Mountain Lake Revisited: Impacts of Invasion on Native Symbiotic Systems

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
In
Biological Sciences

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May 2nd, 2018
Blacksburg, Virginia

Keywords: invasive species, symbiosis, crayfish

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Academic Abstract

Organismal invasions have repeatedly been cited as both a driving force behind global change and beneficiaries of that change. Although many drivers of these invasions have been well studied, few studies have addressed invasions through the perspective of native symbiont communities. In the Mountain Lake region of Virginia, crayfish host diverse assemblages of obligate cleaning symbionts known as branchiobdellida. This cleaning symbiosis has been found to result in significant fitness benefits for native crayfish. Historical survey work showed that invasive crayfish, known to be intolerant of symbionts, were introduced into the region by the 1960s. I carried out an extensive regional survey to determine how this invasion has progressed and what impacts it is having on the native crayfish-branchiobdellida symbiosis. Survey results show that invasive crayfish have successfully spread throughout the region, resulting in the displacement of native crayfish. Additionally, findings suggest that invasion results in significantly reductions in abundance and richness in native symbiont communities. To determine mechanisms contributing to observed impacts on native symbionts, I carried out a study that simulated displacement of native crayfish by invasive crayfish in a controlled setting. This study found that as native crayfish are increasingly displaced by invasive crayfish, both symbiont dispersal and survival are negatively affected. This potential loss of symbiosis caused by invasion may reduce symbionts on native crayfish below abundances necessary for fitness benefits, exacerbating the negative impacts of invasions and presenting a major conservation issue in invaded systems.

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Public Abstract

Introductions of non-native organisms are widely recognized for the negative impacts they have on native biodiversity. Although ongoing study has been directed at understanding many of the factors contributing to invasions, few studies address invasion through the perspective of native symbionts. In the Mountain Lake region of Virginia, crayfish host multiple species of small worms, known as crayfish worms, that provide cleaning services to their crayfish host. These services have been found to provide health benefits to native crayfish. Historical surveys found that invasive crayfish, known to be intolerant of crayfish worms, were introduced into the region by the 1960s. To determine how far invasive crayfish have spread and what impacts they are having on native organisms, I carried out a survey of the region. This survey found that invasive crayfish are now widespread in the region. Additionally, findings suggest that the introduction of invasive crayfish results in reductions in both the number of worms and worm species present. These reductions may be significant enough to prevent health benefits given to native crayfish from occurring. In addition to this survey, I carried out a study under controlled settings to determine what causes negative impacts on native crayfish worms. This study found that as invasive crayfish displace native crayfish, crayfish worms are increasingly killed due to the intolerance of invasive crayfish to the worms. These findings suggest symbiosis should be included in studies of invaded systems if we are to fully understand the introduction and spread of invasive species.

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Acknowledgements

I would like to thank Dr. Bryan L. Brown for providing exceptional mentorship and for allowing me the intellectual freedom to develop my research towards the areas that most interested me. I would like to thank my committee members, Dr. Emmanuel A. Frimpong and Dr. John E. "Jeb" Barret, for their invaluable feedback in developing my thesis. I thank Kevin Eliason for allowing me to tap into his GIS expertise and Phil McElmurray for his constant willingness to help tackle the data at a moment's notice. I thank all the undergraduates who gave up their valuable time to help collect crayfish, especially Brandon Guevara and Lauren Krauss. Finally, my endless thanks and love to my wife Yvonne for tolerating preserved specimens in the basement that probably belong somewhere else, the constant presence of dirty field gear in the car trunk, and for being my constant companion through it all.

Dedication

I dedicate this thesis to my wife Yvonne

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Impacts of Invasion on Symbiotic Systems

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ABSTRACT

Organismal invasions have repeatedly been cited as both a driving force behind global change and beneficiaries of that change. Although many drivers of these invasions have been well studied, few studies have addressed invasions through the perspective of native symbiont communities. Conceptually, we know that the introduction of invasive hosts presents a potential opportunity to native symbionts. Invasive hosts could benefit native symbionts through providing a novel host environment that supports them or invasive hosts could be intolerant of native symbionts resulting in negative impacts on native symbiont communities. In the Mountain Lake region of Virginia, crayfish host diverse assemblages of obligate cleaning symbionts known as branchiobdellida. In this region, an invasive crayfish host has been introduced and is interacting with the native host-symbiont system. Previous studies suggest that this invasive host may be less tolerant of symbionts than native hosts. To determine what impacts invasion is having on the native host-symbiont system, I carried out an extensive regional survey to determine impacts of invasion under natural conditions, and carried out a complementary host displacement study in controlled settings to determine mechanisms contributing to observed impacts. Findings suggest that invasion results in significantly reduced native symbiont community abundance and species richness, with impacts increasing as the proportion of invasive hosts at a site increases. These impacts appear to be driven by invasive hosts that are intolerant of symbiont activity. Due to this intolerance, both symbiont dispersal and survival are reduced as invasive hosts progressively displace native hosts. Results strongly suggest that negative impacts from invasion imperil the integrity of native host-symbiont systems. Given that many native hosts accrue significant fitness benefits from their relationships with native symbionts, loss of symbiosis may produce a positive feedback loop that decreases invasion resistance of native species, exacerbates the effects of invasions, and presents a major conservation issue in invaded systems.

Introduction

Negative impacts associated with the introduction of invasive species have been recognized for some time (Pimentel et al. 2005, Strayer 2010, Vilà Montserrat et al. 2011). The introductions are now viewed as both a result of, and a contributor to, global change and resultant negative impacts on native diversity (Didham et al. 2005, MacDougall and Turkington 2005, Hellmann et al. 2008). With the growing interconnectedness of our world, these introductions and the negative impacts they bring have become increasingly common. No two invasions are exactly the same. Some invasions result in the introduction of exotic species that establish in one location without initially expanding significantly from point of introduction (Rilov et al. 2004, Crooks, 2005). Others are characterized by explosive range expansion in the novel environment (Aldridge et al. 2004, Siegert et al. 2014). Understanding why invasions reach these outcomes requires an understanding of not only the biology of the invasive species itself, but also the numerous forces that act upon and are acted upon by the invasive species in the invaded system.

The successful establishment of non-native species in novel environments often involves direct interactions between invasive and native species with no evolutionary history of co-occurrence. The result of these interactions can vary based on the specific ecology of the system: (1) introduced organisms may facilitate native species (Rodriguez 2006), (2) interactions may result in evolutionary responses in native organism adapting to pressures from invaders (Phillips and Shine 2004, Carroll et al. 2005, Goergen et al. 2011), or (3) native organisms are extirpated as a result of these interactions (Sax and Gaines 2008, Roy et al. 2012). Interactions are not limited to direct competition between invasive and native species though. Often a variety of indirect effects related to invasion are actually more impactful on both the success of invasive species or the ability of native species to persist when impacted by invasion (White et al. 2006). These indirect effects are ways in which invasive species alter the invaded environment, which in turn affected native species. Understanding the role these indirect effects play in the invasion process is critical in understanding how and why some invasions succeed while others fail. Although the roles of many indirect effects play in the invasion process have been well studied (Mortensen et al. 2008, Kimbro et al. 2009, Walsh et al. 2016) others, such as the role of symbiosis in invasions, have received less attention.

Effects of symbiosis are ubiquitous in invaded systems due to the prolific nature of these interactions. When invasive species enter novel systems, non-analog symbiotic systems are created through interactions between invasive and native hosts and their symbionts. Due to difficulties inherent in collecting empirical data on these effects, most recent research is conceptual in nature and based largely on the enemy release hypothesis as it relates to parasites, or studies on impacts of naturally altered symbiosis on native organisms (Traveset and Richardson, 2010, Dunn et al. 2012, Asland et al. 2015, Dunn and Hatcher 2015). Results from these studies suggest that invasive hosts may benefit from the potential inability of parasites to transfer to novel invaded systems alongside their hosts. Once introduced to novel systems, these invasive hosts may have decreased tolerances of native symbionts compared to native hosts (Torchin et al. 2003, Roy et al. 2011). Findings from studies on the natural loss of mutualists suggest that the breakdown of mutualisms can result in widespread negative impacts on native

systems (Robertson et al. 2001, Palmer et al. 2008). However, few studies have considered invasion from the perspective of native symbionts.

From the perspective of a symbiont, the introduction of an invasive species represents opportunity. This opportunity is highly dependent on the tolerance of symbionts expressed by the novel host. Depending on the biology of the newly introduced species, it may present a novel host environment that supports the expansion of native symbiont communities, an inferior environment that negatively impacts symbiont communities, or a host environment that does not result in any significant impacts on symbiont communities. Both these outcomes have the potential to significantly affect native symbiotic systems.

Successful colonization of an invasive host by native symbionts may result in either fitness benefits for the invasive host, allowing it to more easily establish itself in the novel environment, or negative effects that prevent them from successfully establishing dependent on the nature of the symbiosis. If an invasive host species does not tolerate native symbionts, failed colonization by native symbionts may result in negative impacts on the local symbiont community. This could result in reduced parasite loads on native hosts or the loss of fitness benefits that native hosts may have derived from mutualisms (Mastitsky et al. 2010, Mestre et al. 2015). Depending on the nature of these impacts on native symbionts, they may aid native hosts in resisting displacement or aid invasive species in spreading in novel environments.

In aquatic systems an example of symbionts aiding an invasion can be observed in crayfish and their symbiont *Aphanomyces astaci*, a water mold that causes Crayfish Plague. Although North American crayfish are resistant to effects from this disease, native crayfish where North American crayfish are introduced have no such resistance and suffer high mortality from the disease (Unestam and Weiss 1970, Oidtmann et al. 2002, Holdich 2003). Due to the nature of *A. astaci*, its introduction and spread is possible without a North American crayfish host (Reynolds 1988, Reynolds 1997, Oidtmann et al. 2002). These symbiont introductions both prior to and alongside host invasion have been linked to native crayfish population declines that reduce competition and contribute to the successful spread of invasive North American crayfish (Holdich and Reeve 1991, Diéguez-Uribeondo et al. 1997, Filipová et al. 2013)

In the Mountain Lake region of Virginia, USA, the introduction and subsequent spread of a number of invasive crayfish is ongoing. Currently, the potential impacts of invasion on the local crayfish symbiotic system and the mechanisms behind these impacts are unknown. I hypothesize that the introduction and spread of invasive crayfish is resulting in negative impacts on the local symbiont community throughout the Mountain Lake region of Virginia, USA. Due to the positive effects this symbiosis has on native crayfish hosts (Brown et al. 2002), negative impacts on native symbiont communities may contribute to the successful spread of invasive crayfish and localized extirpation of native crayfish in the region.

Materials and Methods

Study System

The Mountain Lake region of Virginia hosts a diverse assemblage of both crayfish and crayfish symbionts, the most ubiquitous of which are the branchiobdellida (Hobbs et al. 1967). These leach-like annelid worms are obligate symbionts of crayfish, appearing to be dependent on a crayfish host for both dispersal through direct crayfish to crayfish contact and reproduction (Young 1966, Brown et al. 2002, Gelder 2010, Skelton et al. 2013, Creed et al. 2015). This symbiosis can consist of a single symbiont species on multiple host species or multiple symbionts on a single host depending on the local symbiont species pool and the "quality" of available hosts. This host quality is most closely related to the size and species of the crayfish. Older and larger crayfish have significantly higher tolerance of symbionts than younger, smaller crayfish that actively control symbiont abundances through cleaning behaviors that regulate symbiont abundance (Farrell et al. 2014, Skelton et al. 2014, Thomas et al. 2016). This relationship between symbiont tolerance and host size is believed to be related to the molting periodicity of the crayfish host. Host molting likely reduces potential symbiont food sources, resulting in increased parasitism. As molting occurrence is inversely related to crayfish size, larger crayfish experience increased buildup on their carapace and are more dependent on symbionts for cleaning services (Skelton et al. 2014, Thomas et al. 2016). This cleaning has been shown to provide large crayfish in the genus *Cambarus* with significant health benefits (Brown et al. 2012, Skelton et al. 2015). Not all crayfish species appear to derive these benefits from worm symbionts. Crayfish in the genus *Faxonius*, formerly *Orconectes* (Crandall and De Grave, 2017), appear to exhibit a lack of symbiont tolerance (Brown and Creed 2004). Ectosymbiont activity immediately elicits increased cleaning behavior from *Faxonius* crayfish (Farrell et al. 2014). Larger branchiobdellida species are less able to find shelter from these cleaning behaviors, resulting in their removal from local symbiont community. When worm populations decrease, densities that positively influence *Cambarus* health are not reached (Brown et al., 2012). As many invasive crayfish belong to the genus *Faxonius*, these negative impacts on native symbiont communities may play a role in the successful establishment of invasive crayfish and loss of native crayfish diversity.

In the Mountain Lake region of Virginia, an ongoing invasion by multiple species of introduced *Faxonius* crayfish provides us an opportunity to examine more closely how invasion affects native symbiotic systems. In the mid-20th century, surveys determined that the crayfish *Faxonius cristavarius* (Taylor, 2000) had become established in the Mountain Lake region of Virginia. This establishment likely occurred through a combination of the recorded dumping of *F. cristavarius* into Mountain Lake by students at the University of Virginia's Mountain Lake Biological Station in 1933 and bait dumping by fisherman that collected their bait in West Virginia where the species is native (Hobbs and Walton 1966, Hobbs et al. 1967). By 1960, *F. cristavarius* was collected from a nearby stream that was unconnected to Mountain Lake. Study of this invasion showed a transition from a crayfish community consisting of 22 native crayfish f and 12 *F. cristavarius* to 94 *F. cristavarius* and one native crayfish by 1965 (Hobbs and Walton 1966). Hobbs et al. (1967) also believed that a third native crayfish species was likely present at this site prior to introduction of *F. cristavarius* and had been locally extirpated prior to the 1960

collection. An additional survey at this site in 1966 found that *F. cristavarius* continued to dominate the local crayfish community and that two native crayfish species were present in low abundance. Based on these findings Hobbs et al. (1967) stated the following: "With the apparent expulsion of most of the *C. I. longulus* population by 1965, the supposed eradication of *C. acuminatum* prior to 1960, and the apparent inroad on the *C. b. bartonii* population during the intervening 5-year period, the possibility of a rapid spread of *0. Juvenilis* (now *F. cristavarius*) in the James drainage system might well be anticipated." Based on sampling by the Brown Lab, it is known that both *F. cristavarius* and *Faxonius virilis* (Hagen 1870) have become well established in all three river basins in the Mountain Lake region of Virginia.

Dispersal Study

To better understand how the displacement of native *Cambarus* crayfish by invasive *Faxonius* crayfish affects symbiont communities, I simulated the gradual displacement of native crayfish by invasive crayfish in artificial streams. For these studies I used FrigidUnits, Living Stream setups with dimensions of 84" x 24" x 22". These setups allowed me to maintain simulated flow and a constant temperature of 65° F.

Displacement was simulated using the following crayfish compositions: (1) 8 native-0 invasive (2) 6 native-2 invasive (3) 4 native-4 invasive, (4) 2 native-2 invasive, and (5) 0 native-8 invasive. Each composition was simulated in a single Living Stream unit with each composition replicated four times. Native crayfish were represented by the crayfish *Cambarus appalachiensis* (Loughman et al. 2017) while invasive crayfish were represented by *F. cristavarius*. Crayfish host size and sex were controlled to minimize confounding effects. I used the branchiobdellida *Cambarincolla ingens* as my representative symbiont as this large worm is the dominant symbiont in the native symbiont community.

At initiation each individual crayfish was marked with colored polymer lacquer to enable us to track dispersal between hosts. Fifteen symbionts were placed on a native host in each artificial stream. In artificial streams representing full displacement of native crayfish, an invasive host was chosen at random to be the initial host. This initial symbiont abundance was higher than normally observed in nature so as to stimulate dispersal away from the initial host and compensate for symbiont mortality. Observations on symbiont abundance per host and survival per artificial stream were made every day for three days to capture early dispersal off the initial host. Subsequent observations were made every three days due to symbiont dispersal slowing as symbiont abundance was reduced below elevated levels.

Results were analyzed by determining symbiont dispersal and survival as related to the percent of the crayfish community composed of invasive crayfish. Symbiont dispersal was determined as the proportion of worms that had dispersed away from the original host and onto a new host out of the original number of worms. Symbiont survival was determined as the proportion of worms that were still surviving at each observation out of the original number of worms.

To determine if degree of crayfish invasion affected symbiont dispersal and survival, a linear mixed model was used in a repeated measures analysis. This analysis was conducted with the *lme()* function in the R package *nmle* (R Core Team. 2016). In the mixed model, % invasion and

days since initiation of the experiment were included as fixed effects and experimental unit as a random effect to control for the inherent autocorrelation introduced by repeated measures. Model residuals were examined for deviations from normality and heteroscedasticity using residual plots and found them satisfactory.

Field Survey

To determine impacts of invasion on symbiotic systems under natural conditions, I sampled crayfish communities at 74 sites across the Mountain Lake region of Virginia. Sampled sites represented all three river basins in the Mountain Lake region with 22 sites in the James River Basin, 43 sites in the New River Basin, and 8 sites in the Roanoke River Basin. At each site, crayfish, I sampled the local host-symbiont community using dip nets in suitable habitat. The sampling period at each site was controlled to allow for direct comparisons between collected crayfish and branchiobdellida from each sampled site per unit collecting effort. After collection, I determined crayfish host size and species. To analyze the relationship between crayfish size and worm diversity, I used total carapace length. This length is a measure from the tip of the rostrum to the posterior edge of the cephalothorax and is directly related to the size of the crayfish. For each sampled site I calculated what percent of collected crayfish were invasive to examine impacts of invasion on symbiont diversity. All branchobdellida on collected crayfish were enumerated to species.

To examine the effects of invasion on native symbiont communities, a multiple regression model on two response variables, symbiont richness and symbiont abundance, was used. The two predictors were % invasion and carapace length. Carapace length was included as a second factor because symbiont abundance in both locally occurring crayfish genera varies predictably with host size (Brown and Creed 2004). The distribution of residuals in both models was examined; while residuals for the model of richness were satisfactory, I decided to square-root transform abundance to better meet the assumptions of multiple regression. To visualize the change in the response variable x host size relationship, % invasion was binned in 20% intervals, though the % invasion variable was analyzed continuously in the multiple regression.

Results

Dispersal Study

Complete displacement of native host crayfish by invasive crayfish under controlled settings resulted in both decreased dispersal between crayfish hosts (Figure 1) and decreased symbiont survival (Figure 2) by the end of the displacement study. Displacement appeared to initially promote the dispersal of symbionts, before dropping precipitously at full displacement of native crayfish. Unlike initial positive effects on symbiont dispersal, the displacement of native hosts appears to result in progressively reduced symbiont survival. Displacement of native crayfish by invasive crayfish was found to have significant effects (Table 1) on both symbiont transfer between hosts (Figure 3) and survival (Figure 4) over the length of the experiment. Symbiont dispersal followed a pattern of decreased dispersal with increased native host displacement by invasive hosts. One hundred percent displacement of native hosts resulted in the complete

cessation of symbiont dispersal. Symbiont survival was also significantly affected by the presence of invasive host, though these affects did not become apparent until complete loss of native hosts.

Field Study

Multiple regression showed increases in the percent of invasive hosts in host community had significant negative effects on symbiont abundance (Table 2) and richness (Table 3). Crayfish collected from sites with fully intact native crayfish communities hosted symbiont communities with both high and variable abundances and richnesses. As invasive crayfish displaced native hosts, abundance and richness decreased and became less variable (Figure 5). Invasion also resulted in a decoupling of both abundance (Figure 6) and richness (Figure 7) from host size. A significant relationship between size and symbiont community composition was apparent in crayfish at low to moderately invaded sites (0% – 40% invasive hosts). As invasive hosts continued to displace native hosts, this relationship weakened, though was still observable even at heavily invaded sites (80%-100% invasive hosts).

Discussion

Evidence from this study suggest that invasion has clearly had negative effects on the crayfish-branchiobdellida symbiotic system in the Mountain Lake region of Virginia. As a direct result of invasion both symbiont community abundance and richness have significantly decreased. These negative impacts on symbiont communities appear to be driven by a decreased symbiont tolerance exhibited by invasive crayfish compared to natives (Farrell et al. 2014). Invasive crayfish in the genus *Faxonius* have previously been found to exhibit anti-microbial hemolymph making mutualisms with worm symbionts somewhat redundant and leaving commensalism or parasitism as the more likely outcomes of symbiosis (Farrell et al. 2014). This resistance may result in decreased potential benefits *Faxonius* crayfish can derive from the symbiosis and may help explain increased cleaning behaviors they exhibit in response to symbionts.

Symbionts in this system do not appear to select for host species quality due to dispersal increasing with intermediate invasion, but survival decreasing along the host displacement gradient. Initial increased dispersal is likely due to life history differences between native *Cambarus* and invasive *Faxonius* crayfish. Native crayfish are typically less mobile, spending more time stationary under rocky substrate than *Faxonius* crayfish (Loughman et al. 2013, Anastácio et al. 2015, Hirsch et al. 2016). This increased movement by invasive hosts may provide more opportunity for non-selective symbionts to disperse between as crayfish worms requiring direct host to host contact for dispersal to occur. However, increased dispersal does not translate into increased survival. With progressive displacement of native hosts, symbionts are increasingly likely to disperse onto an intolerant invasive host. This mechanism may explain why symbiont dispersal appeared to significantly decrease when total displacement of native hosts occurred. When symbionts dispersed across fully invasive host communities they may have been removed too quickly for dispersal success to be recorded.

As intolerant invasive hosts increasingly displace tolerant native hosts, typical drivers of symbiont community composition such as host size appear to become less important. This displacement results in a decoupling of the relationship between host size and both symbiont abundance and richness. Crayfish collected at sites dominated by native hosts followed a trend that larger crayfish hosted increasingly abundant and rich symbiont communities due to increased benefits from the symbiosis. Crayfish from sites dominated by non-native hosts exhibit a significantly weaker relationship between host size and both symbiont abundance and richness. These negative impacts from invasion are also apparent on the variance of symbiont community measures. Observed impacts on variance are likely related to decreased influences of ontogenetic shifts that have been documented in previous studies (Skelton et al. 2014, Thomas et al. 2016). Native *Cambarus* crayfish clearly show such shifts with older, larger native crayfish exhibiting reduced symbiont control behaviors with decreased molting periodicity and increased potential benefits of the symbiosis (Skelton et al. 2014). Sites impacted by invasion did exhibit reduced variance, likely due to large invasive hosts having low symbiont tolerance. Reductions in symbiont community measures appeared to reflect that as hosts increasingly displace native hosts, the integrity of the native symbiotic system is put at risk. These negative impacts on the Mountain Lake Region's symbiotic system are not reflective of all invasions though.

In plant systems especially, invasion has been found to benefit native symbiont communities in some cases. Due the nature of invasions, introduced hosts may lose their natural symbionts during the invasion process (Colautti et al. 2004). Evidence suggest that in such cases, invasive hosts that derive fitness benefits from symbioses may not be able to successfully spread in novel systems (Simonsen et al. 2017). This presents an opportunity for symbionts native to the invaded system. Invasive hosts that are tolerant to symbiont colonization present as figurative "green pastures" into which native symbionts can expand without competition from already established symbiont communities. Depending on the nature of the invasive host, colonization by these native symbionts may convey fitness benefits to invasive hosts, promoting their spread (Zhao et al.2013) or symbiont establishment may negatively impact invasive hosts and limit their spread (Prider et al. 2009). From the perspective of native symbionts, either host outcome is beneficial.

In symbiotic systems such as the one reported here, introductions actually result in a disruption of native symbiotic systems (Traveset and Richardson 2014). These disruptions are not all alike though. Unlike in the crayfish host-symbiont system, many negative impacts on native symbiont communities appear to be due to invasive hosts altering the local environment. This altering is most easily observed in plant systems where invasive hosts alter the chemistry of surrounding soils. This altered soil chemistry results in an environmental conditions that native symbionts cannot tolerate and result in simplified symbiotic systems (Roriguez 2006, Day et al. 2015, Dickie et al. 2017). Through disrupting native mutualisms, invasive species may be able to indirectly impact native hosts and promote the success of their own future spread (Meinhardt and Gehring 2012). Not all negative impacts on native symbionts translate to negative impacts on native hots though. Invasive hosts that are intolerant to native parasites may actually cause a dilution effect. By removing native parasites from the local environment, invasion may actually translate to fitness benefits to native hosts that are then affected by decreased parasite loads

(Nelson et al. 2015, Gendron and Marcogliese 2017). Although effects on native hosts vary greatly between these two potential outcomes, both result in negative impacts on native symbionts.

When invasion occurs, native symbiont communities are often forgotten both as organisms impacted by invasion and as factors contributing to the potential success of these invasions. Findings appear to show that the spread of invasive crayfish in the Mountain Lake Region is having negative impacts on native symbiont communities. These impacts are resulting in significantly less abundance and rich symbiont communities as invasive hosts increasingly displace native hosts. Findings suggest that native crayfish hosts are also experiencing negative impacts from invasion, but the role symbiosis plays in these impacts remains unclear. Are native hosts more easily displaced by invasion as their mutualism with native symbionts weakens? Answering this question is critical if we are to understand what impact invasion is truly having on the Mountain Lake Region.

Figures and Tables

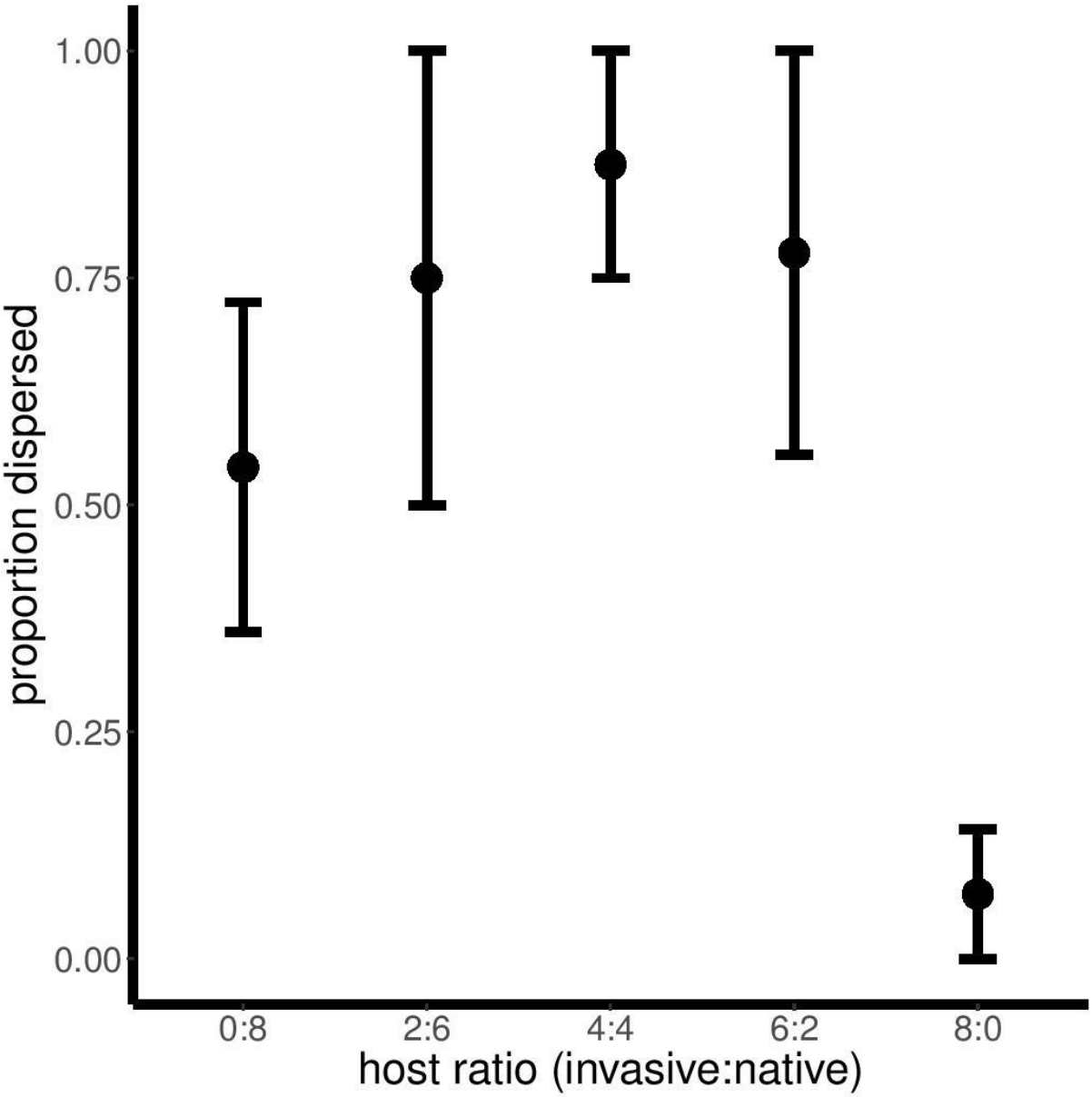

Figure 1: Proportion of symbionts that dispersed from the original host as native crayfish are progressively displaced from a fully native host community (0:8) to a fully invasive host community (8:0). Error bars represent standard error for the four replicates carried out at each host composition. Points represent the proportion of symbionts that had dispersed by the final day of the experiment.

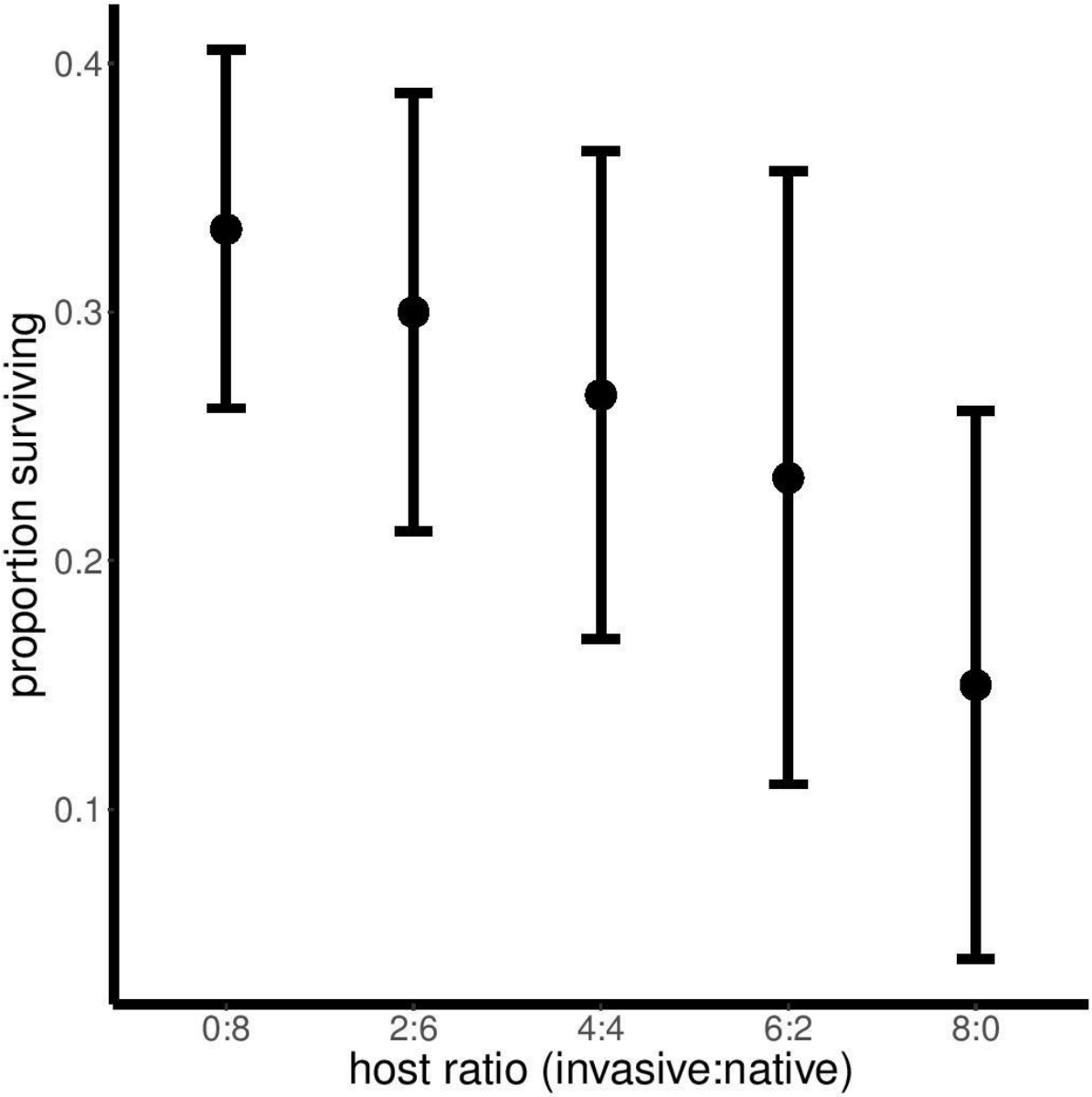

Figure 2: Proportion of symbionts that survived to the final day of the displacement study as native crayfish are progressively displaced from a fully native host community (0:8) to a fully invasive host community (8:0). Error bars represent standard error for the four replicates carried out at each host composition. Points represent the proportion of symbionts that had survived to the final day of the experiment.

Table 1: Summary of results from linear mixed model examining significance of relationships between both % invasion and days since initiation of experiment on symbiont survival and transfer between hosts.

Response Variable	% Invasion	Days Since Initiation	Interaction
Symbiont Survival	<.0001	<.0001	0.1448
Symbiont Transfer	<.0001	0.0166	0.3683

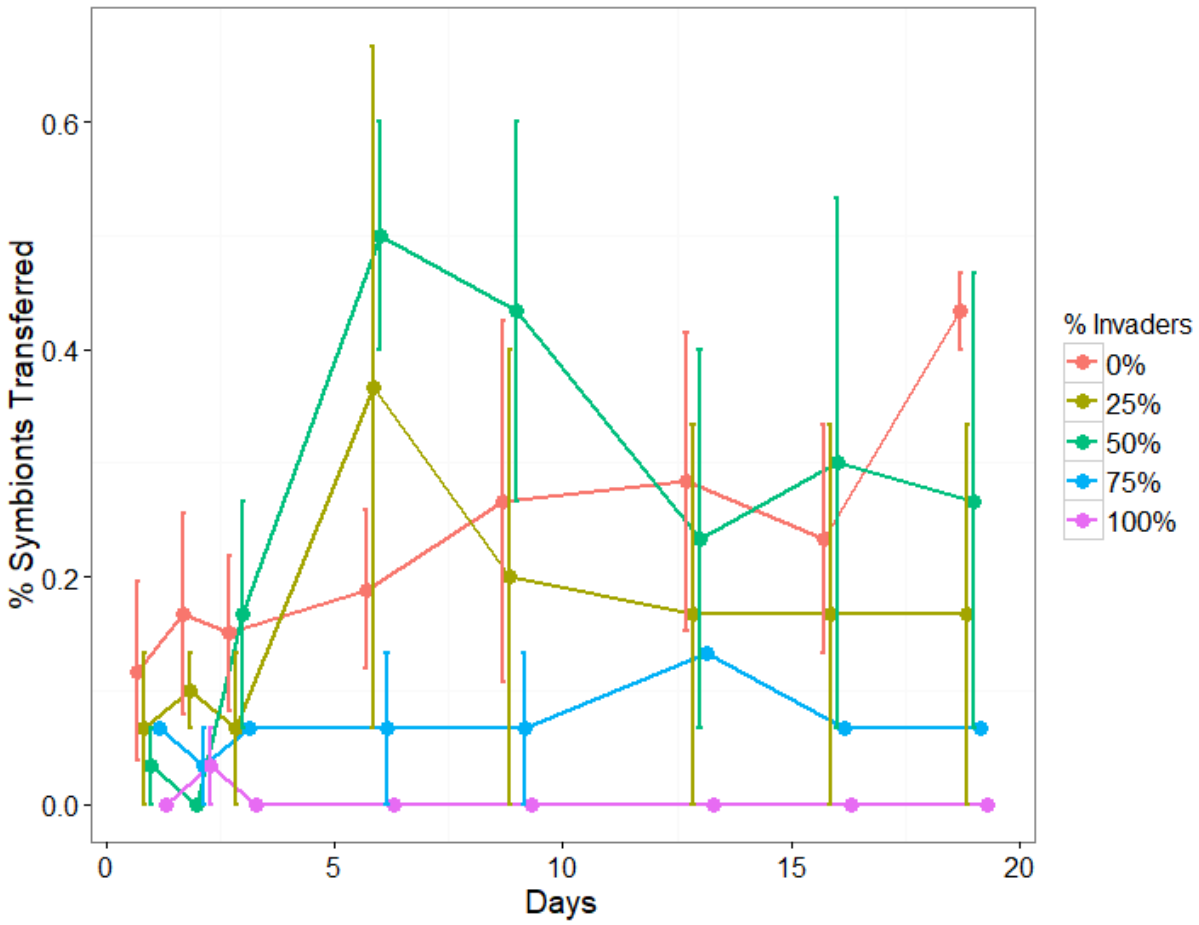

Figure 3: Percent of symbionts out of the original 15 symbionts that dispersed over the length of the experiment. Each line represents four replicate crayfish-symbiont communities at the designated composition. Error bars represent the standard error of the four replicates. Points were jittered by a random increment of up to +/- 0.75 days to decrease overlap between error bars and increase readability.

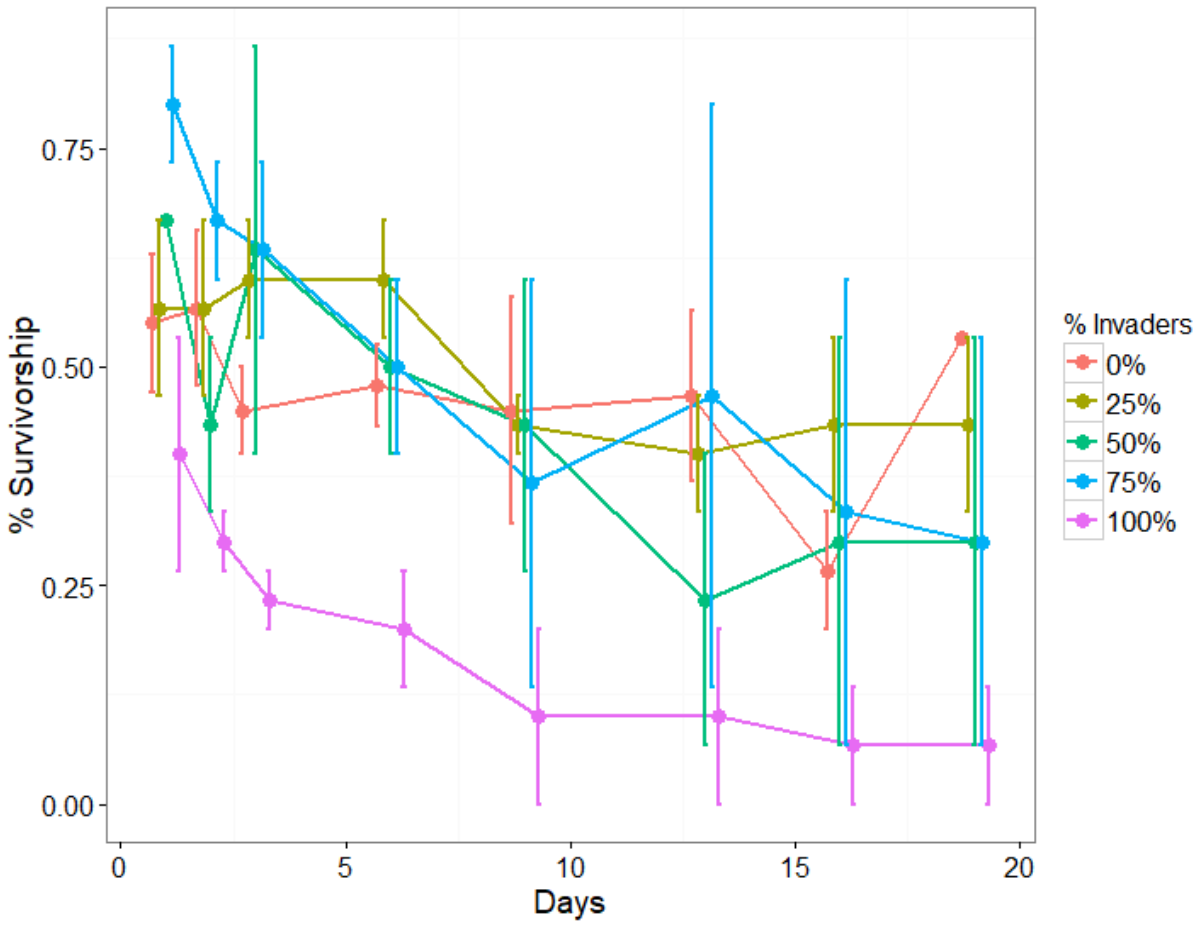

Figure 4: Percent of symbionts out of the original 15 symbionts that survived the length of the experiment. Each line represents four replicate crayfish-symbiont communities at the designated composition. Error bars represent the standard error of the four replicates. Points were jittered by a random increment of up to +/- 0.75 days to decrease overlap between error bars and increase readability.

Table 2: Summary of results from multiple regression estimating the relationship between carapace length and symbiont abundance as invasion impacts increase.

% Invaded	r^2	p-value	Slope
0%	0.4501	<.0001	0.22377
>0%,<20%	0.4176	<.0001	0.20536
>20%,<40%	0.4193	<.0001	0.22383
>40%,<60%	0.2822	<.0001	0.14796
>60%,<80%	0.2138	0.0058	0.07774
>80%,≤100%	0.3001	0.000825	0.05624

Table 3: Summary of results from multiple regression estimating the relationship between carapace length and symbiont richness as invasion impacts increase.

% Invaded	r^2	p-value	Slope
0%	0.375	<.0001	0.11374
>0%,<20%	0.434	<.0001	0.16716
>20%,<40%	0.2299	<.0001	0.10908
>40%,<60%	0.1906	<.0001	0.05693
>60%,<80%	0.1885	0.00958	0.05625
>80%,≤100%	0.1389	0.0222	0.02942

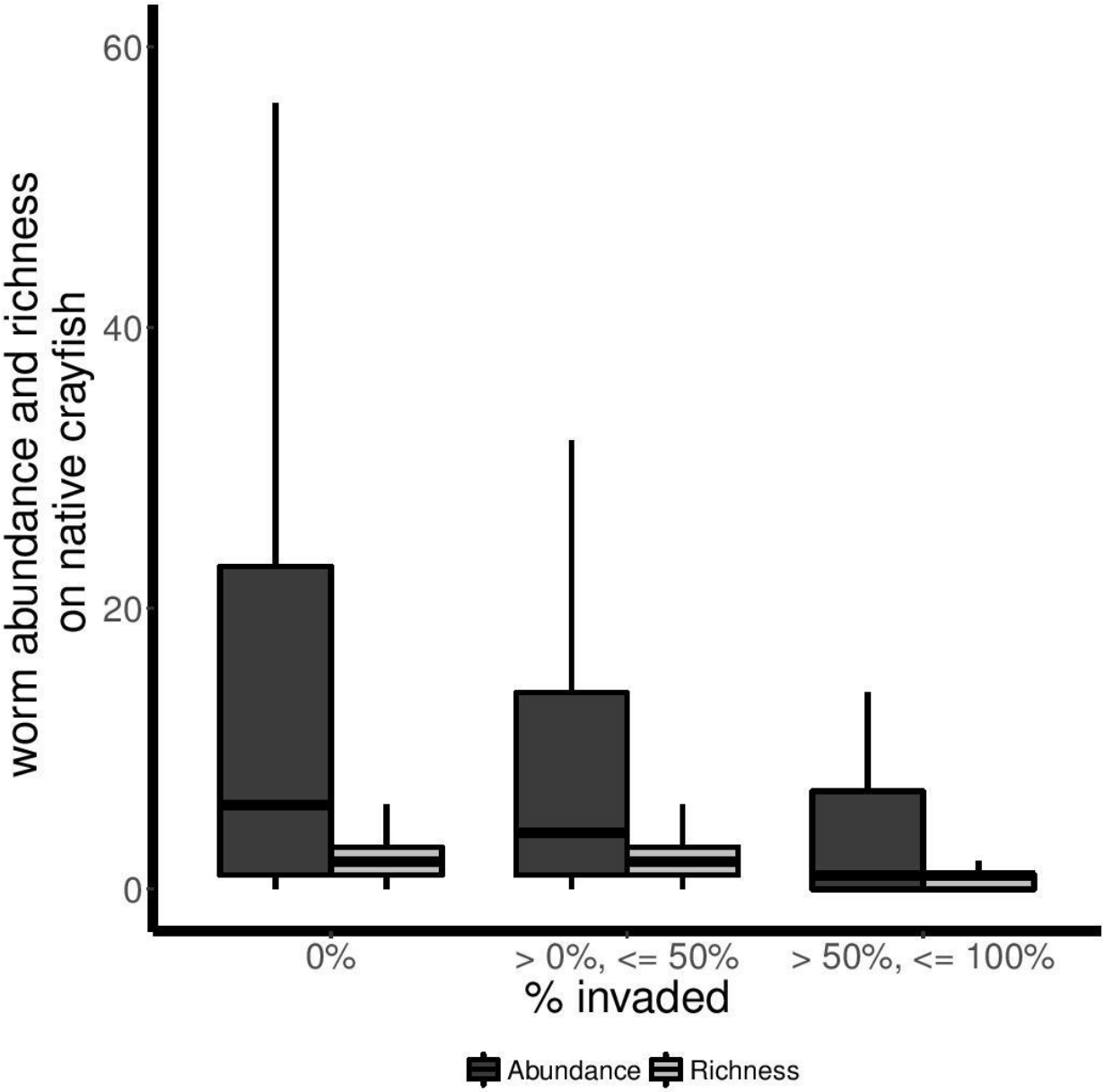

Figure 5: Symbiont abundance and richness on crayfish hosts collected from sites with no impacts from invasive (0% of collected crayfish), moderate impacts (>0%-50% of collected crayfish, and high impacts (>50%-100% of collected crayfish). Lower hinge represents median of 25th percentile of collected crayfish at each invasion level. Upper hinge represents median of 75th percentile. Horizontal line represents median of 50th percentile. Upper and lower whisker represent 1.5 X interquartile median of 75th percentile and 25th percentile respectively. Outliers are present in analysis, but removed from figure for readability.

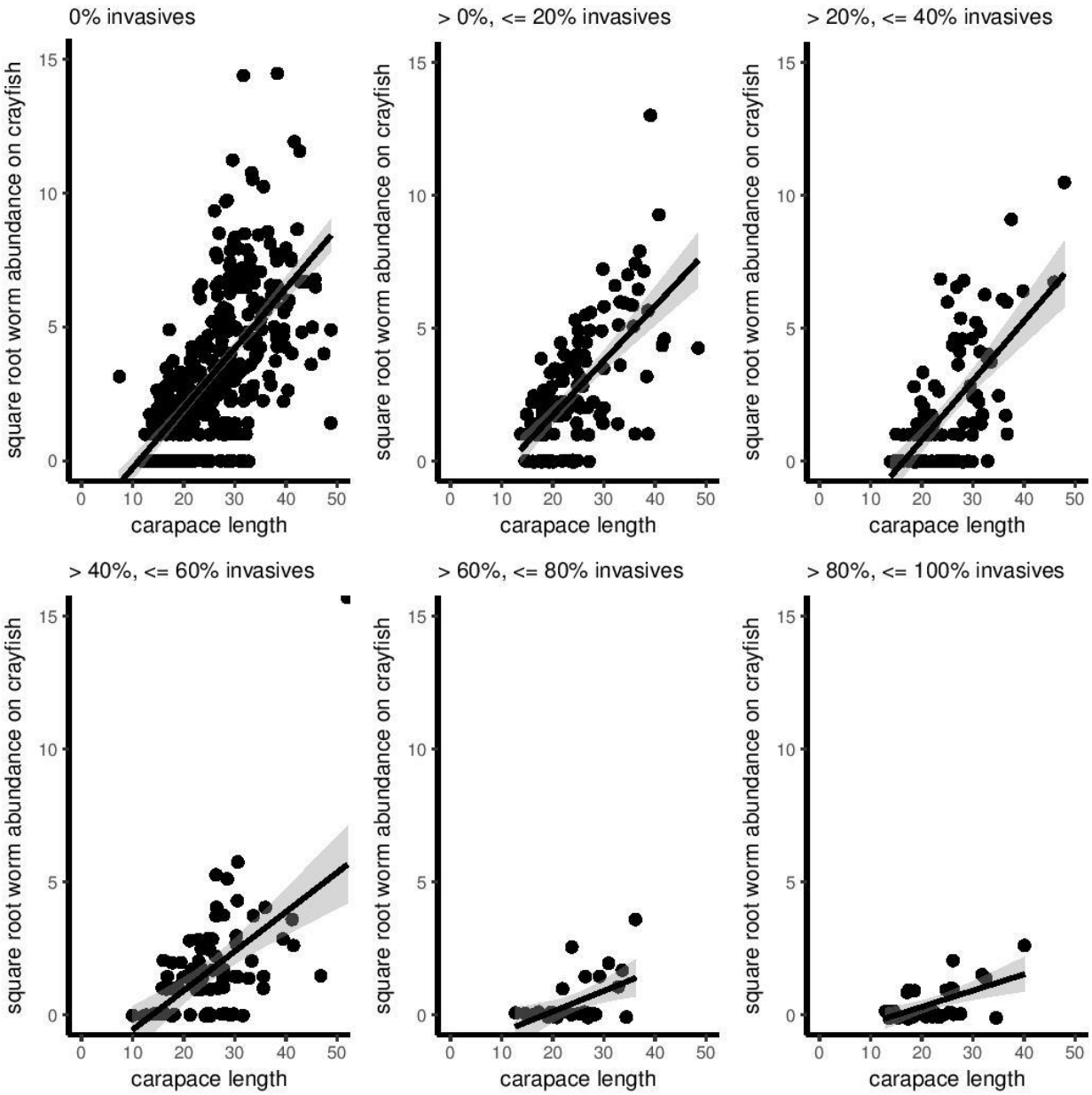

Figure 6: Relationship between the square root of symbiont abundance and carapace length of individual crayfish hosts as sites are increasingly impacted by invasion. Points represent the symbiont community of an individual crayfish of a given size, impacted by invasion by the degree it is grouped with.

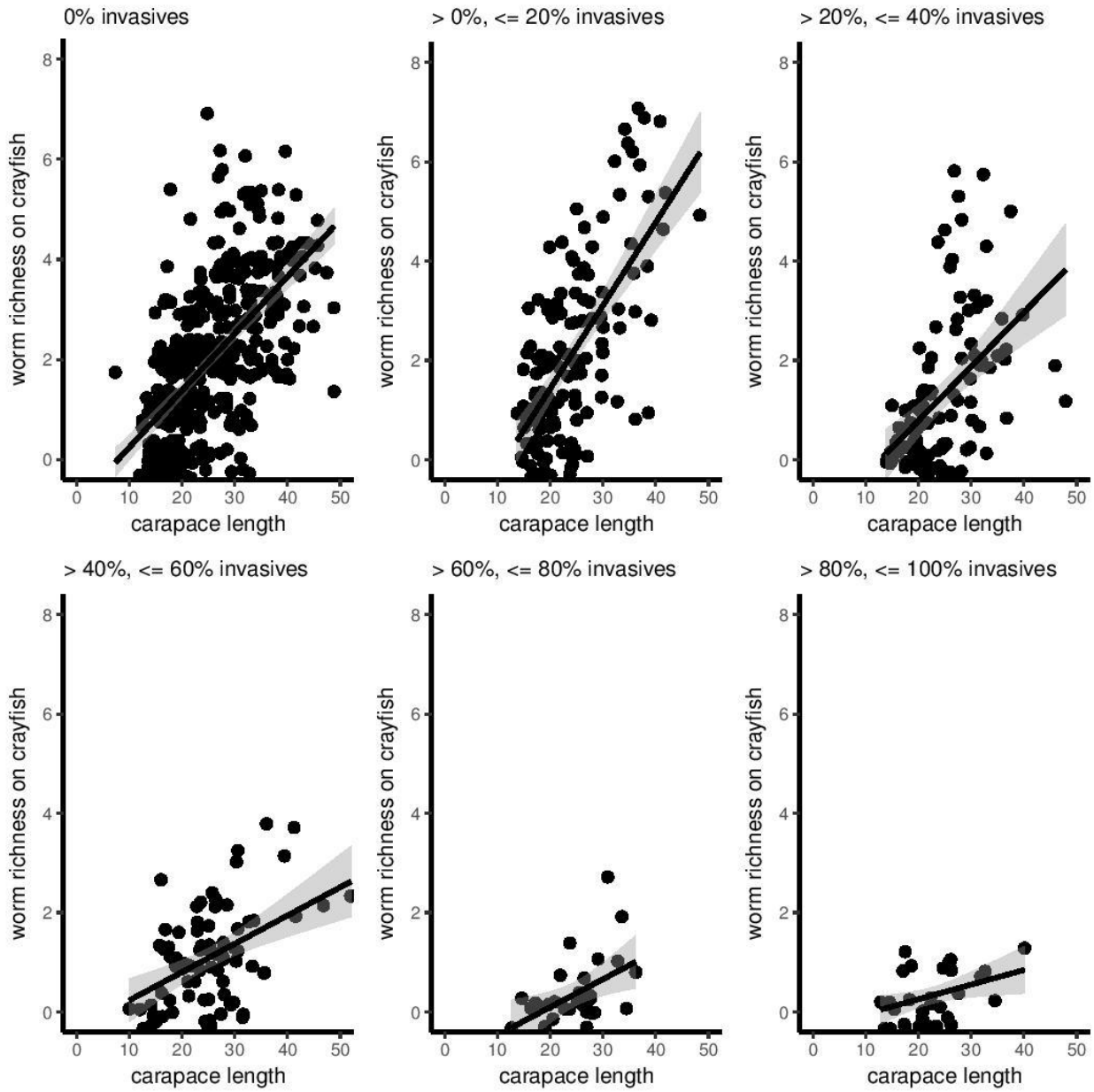

Figure 7: Relationship between the square root of symbiont richness and carapace length of individual crayfish hosts as site are increasingly impacted by invasion. Points represent the symbiont community of an individual crayfish of a given size, impacted by invasion by the degree it is grouped with.

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Mountain Lake Revisited: Impacts of Invasion on Native Symbiotic Systems

Impacts of Invasion on the Crayfish and Branchiobdellida of the Mountain Lake Region of Virginia

Spencer Sullivan Bell

ABSTRACT

The Mountain Lake region of Virginia stretches across three counties in south West Virginia and is drained by three major river basins: the James, New, and Roanoke River basins. Historical survey efforts found that this region hosts a diverse assemblage of both crayfish and branchiobdellida. These surveys also recorded early introductions and impacts from a species of invasive crayfish. Fifty years after these historical surveys, I carried out a regional survey to determine how this invasion has progressed and what impact it is having on native communities. Findings show that the invasive crayfish *Faxonius cristavarius* has successfully spread throughout all three basins in the Mountain Lake region with severe impacts on both native crayfish and branchiobdellida communities. In addition to the spread of *F. cristavarius*, I determined that the invasive crayfish *Faxonius virilis* is now established sporadically throughout the region. Impacts from these invasions appear to include range loss experienced by multiple native crayfish species and reduced branchiobdellida community abundance and richness as previously reported. Currently, some areas of the Mountain Lake Region appear to be protected from the spread of invasive crayfish by natural barriers and flow conditions. These barriers to the spread of invasion currently ensure the continued persistence of natural crayfish communities in such reasons, but their protection is precarious. The same human activities related to bait dumping that brought these invasive species could bypass protective barriers and continued the spread of invasions in the future. These findings contribute to a rich history of recording the unique diversity of the Mountain Lake Region and hope they serve as an inspiration for future efforts to continue such work into the future.

Introduction

The Mountain Lake Region of Virginia consists of approximately 1300 square km in Craig, Giles, and Montgomery Counties, VA centered around Mountain Lake on Salt Pound Mountain. Sitting fully within the Valley and Ridge topographic region, elevation ranges from 396 m at New Castle, Craig County to 1329 m at Bald Knob on Salt Pound Mountain in Giles County (Figure 1). Due the Eastern Continental Divide, several major watersheds can be found in the region. The New River watershed drains to the Ohio and Mississippi Rivers before ultimately flowing into the Gulf of Mexico while both James and Roanoke River watersheds drain to the Atlantic Ocean. This number of distinct watersheds and the topographical variation within each watershed contribute to the diversity of aquatic species that can be found in the Mountain Lake Region.

Thanks in part to the proximity of both the University of Virginia's Mountain Lake Biological Station and Virginia Tech's main campus, a rich history of scientific study has been established in the Mountain Lake Region. This research has ranged from studies on the formation and geology of Mountain Lake itself (Hutchinson et al. 1932, Cawley et al. 2001, Roningen and Burbey 2012) to surveys of the region's species diversity (Burton and Odum 1945, Graff 1947, Bovee 1960, Wagner and Wagner 1966) and ecology (Studlar 1982, Schultheis et al 2002, Kelly and Holub 2008). Most helpful for the purposes of this study was survey work carried out by Horton H. Hobbs Jr., Perry C. Holt, and Margaret Walton related to the crayfish and crayfish symbionts of the Mountain Lake Region (Hobbs et al. 1967).

As a part of this survey, Hobbs et al. (1967) sampled across all three river basins in the Mountain Lake Region to determine the composition of each basin community of crayfish, branchiobdellida, and ostrocods. Not only did this work establish the distribution for each organismal group, it also observed and commented on the initial introduction of the invasive crayfish *Faxonius cristavarius* (formerly *Orconectes juvenilis*) into the Mountain Lake Region. Based on subsequent crayfish community change after the introduction of *F. cristavarius* into Mountain Lake proper in 1933 and Potts Creek in nearby West Virginia in the late 1950s, Hobbs et al. (1967) stated the following: "With the apparent expulsion of most of the *C. I. longulus* population by 1965, the supposed eradication of *C. acuminatus* prior to 1960, and the apparent inroad on the *C. b. bartonii* population during the intervening 5-year period, the possibility of a rapid spread of *0. juvenilis* in the James drainage system might well be anticipated."

Following the survey efforts by Hobbs et al. 1967, no survey of the crayfish and crayfish symbionts of the Mountain Lake region of Virginia in its entirety has been conducted. Subsequent, untargeted sampling in the Mountain Lake Region has found that *F. cristavarius* is now present at sites unreported by Hobbs et al. (1967). Here I report on a survey of all three basins in the Mountain Lake Region of Virginia to determine how widespread the invasion by *F. cristavarius* has become, and to determine how this invasion has impacted symbiont communities in the region.

Materials and Methods

Seventy-four sites were sampled for crayfish and branchiobdellida across the Mountain Lake Region of Virginia (Table 1). Twenty-two sites were sampled in the James River basin, 43 in the New River basin, and 8 sites in the Roanoke River basin. These sites were selected to replicate survey efforts carried out by Hobbs et al. (1967) as closely as possible. At each site collections were made through dip netting in suitable habitat for a controlled amount of time to allow for direct comparisons between collections. Collected samples were preserved in 70% ethanol and returned the laboratory for identification of both crayfish and branchiobdellida using regional morphological keys from Hobbs et al. (1967) as well as species descriptions for species described since historical survey efforts by Taylor (2000) and Loughman et al. (2017).

Results

Crayfish and their branchiobdellida symbionts

Freshwater crayfish are a highly group of highly diverse crustaceans in the order Decapoda with a worldwide distribution, occurring on every continent except for mainland Africa and Antarctica (Taylor et al. 2007). Over 450 of the 600+ described species of crayfish occur in North America with diversity centered in the southern Appalachian Mountains (Taylor et al. 1996, Taylor et al. 2007, Crandall and Buhay 2008, Crandall and De Grave 2017).

Crayfish host diverse assemblages of ectosymbiotic worms in the Order Branchiobdellida. This diversity consists of approximately 140 species of branchiobdellida, commonly referred to as crayfish worms (Gelder et al. 2002, Gelder and Williams 2011). These worms are obligate symbionts of crustaceans, primarily astacoidean crayfish, requiring a host for reproduction (Young 1966, Gelder 2010, Skelton et al. 2013, Creed et al. 2015). This symbiosis is common throughout much of the Holarctic including the Americas, East Asia, and the Euro-Mediteranian region (Gelder 1999, Fard and Gelder 2011). Due in part to a relatively high number of studies initiated by the late Perry C. Holt at Virginia Tech, nearly half of all described Branchiobdellida species occur in the southern Appalachian region (Hobbs et al. 1967, Holt 1969, Holt and Opell 1993).

Historically, crayfish worms were viewed as ectocommensals or ectoparasites that provided no significant benefits to their crayfish host (McManus 1960, Holt 1963, Young 1966, Bishop 1968, Grabda and Wierzbicka 1969). More recently, empirical studies have shown that this symbiosis is more complex with interactions ranging from parasitism mutualism depending on the condition of the crayfish host and symbiont community (Brown et al. 2002, Brown and Creed 2004, Lee et al. 2009, Brown et al. 2012).

Crayfish of the New River Basin

In the New River Basin, *Cambarus bartonii* is the only crayfish found in headwater streams. As stream size increases, *Cambarus appalachiensis* becomes the dominant crayfish with *C. bartonii* present in marginal habitat at low abundances. Historically, *C. appalachiensis* may have co-occurred with *Cambarus chasmodactylus* in the New River, but no specimens of *C. chasmodactylus* were collected during this study and recent observations of the species in the Mountain Lake Region are limited (Russ et al. 2016). *Faxonius cristavarius* introduced into Mountain Lake in 1933 do not appear to have successfully spread to nearby streams in the New River Basin. *Faxonius cristavarius* has successfully established in other portions of the basin, likely as a result of bait bucket introductions in the New River first reported to have occurred in the mid-1960s and the movement of crayfish upstream into tributaries of the New River (Hobbs et al. 1967).

Crayfish of the James and Roanoke River Basins

In the James and Roanoke Basins, *C. bartonii* inhabits all headwater habitat until streams become large enough for alternating riffle-pool habitat to occur. In these larger streams *Cambarus longulus* inhabits riffle habitat and *C. bartonii* is pushed to habitat on the edges of riffles and into pool habitat. Historically, as the alternating pool and riffle habitat became more pronounced *Cambarus acuminatus* displaced *C. bartonii* in riffle edge and pool habitat with *C. bartonii* present in marginal habitat (Hobbs et al. 1967). *Cambarus accuminatus* also displaced *C. longulus* in low elevation in riffle habitat with large rocks that *C. acuminatus* was able to utilize (Hobbs et al. 1967). With the spread of *F. cristavarius* as predicted by Hobbs et al. (1967), *C. acuminatus* appears to have experienced significant range loss in both Atlantic basins. No *C. acuminatus* were collected in the James River Basin and four individuals from two sites were collected in the Roanoke River Basin. *Faxonius cristavarius* is now established in both basins and appears to be quite prolific in the Roanoke River Basin. More recently, *F. virilis* has become established in both basins though its spread appears to be limited.

Crayfish

Genus *Cambarus*

Cambarus acuminatus, Faxon 1884 (Acuminate Crayfish)

Range: Limited to Atlantic Slope drainages from the Schuylkill River basin in Pennsylvania south to the Saluda River basin in South Carolina with specimens collected in Pennsylvania, Maryland, Virginia, North Carolina, and South Carolina (Hobbs, 1989; Lieb et al. 2008).

Specimens Examined: 4 specimens were collected from sites 46 and 57 in the Roanoke River basin.

Crayfish Associates: Collected with *C. bartonii* at site 46; *C. longulus* at sites 46 and 57; *F. cristavarius* at sites 46 and 57; *F. virilis* at site 46.

Branchiobdellida Associates: Collected hosting *Ankyrodrilus koronaeus* at site 57; *Cambarincola branchiophila* at site 46; *Cambarincola fallax* at site 57; *Cambarus philadelphica* at sites 46 and 57.

Remarks: *Cambarus acuminatus* historically occurred in pool and riffle habitat in larger streams in the James and Roanoke River basins. Hobbs et al. (1967) predicted that invasion by *F. cristavarius* contributed to the localized extirpation of *C. acuminatus* due to competitive exclusion based on observations in the field. Survey efforts presented here appear to show that *C. acuminatus* has experienced severe range loss in the Mountain Lake region due to the expansion of *F. cristavarius*. These findings are mirrored in the James River basin in nearby West Virginia where *C. acuminatus* was once found, but is no longer present (Hobbs et al. 1967, Loughman et al. 2009).

Cambarus appalachiensis, Loughman 2017 (Conhaway Crayfish)

Range: Upper New and middle New River basins in Virginia and the Upper New, Middle New, Lower New, upper Kanawha, Gauley, and Greenbrier River basins in West Virginia (Loughman et al. 2017).

Specimens Examined: 299 specimens were collected from sites 3, 8, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 41, 42, 43, 44, 45, 49, 50, 52, 53, 54, 55, 58, 70, 71, 72, and 73 in the New River Basin.

Crayfish Associates: Collected with *C. bartonii* at sites 27, 28, 29, 30, 32, 35, and 49; *F. cristavarius* at sites 36, 37, 38, 43, 44, 49, 50, 52, 53, 54, 71, 72, and 73.

Branchiobdellida Associates: Collected hosting *A. koronaeus* at sites 8, 23, 52, and 70; *Ankyrodrilus legaeus* at site 72; *B. illuminatus* at sites 3, 22, 31, 32, 34, 38, 42, 43, 45, 49, 50, and 52; *C. branchiophila* at sites 8, 25, 33,34, 36, 37, 38, 39, 41, 43, 49, 50, 58, and 70; *C. fallax* at sites 3, 8, 22, 23, 25, 28, 29, 30, 31, 32, 33, 36, 37, 38, 39, 41, 42, 43, 45, 49, 50, 52, 53, 54, 58, 70, 71, and 73; *Cambarincola heterognatha* at sites 3, 28, 33, 36, 37, 42, 43, 45, 49, 50, 52, 53, and 54; *Cambarincola ingens* at sites 3, 8, 25, 32, 33, 36, 37, 38, 39, 41, 42, 43, 45, 49, 50, 52, 53, 54, 58, 70, 71, and 73; *C. philadelphica* at sites 3, 23, 27, 29, 30, 33, 35, 37, 38, 45, 52, 53, 54, 55, 71, and 73; *Pterodrilus alcicornus* at sites 3, 8, 22, 23, 25, 29, 31, 32, 33, 34, 36, 37, 38, 39, 41, 42, 43, 45, 49, 50, 52, 53, 54, 58, and 70; *Xironogiton instabilius* at sites 32, 33, 37, 43, and 59.

Remarks: *Cambarus appalachiensis* was historically identified as *Cambarus sciotensis*, but was recently described as a new species by Loughman et al. (2017) due to a disjunct distribution and significant morphological differences between the species. *Cambarus appalachiensis* is the dominant crayfish in most mid-size and large tributaries of the New River. *Faxonius cristavarius* displaces *C. appalachiensis* in portions of the New River basin impacted by

development and disturbance, but much of the basin is protected from such issues due to the Jefferson National Forest.

Cambarus bartonii, Hay 1902 (Appalachian Brook Crayfish)

Range: Wide ranging in both Atlantic and Gulf Slope drainages from New Brunswick and Quebec in the north to Alabama and Georgia in the south (Hobbs 1989, Taylor et al. 2005)

Specimens Examined: 244 specimens were collected from sites 4, 6, 7, 9, 10, 11, 12, 13, 14, 16, 20, 60, and 62in the James River Basin 1, 2, 21, 26, 27, 28, 29, 30, 32, 40, 49, 59, 63, and 65 in the New River Basin and sites 46, 47, 48, 56, 67, 68, and 69 in the Roanoke River Basin

Crayfish Associates: Collected with *C. acuminatus* at site 46; *C. appalachiensis* at sites 27, 28, 29, 30, 32, and 49; *C. longulus* at sites 6, 9, 16, 20, 46, 47, 48, 60, 62, 67, 68, and 69; *F. cristavarius* at sites 7, 16, 20, 46, 47, 49, 60, 67, 68, and 69; *F. virilis* at sites 46 and 67.

Branchiobdellida Associates: Collected hosting *A. koronaeus* at sites 13 and 62; *B. illuminatus* at sites 2, 9, 10, 11, 12, 13, 21, 26, 27, 35, 40, 51, 56, 59, 60, 61, 62, 63, 66, and 67; *C. branchiophila* at sites 11 and 68; C. fallax at sites 25, 26, 28, 29, 30, 32, 49, and 56; *C. heterognatha* at sites 4, 9, 11, 13, 20, 28, 35, and 61; *Cambarincola holostoma* at sites 9, 11, and 13; *C. ingens* at sites 13, 32, 40, and 61; *C. philadelphica* at sites 1, 2, 4, 6, 9, 10, 11, 12, 13, 16, 20, 21, 26, 27, 35, 47, 48, 51, 56, 59, 60, 61, 62, 63, 67, and 69; *P. alcicornus* at sites 25, 26, 32, and 49; *X. instabilius* at sites 4, 6, 9, 10, 11, 32, 35, and 63.

Remarks: *Cambarus bartonii* is the most geographically widespread species in the region, occurring in the headwater streams of all three major drainages in the Mountain Lake region. As invasive crayfish appear to be unable to spread into these headwater streams, *C. bartonii* may be somewhat protected from effects of invasion compared to other native crayfish species. This protection is solely due to physical barriers to the spread of invasion as Hobbs et al. (1967) found that *F. faxonius* displaced *C. bartonii* in Mountain Lake proper after its introduction into the lake in the early 1930s.

Cambarus longulus, Girard, 1852 (Atlantic Slope Crayfish)

Range: Limited to Atlantic drainages including the Roanoke and Yadkin-Pee Dee River basins in North Carolina, the James and Roanoke River basins in Virginia, and the James River basin in West Virginia.

Specimens Examined: 182 specimens were collected from sites 5, 6, 9, 16, 18, 20, 60, 61, 62, 64, and 65 in the James River Basin and sites 48, 57, 67, 68, and 69 in the Roanoke River Basin.

Crayfish Associates: Collected with *C. acuminatus* at site 57; *C. bartonii* at sites 6, 9, 16, 20, 48, 60, 62, 67, 68, and 69; *F. cristavarius* at sites 5, 16, 18, 20, 57, 60, 61, 64, 65, 67, 68, and 69.

Branchiobdellida Associates: Collected hosting *B. illuminatus* at sites 9, 16, 57, 64,65, and 74; *C. branchiophila* at sites 6, 46, and 47; *C. fallax* at sites 57, 65, 67, and 69; *C. philadelphica* at sites 6, 9, 16, 18, 20, 46, 47, 48, 57, 64, 67, 68, and 74; *P. alcicornus* at site 57; *X. instabilius* at site 6.

Remarks: *Cambarus longulus* inhabits most riffle habitat in the James and Roanoke Basins in high elevation streams large enough for alternating riffle-pool habitat to occur. Historically, as stream size increased *C. acuminatus* presence in streams increased and the two species began to directly compete. As large rocks became more prevalent in riffle habitat, *C. acuminatus* encroached into riffle habitat usually dominated by *C. longulus* and could displace the species. Due to *C. longulus*' apparent restriction to riffle habitat dominated by small substrate, Hobbs et al. (1967) suggested this species is the most ecologically restricted stream dwelling crayfish in the Mountain Lake Region.

Genus *Faxonius*

Faxonius cristavarius, Taylor 2000 (Spiny Stream Crayfish)

Range: Gulf Slope basins from the upper Cumberland River basin in southeastern Kentucky to the upper New River basin in North Carolina. The species has been recorded in Kentucky, Ohio, West Virginia, Virginia, and North Carolina (Taylor et al. 2000).

Specimens Examined: specimens were collected from sites 5, 7, 16, 17, 18, 19, 20, 60, 61, 64, and 65 in the James River Basin sits 36, 37, 38, 43, 44, 49, 50, 52, 53, 54, 71, 72, and 73 in the New River Basin sites 46, 47, 57, 67, 68, and 69 in the Roanoke River Basin.

Crayfish Associates: Collected with *C. acuminatus* at sites at sites 46 and 57; *C. appalachiensis* at sites 36, 37, 38, 43, 44, 49, 50, 52, 53, 54, 71, 72, and 73; *C. bartonii* at sites 7, 16, 20, 46, 47, 49, 60, 67, 68, and 69; *C. longulus* at sites 5, 16, 18, 20, 57, 60, 61, 64, 65, 67, 68, and 69; F. virilis at sites 46 and 67.

Branchiobdellida Associates: Collected hosting *A. legaeus* at site 72; *A. koronaeus* at site 46; *B. illuminatus* at sites 17 and 71; *C. branchiophila* at sites 36, 37, 43, 46, 47, and 68; *C. fallax* at sites 36, 38, 49, 50, 71, and 73; *C. heterognatha* at sites 20, 38, and 61; *C. ingens* at sites 43 and 49; *C. philadelphica* at sites 7, 19, 20, 47, 52, 60, 61, 64, 67, 71, and 73; *P. alcicornus* at sites 17, 36, 37, 38, 43, 49, 50, and 73; *X. formosus* at site 67.

Remarks: *Faxonius cristavarius* was historically identified as *Orconectes juvenilis*, but was described as a new species by Taylor (2000). This species has become widespread in the Mountain Lake region since initial reporting by Hobbs et al. (1967). This spread appears to be contributing to range loss of native crayfish in the Mountain Lake region. Similar range loss of native crayfish affiliated with the spread of *F. cristavarius* has been observed in the upper New River basin in North Carolina (Robert Creed personal communication).

Faxonius virilis, Hagen 1870 (Virile Crayfish)

Range: Native to the Missouri, upper Mississippi, lower Ohio, and Great Lake drainages (Benson 2015).

Specimens Examined: 6 specimens were collected from site 15 in the James River Basin and sites 46 and 67 in the Roanoke River Basin.

Crayfish Associates: Collected with *C. acuminatus* at site 46; *C. bartonii* at sites 46 and 67; *C. longulus* at sites 46 and 67; *F. cristavarius* at sites 46 and 67.

Branchiobdellida Associates: Collected hosting *A. koronaeus* at sits 46 and 67; *B. illuminatus* at site 15; *C. philadelphica* at sites 46 and 67.

Remarks: *Faxonius virilis* has historically been harvested in its native range and sold outside its native range as live fishing bait. Through the dumping of unused fishing bait, *F. virilis* has become established widely outside its native range (Lodge et al. 2000, Taylor et al. 2005, DiStefano 2009, Kilian et al. 2011). In the Mountain Lake Region, introduction of this species appears to have been recent as it was not collected in sampling efforts carried out by Hobbs et al. (1967). *Faxonius virilis* is currently present in the main stem of the New River and in scattered locations in the James and Roanoke River basins.

Branchiobdellida

Genus *Ankyrodrilus*

Ankyrodrilus koronaeus, Holt 1965

Specimens Examined: 1,640 specimens were collected from sites 13 and 62 in the James River basin; sites 8, 23, and 70 in the New River basin; sites 46, 47, 57, and 67 in the Roanoke River basin.

Crayfish Hosts: Collected on host species *C. acuminatus*, *C. appalachiensis*, *C. bartonii*, *C. longulus*, *F. cristavarius*, and *F. virilis*.

Ankyrodrilus legaeus, Holt 1965

Specimens Examined: 15 specimens were collected from site 72 in the New River basin.

Crayfish Hosts: Collected on host species *C. appalachiensis* and *F. cristavarius.*

Genus *Bdellodrilus*

Bdellodrilus illuminatus, Moore 1894

Specimens Examined: 586 specimens were collected from sites 6, 9, 10, 11, 12, 13, 15, 16, 17, 60, 61, 62, 64, 65, and 74 in the James River basin; sites 2, 5, 21, 22, 26, 27, 32, 33, 35, 38, 40,

45, 49, 50, 51, 52, 54, 59, 63, 66 in the New River basin; sites 56, 57, and 67 Roanoke River basin.

Crayfish Hosts: Collected on host species *C. appalachiensis*, *C. bartonii*, *C. longulus*, *F. cristavarius*, and *F. virilis*.

Genus *Cambarincola*

Cambarincola branchiophila

Specimens Examined: 332 specimens were collected from sites 6 and11 in the James River basin; sites 8, 25, 31, 34, 36, 37, 38, 39, 41, 42, 43, 49, 50, 58, 70, and 71 in the New River basin; sites 46, 47, and 68 in the Roanoke River basin.

Crayfish Hosts: Collected on host species *C. acuminatus*, *C. appalachiensis*, *C. bartonii*, *C. longulus*, and *F. cristavarius*

Cambarincola fallax, Hoffman 1963

Specimens Examined: 2,821 specimens were collected from site 65 in the James River basin; sites 3, 8, 22, 23, 25, 26, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 41, 42, 43, 45, 49, 50, 52, 53, 54, 58, 70, 71, and 73 in the New River basin; sites 56, 57, 67, and 69 in the Roanoke River basin.

Crayfish Hosts: Collected on host species *C. accuminatus*, *C. appalachiensis*, *C. bartonii*, *C. longulus*, and *F. cristavarius*.

Cambarincola heterognatha, Hoffman1963

Specimens Examined: 351 specimens were collected from sites 4, 9, 11, 20, and 61 in the James River basin; sites 3, 28, 33, 35, 36, 37, 38, 42, 43, 45, 49, 50, 52, 53, 54, and 59 in the New River basin.

Crayfish Hosts: Collected on host species *C. appalachiensis*, *C. bartonii*, and *F. cristavarius*.

Cambarincola holostoma, Hoffman 1963

Specimens Examined: 137 specimens were collected from sites 9, 11, and 13 in the James River basin.

Crayfish Hosts: Collected on host species *C. bartonii*.

Cambarincola ingens, Hoffman 1963

Specimens Examined: 389 specimens were collected from sites 3, 8, 13, 25, 27, 31, 32, 33, 36, 37, 38, 39, 40, 41, 42, 43, 45, 49, 50, 52, 53, 54, 58, 70, 71, and 73 in the New River basin.

Crayfish Hosts: Collected on host species *C. appalachiensis*, *C. bartonii*, and *F. cristavarius*.

Cambarincola philadelphica, Leidy 1851

Specimens Examined: 1,425 specimens were collected from sites 4, 6, 9, 10, 11, 12, 13, 16, 18, 19, 20, 60, 61, 62, 64, and 74 in the James River basin; sites 1, 2, 3, 21, 23, 26, 27, 29, 30, 33, 35, 37, 45, 51, 52, 53, 54, 55, 59, 63, 71, and 73 in the New River basin; sites 46, 47, 48, 56, 57, 67, 68, and 69 in the Roanoke River basin.

Crayfish Hosts: Collected on host species *C. acuminatus*, *C. appalachiensis*, *C. bartonii*, *C. longulus*, *F. cristavarius*, and *F. virilis*

Genus *Pterodrilus*

Pterodrilus alcicornus, Moore 1895

Specimens Examined: 2,236 specimens were collected from site 17 in the James River basin; sites 3, 8, 22, 23, 25, 26, 29, 31, 32, 33, 36, 37, 38, 39, 41, 42, 43, 45, 49, 50, 52, 53, 54, 58, 70, and 73 in the New River basin; site 57 in the Roanoke River basin.

Crayfish Hosts: Collected on host species *C. appalachiensis*, *C. bartonii*, *C. longulus*, and *F. cristavarius*.

Genus *Xironodrilus*

Xironodrilus formosus, Ellis 1918

Specimens Examined: 2 specimens were collected from site 67 in the Roanoke River basin.

Crayfish Hosts: Collected on host species *F. cristavarius*.

Genus *Xironogiton*

Xironogiton instabilius, Moore 1894

Specimens Examined: 260 specimens were collected from sites 4, 6, 9, 10, and 11 in the James River basin; sites 32, 33, 35, 37, 43, 49, and 63 in the New River basin.

Crayfish Hosts: Collected on host species *C. appalachiensis*, *C. bartonii*, and *C. longulus*.

Discussion

Spread of Invasive Crayfish in the Mountain Lake Region

Collection efforts directed at determining how an ongoing invasion has affected native communities of both crayfish and their symbiotic worms have shown that predictions made by Hobbs et al. (1967) have largely been supported. Not only has *F. cristavarius* successfully spread throughout the James River basin as predicted, but also through the Roanoke River basin and portions of the New River basin. In addition to the successful spread of *F. cristavarius*, the invasive crayfish *F. virilis* has been introduced into the region and presents a new potential threat native diversity.

Initial introductions of both species appear to be related to their use as fishing bait. Hobbs et al. (1967) noted that *F. cristavarius* may have spread into the Mountain Lake Region from West Virginia naturally, but suggested that occurrence patterns of this species made natural spread an unlikely explanation. The initial introduction of *F. cristavarius* into the region of Mountain Lake proper in 1933 is known to have been due to the dumping of scientific collections (Hobbs et al. 1967). Researchers based at the Mountain Lake Biological Station followed this introduction and noted that *F. cristavarius* displaced not only the native *C. bartonii* that occurred in Mountain Lake, but also *C. acuminatus* that were also dumped into the lake in the early 1930s. The sources of subsequent introductions are less clear, but these introductions were noted to have occurred near popular fishing spots and were not contiguous with *F. cristavarius*' native range (Hobbs et al. 1967). Invasion by *F. virilis* appears to be a more recent occurrence with occurrence patterns that also suggest an origin in the region due to bait dumping. This dumping has resulted in the establishment of *F. virilis* in Maryland (Kilian et al. 2010), Pennsylvania (Lieb et al. 2011), West Virginia (Loughman and Welsh 2010), Tennessee (Dunn, 2010), and North Carolina (Simmons and Fraley 2010), all states bordering Virginia. Locally, populations occur in water bodies where recreational fishing occurs and streams that empty these bodies (Hungry Mother Lake in Marion,Virginia and Virginia Tech Duck Pond/Stroubles Creek in Blacksburg, VA). *Faxonius virilis* has also become established in a few isolated locations in the James and Roanoke River basins.

Although all native crayfish that historically occurred in the region are still present, *C. acuminatus* has experienced significant range loss. Survey work in the 1950s-1960s found that *C. acuminatus* was present at multiple sites in Craig Creek in the southern portion of the James River basin, two sites in Johns Creek in the northern portion of the Jams River basin, and multiple sites in the North Fork of the Roanoke River in the Roanoke River basin (Hobbs et al. 1967). Recent survey work found *C. acuminatus* at no sites in the James River basin and only two sites in the North Fork of the Roanoke in the Roanoke River basin. At both these sites in the Roanoke River basin, *C. acuminatus* co-occurs with invasive crayfish. In the New River basin, *C. appalachiensis* has been displaced by *F. cristavarius* in multiple sites in Toms Creek in the southern portion of the basin, but displacement is limited at sites in the central and northern portions of the basin. *Cambarus bartonii* and *C. longulus* are still well represented in the Mountain Lake region, but have been displaced by *F. cristavarius* in parts of both the James and Roanoke River basins.

The continued persistence the native crayfish community in the Mountain Lake Region may be explained in part by the apparent inability of invasive crayfish to spread into some regional streams, and inability of invasive crayfish to completely displace native crayfish in other streams (Figure 2). Contributing to this inability of invasive crayfish to successfully spread are natural barriers caused by the geography of the Mountain Lake Region. Such natural barriers have been found to prevent the spread of invasive crayfish upstream and lead to attempts to utilize man-made barriers to prevent the spread of invasive crayfish (Kerby et al. 2005, Dana et al. 2011, Frings et al. 2013).

In the New River basin, a karst landscape results in streams that originate in cave systems and flow belowground for significant distances, as observable in Sinking Creek, a mid-sized stream in the central portion of the New River basin. This stream disappears underground and has no permanent surface flow that connects with the main stem of the New River where invasive crayfish are known to occur. This biogeographic phenomenon results in a physical barrier to upstream spread that cannot be naturally bypassed. Other barriers to the spread of invasive crayfish are observable in Little Stony Creek in the central portion of the New River basin. This stream flows directly into the main stem of the New River with invasive crayfish present at sites near the New River confluence. Moving upstream, away from this confluence, Little Stony Creek is characterized by high flow riffle habitat with large rocks and a 69-foot waterfall that blocks upstream movement by invasive crayfish. No invasive crayfish were collected at either of these New River basin sites above these natural barriers.

Other factors related to unsuccessful spread and establishment of invasive crayfish in parts of the Mountain Lake Region may be related to the maintenance of natural stream and riparian habitat as well as the natural drying of headwater streams. Habitat disturbance in the form of altered stream morphology or the removal of riparian plant growth has been found to promote the spread of invasive species in aquatic systems (Light 2003, Marvier et al. 2004, Puth and Allen 2004). In headwater streams especially, the maintenance of seasonal flow regimes may prevent invasive organisms that are unable to tolerate stream draw down from persisting (Larson et al. 2009).

The James River basin contains Craig Creek in the southern portion of the basin and is heavily impacted by agricultural development and bridge construction, while Johns Creek in the north is protected from most development by the Jefferson National Forest. Downstream portions of Craig Creek are crossed by highway bridges that result in unnatural pool habitat where flow bottoms out and high amounts of sediment settle. At sites with these conditions, invasive crayfish have largely displaced native crayfish. The headwaters of Craig Creek are in the Jefferson National Forest which provides some protection from such disturbances. Downstream disturbances may be contributing to the occurrence of invasive crayfish at these upstream sits though. Man-made pool habitat may facilitate the movement of invasive crayfish upstream to invaded headwaters in Craig Creek similar to what has been observed with man-made reservoirs (Havel et al. 2005). Observed spread into Craig Creek's headwaters appear to only be seasonal though. Sampling in late summer when these headwater streams are reduced to scattered pools of water show that invasive crayfish have moved downstream where more permanent water is present. As native crayfish readily dig burrows into the stream bed to access water during

periods of low flow, these low flow periods may prevent the total displacement of native crayfish in headwater streams.

Due to these various natural barriers, large portions of the Mountain Lake Region may remain unimpacted by invasive crayfish in the future. Many of these barriers provide only a precarious defense to invasion. The establishment of invasive crayfish relies only on the dumping of crayfish collected in a local stream impacted by invasion into a stream without invasive crayfish. Portions of Sinking Creek in the New River basin may be especially vulnerable to such introductions as land use surrounding this stream is similar to that surrounding the heavily invaded Craig Creek in the James River basin. Other areas of concern include the establishment of additional invasive crayfish into the Mountain Lake Region. A likely candidate for introduction is the Red Swamp Crayfish (*Procambarus clarkii*). This species has been occasionally reported as occurring in Stroubles Creek in the southern portion of the New River basin. *Procambarus clarkii* is readily available for purchase from online suppliers or at pet shops and is often used in primary school science classes as a study organism. If *P. clarkii* are not properly disposed of, establishment is not unlikely as this species has wide biological tolerance which has enabled it to become one of the most widespread invasive crayfish globally.

Impacts of Invasion on Mountain Lake Branchiobdellida Community

All crayfish worm species that Hobbs et al. (1967) collected in their survey efforts are still present in the Mountain Lake Region, but sites with invasive *Faxonius* crayfish do appear to have less abundant symbiont communities. Brown and Creed (2004) found that symbionts were much more abundant on native *Cambarus* crayfish than on *F. cristavarius* in the upper New River basin in North Carolina and found that worms appeared to select native hosts over invasive hosts in controlled host selection trials. Based on these findings they suggested four possible explanations: (1) symbiont preference for *Cambarus* crayfish was due to *Cambarus* host being the most abundant hosts, (2) behavioral differences in *Cambarus* hosts promote symbiont colonization, survival, or reproduction over symbionts on *F. cristavarius* hosts, (3) native *Cambarus* hosts provide a more beneficial cost/benefit relationship than provided by invasive *F. cristavarius* hosts, or (4) symbionts may actively select *Cambarus* crayfish as hosts over *F. cristavarius*. Brown and Creed (2004) found the first explanation unlikely due to worm abundances on each host species not shifting when invasive crayfish outnumbered native crayfish, but found the other explanations difficult to evaluate. If native worm communities are negatively impacted by the establishment of invasive crayfish as suggested Brown et al. (2004) then native crayfish may not derive benefits from the symbiosis furthering the negative impacts of invasion (Brown et al. 2002, Brown et al. 2012, Skelton et al. 2013).

In conclusion, an invasion of non-native crayfish first reported by Hobbs et al. (1967) has continued to spread over the past 50 years. As predicted by Hobbs et al. (1967) this invasion has now expanded throughout all three basins in the Mountain Lake region of Virginia. My findings suggest negative impacts from this invasion are affecting both the native crayfish and symbiont communities. Spread of invasive species appears to be directly related to the significant range loss of the native crayfish *C. acuminatus* in the James and Roanoke basins as well as *C. appalachiensis* in some portions of the New River basin. Although the native symbiont

community of the Mountain Lake region appears to remain intact, significant negative impacts on this community related the introduction of invasive species are now known as reported in the previous chapter. I expect these negative impacts on native crayfish and branchiobdellida communities to continue to worsen as invasive crayfish continue to spread throughout the region. Although some native species are currently protected by natural barriers to invasion, these barriers may be bypassed through the same active human transportation that brought these introduced species into the region. The rate of spread and severity of impacts from this invasion are difficult to predict. I hope that periodic survey efforts in the Mountain Lake Region will continue so as to maintain a historical record of this regions unique diversity.

Figures and Tables

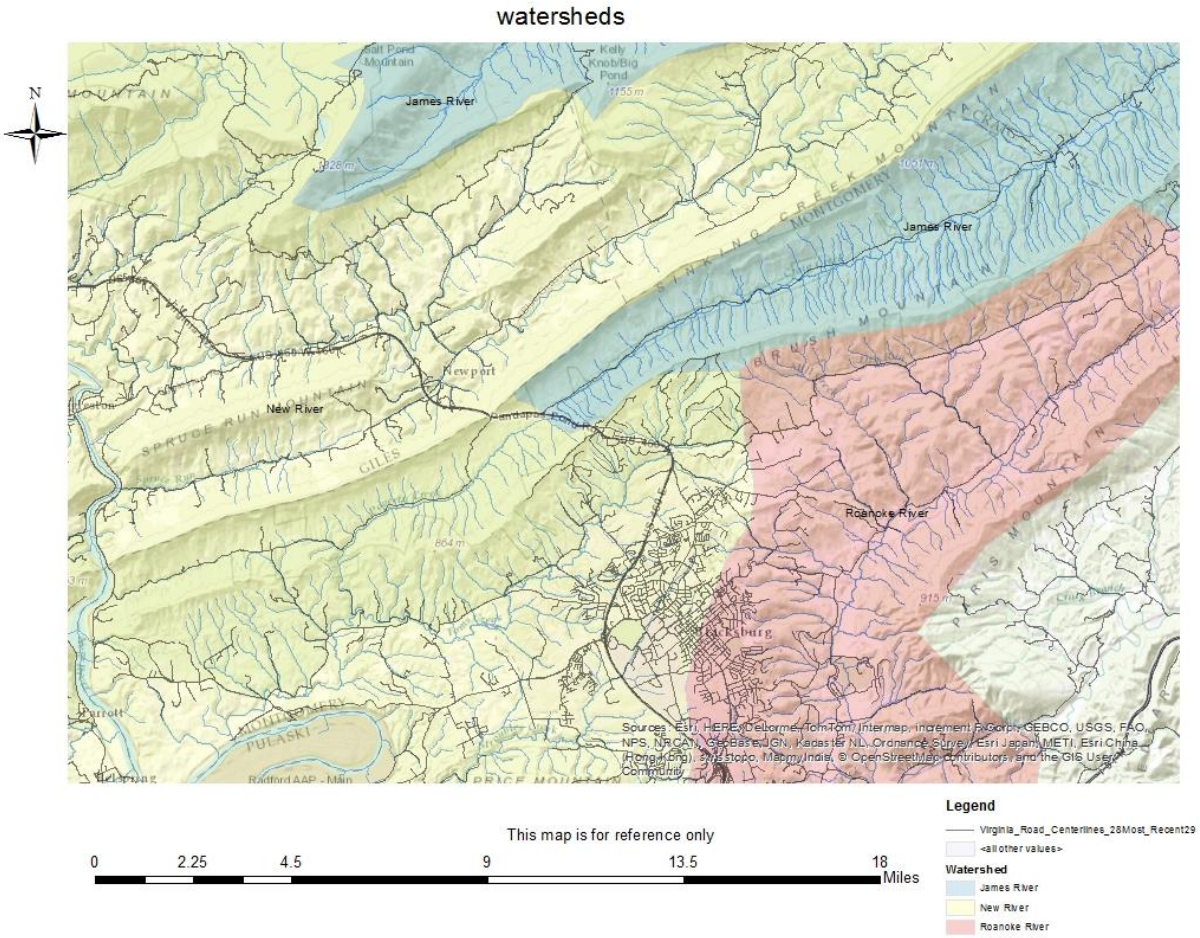

Figure 1: Map of the Mountain Lake region of Virginia. This region extends across three river basins (James River basin, New River basin, and Roanoke River basin) in three counties of Virginia (Craig County, Giles County, and Montgomery County) and is centered around Mountain Lake on Salt Pound Mountain in Giles County.

Table 1: Table of sites sampled for crayfish and branchiobdellida.

Site #	Latitude	Longitude	Basin
1	37°24.074	80°30.384	New
2	37°24.264	80°30.564	New
3	37°26.387	80°30.973	New
4	37°28.388	80°25.214	James
5	37°25.771	80°22.896	James
6	37°23.471	80°27.759	James
7	37°24.942	80°23.377	James
8	37°18.184	80°29.142	James
9	37°27.610	80°21.159	James
10	37°28.013	80°20.406	James
11	37°29.169	80°17.234	James
12	37°30.303	80°11.919	James
13	37°29.651	80°06.436	James
14	37°27.935	80°06.423	James
15	37°24.845	80°09.743	James
16	37°24.079	80°12.077	James
17	37°23.750	80°12.782	James
18	37°22.532	80°15.371	James
19	37°20.177	80°19.656	James
20	37°19.626	80°20.938	James
21	37°22.344	80°31.515	New
22	37°22.389	80°40.367	New
23	37°18.704	80°30.938	New
24	37°18.776	80°30.567	New
25	37°18.567	80°30.069	New
26	37°18.973	80°29.795	New
27	37°17.812	80°29.657	New
28	37°16.443	80°32.644	New
29	37°15.972	80°34.857	New
30	37°15.948	80°36.041	New
31	37°18.256	80°32.375	New
32	37°20.200	80°37.942	New
33	37°20.460	80°37.345	New
34	37°22.026	80°40.556	New
35	37°23.550	80°38.465	New
36	37°23.746	80°39.571	New
37	37°23.604	80°39.804	New
38	37°24.143	80°38.931	New
39	37°12.807	80°46.832	New
40	37°16.109	80°41.609	New

41	37°16.269	80°41.657	New
42	37°17.408	80°42.310	New
43	37°19.162	80°37.780	New
44	37°19.606	80°49.044	New
45	37°25.740	80°32.736	New
46	37°11.382	80°21.414	Roanoke
47	37°11.106	80°21.065	Roanoke
48	37°14.645	80°21.714	Roanoke
49	37°25.192	80°33.365	New
50	37°25.080	80°36.817	New
51	37°22.822	80°34.584	New
52	37°25.507	80°33.041	New
53	37°14.116	80°31.474	New
54	37°14.270	80°31.493	New
55	37°13.951	80°32.350	New
56	37°17.856	80°20.412	Roanoke
57	37°18.411	80°15.770	Roanoke
58	37°12.488	80°46.006	New
59	37°22.052	80°33.612	New
60	37°18.300	80°25.557	James
61	37°19.505	80°21.650	James
62	37°28.967	80°07.830	James
63	37°22.763	80°34.068	New
64	37°25.814	80°21.825	James
65	37°26.598	80°20.409	James
66	37°22.476	80°31.537	New
67	37°11.727	80°19.526	Roanoke
68	37°11.992	80°18.933	Roanoke
69	37°14.260	80°16.159	Roanoke
70	37°20.491	80°24.651	New
71	37°13.628	80°29.810	New
72	37°14.725	80°27.402	New
73	37°14.281	80°28.407	New
74	37°26.273	80°23.486	James

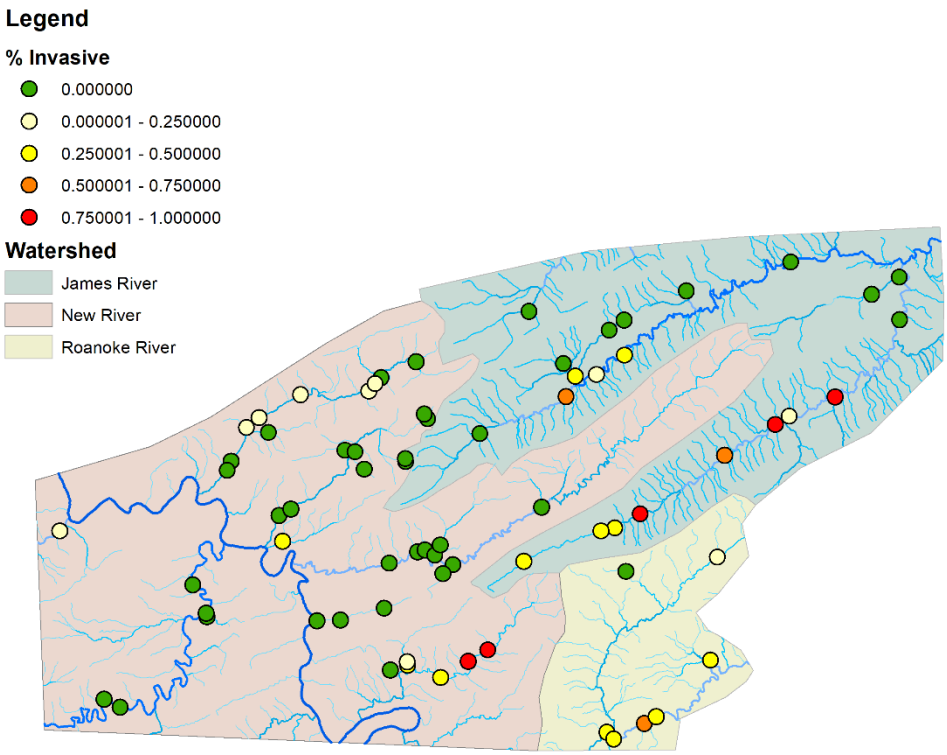

Figure 2: Map of the Mountain Lake region of Virginia showing the percent of collected crayfish that were invasive species at each sampled site. Sites impacted by agricultural and urban development in the southern portions of all three basins represent areas where invasive species have most successfully spread. Sites protected by natural barriers to dispersal and more natural sites protected by the Jefferson National Forest and seasonal flow patterns in the northern portions of all three basins are less readily invaded.

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Conclusions

Historical survey efforts carried out by Hobbs et al. (1967) found that the invasive crayfish *F. cristavarius* was introduced into the Mountain Lake region of Virginia by the mid-20th century. Based on early study of this invasion, Hobbs et al. (1967) predicted that this invasive crayfish would likely spread throughout the region in the future. My survey findings presented in this thesis support those predictions. *Faxonius cristavarius* has been found to have spread throughout much of the region, especially in the James and Roanoke River basins. Additionally, another invasive crayfish has also become established in the region. *Faxonius virilis* currently has a more limited introduced range than *F. cristavarius*, but this may be representative of a later invasion date for that species.

One of the impacts of this invasion reported by Hobbs et al. (1967) appears to be direct competition between invasive and native crayfish that contributes to the loss of native crayfish diversity. Invasion appears to have directly contributed to the localized extirpation of the native crayfish *C. accuminatus* in the James River Basin as well as range in the Roanoke River Basin. Other native species do appear to be experiencing range loss where invasive crayfish have successfully spread, but are apparently continuing to persist in streams that are not as heavily impacted by invasion. As invasive crayfish continue to spread in the region, impacts on local crayfish populations may be increasingly negatively impacted.

One of the areas related to this invasion unaddressed by Hobbs et al. (1967) is how this invasion may impact native symbiont diversity. Previous findings have suggested that the invasive crayfish *F. cristavarius* is significantly less tolerant of symbionts than native crayfish (Brown and Creed 2004, Farrell et al. 2014). How this intolerance impacted native symbiont communities on the landscape were unknown though prior to this study. To explore mechanisms behind how this symbiont intolerance by invasive crayfish impacts native symbionts, I carried out a displacement study that simulated displacement of native crayfish hosts by invasive crayfish hosts in a controlled setting. This study found that as invasive crayfish increasingly displace native crayfish, symbiont survival significantly decreases. Symbiont dispersal appears to increase in the early stages of invasion, but significantly decrease in later stages of invasion.

To determine what impacts these mechanisms are finding throughout the Mountain Lake Region, I compared the richness and abundance of native symbiont communities at sites variously impacted by invasion. These findings suggest that invasion has significant negative impacts on both these measures of native symbiont communities. As invasive crayfish increasingly become established, both symbiont abundance and richness increasingly decrease. Further, invasion appears to result in weakening of the relationship between crayfish size and the abundance and richness of the symbiont community supported by that crayfish. This is significant as previous findings related to this system have found crayfish size to be a major driver behind symbiont diversity on native crayfish. The weakening of this relationship suggests that as invasion increases, symbiont communities supported by large native crayfish are no longer maintained. As large native crayfish are dependent on these symbionts for fitness benefits, my findings suggest that invasion may result in a collapse of this native mutualistic system. The significance of this potential collapse warrants further study. Specifically, is the loss native symbionts simply

another casualty of invasion or is the loss of fitness benefits given to native crayfish by this symbiosis significant enough to give invasive crayfish a competitive edge as they continue to spread in the region?

Appendix A: Remarks on the Identification of the Crayfish of the Mountain Lake region of Virginia

Identification of regional crayfish from mixed communities follows a logical step process: (1) Identify river basin (James, New, Roanoke) that crayfish were collected from, (2) Identify genus of collected crayfish (*Cambarus* or *Faxonius*), (3) Focus on morphological characteristics described below that are specific to co-occurring crayfish.

Identification of crayfish genera:

Two genera of crayfish occur in the Mountain Lake Region of Virginia: *Cambarus* (includes all locally native crayfish) and *Faxonius* (includes all locally invasive crayfish). Diagnosis between the two genera can be made through comparison of either gonopod morphology in male crayfish or rostrum morphology in both male and female crayfish. *Cambarus* crayfish are characterized by a male gonopod ending in two terminal elements bent approximately 90 degrees from the main shaft as well as a rostrum ending in single terminal point. *Faxonius* crayfish are characterized by a male gonopod ending in two, straight terminal elements as well as a rostrum with accessory spines and ending in three points.

New River Basin:

Cambarus appalachiensis and *C. bartonii* are the most difficult crayfish to differentiate locally. Crayfish collected from headwater streams can be identified as *C. bartonii* with some certainty, but *C. bartonii* does co-occur with *C. appalachiensis* in larger streams. Identification of these two species when they co-occur is most made through comparison of rostrum morphology. The rostrum of *C. bartonii* can be characterized is short and broad, whereas the rostrum of *C. appalachiensis* is longer and less broad. Distinguishing between the two species may require comparing multiple specimens as distinguishing between smaller specimens can be difficult.

When identifying locally collected crayfish in the genus *Faxonius*, species level differences are most easily observed in large, male crayfish. Male *F. cristavarius* are characterized by gonopods that closely fit the description of a typical *Faxonius* gonopod. In male *F. virilis*, the anterior terminal element of the gonopods are observably longer. This morphology could be described appearing like blades of grass blown over in the wind as opposed to the straight terminal elements of *F. cristavarius*. In addition to gonopod structure, coloration and chelae morphology can be used to identify large *Faxonius* crayfish. Both *F. cristavarius* and *F. virilis* are often colored in various shades of greens and browns, but large *F. virilis* can always be identified through having greenish-blue chelae covered in large yellow tubercles. This morphology is not present in large *F. cristavarius*. If collected crayfish are small and/or female, areola width can be used to differentiate local *Faxonius* crayfish with some success. *Faxonius cristavarius* are characterized by an "open" areola while *F. virilis* are characterized by a "closed" areola. The determination of whether the areola is open or closed can be made by observing the dorsal carapace of the crayfish. If a gap is present on the dorsal carapace with the appearance of an "I" the areola is open, if no gap is present the areola is closed. Areola morphology should be used only if all other character identifications have failed as there is some plasticity with this morphology that may confuse those unexperienced with crayfish morphology.

James and Roanoke Basins:

Although multi-species collections are more common in the James and Roanoke Basins, the crayfish present in these basins are more diagnostically distinct than in the New Basin. *Cambarus longulus* can usually be easily identified using the diagnostic tuft of setae on the fixed finger of their chelae as the identifying trait. Sometimes this trait may not be present though, usually in small individuals. In such cases, rostrum morphology can be used to effectively identify all *Cambarus* crayfish in the James and Roanoke Basins. *Cambarus acuminatus* is characterized by a moderately narrow rostrum that is acuminate in shape, *C. bartonii* is characterized by a short, broad rostrum, and *C. longulus* is characterized by a rostrum with thickened margins with significant narrowing towards the point of the rostrum. If unable to identify a specimen using rostrum morphology, the presence of a cervical spine can be diagnostic. *Cambarus acuminatus* usually has cervical spines while *C. longulus* rarely has such spines and *C. bartonii* never has cervical spines.

Identification of *Faxonius* crayfish in the James and Roanoke Basins focuses on the same morphology as in the New Basin.