Invasive Hosts and their Context-Dependent Relationships with Native Symbionts

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Academic Abstract

Symbiotic relationships display plasticity through time, depending on a variety of factors that include host properties, symbiont densities, and environmental conditions. Invasive species can affect symbiotic relationships by introducing invasive symbionts, reducing the population of native symbionts, or competing for native symbionts as a resource. There is an established symbiotic relationship between crayfish and annelid worms in the order Branchiobdellida. Branchiobdellidan worms can have a mutualistic cleaning symbiosis with crayfish, or at times become parasitic and feed on crayfish gill tissue if nutrients on the host are low. With the introduction of invasive crayfish in the Southern Appalachians in Virginia, branchiobdellidan worm populations have sharply declined due to invasive crayfish being less competent hosts for the symbionts. However, degree of competency as a host may differ among invasive species to, as invasive hosts have their own unique context-dependent symbiotic relationships. To investigate how symbiotic relationships differ between invasive hosts, I encouraged symbiotic relationships between invasive hosts *Faxonius virilis* and *Faxonius cristavarius* and native symbionts *Cambarincola ingens*. In two experiments spanning several months, I observed changes in growth rates of hosts and damage to gill tissues over varying levels of symbiont exposure. One species of invasive host, *F. cristavarius*, had increased growth rates when exposed to native symbionts at low symbiont densities, while for the other invasive host, *F. virilis*, growth rates and gill chamber damage was not impacted by the presence of symbionts. I also compared an invasive host *F. cristavarius* to a native host *Cambarus appalachiensis* to measure the response of growth rate, symbiont damage to gills, and behavior of worms across a
gradient of symbiont exposure. The native host’s growth rates increased over time, but not due to an effect of symbionts. However, the invasive host exhibited effects from parasitism when symbiont densities were high. My findings suggest that invasive hosts can have their own unique context-dependent relationship with native symbionts. Because there is no one-size-fits-all rule for invasive hosts, when invasive hosts enter a region, new symbiotic relationships can be formed that are beneficial for invasive hosts and native symbionts. Invasive hosts or native symbionts could also be rejected by the other which may lead to decreases in either of their populations.
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**General Audience Abstract**

Symbiotic relationships are relationships between two or more organisms lasting for long periods of time and are often associated with proximity or touch. In symbiotic relationships there can be a host and a symbiote. The difference between the host and symbiont can be found in their roles such as protection from predators or parasites or by providing nutrients or transportation and the difference in size with the host being larger. Symbiotic relationships are not static and can change over time due to a variety of reasons, such as host size, symbiont abundance, or nutrient availability. The introduction of harmful non-native species, otherwise known as invasive species, can disrupt symbiotic relationships across ecosystems. Invasive species can introduce non-native symbionts, and also can become potential hosts for native symbionts. The relationship between crayfish and Branchiobdellidan worms, an order of small, segmented worms, has been established over decades of research as a useful system for studying symbiosis. Branchiobdellidan worms can provide a beneficial cleaning service by removing harmful symbionts or bacteria from their crayfish host. Alternatively, they can become parasites and feed on crayfish gills if nutrients are not available on the host. Introduced invasive crayfish can decrease the population of brachiobdellidan worms within the Southern Appalachians in Virginia. However, an established relationship between native symbionts and invasive crayfish hosts has not been studied. To investigate the effects of a symbiotic relationship over the time span of several months between invasive hosts and native symbionts, I experimentally reduced the ability of invasive hosts to remove branchiobdellidan symbionts to allow native branchiobdellidan worms time to acclimate on to invasive crayfish and establish a symbiotic
relationship. In two experiments over several months, I recorded changes in host growth rates and gill damage. Invasive hosts had an increased growth rate when there was a low abundance of worms. I also compared an invasive host to a native host to see how changes in growth rates, gill chamber damage, and locations of worms on their host may differ. The native host’s growth rates increased, but the invasive host had a negative growth rate when worm densities were too high. My findings suggest invasive hosts can have their own unique symbiotic relationship with native symbionts. When invasive hosts are introduced to a region, native symbiont populations may either decrease or native symbionts may find compatible invasive hosts. By examining relationships between native symbionts and invasive hosts, we can understand how invasions may influence symbiotic relationships and how other organisms are affected in the ecosystem.

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Chapter 1: The Impact of Invasive Species on Native Symbiont and Host Relationships

Abstract

Community interactions are a driving part of every ecosystem. A major component of community interactions are symbiotic relationships. Symbiosis can be found in every multicellular organism on the planet. However, there are still gaps in knowledge on how symbiotic relationships are affected by disruptive changes in an ecosystem. One example of a disruptive change is when invasive species affect symbiotic relationships in an ecosystem. However, this area of research has seen scant attention. With the introduction of invasive species to new regions, it is vital to understand how invasive hosts affect native symbiont and native host communities. To further research how invasive species affect symbiotic relationships, I used the model system of crayfish and branchiobdellidan worms. Crayfish and branchiobdellidan worms have an established context-dependent cleaning symbiosis. Branchiobdellidan worms can clean crayfish exoskeletons by feeding on bacteria or other symbionts living on the crayfish host, and crayfish can have larger growth rates in response to the cleaning. Invasive crayfish can be used to understand how invasive hosts affect native communities on a broader scale. Along with studying how individual organisms affect ecosystems, it is important to relay that information to local communities to aid with management strategies and policies for handling invasive species.
Symbiosis

Symbiosis is an integral part of life for every multicellular organism on the planet because all metazoans have close, intimate interactions with other organisms regularly (Gilbert et al. 2012). In addition, symbiosis can aid in the creation of organisms' immune systems and the development of organisms at multiple life stages (Gilbert et al. 2012). Symbiosis is the relationship between two or more organisms defined as host and symbionts and can be determined by close physical interactions or touch (Silknetter et al. 2020). Symbiosis can be classified into three main categories: mutualism, in which both host and symbiont benefit, commensalism, in which one party benefits and the other is unaffected, and parasitism, in which one party benefits while the other party is harmed (Silknetter et al. 2020).

By being integral to every multicellular organism, symbiosis can shape biodiversity in a variety of ways (Chomicki et al. 2022). Biodiversity can be shaped by protective symbiosis in which a symbiont aids in defense of their host from pathogens or predators (Leclair et al. 2017). In addition, microbial symbionts can adapt to environmental stressors such as thermal stress on coral hosts and recolonize, changing the composition of symbionts on their hosts (Maher et al. 2020). Aside from shaping biodiversity, another key characteristic of many symbioses is that the relationships have degrees of plasticity. Many symbioses can span a range of outcomes, not just from mutualism to parasitism or commensalism, but also from parasitism to mutualism, or from commensalism to mutualism (Karst et al. 2008). Context dependency refers to the change in the outcome of symbiotic relationships depending on the context, both biotic and abiotic (Chamberlain et al. 2009). The change in outcomes of symbiotic relationships can be affected by
factors such as maturation of the host, density of symbionts, and environmental conditions (Skelton et al. 2016; Klepzigl & Six 2004). Environmental conditions, such as nutrient availability, can determine the outcome of symbiotic relationships for both host and symbiont. For example, bark beetles have a mutualistic relationship with fungal species as adults, but as larvae the beetles and fungi both compete for nutrients from a host tree (Klepzigl & Six 2004). The availability of resources and functional groups of both host and symbiont affect the symbiotic relationship as well. The relationship between plants and mycorrhizal fungi is dependent on the soil composition and the functional traits of both the plant and fungi (Hoeksema et al. 2010). Symbiont density, maturation of host, and nutrient availability can all be observed to change the outcomes of protective symbiosis. Depending on the size of the host or presence of parasites, defensive symbionts can either aid their host or become parasitic themselves (Hopkins et al. 2017).

One well-studied symbiotic relationship occurs between crayfish and branchiobdellidan worms (Skelton et al 2013). Crayfish and branchiobdellidan worm relationships can shift because of a wide variety of contextually-dependent factors, from attributes such as size of host, symbiont density, availability of nutrients, environmental conditions, and invasiveness of either host or symbiont (Brown et al. 2012; Thomas et al. 201; Skelton et al, 2016; Creed et al. 2022). One context that has been scarcely explored is the landscape-scale composition of hosts, especially if the landscape includes invasive species that are ill adapted to native symbionts.
Invasive Hosts and Symbiosis

Invasive species are an ever-growing problem plaguing society. Invasive species are the result of biological invasions, which refer to the introduction of organisms to an environment through human activity (Richardson et al. 2010), and which have an impact that negatively affects humans (Richardson et al. 2010). Some of the potential impacts from invasive species are harm to indigenous species, and the loss of ecosystem services (Lipták et al. 2023). These losses result in damages up to billions of dollars globally due to a loss of ecosystem services, and efforts to control the effects of invasive species (Mcneely 2001).

With the introduction of invasive species, there are still questions about how invasive species shift host-symbiont dynamics in an ecosystem. Invasive plant species that have symbiotic relationships with fungi can reduce species richness and diversity of other fungi and plants in their system (Sokornova et al. 2022). Invasive plants will also change the symbiotic networks in soil and feeding habits of grazers in the area, putting their native counterparts at a disadvantage (Coats & Rumpho 2014). Invasions focused on animals can also lead to dire consequences for the ecosystem. Invaders may form symbiotic relationships with other introduced species causing a potential cascade of effects. When invasive ants and aphids interact with each other they can form a mutualistic relationship that can damage native plant species (Holt et al. 2022). Invaders can outcompete native species for resources and change the landscape and biodiversity of an area. As more invaders are introduced the population of native hosts and symbionts can be reduced over time (Creed et al. 2022). Not all invasions can lead to decreases in communities of native symbionts. Depending on the competency of the invasive host, native symbiont and invasive host populations could increase or decrease over time (Creed et al. 2022). With the
problems invasive species bring, it is important to look at study systems that can allow scientists to understand the impacts of invasive species on a broader scale. To attempt a better understanding of how invasive species affect symbiotic relationships, crayfish and branchiobdellidan worms can be used as a study system.

*Crayfish-Branchiobdellidan Symbiosis*

Crayfish and branchiobdellidan worms have a documented context-dependent symbiotic relationship, which is now changing due to the introduction of invasive species. Branchiobdellidan worms are annelids and belong in the class Clitellata that primarily live on crayfish (McManus 1960), although in tropical regions they can be found on other large freshwater crustaceans (Brinkhurst, & Gelder 2001). Branchiobdellidan worms have historically been referred to as commensals on crayfish hosts (Young 1966). Most Branchiobdellidan worms need a live crayfish host to live and reproduce on (Young 1966; Creed et al. 2015). More worms are found on larger crayfish, as more eggs and cocoons have been seen on larger crayfish due to the larger surface area available (Creed et al. 2015). Within the genus *Camboricola*, some studies have found the worms to have a commensal relationship with their crayfish hosts (Keller 1992). Despite their longstanding label of “commensals”, the crayfish-branchiobdellidan relationship is context-dependent and outcomes can be mutualistic or facultatively parasitic (Brown et al. 2012; Lee et al. 2009). Crayfish and branchiobdellidan worms can engage in a mutualistic cleaning symbiosis (Brown et al 2002). Depending on the density of the worm species *C. ingens*, the crayfish species *Cambarus chasmodactylus* were shown to significantly increase in size and have decreased mortality when there were higher densities of symbionts (Brown et al 2002). Later studies would further elucidate the intricate relationship between crayfish and branchiobdellidan worms. Branchiobdellidans can be seen in mutualistic relationships with several species of
crayfish, under both laboratory and field conditions (Brown et al. 2002; Brown et al. 2012; Lee et al. 2009; Ames et al. 2015; Thomas et al. 2016). However, their relationship is more complex than originally thought. The density of worms may vary on different crayfish species due to host selection behavior (Brown & Creed 2004). Mutualistic worms may be found on one crayfish species, but may not be as prevalent on other species, and the degree of mutualism on one species may change over time (Brown & Creed 2004). The relationship between crayfish and branchiobdellidan worms can change from commensal to mutualistic based on environmental factors affecting the nutrients available for branchiobdellidan worms and the potential for fouling of host gills, which the branchiobdellidans can alleviate (Lee et al. 2009). A factor that may influence their relationship can be size of the crayfish, as smaller crayfish will remove worms at higher rates compared to adults of the same species (Skelton et al. 2014). Along with the size of the crayfish, the species of worm impacts their symbiotic relationship. Younger crayfish may have different worm species compared to older crayfish (Skelton et al 2016). At times the relationship between worms and crayfish can cross the border into a parasitic relationship when, at high densities, worms can switch from cleaning symbiosis to attacking crayfish gill tissues (Brown et al. 2012). Crayfish exhibit grooming as a behavior aimed at controlling over infestations of branchiobdellidans (Farrel et al. 2014). Grooming behavior varies between different crayfish species. *Faxonius cristavarius*, an invasive crayfish to the Southern Appalachians in Virginia grooms at higher rates compared to a native crayfish species *Cambarus chasmodactylus* (Farrell et al. 2014). A dynamic that changes the complexity of the crayfish and branchiobdellidan worm relationship is the involvement of invasive species.

Crayfish can act as prominent invaders once introduced. Invasive crayfish have the potential to reproduce quickly and outcompete previously established species for resources (James et al.
2016). If left unchecked, invasive crayfish can quickly spread through an aquatic system, shown by the *Faxonius rusticus* invasion of the John Day River Basin (Messager et al. 2018). Crayfish invasions are severe enough for multiple management strategies to have little effect on the introduced crayfish (Gherardi et al. 2011). Invasive crayfish are detrimental to the ecosystem and can cause financial burdens to local governments but have unique relationships with branchiobdellidan worms. Introduced crayfish can carry branchiobdellidan worms that are also non-native to their new region (Vedia et al. 2015). Newly introduced worms then may transfer to other native and non-native crayfish (Hunt et al. 2018). Invasive crayfish can act as worm carriers, but there is evidence of invasive crayfish negatively affecting worm populations. *F. cristavarius*, an invasive crayfish to the Southern Appalachians in Virginia grooms off native worms at higher rates compared to native crayfish (Farrel et al. 2014). *F. cristavarius* serving as non-compatible hosts negatively affect native crayfish by decreasing the density of worms that benefit native crayfish species (Creed et al 2022). However, because each individual invasion event is different, there are multiple pathways showing the effects of invasive species on native symbionts (Creed et al. 2022). The effects of invasive species on native symbionts may range from either being positive, neutral, or negative (Creed et al 2022). At the present time, there is no one-size-fits-all solution for how to handle invasive crayfish and branchiobdellidan worms. The next steps for researchers and citizens would be to utilize tools for invasive species management and science education.

**Citizen Science & Invasive Species**

The crayfish-branchiobdellidan system is not only an excellent model for the study of symbiosis, it is also potentially useful to involve communities with science that affects their daily lives.
Invasive species are a direct cause of human effects and reduce native species diversity in their introduced systems. Working with local communities can be a way to reduce the effects of invaders and increase science education. Science communication is a great way to spread awareness of ecological issues to the public. Scientists can improve their communication skills, while general audiences' knowledge of science can increase through science communication programs (Clark et al. 2016). Along with programs to help scientists with communication skills, citizen science can help aid public participation and provide useful data measurements (Dickinson et al. 2012). Citizen science programs can be used to help survey invasive species in areas, increasing public awareness of invasive species (Gallo & Waitt 2011). By increasing public knowledge on invasive species, citizens can more accurately identify native or invasive species (Lipták et al. 2023). Citizens who recognize invasive species are more likely to wish to protect native species (Lipták et al. 2023). Incorporating the public into scientific work is a way to make science more approachable and raise awareness for ecological issues. By making science more accessible, it can be easier to detect invasive species and to protect endangered organisms. Citizens can appreciate the usefulness of the crayfish-branchiobdellidan worm system, while not knowing every intricate detail. Citizen science can act as a platform that increases the understanding of how invasive species affect native host and symbiont relationships.

Conclusion

Symbiosis is an integral part of every ecological system. The model system of crayfish and branchiobdellidan worms showcases the complexity of symbiotic relationships and how context dependent they may be. Invasive species affect the relationship between crayfish and branchiobdellidan worms. The addition of invasive crayfish can have negative effects, with
invasive crayfish reducing native symbiont populations through grooming. Not every invasive crayfish is detrimental to branchiobdellidan worms, as some crayfish may carry their own non-native worms and transfer them when introduced. Since there are multiple outcomes for invasive hosts, it is best to come up with management strategies on how to handle recent and incoming invasions. Getting local communities involved can be beneficial for reducing the effects of invasive crayfish. Teaching communities how to identify invasive crayfish, and the impact they have on a model symbiotic system, will help both scientists and the general public in the end.
Chapter 2: Native Symbionts and Their Impact on Invasive Hosts

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Abstract

Symbiotic relationships can be found in organisms across the globe. Symbiotic relationships are not static, and can change depending on availability of nutrients in the environment, size of the host, and the abundance of symbionts. Due to invasive species becoming more prevalent in ecosystems, the effect of invasive hosts on native hosts and symbionts is beginning to be investigated. In the Southern Appalachians in Virginia there is an established symbiotic relationship between crayfish and branchiobdellidan worms. Crayfish and branchiobdellidan have a context-dependent symbiotic relationship that changes over time due to multiple variables. Invasive crayfish have been introduced into the Southern Appalachians in Virginia within the last century. The invasive crayfish have reduced native symbiont populations over time, but no studies have been conducted on the effects of an established symbiotic interaction between the invasive hosts and native symbionts. To investigate the long-term effects, I conducted two studies over several months examining if there is change in growth rates of invasive hosts, if hosts suffered gill damage from the presence of symbionts, and if native symbiont behavior changed depending on the species of host. In order to ensure a symbiotic relationship between native symbionts and invasive hosts I gave half the hosts dactyl ablations which are small incisions that remove the dactyl from the first pair of walking legs. My findings suggest that the context-dependent relationship changed between each species. The invasive species *F. cristavarius* had an increase in growth when having four branchiobdellidan worms and dactyl ablations. However, *F. cristavarius* with six branchiobdellidan worms and
dactyl ablations had a decrease in growth over time. The invasive species *F. virilis* had no impactful changes from having branchiobdellidan worms or dactyl ablations. The native species *C. appalachiensis* had similar increases in growth rates with and without brachniobdellidan worms and no dactyl ablations. *C. appalachiensis* with dactyl ablations had a higher growth rate without branchiobdellidan worms compared to when having branchiobdellidan worms. Because symbiotic relationships vary among hosts, it is important to understand how relationships with invasive hosts differ from those with native hosts. Invasive species may affect symbiotic relationships at larger spatial scales, so it is crucial to know how their relationships change between hosts.
**Introduction**

Symbiosis is an integral part of life for every multicellular organism on the planet because all multicellular organisms have close, intimate interactions with other organisms regularly (Gilbert et al. 2012). By being integral to every multicellular organism, symbiosis can shape biodiversity in a variety of ways (Chomicki et al. 2022). Symbionts can aid hosts in terms of survival by aiding in defense of their host from pathogens or predators, transferring nutrients, and by providing cleaning services to their hosts (Leclair et al. 2017; Hopkins et al 2017; Ferrier-Pagès & Leal 2018; Brown et al. 2002). However, many symbioses are context dependent. Context dependency refers to the change in the outcome of symbiotic relationships depending on both biotic and abiotic contexts (Chamberlain et al. 2009). Factors like size of the host, presence of symbionts, or nutrients available in the environment, can determine whether symbionts either aid their host or become parasitic (Skelton et al. 2016; Klepzigl & Six 2004). Due to the negative effects invasive species bring to new environments, there is growing concern on how invasive species affect symbiotic relationships, making it vital to understand how invasions affect the outcomes of symbiotic relationships.

The introduction of invasive species can shift the dynamics of symbiotic relationships, as well as the interactions between species in an ecosystem. Invasive plant species interacting with symbiotic fungi can reduce species richness and diversity of other fungi and plants in their system (Sokornova et al. 2022). Invasive plants will also change the symbiotic networks in soil and feeding habits of grazers in the area, putting their native counterparts at a disadvantage (Coats & Rumpho 2014). Animal invasions can also lead to dire consequences for an ecosystem. Invaders may form symbiotic relationships with other introduced species causing a
potential cascade of effects. When invasive ants and aphids interact with each other, they can form a mutualistic relationship that can damage native plant species (Holt et al. 2022). Invaders can outcompete and even remove resources, such as symbionts, from native species. As more invaders are introduced, the population of native hosts and symbionts can be reduced over time (Creed et al. 2022; Bell et al In revision). Not all invasions necessarily lead to decreases in communities of native symbionts. Native symbionts can hinder or aid in the success of an invasion depending on the level of compatibility with an invasive host, allowing for invasive hosts to successfully invade or to be warded off (Creed et al. 2022; Traveset & Richardson 2014). In some circumstances, native symbionts can have little to no effect on the invader, leading to native symbiont populations declining (Creed et al. 2022). However, these types of landscape-scale impacts of invasive hosts on native host-symbiont relationships are ultimately a product of the organism-scale interactions between hosts and symbionts.

To attempt a better understanding of how invasive species affect the symbiotic relationship between host and symbiont, I performed experiments using the symbiotic relationship between crayfish and branchiobdellidan worms. Specifically, I wanted to encourage a symbiotic relationship between invasive species and native symbionts and examine the outcomes of this interaction relative to the outcomes in a native host-native symbiont relationship. Crayfish and branchiobdellidan worms have a context-dependent relationship that changes depending on the size of the host, the density of symbionts, and environmental conditions (Skelton et al. 2016; Lee et al. 2009). Crayfish growth rates can increase along with reduced mortality rates under mutualistic conditions with branchiobdellidan worms (Brown et al. 2002). However, preliminary
evidence suggests that the crayfish-branchiobdellidan relationship changes when non-native hosts are introduced.

Invasive crayfish in the Southern Appalachians in Virginia have unique relationships with branchiobdellidan worms. Invasive crayfish can host native branchiobdellidans, but there is evidence of invasive crayfish negatively affecting worm populations, primarily because invasive crayfish groom off native worms at higher rates compared to native crayfish (Farrel et al. 2014). The mechanism of crayfish grooming produces impacts beyond just the pairwise host-symbiont interaction. Once introduced, invasive crayfish can reduce both abundance and diversity of native symbionts at whole-stream and whole-landscape scales, negatively affecting both native hosts and symbionts (Bell et al. In revision).

To further study the effects of invasive hosts on the crayfish-branchiobdellidan symbiosis, I conducted two experiments. The first study encouraged a symbiotic relationship between the native branchiobdellidan worm *Cambarincola ingens* and two invasive host species: *Faxonius virlis* and *Faxonius cristavarius*. The second study examined a native crayfish species, *Cambarus appalachiensis*, and an invasive crayfish species, *F. cristavarius* while encouraging a symbiotic relationship with *C. ingens*. These studies were designed to examine whether invasive crayfish have similar growth rate responses when interacting with native symbionts when compared to native crayfish. I also looked for direct evidence of parasitism by measuring gill scarring due to a potential parasitic effect, and I examined whether branchiobdellidan worm locations on the host would differ depending on the host species and experimental treatments.
My hypotheses include: 1) invasive crayfish with native symbionts can have increased growth rates responses when similar to native crayfish, 2) crayfish with higher counts of gill damage are direct evidence of parasitic behavior from native symbionts, and 3) the location of branchiobdellidan worms would change depending on the species of host and the experimental treatment given to the host.

**Materials & Methods**

**Study System:**

Branchiobdellidan worms are annelids and belong in the class Clitellata that primarily live on crayfish in temperate regions (McManus 1960). Most Branchiobdellidan worms need a live crayfish host on which to live and reproduce (Young 1966; Creed et al. 2015). The number of worms and their cocoons increases predictably with host size, with larger crayfish hosting hundreds of worms (Creed et al. 2015). Branchiobdellidan worms were historically considered to be commensals on their crayfish hosts (Young 1966; Keller et al. 1992). However, their relationship is more complex than originally thought. The relationship between crayfish and branchiobdellidan worms can change depending on environmental factors, such as fouling pressure (Lee et al. 2009). Factors that may influence their relationship can be size of the crayfish, as smaller crayfish will remove worms at higher rates compared to adults of the same species (Skelton et al. 2014), and environmental conditions such as habitat and availability of nutrients (Lee et al. 2009; Ames et al 2015). Along with the size of the crayfish, the species of worm impacts their symbiotic relationship. At times the relationship between worms and crayfish can cross the border into parasitic, when at high densities worms can switch from cleaning symbiosis to attacking the crayfish’s gill chamber (Brown et al. 2012). Crayfish
exhibit grooming as a behavior aimed at removing excessive amounts of worms (Farrel et al. 2014). Grooming behavior may vary between the species and age of the crayfish (Skelton et al. 2016; Thomas et al. 2016). *F. cristavarius*, an invasive crayfish to the Southern Appalachians in Virginia, grooms at higher rates compared to *C. chasmodactylus* (Farrell et al. 2014).

A dynamic that changes the complexity of the crayfish and branchiobdellidan worm relationship is the involvement of invasive species. The invasive species *F. cristavarius* serves as a non-compatible host and negatively affects native crayfish by decreasing the density of worms that benefit native crayfish species (Creed et al. 2022; Bell et al. In revision). However, because each individual invasion event is different, there are multiple pathways showing the effects of invasive species on native symbionts (Creed et al. 2022). The effects of invasive species on native symbionts may range from either being positive, neutral or negative. Introduced crayfish may also carry branchiobdellidan worms that are also non-native to their new region (Vedia et al. 2015). Newly introduced branchiobdellidan worms then have the capability to transfer to other native and non-native crayfish (Hunt et al. 2018). Invasive crayfish allow us to examine how a well-documented symbiotic model relationship can be affected by invasions and see how other symbiotic relationships can potentially be impacted.

Crayfish and branchiobdellidan worms prove to be an excellent study system for symbiosis. Crayfish are abundant across multiple continents, and multiple species of branchiobdellidan worms can inhabit a crayfish host (Skelton et al. 2016). Aside from the abundance of host and symbiont, the model system is easy to manipulate. Branchiobdellidan worms can be removed live from crayfish to manipulate the density of symbionts, and crayfish can be chemically
cleaned using MgCl$_2$ to remove all branchiobdellidan worms from the host to allow for a multitude of experiments (Brown et al 2002; Brown and Creed 2004). Along with symbiont density manipulation, crayfish and branchiobdellidan worms can be comfortably housed in aquaria, making laboratory-based experiments feasible. Because of the advantages of using crayfish and branchiobdellidan worms as a model system, I used them to examine the effects of a long-term symbiotic relationship with invasive hosts and native symbionts.

**Study 1: Invasive Crayfish Interactions with Native Symbionts**

I manipulated the branchiobdellidan worm *C. ingens* on two crayfish species, *F. cristavarius* and *F. virilis*, to understand how both invasive species are affected by the native worm symbionts in the region. Both species of crayfish have heavily invaded the Southern Appalachians in Virginia.

Using aquaria in the laboratory, I examined the effects of prolonged *C. ingens* exposure on the host crayfish to see if growth rates, gill tissue damage, and mortality rates would change due to the presence of four *C. ingens*. I collected crayfish (N=24) from Stroubles Creek in Newport, VA (Giles County) USA from March-June of, 2022, by flipping over large rocks and using dip nets to collect crayfish that were shallowly burrowed. Once the crayfish were collected, the crayfish were randomly assigned to one of two treatments: treatment with dactyl ablations (N=12) and a treatment without ablations (N=12). Dactyl ablations are small incisions that remove the dactyl from the first pair of walking legs. They are designed to reduce the ability of the crayfish to groom off the worms by negating the “pinching” ability of the two legs most used for grooming (Skelton et al. 2016). I collected branchiobdellidan worms (N=96) for use in the study from a large quantity of native crayfish found in Sinking Creek in Newport, VA, USA in between May-June of, 2022, by capturing native crayfish and removing the worms with forceps in a lab.
environment. I used 60 seconds of immersion in 10% MgCl₂ to remove any branchiobdellidan worms from experimental crayfish prior to the experiment (Brown et al. 2002; Brown and Creed 2004). For worm exposures, I distributed 4 worms along each of the 24 invasive hosts dorsal and ventral surfaces and gave the worms 10 minutes to acclimate on their new hosts before placing the crayfish into individual aquariums. At the start of the study, crayfish ranged from a carapace length (CL) of 24.75-33.57mm for *F. cristavarius* and 31.86-62.23mm for *F. virilis*, and a blotted wet mass (BWM) ranging from 4.29-10.55g for *F. cristavarius* and 8.32-82.08g for *F. virilis*. Each aquarium (N=24) contained 38 liters of tap water cleaned by bubbling and a bottom layer covered with local stream sediment. Water was added every six weeks to top off the aquaria, crayfish were fed five shrimp pellets every week, room temperature was at 19.44 °C, and the light and dark cycle of the room fluctuated depending on the amount of natural light coming into the lab environment. For each treatment, I used 6 replicates having a total of 24 hosts in the experiment. Crayfish were initially measured and inoculated with worms on June 9th and June 14th. Every two weeks, I assessed current levels of branchiobdellidan infestation and measured crayfish CL and BWM. I repeated these measurements three more times (July 7th and 12, August 4th and 9th, and September 1st and 6th) for a total experiment length of 89 days. The change in percent BWM was analyzed using a time series model that compensated for missing data and unequal samples sizes; I used a linear mixed model with ablation, symbiont density treatment, and time as fixed effects, and a random effect of individual in R statistical software with the package nlme and the function lme (R Development Core Team 2023).
Study 2: Native and Invasive Crayfish with Native Symbionts

When comparing the symbiotic relationship with branchiobdellidans between native and invasive crayfish, I used similar methodology as the previous study aside from a few differences. I collected crayfish (N=20) from Stroubles Creek in Newport, VA, USA in between March-April of, 2023 and (N=20) crayfish from Sinking Creek in Newport, VA, USA in March, 2023. Crayfish hosts for the experiment were collected by flipping over large rocks and using dip nets to collect crayfish that were shallowly burrowed. Crayfish were randomly assigned to one of four treatments: branchiobdellidan worms with dactyl ablations (N=10), branchiobdellidan worms without dactyl ablations (N=10), no branchiobdellidan worms and dactyl ablations (N=10), and no branchiobdellidan worms and no dactyl ablations (N=10). I collected branchiobdellidan worms (N=120) for use in this study from a large quantity of native crayfish found in Sinking Creek in Newport, VA, USA between April-May, 2023, by capturing native crayfish and removing the worms with forceps in a lab environment. I used 60 seconds of immersion in 10% MgCl$_2$ to remove any branchiobdellidan worms from experimental crayfish prior to the experiment (Brown et al. 2002; Brown and Creed 2004). For worm exposures, I distributed 6 worms along 20 hosts dorsal and ventral surfaces, and gave the worms 10 minutes to acclimate on their new hosts before placing the crayfish into individual aquariums. I used the native species *Cambarus appalachiensis* to compare the differences between a native and invasive crayfish under prolonged symbiont exposure. Next, I limited the size range of crayfish used during the experiment. I chose a size range to have crayfish large enough to maintain worms without grooming and to molt within the time span of the experiment (Skelton et al. 2016). *C. appalachiensis* was between the CL of 32-38 mm and *F. cristavarius* was between 24-30 mm. Each aquarium (N=40) contained 38 liters of tap water cleaned by bubbling and a bottom layer
covered with local stream sediment. Water was added every six weeks to top off the aquaria, crayfish were fed five shrimp pellets every week, room temperature was at 19.44 °C, and the light and dark cycle of the room fluctuated depending on the amount of natural light coming into the lab environment. I increased the total number of worms in the symbiont-added treatment from 4 to 6 to see if a higher symbiont count would affect the symbiotic relationship between the crayfish and worms. Previous experiments with *Cambarus* have shown 6 to be the *C. ingens* density that produced the most positive effect on growth (Brown et al. 2002 and 2012). For each treatment, we used 5 replicates instead of 6 used in the previous experiment, having a total of 40 hosts in the experiment. The experiment lasted for a four-month period. However, the frequency of measurements and worm counts were twice as frequent, as I recorded worm counts every week, and measured CL and BWM every two weeks. The initial worm additions and measurements took place on May 25th and 26th with subsequent measures on (June 8th and 9th, June 22, and 23rd, July 6th, July 20th, August 3rd, August 17, August 31st, and September 14th). The change in percent BWM was analyzed using a time series model that compensated for missing data and unequal samples sizes; I used a linear mixed model with dactyl ablation, symbiont density treatment, crayfish species, and time as fixed effects, and a random effect of individuals in R statistical software with the use of the package nlme and the function lme (R Development Core Team 2023). I had four gravid females during this experiment, and thus I performed data analyses with and without gravid females to ensure they didn’t overly affect the results.

I measured worm locations on the host for both species of crayfish once a week starting from June 22nd to September 14th (Figure 1). I used the proportion of worms present on each site on
the host at 28, 56, and 112 days in a Principal Components Analysis with the princomp function in R statistical software (R Development Core Team 2023). At the end of the experiment, crayfish were sacrificed to determine if branchiobdellidan worms had damaged host gills. Gill damage was assessed by examining crayfish’s gill chamber beneath their carapace. I counted the individual melanized areas shown on the gill chamber with the use of a stereo-microscope, and gill damage was then separated into two categories depending on the level of damage. The two categories were scar damage and filament damage. Scar damage was indicated by small brown and red marks on the gills that are characteristic of worms feeding on the host’s gills (Figure 2). Filament damage was characterized by large red and black streaks going across the gills from unknown causes of damage (Figure 3). Three *F. cristavarius* were omitted from the analysis due to excessive body damage or from being misplaced during study. Gill damage was analyzed using linear models with ablations, crayfish host species, and the abundance of worms as a factor in R statistical software with the use of the and the function lm (R Development Core Team 2023).

**Results**

**Study 1: Growth experiments**

Branchiobdellidan worms influenced the growth rates of crayfish in both experiments. I documented two mortalities during the duration of study 1 (Table 1). In study 1, there was no evidence (p-value = 0.8319) that the invasive host *F. virlis* growth was affected by dactyl ablations (Figure 4a). There was strong evidence (p-value = 0.0420) that the invasive host’s, *F. cristavarius*, growth rates were influenced by worm density and the treatment of dactyl ablations based off the higher growth rates of the treatment group with dactyl ablations (Table 2; Figure
4b). *F. virlis* with dactyl ablations had the highest growth rates in study 1. *F. virlis* without dactyl ablations and *F. cristavarius* with dactyl ablations also showed positive growth, just not with as high a rate as *F. virlis* with dactyl ablations. *F. cristavarius* without dactyl ablations showed little to no growth in study 1.

**Study 2: Growth experiments**

During the duration of study 2 seven mortalities were recorded (Table 1). In study 2, the native species *C. appalanchensis* with dactyl ablations and worms had the lowest rate of growth, while hosts with dactyl ablations and without worms showed an increase in growth (Figure 5a). *C. appalanchensis* without dactyl ablations grew at a similar rate with and without worms (Figure 5b). The absence of dactyl ablation did not influence the invasive species *F. cristavarius*’ growth rates (Figure 6a). However, the treatment of dactyl ablation, branchiobdellidan worms, and time did affect *F. cristavarius* growth (p-value = 0.0015), (Table 3) as growth rates increased without branchiobdellidan worms and growth rates decreased branchiobdellidan with worms (Figure 6b).

**Study 1 & 2: Gill Scarring**

There was little evidence of branchiobdellidan worm density influencing the number of gill scars; however, the result varied depending on the species of host (Table 4). Crayfish in the no branchiobdellidan worm treatment had less scar damage (*C. appalanchensis* without dactyl ablations scar damage mean = 37.67, *C. appalanchensis* with dactyl ablations scar damage mean = 24.80, *F. cristavarius* without dactyl ablations scar damage mean = 10.00, *F. cristavarius* with dactyl ablations scar damage mean = 9.00) compared to crayfish with branchiobdellidan worms.
(Figure 7a; Figure 7b). *F. cristavarius* without dactyl ablations had a similar amount of scar damage with or without dactyl ablations. When worms were present throughout the experiment, *F. cristavarius* had higher amounts of scar damage with dactyl ablations. *C. appalanchensis* with and without worms had higher amounts of scar damage without dactyl ablations. *F. virilis* without dactyl ablations had higher amounts of scar damage. There was no statistical evidence of either experimental treatment influencing filament damage (Table 4; Figure 8a; Figure 8b).

**Study 2: Worm Location on Host**

Different species of crayfish had worms colonize on different areas of their bodies, depending on the species and treatment of dactyl ablations. A PCA 28 days after the experiment began showed that 91% of the variance was explained by the first two axes (Figure 9). Ventral abdomen sites were weighted heavily on Component 1, while carapace and cephalothorax were weighted heavily on Component 2. The branchiobdellidan worm locations were separated more by host species, and the dactyl ablations had little effect on where the branchiobdellidans were found at the beginning of the study. A PCA 56 days after the experiment began showed that 98% of the variance was explained by the first two axes (Figure 10). Ventral abdomen sites were weighted heavily on Component 1, and the cephalothorax sites were weighted heavily on Component 2. Worm locations for both species were no longer heavily weighed by the carapace, but locations were still grouped largely by species rather than treatment. A PCA 112 days after the experiment began showed that almost all of the variance was explained by the first two axes (Figure 11). Ventral abdomen sites were weighted heavily on Component 1, and Component 2 showed branchiobdellians would be found around the cephalothorax. By the end of the study *C. appalanchensis* with dactyl ablations had branchiobdellidans appear on different locations when compared to the rest of the treatment groups.
Discussion

There was evidence that symbiosis with branchiobdellidans resulted in mutualistic outcomes for at least one invasive crayfish species, *F. cristavarius*. Higher growth in the presence of worms indicated invasive crayfish can receive benefits from native symbionts. Supporting my hypothesis that invasive crayfish with native symbionts can have increased growth rates responses when similar to native crayfish. The difference in growth rates between native and invasive hosts was noticeable. The native crayfish host *C. appalachiensis* without dactyl ablations had high growth rates with and without worms compared to *C. appalachiensis* with dactyl ablations. *C. appalachiensis* with dactyl ablations showed stark difference in growth rates when worms were present. The treatment group with dactyl ablations and 6 worms had the lowest growth rate out of the native host treatment groups. The aquaria environment of *C. appalachiensis* potentially may have reduced the potential of gill fouling, as the higher the gill fouling, the more likely cleaning will occur. By reducing the potential benefit of the worms, the crayfish-worm relationship may become more commensal than mutualistic (Lee et al. 2009).

The difference in growth rates for invasive crayfish hosts varied depending on the treatment group. *F. cristavarius* without dactyl ablations had low growth rates with and without worms. *F. cristavarius* with dactyl ablations had low growth rates, while without worms, growth rates were higher, as in both experiments, there were differences in growth rates, but the difference depended on the abundance of worms present. The presence of worms on *F. cristavarius* had a positive influence on growth rates at lower levels of worms, but a higher abundance of worms resulted in a negative growth rate, suggesting a weak parasitic effect. During the duration of the
second study, *F. cristavarius* with dactyl ablations molted several times, with some crayfish molting more than once. Suffering partial or full limb loss can increase molting rate of crustaceans, and often this molting will focus on limb regeneration instead of growth (Fujaya et al. 2020). Therefore, it is possible that the ablation treatment actually stimulated molting. However, given that the treatment of dactyl ablations was applied in both studies, the difference in outcome appears to depend on worm levels, with *F. cristavarius* benefitting from a small number of worms, but experiencing a parasitic effect with larger numbers of worms.

The tolerance invasive crayfish have for native branchiobdellidans is lower than their native counterparts (Farrel et al. 2014; Creed et al. 2022). Native crayfish within the Southern Appalachians in Virginia can get benefits while having up to ten *C. ingens* worms (Brown et al. 2012). Evidence for invasive crayfish having their own threshold for native branchiobdellidans was seen by examining their gill chamber, which supported my hypothesis that crayfish with higher counts of gill damage are direct evidence of parasitic behavior from native symbionts. A high presence of gill scar damage on a crayfish is evidence for branchiobdellidan worms feeding on crayfish gill tissue, shifting their relationship from mutualism or commensalism to parasitism (Brown et al. 2012). Without worms, invasive crayfish had a similar amount of gill scar damage with and without dactyl ablations. This damage can likely be attributed to gill scar damage being obtained in the field before the start of the experiments. Invasive crayfish with dactyl ablations had higher amounts of gill scar damage, indicating that branchiobdellidans were actively feeding on the gills of invasive hosts. Both *F. virlis* and *F. cristavarius* had higher gill scar damage with dactyl ablations, but *F. cristavarius* was observed under multiple worm levels. On the other hand, native crayfish with and without the presence of worms had more gill scar damage without
dactyl ablations. The native species *C. appalachensis* can house multiple species of branchiobdellidan worms in their natural habitat, and native crayfish may have received gill scar damage before the start of the study. Native and invasive crayfish having varying amounts of gill scar damage is evidence for invasive hosts having their own threshold tolerance for branchiobdellidan worms. A possible explanation may be due to the invasive hosts having different levels of thickness for their exoskeletons, leaving invasive crayfish more susceptible to parasitism if their gill chambers are easier to access.

Branchiobdellidan worms also behaved differently on different host species, as indicated by the positions that they occupied on the host. Branchiobdellidan worms can be found at virtually any microhabitat on a crayfish, with some branchiobdellidans specializing on particular body regions (Skelton et al. 2015). The abundance of worms in each microhabitat may change depending on dispersal rates of symbionts and on the size of the host (Skelton et al. 2015). The species of host can alter where worms arrange themselves on a crayfish host because of differences in the size and grooming behavior of different species. When comparing native and invasive crayfish, the locations of branchiobdellidan worms differed between crayfish species, giving evidence that my hypothesis that the location of branchiobdellidan worms would change depending on the species of host and the experimental treatment given to the host was supported. The invasive host *F. cristavarius* had worms appear in similar areas for most replicates. By grooming at higher rates than native hosts (Farrel et al. 2014), *F. cristavarius* would have a reduced number of branchiobdellidan worms, indicating that host grooming likely heavily influenced branchiobdellidan worm location. Native hosts have worms appearing in a variety of locations on the body. The effects of dactyl ablations did not appear to impact the location of worms until the
end of the study. Native hosts with dactyl ablations had worms in different locations compared to the rest of the treatments. Native hosts with dactyl ablations were able to keep their worms for the longest duration, providing evidence for native and invasive hosts having different levels of suitability for native symbionts.

Invasive crayfish have unique context-dependent relationships with branchiobdellidan worms. *F. cristavarius* growth rates changed dramatically depending on the abundance of symbionts during a long period of exposure. *F. cristavarius* also had higher gill scar damage with worms and dactyl ablations when compared to the native host *C. appalanchesésis* and another invasive host *F. virilis*, which had higher scar damage with worms and no dactyl ablations. Even the location of worms was dependent on whether worms were on a native or invasive host. My study has given insight on how long-term exposure of native symbionts on invasive hosts can vary depending on host behavior, abundance of symbionts, and experimental treatments to maintain symbiosis.

Symbiosis is an integral part of every ecological system. The model system of crayfish and branchiobdellidan worms showcases the complexity of symbiotic relationships and how context dependent they may be. The addition of invasive crayfish into a system can have negative effects on branchiobdellidans, with invasive crayfish reducing native symbiont populations through grooming (Bell et al. *In revision*). Native symbionts can negatively impact invasive crayfish by damaging their gills when their abundance is high. However, at lower symbiont populations, invasive crayfish can experience similar mutualistic results that native crayfish have been documented to receive. Even though invasive hosts can receive benefits, the risk of parasitism from an abundant population may not be worth the risk. In response, invasive
hosts may reduce populations of native symbionts by grooming or predating them to eliminate potential threats to their well-being. The difference in symbiotic relationships for native and invasive crayfish gives us insight into how invasive hosts can affect symbiotic relationships in other model systems. Identifying mechanisms in which invasive hosts can reduce native symbiont populations can aid in understanding if invasive species will be suitable hosts for native symbionts. However, every invasion is different, it is imperative to recognize there is no one-size-fits-all rule. Invasive hosts can introduce their own non-native symbionts into a new region or be unbothered by native symbionts entirely. Invasive hosts should be looked at individually, for they all have their own unique context-dependent relationship with organisms around them.
### Tables and Figures

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<tr>
<th>Species</th>
<th>Treatment</th>
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Table 1. Summary of mortalities recorded from Study 1 and Study 2.
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Table 2. Summary of results from linear mixed models examining the relationship between invasive hosts’ growth rates with native symbionts. The treatment of dactyl ablations and time since the experiment started were used.
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Table 3. Summary of results from linear mixed models examining the relationship between invasive and native hosts’ growth rates with the treatment of native symbionts, dactyl ablations, and the time since the experiment started being used.
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Table 4. Summary of results from linear models examining the relationship between invasive and native hosts’ gill chamber scar and filament damage with the treatment of native symbionts and dactyl ablations.
Figure 1. Diagram of locations on the dorsal and ventral sides of a crayfish where branchiobdellidan worms were found on their crayfish hosts.
Figure 2. Crayfish gill chamber scar damage shown by the dark melanated tips where damaged occurred.
Figure 3. Crayfish gill chamber filament damage shown by large black streaks appearing on the crayfish’s gills.
Figure 4a. Percent Mass change of *F. virilis* during a 89-day study examining the effects of branchionellidan worms and dactyl ablations on the growth and mortality of crayfish. Error bars represent the standard error of the six replicates. Points were jittered by a random increment to decrease overlap between error bars and increase readability.

Figure 4b. Percent Mass change of *F. cristavarius* during a 89-day study examining the effects of branchionellidan worms and dactyl ablations on the growth and mortality of crayfish. Error bars represent the standard error of the six replicates. Points were jittered by a random increment to decrease overlap between error bars and increase readability.
Figure 5a. Percent Mass change of *C. appalachiensis* during a 112-day study examining the effects of branchiondellidan worms and dactyl ablations on the growth and mortality of crayfish. Error bars represent the standard error of the five replicates. Points were jittered by a random increment to decrease overlap between error bars and increase readability.

Figure 5b. Percent Mass change of *C. appalachiensis* during a 112-day study examining the effects of branchiondellidan worms on the growth and mortality of crayfish. Error bars represent the standard error of the five replicates. Points were jittered by a random increment to decrease overlap between error bars and increase readability.
Figure 6a. Percent Mass change of *F. cristavarius* during a 112-day study examining the effects of branchionellidan worms and mortality of crayfish. Error bars represent the standard error of the five replicates. Points were jittered by a random increment to decrease overlap between error bars and increase readability.

Figure 6b. Percent Mass change of *F. cristavarius* during a 112-day study examining the effects of branchionellidan worms and dactyl ablations on the growth and mortality of crayfish. Error bars represent the standard error of the five replicates. Points were jittered by a random increment to decrease overlap between error bars and increase readability.
Figure 7a. The amount of gill chamber scar damage of native and invasive hosts with the effects of dactyl ablations. Individual species are listed as CA for *C. appalachiensis*, FC for *F. cristavarius*, and FV for *F. virilis*. Error bars represent the standard error between individual species and treatment.

Figure 7b. The amount of gill chamber scar damage of native and invasive hosts with the effects of dactyl ablations and branchiobdellidan worms. Individual species are listed as CA for *C. appalachiensis*, FC for *F. cristavarius*, and FV for *F. virilis*. Error bars represent the standard error between individual species and treatment.
Figure 8a. The amount of gill chamber filament damage of native and invasive hosts with the effects of dactyl ablations. Individual species are listed as CA for *C. appalachiensis*, FC for *F. cristavarius*, and FV for *F. virilis*. Error bars represent the standard error between individual species and treatment.

Figure 8b. The amount of gill chamber filament damage of native and invasive hosts with the effects of dactyl ablations, and branchiobdellidan worms. Individual species are listed as CA for *C. appalachiensis*, FC for *F. cristavarius*, and FV for *F. virilis*. Error bars represent the standard error between individual species and treatment.
Figure 9. A principal component analysis of the location of branchiobdellidan worms on crayfish 28 days after the start of study 2. The color represents the species of crayfish host, and the shape represents having or not having the treatment of dactyl ablations. The closer together the points are, the more similar worm locations would be on individual hosts.
Figure 10. A principal component analysis of the location of branchiobdellidan worms on crayfish 56 days after the start of study 2. The color represents the species of crayfish host, and the shape represents having or not having the treatment of dactyl ablations. The closer together the points are, the more similar worm locations would be on individual hosts.
Figure 11. A principal component analysis of the location of branchiobdellidan worms on crayfish 112 days after the start of study 2. The color represents the species of crayfish host, and the shape represents having or not having the treatment of dactyl ablations. The closer together the points are, the more similar worm locations would be on individual hosts.
Supplemental Figure 1. The average number of branchiobdellidan worms on *F. virlis*, *F. cristavarius*, and *C. appalachiensis* during a 89 and 112 day aquarium experiment.
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