted into the Master's program in the Department of Biology at Appalachian State University. Cait studied crayfish symbionts and biological invasions under the mentorship of Dr. Robert P. Creed. Cait will graduate in May 2023 with a master's degree in biology. She has accepted a job offer as a field biologist and environmental consultant in Knoxville, Tennessee at Environment and Archaeology, LLC.