

NATURAL HISTORY AND ECOLOGICAL OBSERVATIONS OF A POPULATION OF  
CONHAWAY CRAYFISHES AND THEIR SYMBIOTIC BRANCHIOBDELLIDAN ASSOCIATES

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## ABSTRACT

Crayfish throughout the holarctic are found in association with an order of worms known as branchiobdellidans. This relationship has been confirmed as a cleaning symbiosis in several species. The Conhaway crayfish, *Cambarus appalachiensis*, is a species of crayfish endemic to the New River Basin in Virginia and West Virginia. We studied a population of *C. appalachiensis* in Sinking Creek in Newport, VA from March 2017 until February 2018. We collected morphological data and quantified the branchiobdellidan communities on 986 individuals, and kept note of egg brooding and young of year throughout the study period. The life cycle of *C. appalachiensis* was found to be similar to other large-bodied species of *Cambarus* crayfish. Molting occurred throughout the year, peaking in the months of April and September. This molting served as a disturbance effect to the symbiotic branchiobdellidan community and reset community assembly. The worm communities on larger, recently molted crayfish more closely resembled the less diverse communities on smaller crayfish. Most worms on recently molted crayfish were ones that we know are early colonizers. This thesis work provides the first life history information on a newly described species of *Cambarus* crayfish and provides both seasonal data on its branchiobdellidan associates and one of the first empirical examples of host ontogeny acting as a disturbance on a symbiotic community.

# NATURAL HISTORY AND ECOLOGICAL OBSERVATIONS OF A POPULATION OF CONHAWAY CRAYFISHES AND THEIR SYMBIOTIC BRANCHIOBELLIDAN ASSOCIATES

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## GENERAL AUDIENCE ABSTRACT

Crayfish throughout North America and Eurasia are the symbiotic partners to a number of small worms. This relationship has been confirmed as a cleaning symbiosis for several crayfish, similar to the cleaning stations at a coral reef. The Conhaway crayfish is a species of crayfish found in the New River Basin in Virginia and West Virginia. We studied a population of Conhaway crayfish in Sinking Creek in Newport, VA from March 2017 until February 2018. We collected data on the physical attributes of the crayfish, quantified the worms present on 986 individuals, and kept note of female crayfish with eggs and baby crayfish throughout the study period. The life cycle of the Conhaway crayfish was found to be similar to other large-bodied species of closely related crayfish. Molting, where the crayfish loses its shell and grows a new one, occurred throughout the year, peaking in the months of April and September. This molting served as a disturbance effect to the symbiotic worms, similar to how a wildfire might displace animals in a grassland ecosystem. The type and number of worms found on larger, recently molted crayfish more closely resemble the type and number of worms found on smaller crayfish. This thesis work provides the first information on the physical and reproductive attributes of a newly described species of crayfish and provides both seasonal data on its symbiotic worms and one of the first empirical examples of host growth and aging acting as a disturbance to symbiotic organisms living on that host.

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## ON THE HISTORY OF CRAYFISH AND BRANCHIOBDELLIDAN RESEARCH IN THE MOUNTAIN LAKE REGION, WITH NOTES ON THE STUDY SYSTEM

The *Cambarus* crayfish of the Mountain Lake region of Southwest Virginia engage in a cleaning symbiosis with several species of branchiobdellidan worms (Annelida: Branchiobdellida) (Figure 1.1) (Hobbs Jr. et al. 1967). This cleaning symbiosis is similar to other well studied cleaning symbioses, such as coral reef cleaning stations, with an important difference. Because the branchiobdellidan worms attach to the crayfish and consume the gill tissue of the crayfish when deprived of other food, the cleaning symbiosis is context dependent: low to moderate worm loads provide a benefit to the crayfish, while high worm loads become parasitic (Brown et al. 2002).

This area of Virginia has long been an area of focus for research in this system. Horton H. Hobbs Jr. (Figure 1.2), a legendary crustacean taxonomist who is largely credited with establishing the basis for the taxonomy of North American crayfish, headed the Mountain Lake Biological Station from 1956 - 1960 before taking a position at the Smithsonian (Fitzpatrick 1995). At the time of his death in 1994, he was responsible for describing 42% of the known North American crayfish and creating the family *Cambaridae* in which the focus crayfish of this study and the other crayfish east of the North American Great Divide reside (Fitzpatrick 1995).

In the 1940's, Hobbs suggested that one of his students, Perry C. Holt (Figure 1.2), focus on branchiobdellidans (Gelder 2001). Over the course of his prolific career, which he spent as a professor at Virginia Tech, he described 8 genera, 75 species, elevated branchiobdellidans from a family of leeches to their own order, and was the first to stress that branchiobdellidans are not parasites (Gelder 2001). Holt, his mentor Hobbs, and their collaborator Margaret Walton, an ostracod researcher at the Mountain Lake Biological Station, compiled a survey and key to the

crayfish and branchiobdellidans of the Mountain Lake region that we still use today (Hobbs Jr. et al. 1967).

Since my advisor, Bryan Brown, came to Virginia Tech in 2011, our lab has built on the survey work of our scientific predecessors in the region and utilized the crayfish - branchiobdellidan cleaning symbiosis as a model system to study the community ecology of symbioses. Members of the lab have built on the survey work of Hobbs, Holt, and Walton (Skelton et al. 2016a, Bell 2018), studied how the ontogeny and behaviors of the host crayfish affects the branchiobdellidan community (Skelton et al. 2014, 2016b), and examined the effect of invasive crayfish on the local symbiont communities (Bell 2018).

Despite geographically extensive survey work, we had limited data on the seasonal dynamics of the branchiobdellidan communities in the area. Accordingly, for my thesis project, I elected to do a seasonal survey on the branchiobdellidan communities on a single population of crayfish. I selected Sinking Creek at the Newport Recreation Area in Newport, VA as a study site (Figure 1.3). This section of the creek has an incredibly high abundance of crayfish. There is a story, which may well be apocryphal, that there are so many crayfish in this stream reach that Horton Hobbs would regularly visit it to sample dinner. This bounty of crustaceans made it ideal for this type of study, as I was able to collect an adequate number of specimens in the winter, when crayfish are less active. Additionally, the resident crayfish was about to be split off from *Cambarus sciotensis* as a new species *Cambarus appalachiensis* (Loughman et al. 2017), which afforded the opportunity to collect life history data on the crayfish hosts as well as seasonal natural history data on the worms.

The following two chapters document the results of my thesis work. The first, a life history study of *C. appalachiensis* and natural history observations of the branchiobdellidan worms engaged



in a cleaning symbiosis with the Sinking Creek population, adds to the (entirely too small) collection of *Cambarus* life history studies, and provides seasonal dynamics of a branchiobdellidan community that is fairly representative of those found in the region (Hobbs Jr. et al. 1967, Bell 2018). The second, a study of how crayfish molting acts as a disturbance effect on the branchiobdellidan community of the crayfish, provides some of the first empirical evidence of how host ontogeny acts as a disturbance to a symbiotic community.

# LIFE HISTORY OBSERVATIONS OF A POPULATION OF CONHAWAY CRAYFISH (*CAMBARUS APPALACHIENSIS*) AND THEIR SYMBIOTIC BRANCHIOBDELLIDANS

## INTRODUCTION

Stream-dwelling *Cambarus* crayfish in the Mountain Lake region of Southwest Virginia engage in a cleaning symbiosis with several species of branchiobdellidan worms (Annelida: Branchiobdellida) (Hobbs Jr. et al. 1967). This cleaning symbiosis is context dependent: low to moderate worm loads provide a benefit to the crayfish, while high worm loads become parasitic (Brown et al. 2002). Crayfish and branchiobdellidans are distributed together throughout the holarctic (Gelder 1999), and it has been verified as a cleaning symbiosis in several species of crayfish (Lee et al. 2009, Thomas et al. 2013, Ames et al. 2015). The recently described Conhaway crayfish, *Cambarus appalachiensis* (Loughman et al. 2017), is a particularly tolerant host for these worms, having been found in association with ten different branchiobdellidan species in the Mountain Lake region.

Freshwater crayfish are keystone species in stream communities (Creed 1994), and are among the most threatened freshwater organisms (Taylor et al. 2007, Richman et al. 2015). Natural history knowledge is an important building block for future assessments of conservation priorities. Despite the importance of this knowledge, an assessment of life history studies for crayfish found that only 12% of crayfish species in the United States and Canada had published life history data, with the biggest data deficiency being in the high diversity southeastern United States (Moore et al. 2013).

The Conhaway crayfish, *Cambarus appalachiensis* (Loughman et al. 2017), is a species of crayfish occurring in the New River basin in Virginia and West Virginia. It was recently distinguished from *Cambarus sciotensis* (Rhoades 1944) on the basis of both morphological

differences and biogeographical isolation of the New River populations (Loughman et al. 2017). The geographic range of the population is well studied (Hobbs Jr. et al. 1967, Loughman et al. 2009, Bell 2018). In the Mountain Lake region of Southwest Virginia, a historical survey (Hobbs Jr. et al. 1967) found that it co-occurs only with the native crayfish *Cambarus bartonii* (Fabricius 1798), however, a more recent survey (Bell 2018) found that the invasive crayfish *Faxonius cristavarius* (Taylor 2000) has started to move into its range. A previous study of the Conhaway crayfish showed that it has a three-year lifecycle (Roell and Orth 1992).

The taxonomy and distribution of branchiobdellidans in North America is well-studied (Gelder 1996, 1999, Gelder et al. 2002, Govedich et al. 2010), as is their occurrence and association with host crayfish species in the Mountain Lake region (Hobbs Jr. et al. 1967, Skelton et al. 2016a, Bell 2018). There is also a rich library of literature exploring the ecology of the symbiosis between crayfish and branchiobdellidans (Penn 1959, McManus 1960, Young 1966, Grabda and Wierzbicka 1969, Koepp 1975, Brown et al. 2002, Brown and Creed 2004, Lee et al. 2009, Brown et al. 2012, Thomas et al. 2013, Skelton et al. 2014, Ames et al. 2015, Skelton et al. 2016b, Thomas et al. 2016, Bell 2018). However, there are few studies looking at the coupled natural history of crayfish and branchiobdellidans through time (Skelton et al. 2016a).

This Mountain Lake region of Southwest Virginia has long been an area of focus for research in this system. Horton Hobbs, a crustacean taxonomist, and Perry Holt, who described many species of branchiobdellidan, performed surveys of the crayfish and branchiobdellidans in this area in the 1960's with the help of their collaborators (Hobbs Jr. et al. 1967), providing a basis for understanding the natural history of these crayfish and branchiobdellidans. To better understand the natural history of the Conhaway crayfish and the long-term dynamics of their symbiotic branchiobdellidan communities, we sampled a population of *C. appalachiensis*

bimonthly in Sinking Creek at the Newport Recreation Area in Newport, VA from March 2017 until February 2018.

## METHODS

We sampled a site located in Sinking Creek at the Newport Recreational Area in Newport, VA (37.3031420 N, 80.4857037 W). Sinking Creek is a tributary of the New River, and this particular reach consists of a series of riffles, a large pool, a split series of riffles around a small island, and a further series of riffles downstream. The area has a large population of *C. appalachiensis* that are abundant in all parts of the stream reach throughout the year, making it ideal for this type of study. This particular reach of the stream also contains *C. bartonii* in small numbers.

We collected 986 *C. appalachiensis* in Sinking Creek from March 2017 to February 2018 (not including young-of-year, egg-brooding females, or females with attached young-of-year).

Crayfish were sampled from across the stream reach using dip nets after disturbing the substrate and dislodging larger rocks. Our monthly sample sizes ranged from 53 to 104 crayfish. Young-of-year, egg-brooding females, and females with attached young-of-year were noted at the stream but not returned to the lab for sampling. We measured physical attributes of the crayfish, including the carapace length (the distance from the rostrum to the separation between the cephalothorax and the abdomen of the crayfish), the chelae length, the palm width (the width of the chelae at the widest point), the palm length, (length of the chelae up to the joint), the mass, the sex, and an observational measurement of the hardness of the exoskeleton, referred to as exoskeleton hardening stage, or EHS, (Castillo-Escrivà et al. 2013), as a proxy for time since the crayfish last molted. We counted branchiobdellidan worms on living crayfish using a dissecting microscope and identified them according to the key in Hobbs Jr. et al.

(1967). After the crayfish were processed, both crayfish and branchiobdellidans were returned to the stream.

## RESULTS

The 986 *C. appalachiensis* in Sinking Creek from March 2017 to February 2018 (not including young-of-year) had carapace lengths ranging from 11.0 mm to 53.1 mm. Measures of mass, chelae length, palm width, and palm length were all closely tied to carapace length (Figure 2.1). There was a lack of distinct yearly cohort structure based on the frequency diagrams for the size classes of the crayfish (Figure 2.2). We also collected 188 free living young-of-year, 2 egg brooding females, and 7 females carrying their young-of-year. Free living young-of-year were found throughout the year, with the exception of August 2017 and September 2017; egg brooding females were found in July 2017, and females carrying their young-of-year were found in August 2017, October 2017, January 2018, and February 2018 (Figure 2.3).

The sex ratio of our sampled crayfish was slightly biased towards females (518 females, 468 males), though the sex ratio skewed towards males during the summer months (Figure 2.4). The sex ratio of our crayfish was not significantly different from 1:1 for our whole dataset ( $\chi^2 = 2.5355$ ,  $p = 0.1113$ ). However, the sex ratio of our crayfish was significantly skewed towards females during March 2017 ( $\chi^2 = 4.3478$ ,  $p = 0.03706$ ) and February 2018 ( $\chi^2 = 8.6538$ ,  $p = 0.003264$ ). Form I (reproductive) males accounted for 15.8% of males sampled (74 out of 468), with higher proportions of form I males found during from August 2017 to December 2017 (Figure 2.4). Molting occurred throughout the year, with pronounced peaks in April 2017 and September 2017 (Figure 2.5).

There were four species of worms present on the exoskeleton of the crayfish in this population, *Cambarincola ingens* (Hoffman 1963), *Cambarincola fallax* (Hoffman 1963), *Ankyrodrilus*

*koronaeus* (Holt 1965), and *Pterodrilus alcicornus* (Moore 1894). It should be noted that two species of worms that dwell exclusively in the branchial chamber, *Bdellodrilus illuminatus* (Moore 1893) and *Cambarincola branchiophilus* (Holt 1954), were found at the site, but not included in this study. We made the decision to count the branchiobdellidans on live crayfish in order to study how branchiobdellidans living on the exoskeleton of a crayfish partition their host, and that precluded any sort of quantitatively-accurate sampling of gill-dwelling branchiobdellidans. Both branchiobdellidan worm abundances and the abundances of their cocoons showed seasonal fluctuations. Branchiobdellidan worm abundances were highest in the summer months, an increase that correlated with an increase in the average carapace length of sampled crayfish (Figure 2.6). Branchiobdellidan worm cocoons were most abundant during the summer of 2017 and February 2018, and experienced steep declines during months when a large proportion of the crayfish were molting (Figure 2.6). *C. fallax* and *P. alcicornus* were the most abundant worms in the community, with *C. ingens* and *A. koronaeus* occurring at lower abundances (Figure 2.7).

Of the four worms, *A. koronaeus* was the only one that appeared to inhabit a single specific area of the crayfish, the chelae, exclusively, with the other three worms acting more as spatial generalists (Figure 2.8). 97.3% of *A. koronaeus* sampled were found on the chelae. *C. fallax* was found on all parts of the crayfish, with a plurality, 47.9%, occurring on the mouthparts and antennae. *P. alcicornus* also occurred on all parts of the crayfish, with a plurality, 31.5%, found on the ventral surface of the abdomen and tail fan. *C. ingens* was found on all parts of the crayfish, but seemed to prefer the ventral abdomen and tail fan, where 37.8% were found.

## DISCUSSION

The largest Conhaway crayfish found in our study had a carapace length measuring 53.1 mm, comparable to specimens found of *Cambarus elkensis* (Jones and Eversole 2011), *Cambarus*

*robustus* (Hamr and Berrill 1985), and *Cambarus tenebrosus* (Prins 1968). Much like the life history studies carried out for these crayfish, we found a great deal of overlap among the size classes of the crayfish we sampled. We believe that this lack of distinct cohorts is due to the fact that our crayfish are reproductively active throughout the year. We found young of the year, either free living or on females, in every month except for September. This reproductive behavior is in contrast to the smaller, more quickly reproducing species of *Cambarus* crayfish, such as *Cambarus bartonii*, *Cambarus halli*, *Cambarus hubbsi*, *Cambarus longulus*, and *Cambarus maculatus*, which all mature faster (and to smaller sizes), and display more seasonal reproduction and more distinct size cohorts (Smart Jr. 1962, Hamr and Berrill 1985, Dennard et al. 2009, Larson and Magoulick 2011, Rosenberger et al. 2016).

Our results showed a slightly female biased, but mostly even sex ratio. This finding is consistent with findings for several other species of *Cambarus* crayfish, including a comparative study involving *Cambarus tenebrosus* (Prins 1968), a life history study of *Cambarus elkensis* that found even sex ratios most months with a heavy female bias in August driving an overall bias towards females (Jones and Eversole 2011), a life history study of *Cambarus hubbsi* that found some variation but nothing statistically significant (Larson and Magoulick 2011), and other life history studies of *Cambarus halli* (Dennard et al. 2009), *Cambarus longulus* (Smart Jr. 1962), and *Cambarus robustus* (Corey 1990). Some studies of *Cambarus* crayfish found much more variable sex ratios, including a life history study of *Cambarus maculatus* (Rosenberger et al. 2016), which found much more variable sex ratios that corresponded with water temperature.

Form I males accounted for 15.8% of males sampled, with higher proportions of form I males found from the late summer to early winter (Figure 3b). This trend towards fewer form I males in the spring and early summer was also found in the majority of *Cambarus* life history studies that we reviewed (Hamr and Berrill 1985, Flinders and Magoulick 2005, Jones and Eversole 2011,

Rosenberger et al. 2016). However, some *Cambarus* crayfish displayed different patterns, with form I males of *Cambarus halli* (Dennard et al. 2009), *Cambarus robustus* (Corey 1990), and *Cambarus tenebrosus* (Prins 1968) occurring more frequently in the spring or early summer. Interestingly, while male *Cambarus longulus* appeared to molt into form I around late summer like the males in our study population of *Cambarus appalachiensis*, they stayed in form I until well into the next spring (Smart Jr. 1962).

The population of *Cambarus appalachiensis* we studied molted throughout the year; however, there were two periods of higher molting, one in April 2017 and one in September 2017. This pattern of two large yearly molts in the spring and fall was also seen in *Cambarus longulus* (Smart Jr. 1962), *Cambarus elkensis* (Jones and Eversole 2011), and *Cambarus robustus* (Corey 1990). Several other species of *Cambarus* molted only in the fall (Prins 1968, Hamr and Berrill 1985, Rosenberger et al. 2016). Several authors postulated that molting for *Cambarus* crayfish is triggered by water temperature (Hamr and Berrill 1985, Rosenberger et al. 2016). This environmentally-triggered molting is in contrast to other genera of crayfish, such as *Orconectes* and *Faxonius*, that molt based on reproduction-related biological cues (Hamr and Berrill 1985).

We found that branchiobdellidan abundances were higher in the summer, while still correlating to the average size of crayfish sampled that month. This seasonal variation followed a general pattern previously recorded on host crayfish in the Mountain Lake region of Virginia (Skelton et al. 2016a), as well as findings that larger crayfish host more abundant and diverse branchiobdellidan worm communities (Skelton et al. 2016b). Based on the number of cocoons, most branchiobdellidan reproduction seems to occur from February until July, and seems to be negatively impacted by crayfish molting. *C. fallax* and *P. alcicornus* were the most abundant worms in the community, while *C. ingens* and *A. koronaeus* occurred at lower abundances,



though all worms reached their maximum abundances in late spring and early summer. This pattern follows the uptick in cocoons found in late winter and early spring. Branchiobdellidan location patterns appeared to conform to observations from previous studies (Hobbs Jr. et al. 1967, Brown et al. 2002), with *A. koronaeus* found almost entirely on the chelae of the crayfish, and the other three worms, while found on all locations on the crayfish, chiefly preferred the ventral side of the main body of the crayfish, where they have easy access to the branchial chamber.

While our study contributes life history data for one more species of *Cambarus* crayfish to the collective body of scientific knowledge, there is still much to be done to understand the natural history and ecology of this genus, with life history studies completed for only a small fraction of the species (Moore et al. 2013). Additionally, these crayfish play host to a great many “remarkable” (Moore 1894) symbiotic species (Hobbs Jr. et al. 1967). Symbionts are especially susceptible to extinction during a time when we are losing biodiversity at an accelerating rate (Colwell et al. 2012, Brodie et al. 2014). A better understanding of the natural history of hosts and their symbionts will be invaluable to saving as many species as we are able.

## A STUDY OF DISTURBANCE EFFECTS IN SYMBIOSES USING CRAYFISH MOLTING

### INTRODUCTION

Symbiosis is a ubiquitous life history strategy, with most, if not all, free-living organisms hosting a broad range of mutualists, commensals, parasites, and pathogens (Dimijian 2000). There has recently been a push to incorporate community ecology principles into the study of symbioses in order to take advantage of the robust frameworks developed by community ecologists to understand biodiversity and species distributions across patches, as there is a logical connection between an environmental patch for macroorganisms and a host patch for symbionts (Costello et al. 2012, Mihaljevic 2012, Christian et al. 2015, Johnson et al. 2015). Viewing symbiosis through the lens of community ecology is not a new idea, as previous models for “multi-species metapopulations”, which can be viewed a precursor to metacommunities, have been applied to symbiosis (May and Nowak 1994). More recent community ecology models do not explicitly account for dynamic patches, i.e., patches that move, appear, and disappear (Mihaljevic 2012), a necessary feature for a model to understand symbiotic systems in which hosts are born, disperse between patches themselves, and die. However, more recent theoretical models have sought to account for this ephemerality of patches when bridging the gap between paradigms of community ecology and symbiosis (Seabloom et al. 2015).

An understanding of community assembly and variation is key to the study of the maintenance and variability of biodiversity. The paradigms for understanding community assembly have evolved over the years, from classic niche partitioning (Grinnell 1917, MacArthur 1958) to neutral assembly (Hubbell 2001). Recently, the metacommunity concept has synthesized past ideas to provide a framework for community assembly that can incorporate both local factors, like species interactions and environmental filtering, and dispersal driven processes (Leibold et al. 2004). A metacommunity can best be described as a community of communities, groups of

interacting organisms that can be understood by looking at the inference space at the intersection of organismal equivalence, habitat heterogeneity, and dispersal (Logue et al. 2011).

Traditionally, community ecology has viewed ontogenetic change as a driver of niche partitioning, and of the assembly and structure of communities (De Roos et al. 2008, Miller and Rudolf 2011, Nakazawa 2011). This concept has been extended to symbioses in both theoretical (Karlson and Hurd 1993, Ke and Nakazawa 2018) and empirical frameworks (Soliveres et al. 2010, Skelton et al. 2014, 2016b). Similar to ontogenetic change, there is a long history of viewing disturbance as a major driver in community assembly, both in theoretical and empirical work (Connell 1978, Poff and Allan 1995, Tilman 1999, Chase 2007). Previous research has shown that habitat age has a profound influence on community assembly, on timescales ranging from geological (Rominger et al. 2016) to generational (Sferra et al. 2017). However, the concept of ontogenetic change as a disturbance effect that shapes assembly of symbiotic communities is only now starting to be explored (Costello et al. 2012, Skelton et al. 2014).

We used a freshwater cleaning symbiosis system to explore how ontogenetic changes in hosts can affect symbiont community assembly. Crayfish and their ectosymbiotic annelid worms (Order: Branchiobdellida) engage in a density dependent cleaning symbiosis. These interactions can range from mutualistic to parasitic, with the worms cleaning their hosts at low densities, but damaging their gill tissue at high densities (Brown et al. 2002, 2012). Previous experiments have already shown that host ontogeny affects the worm community on crayfish, as younger crayfish actively groom larger worms off of their carapace, preventing their worm load from becoming parasitic (Skelton et al. 2016b). As the crayfish ages, this grooming behavior decreases dramatically, almost to the point of cessation by the time the animal reaches reproductive age (Skelton et al. 2016b). Molting is a separate kind of host ontogenetic

disturbance. A crayfish needs at least eleven molts to reach reproductive maturity, and thus molts quite frequently, once every several weeks, when the host crayfish is young, decreasing in frequency with the age of the crayfish to once or twice a year (Reynolds 2002). Molting frequency can also be affected by environmental factors or biotic factors (usually reproductive signals) (Hamr and Berrill 1985). This frequent host ontogenetic disturbance provided us with an opportunity to empirically test how these types of host disturbances can affect community assembly. Accordingly, we surveyed the crayfish and worm communities at Sinking Creek in Newport, VA from March 2017 until February 2018 to observe how the worm communities develop on each crayfish after molting.

## METHODS

To get a better understanding of the long-term dynamics of the branchiobdellidan communities on a population of crayfish, we sampled the crayfish *Cambarus appalachiensis* (Loughman et al. 2017) bimonthly in Sinking Creek at the Newport Recreation area in Newport, VA from March 2017 until February 2018. We sampled crayfish from across the stream reach using dip nets, in total netting 986 crayfish during our study. Our monthly sample sizes ranged from 53 to 104 crayfish.

We measured the physical attributes of the crayfish, including the carapace length (the distance from the rostrum to the separation between the cephalothorax and the abdomen of the crayfish), and a gestalt measurement of the carapace hardness. This measurement of carapace hardness (exoskeleton hardening stage, or EHS) was used as a proxy for time since last molt, with a carapace graded as EHS 0/1 (soft exoskeleton), EHS 2 (hard exoskeleton, but with some elasticity), or EHS 3 (very hard exoskeleton) (Castillo-Escrivà et al. 2013). A score of EHS 0/1 indicated that the crayfish molted very recently, a score of EHS 2 indicated the crayfish molted somewhat recently, and a score of EHS 3 indicated that it had been some time since the

crayfish molted. Deriving an exact time since molt is impossible when sampling crayfish in the field, as the re-hardening of the carapace is affected by both the available diet and environmental factors (Aiken and Waddy 1992), but as there is some consistency in those among crayfish in a population, carapace hardness is a serviceable proxy for time since molt for our purposes. For certain analyses, crayfish were divided into three size classes (10mm-20mm, 20mm-30mm, 30mm+) based on grooming behaviors for ranges of carapace lengths (Skelton et al. 2014).

We counted worms on living crayfish using a dissecting microscope. This method had the advantage of allowing us to see the worm location on the host, and how that changed with the makeup of the worm community. We identified 13466 worms on the exoskeletons of the sampled crayfish according to a key developed for the crayfish and symbionts in the Mountain Lake region of Virginia (Hobbs Jr. et al. 1967).

All data were analyzed using R 3.4.4 (R Core Team 2018) and the *vegan* package (Oksanen et al. 2018). We looked at community dynamics of worm communities crayfish of different sizes and with different hardening stages by performing an ordination using non-metric multidimensional scaling (function *metaMDS* in the *vegan* package) calculated community dissimilarity (function *vegdist* in the *vegan* package) with the Gower dissimilarity index (Gower 1971). We chose the Gower dissimilarity index because it accounts for both occurrence and abundance of the species in the community. To validate these results statistically, we performed a PERMANOVA (function *adonis* in the *vegan* package) with the Gower community dissimilarity as a response and the carapace length and carapace hardness as predictors. Additionally, we performed a multivariate test of group dispersion with the Gower community dissimilarity as a response and the carapace length and carapace hardness as predictors (function *betadisper* in the *vegan* package).

We also measured total richness of the worm community as a function of carapace length and carapace hardness. We fit a general linear model (R core functions *lm*, *anova*) to the data with richness as a response and the carapace length and carapace hardness as predictors. Finally, we compared the slopes of these lines using estimated marginal means (function *emtrends* in the *emmeans* package), and looked at the significance of the pairwise comparisons of the slopes (function *CLD* in the *emmeans* package) (Lenth et al. 2019).

## RESULTS

The branchiobdellidan worm community present on the exoskeleton of the crayfish in this population consisted of four species: *Cambarincola ingens* (Hoffman 1963), *Cambarincola fallax* (Hoffman 1963), *Ankyrodrilus koronaeus* (Holt 1965), and *Pterodrilus alvicornus* (Moore 1894). Tracking branchiobdellidan worm abundance through time showed that the total abundance of branchiobdellidans reached its nadir during months when a high proportion of the crayfish were molting (Figure 3.1). There were also differences in community composition. Analysis of the worm community using non-metric multidimensional scaling showed that there are indeed differences in the worm community based on time since molt, with starker differences between the communities on larger crayfish than smaller ones (Figure 3.2). Furthermore, the communities on larger, recently molted crayfish are more similar to communities on smaller crayfish, both consisting largely of the two smaller, early colonizing worms, *C. fallax* and *P. alvicornus* (Figure 3.3). Results from the PERMANOVA showed that carapace length ( $p = 0.001$ ), time since molt ( $p = 0.001$ ), and the interaction between carapace length and time since molt ( $p = 0.031$ ) are all significant predictors for the differences in community composition. We also tested the community dispersion using carapace length and carapace hardness as predictors. Mean dispersion, and thus the mean  $\beta$  diversity of the community (Anderson et al.

2006), increases with time since molt for each range of carapace lengths ( $p < 0.001$ ) (Figure 3.4).

The relationship between richness of the worm community and carapace length of the host crayfish showed that there is a lower richness of worms on crayfish that have molted very recently (Figure 3.5). Richness was significantly related (adjusted  $R^2 = 0.5027$ ,  $p < 0.001$ ) to carapace length ( $p < 0.001$ ), carapace hardness ( $p < 0.001$ ), and the interaction between carapace length and carapace hardness ( $p < 0.001$ ). Estimated marginal means of the slopes of the modeled lines in Figure 5 (95% confidence level used) showed that the slope of the line for crayfish that had molted somewhat recently was significantly different from the slope of the lines for crayfish that had molted very recently and not recently.

## DISCUSSION

Our results demonstrated that molting acts as a disturbance that resets the process of symbiont community assembly on crayfish. While the population of *C. appalachiensis* at our field site at Sinking Creek in Newport, VA molts throughout the year, there are concentrated molting events during the spring (with the most being in April) and fall (with the most being in September). During these periods, the abundance of branchiobdellidan worms is at its lowest. This result matches the observations of early researchers working in this area, who estimated that a crayfish loses up to 75% of its worms when it molts (Hobbs Jr. et al. 1967), as well as previous experimental evidence, which showed deleterious effects on the branchiobdellidan worm populations on crayfish that were induced to molt (Koepp 1975).

Our community level analysis showed crayfish that have recently molted have a branchiobdellidan worm community more similar to crayfish of a smaller size. This dynamic is likely driven by differences in colonization ability among the branchiobdellidan worms (Figure 3.6). Previous research has found that the branchiobdellidan worm communities on smaller

crayfish are characterized by smaller worms that maintain their populations through competition-colonization trade-offs with larger, more predatory worms (Skelton et al. 2016b). The smaller worms (*C. fallax* and *P. alcicornus*) maintain their populations through a combination of their ability to avoid the grooming of their host crayfish and propensity to colonize other crayfish more readily. On the other hand, the larger worms maintain their populations through exploitation of underused “niches” on the crayfish (in the case of the chelae specialist *A. koronaeus*) and competition through intraguild predation (in the case of *C. ingens*). Our results indicate that these worms that characterize communities on smaller crayfish (*C. fallax* and *P. alcicornus*) also characterize the communities on larger crayfish that have molted recently, indicating that their more active dispersal is allowing them to take advantage of these freshly molted host patches. Then, as the carapace hardens, or to put it in the parlance of community assembly, as the patch ages, the community is characterized by larger worms that have a lower colonization ability but are better competitors (*A. koronaeus* and *C. ingens*). Our dispersion analysis showed the same trend. The mean dispersion, and therefore the  $\beta$  diversity, increased with both carapace length and recency of molt. The branchiobdellidan communities on crayfish that had molted very recently had a more similar mean dispersion, and therefore a more similar  $\beta$  diversity, to the branchiobdellidan communities on smaller crayfish. This, combined with the finding that branchiobdellidan communities on crayfish that had molted very recently are more similar to those on smaller crayfish, means that community assembly on larger crayfish that have not molted recently, which is driven more by competition between species of branchiobdellidans than by colonization, results in communities that are both different in composition and more varied between individual hosts than those on crayfish where the assembly of the symbiotic community is driven by colonization. Therefore, as the carapace of the crayfish hardens, the patch ages, and there is the potential for the crayfish to host a higher diversity of different worm assemblages. This relationship is exacerbated by crayfish size, as larger crayfish have more potential species of branchiobdellidan symbionts to draw from, and therefore a higher potential



richness (or  $\alpha$  diversity). So, to use the language of community assembly, since  $\gamma$  diversity is higher on larger host crayfish, potential  $\alpha$  and  $\beta$  diversity are also higher.

The richness of the community of branchiobdellidan worms was significantly lower on crayfish that have molted very recently than on crayfish that have molted somewhat recently or crayfish that have not molted recently. This reduction in richness supports the idea that the branchiobdellidan worm communities on recently molted crayfish are composed of the same few early colonizing species as the branchiobdellidan worm communities on smaller crayfish. There was also a difference in the slope of the relationship between carapace length and branchiobdellidan worm species richness for crayfish that have molted somewhat recently and the slope of the relationship between carapace length and branchiobdellidan worm species richness for crayfish that have molted very recently or not recently at all. We suspect this is due to the predatory behavior of the largest branchiobdellidan worm in our community, *C. ingens* (Skelton et al. 2016b). Given that it can outcompete (mostly by depredating) other worms, we infer that the average higher richness of very large crayfish that have molted somewhat recently can be attributed to the fact that these crayfish, having molted somewhat recently, host worm communities where all four species are present, but *C. ingens* has not yet had the chance to extirpate one or more of the other species of worm through intraguild predation. Conversely, on very large crayfish that have not molted recently, there is a potential for branchiobdellidan richness to be lower, as *C. ingens* may have had the opportunity to extirpate one of the other worms through intraguild predation. This result would suggest that, at least when the potential symbiont community is diverse enough, like on large crayfish, symbiotic communities are subject to the same conditions that have provided support for the Intermediate Disturbance Hypothesis in communities of organisms on a landscape (Connell 1978).

Disturbance effects have long been recognized as a major driver of community assembly across a landscape (Connell 1978, Poff and Allan 1995, Tilman 1999, Chase 2007). There has been a recent flurry of conceptual work striving to consolidate the theoretical underpinnings of community and symbiosis ecology (Costello et al. 2012, Mihaljevic 2012, Christian et al. 2015, Johnson et al. 2015), however, empiricists have yet to take full advantage of these theoretical consolidations. Our study provides what are among the first empirical results showing strong evidence that an ontogenetic change like molting frequency can act as a disturbance effect on a symbiotic community, and are in line with what previous studies have found when applying disturbance-centric community assembly principles to symbiotic systems (Costello et al. 2012, Skelton et al. 2014). Symbiosis is ubiquitous (Dimijian 2000), and still poorly understood. It is clear however, that due to their tightly entwined life histories with their hosts, they are particularly susceptible to reductions in biodiversity like the one currently occurring on a global scale (Colwell et al. 2012, Brodie et al. 2014). Understanding the mechanisms that drive community assembly in symbiotic communities will help us to better understand how symbiont biodiversity is maintained.

FIGURES



FIGURE 1.1

A crayfish (*Cambarus appalachiensis*) pictured with one of its branchiobdellidan associates (*Cambarincola ingens*). Photo by Bryan Brown.



FIGURE 1.2

Horton Hobbs Jr. (photo from the Smithsonian Institution archives, top) and Perry Holt (photo credit unknown, possibly his daughter, bottom), pioneers of crayfish and branchiobdellidan research in the Mountain Lake region.



FIGURE 1.3

Sinking Creek at the Newport Recreation Area in Newport, VA. There is a story, which may well be apocryphal, that there are so many crayfish in this stream reach that Horton Hobbs would regularly visit it to sample dinner.

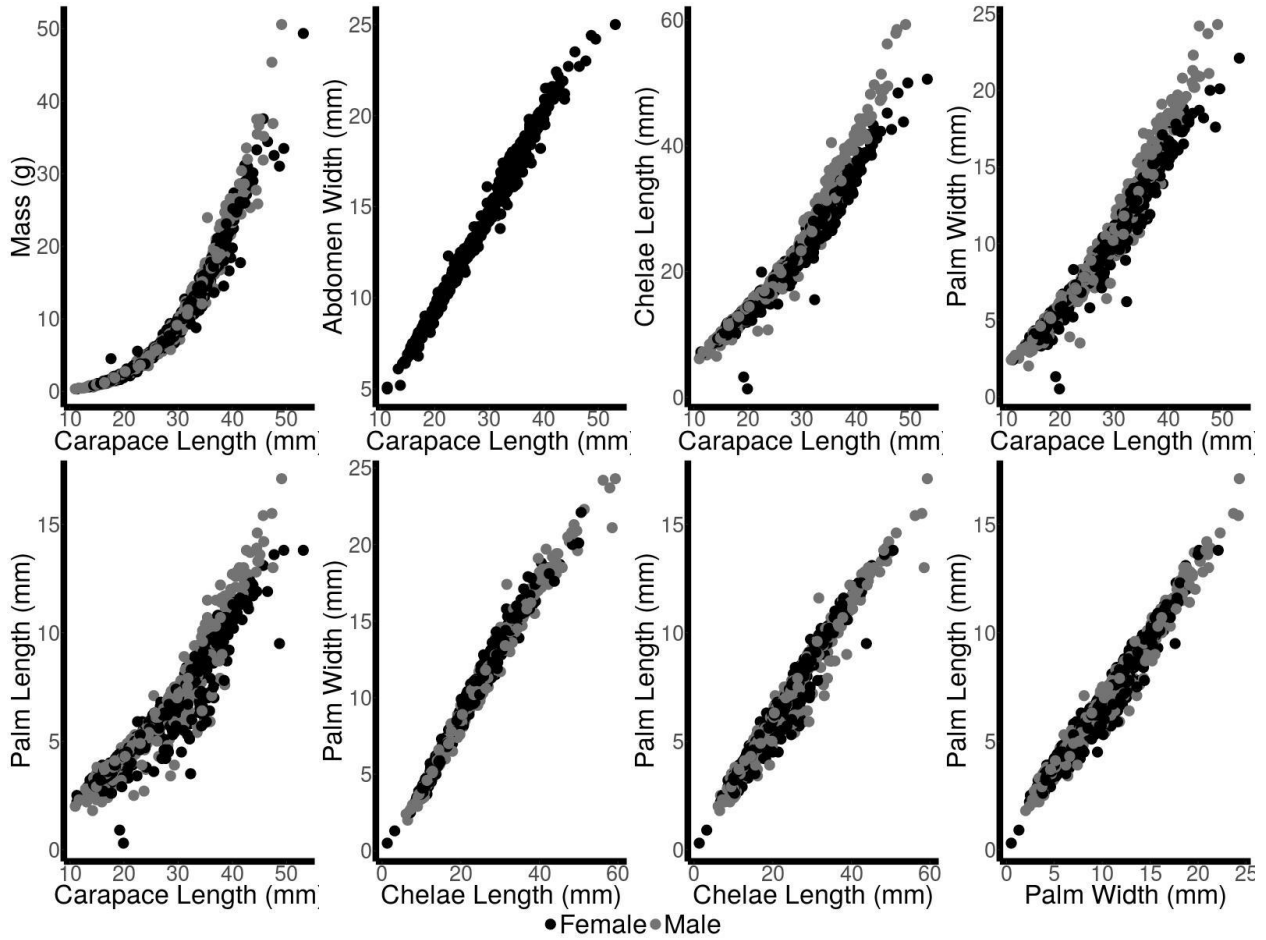


FIGURE 2.1

Relationships between morphological features of *Cambarus appalachiensis*. All relationships appear tightly correlated, though males (grey) typically have larger chelae relative to carapace length than females (black).



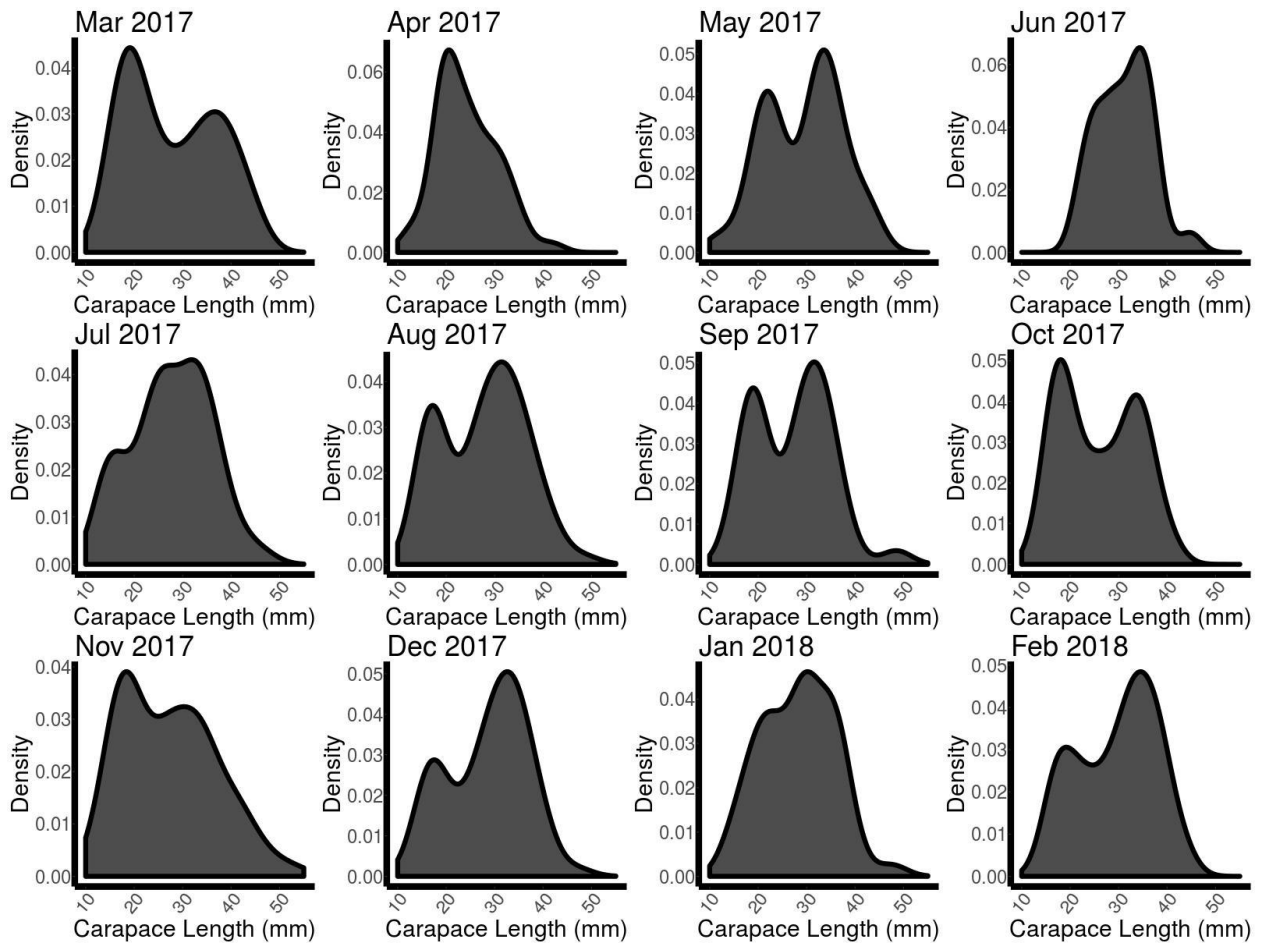


FIGURE 2.2

Frequency distributions for the carapace lengths of *Cambarus appalachiensis* sampled at Sinking Creek. These results do not show any clear age cohorts, and suggest that the crayfish probably reproduces throughout the year.

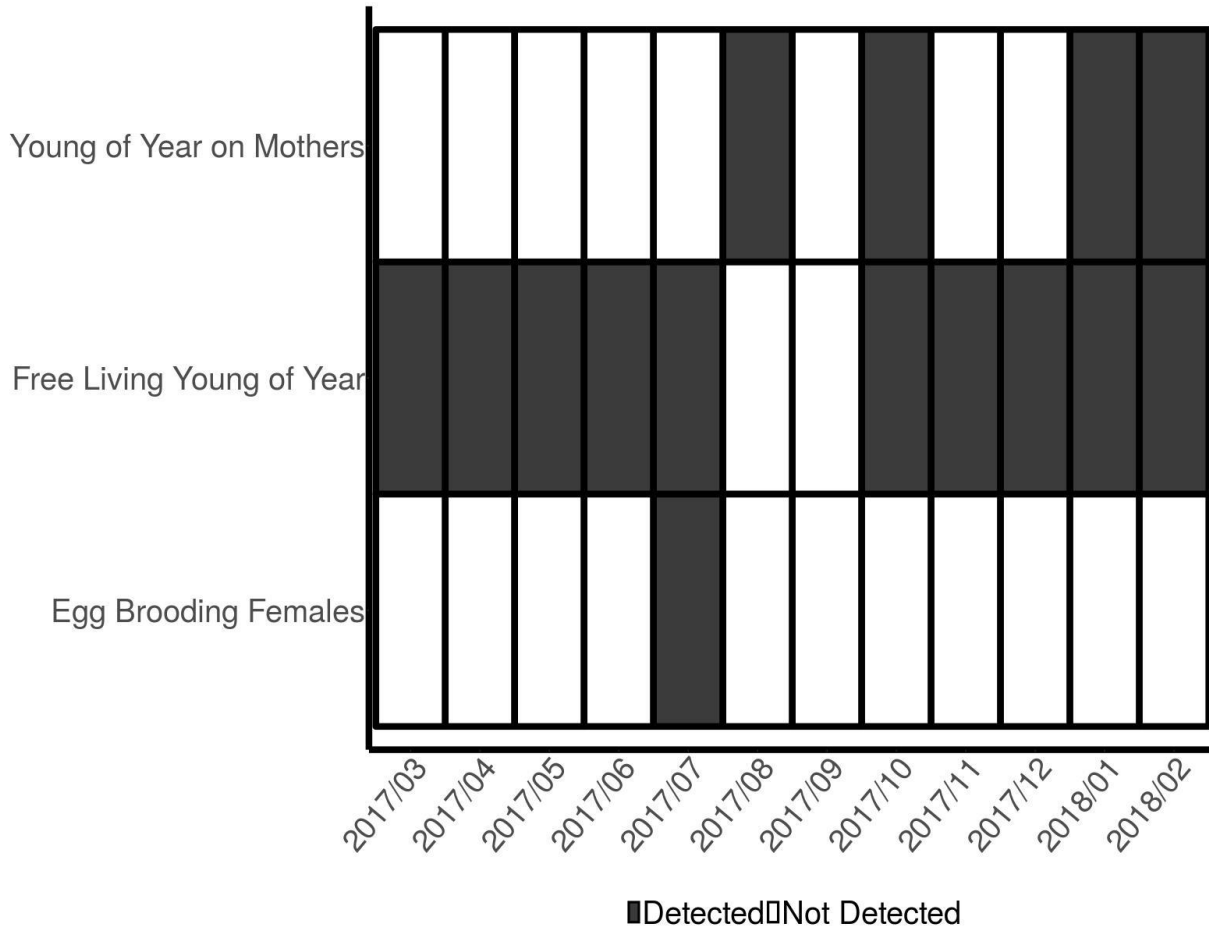


FIGURE 2.3

Record of free living young-of-year, egg-brooding females, and young-of-year on female crayfish. Young-of-year were collected, either free-living or on a female crayfish, every month except for September, which would suggest that *Cambarus appalachiensis* reproduces throughout the year.



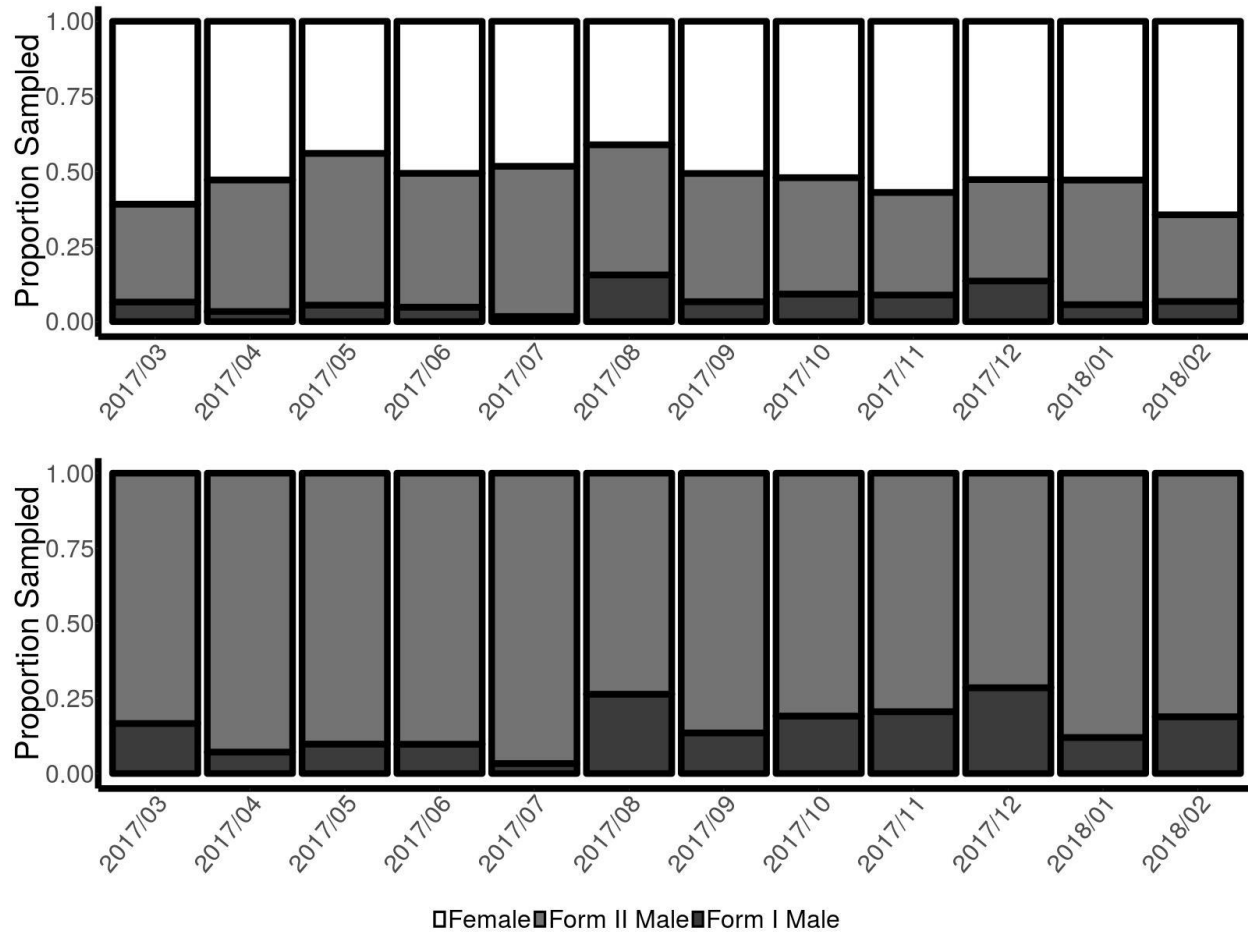


FIGURE 2.4

The sex ratios of sampled *Cambarus appalachiensis* throughout the year (top), and the proportion of males that were form I (reproductive) and form II (non-reproductive) (bottom). Sex ratios did not statistically favor either sex over the entire sampling period, though there were statistically more females in February and March. More form I males were found in the late summer through winter.

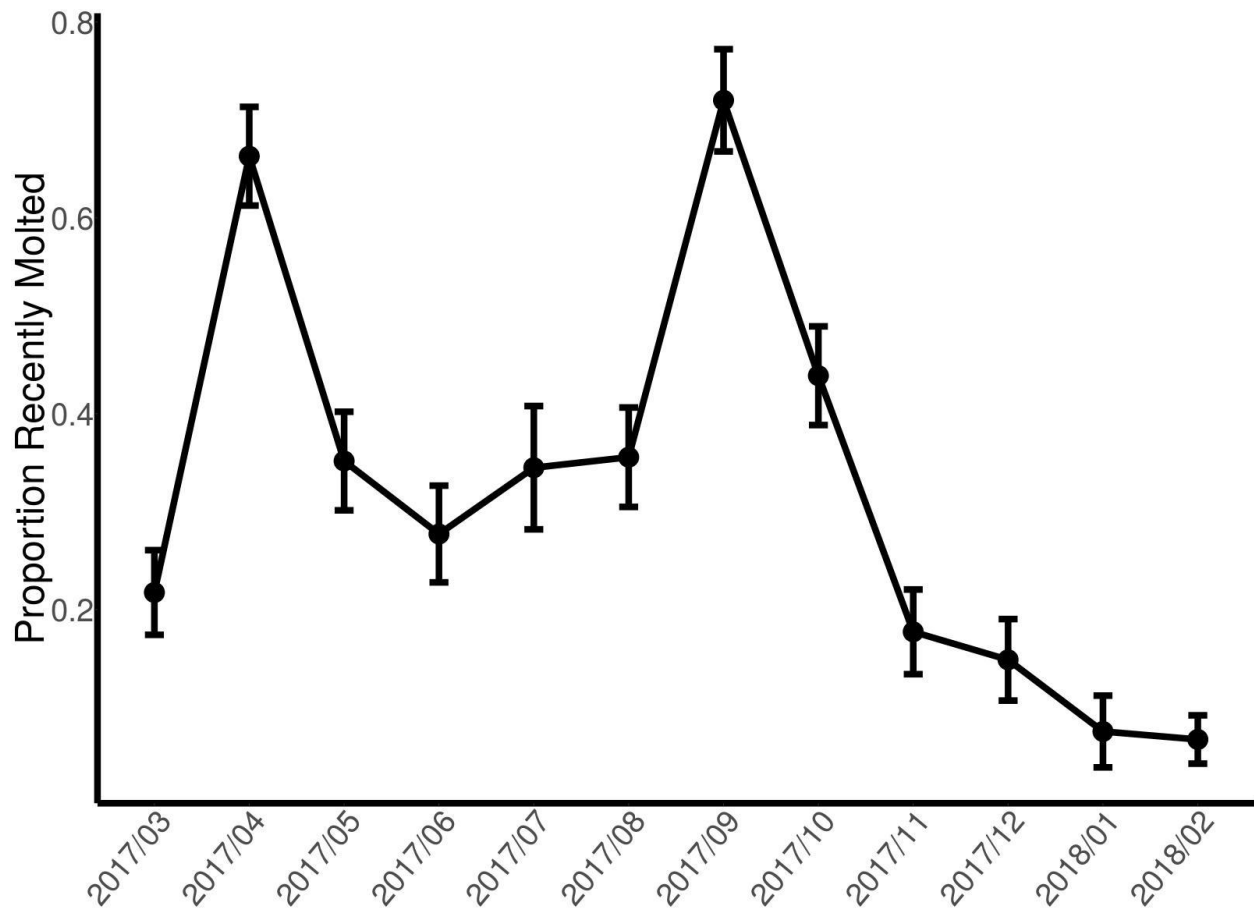


FIGURE 2.5

Molting frequency of *Cambarus appalachiensis* during our study. Peak molting times were in April and September.

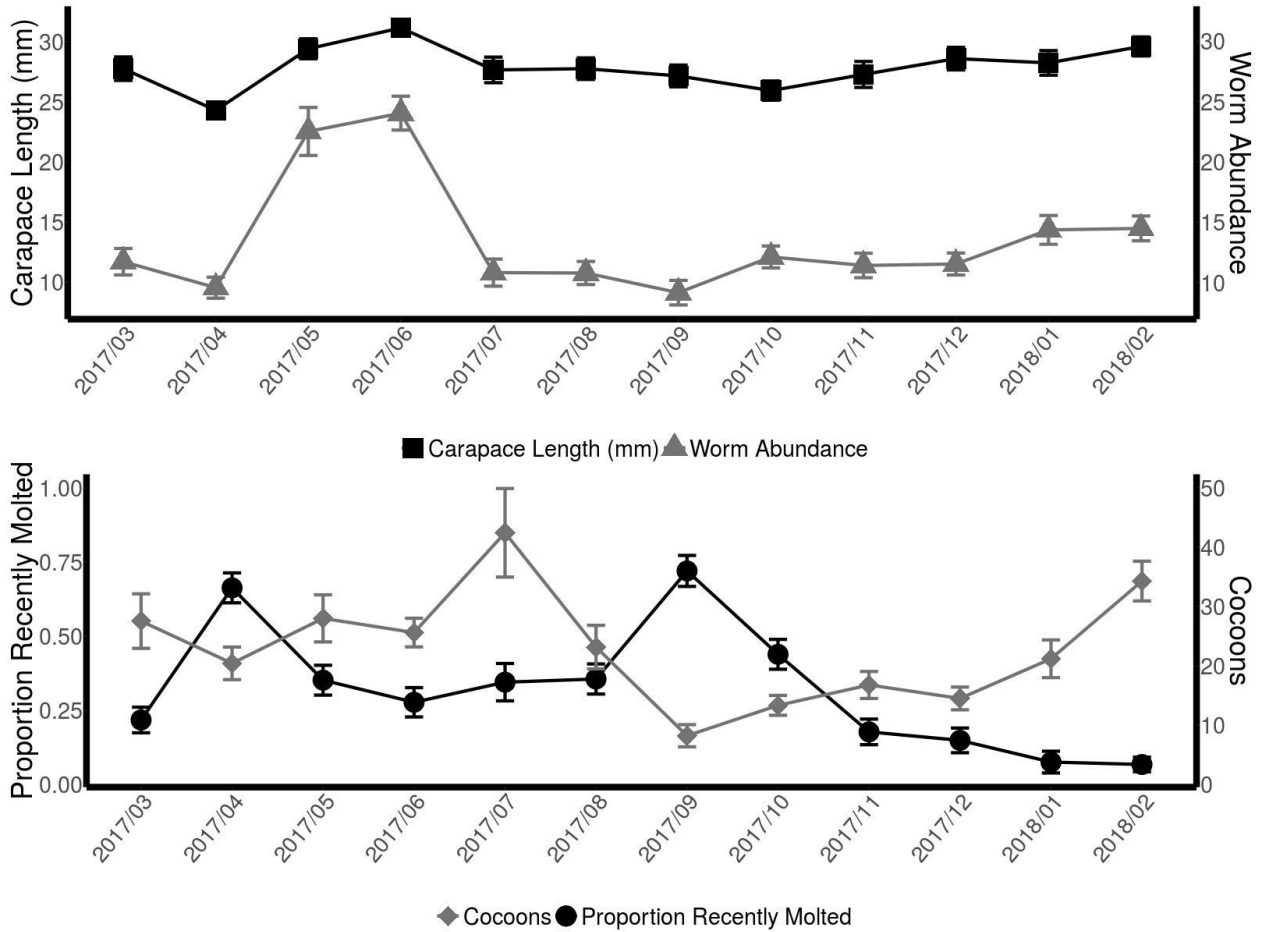


FIGURE 2.6

Branchobdellidan worm abundance was closely correlated with average carapace length of the crayfish, peaking in the summer (top). Branchobdellidan cocoons were most prevalent in the late winter and early spring, and appeared inversely correlated with crayfish molting patterns (bottom).

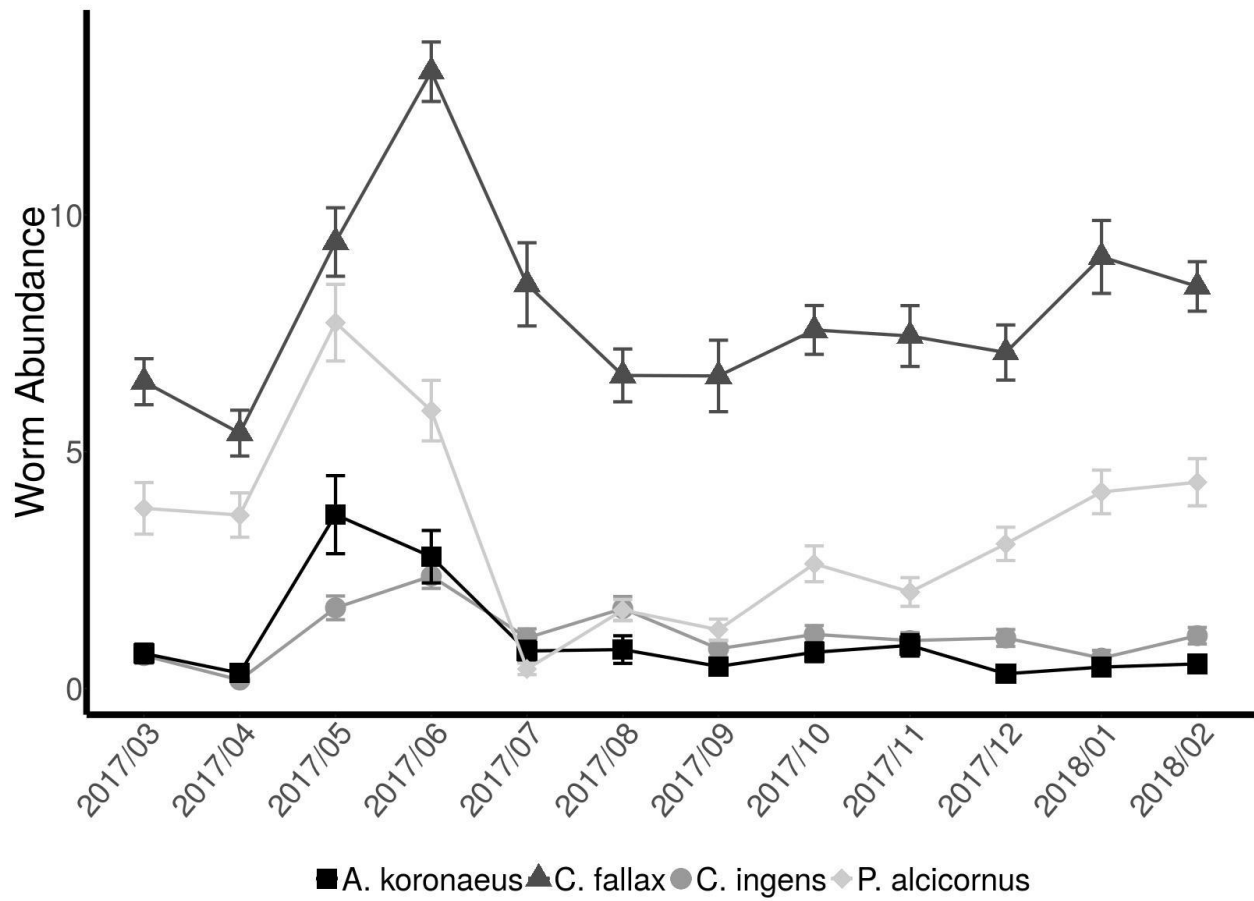


FIGURE 2.7

Abundances of the branchiobdellidans at Sinking Creek throughout the year. *Cambarincola fallax* and *Pterodrilus alcornus* were the most abundant worms in the community, with *Cambarincola ingens* and *Ankyrodrilus koronaeus* occurring at lower abundances.

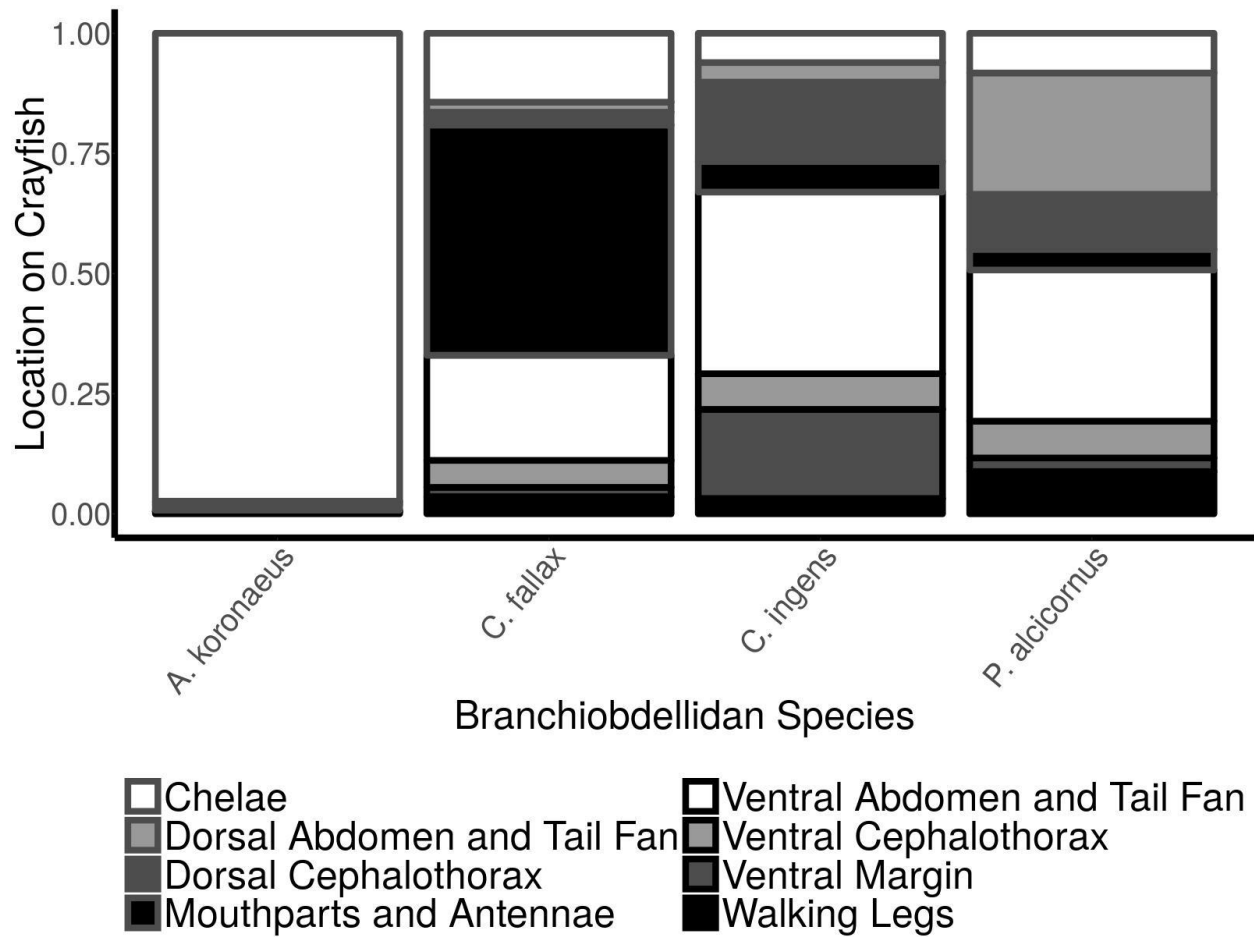


FIGURE 2.8

The locations on the crayfish where each species of worm in our study was predominately found. *Ankyrodrilus koronaeus* was found almost exclusively on the chelae of the crayfish, with the other three worms being largely generalists.

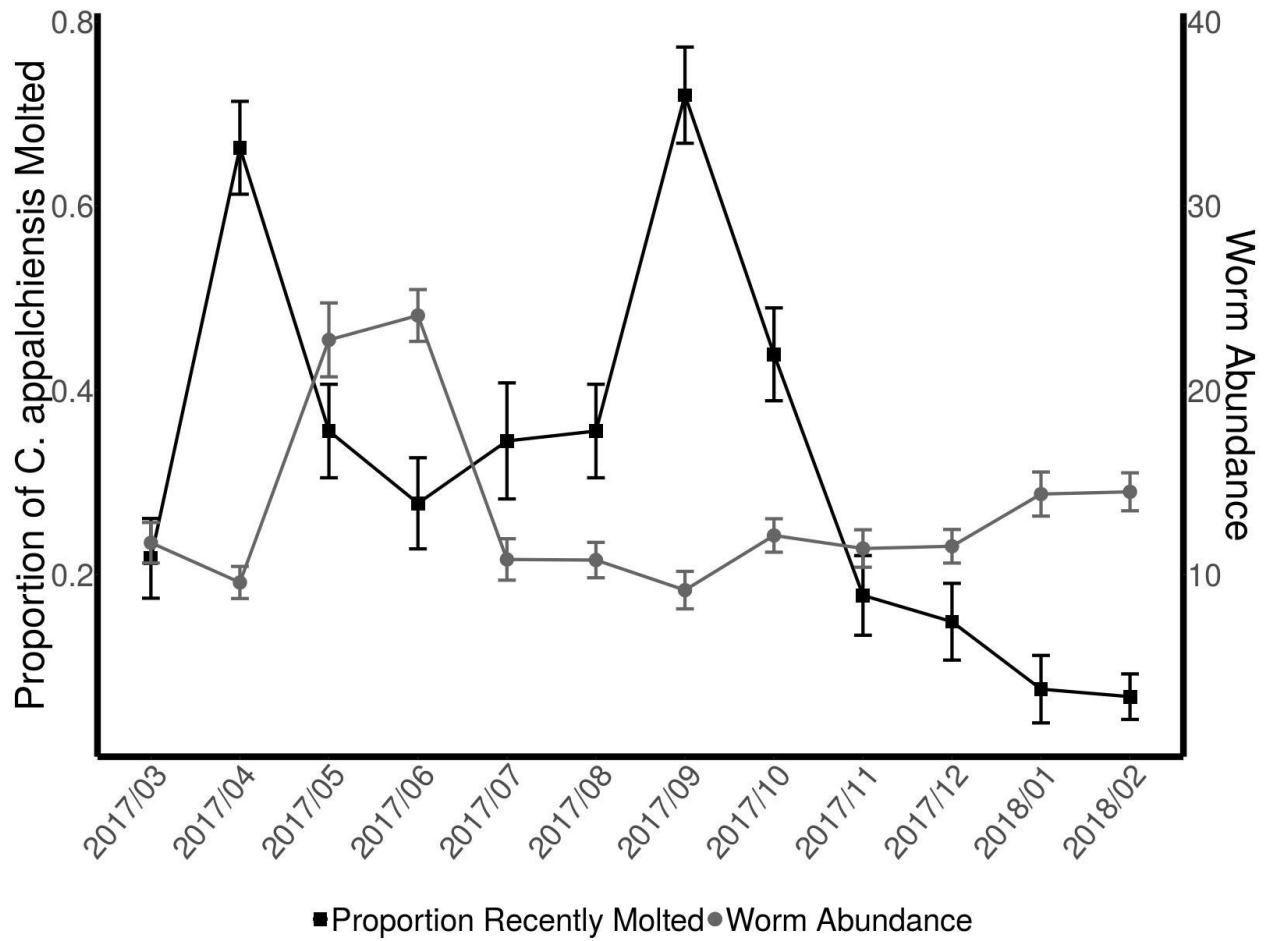


FIGURE 3.1

The proportion of crayfish that molted at the Sinking Creek site from March 2017 until February 2018, along with the average branchiobdellidan worm abundance on each crayfish during that time. There are periods of higher molting frequency in April and September, corresponding to decreases in branchiobdellidan worm abundances.

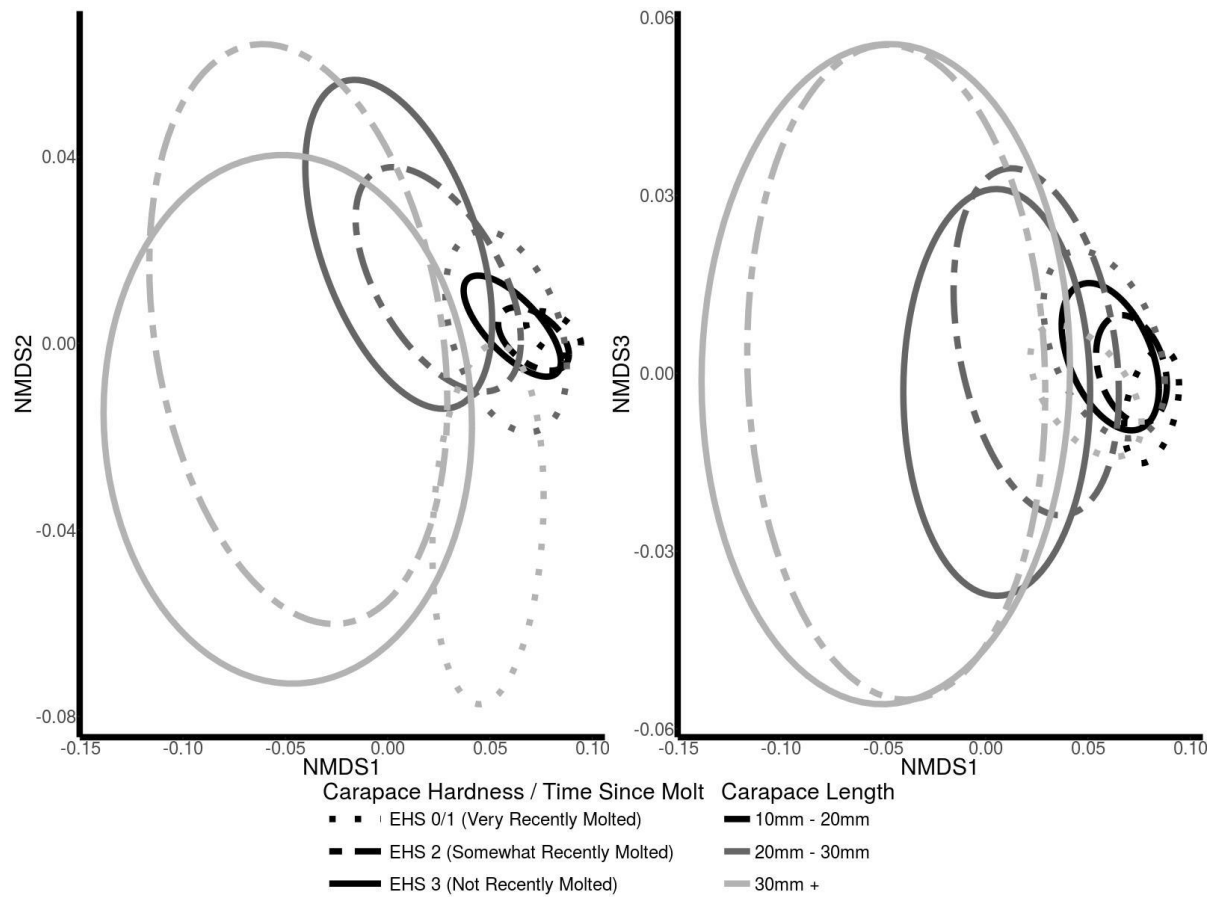


FIGURE 3.2

Non-metric multidimensional scaling of branchiobdellidan worm communities using a Gower distance matrix. Color represents the size category of the crayfish, while the line style represents carapace hardness. A carapace hardness of EHS 0/1 indicated that the crayfish molted very recently, a score of EHS 2 indicated the crayfish molted somewhat recently, and a score of EHS 3 indicated that the crayfish had not molted recently. The circles represent the area around the centroid plus the standard deviation. The closer together two circles are (or the more they overlap), the more similar the branchiobdellidan worm communities on crayfish of those size classes with those carapace hardness. In general, crayfish that have recently molted have a branchiobdellidan worm community more similar to crayfish of a smaller size.

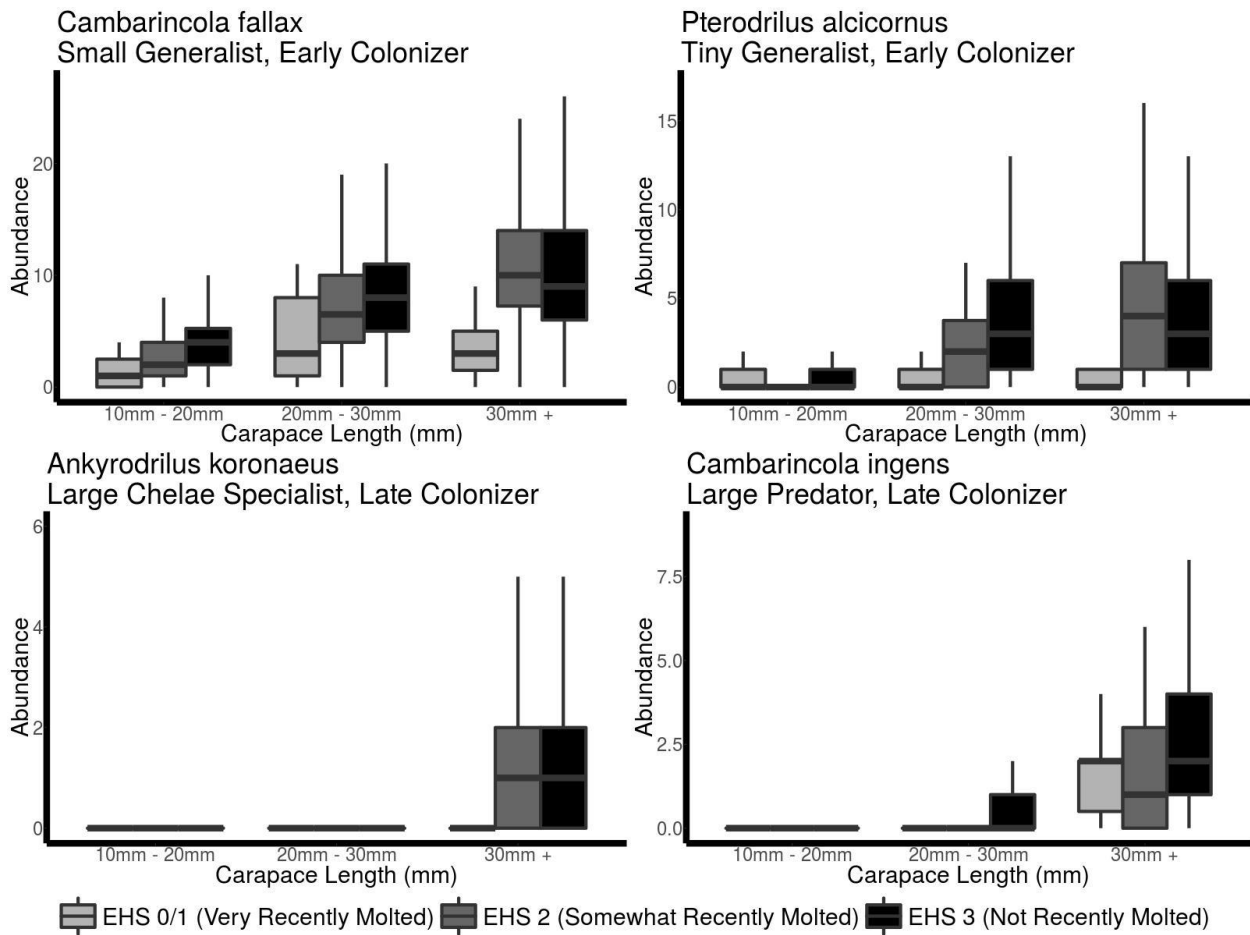


FIGURE 3.3

The abundance of each species of worm on crayfish of each size class and carapace hardness. *Cambarincola ingens* and *Pterodrilus alcornus* occur on crayfish of all sizes and carapace hardnesses. *Ankyrodrilus koronaeus* and *Cambarincola ingens* occur mostly on larger crayfish with carapace hardnesses that indicate that they have not molted very recently.



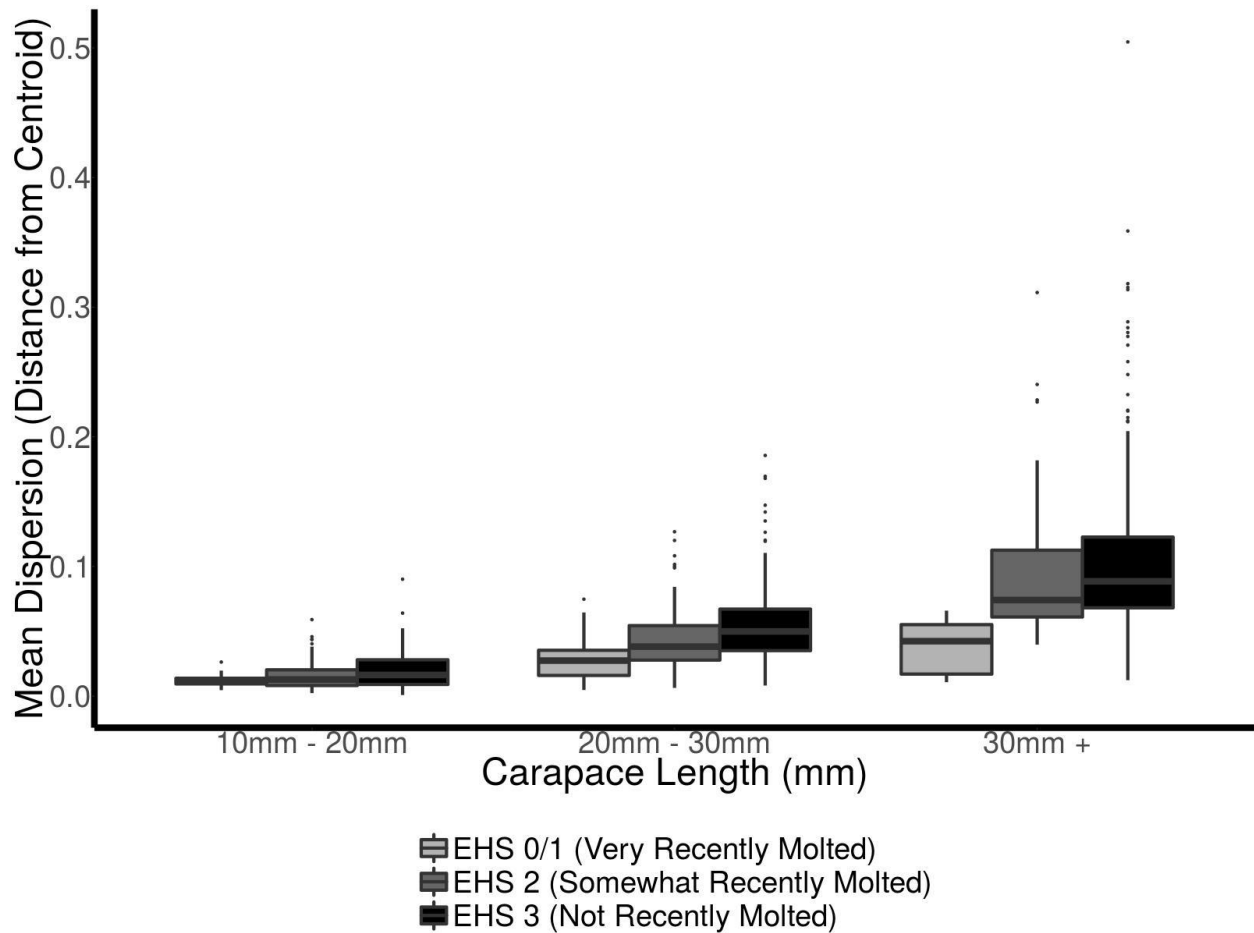


FIGURE 3.4

Mean dispersion (distance from the centroid) for each size class and carapace hardness. For each size class, mean dispersion, and thus the mean  $\beta$  diversity of the community, increases with carapace hardness. A carapace hardness of EHS 0/1 indicated that the crayfish molted very recently, a score of EHS 2 indicated the crayfish molted somewhat recently, and a score of EHS 3 indicated that the crayfish had not molted recently.

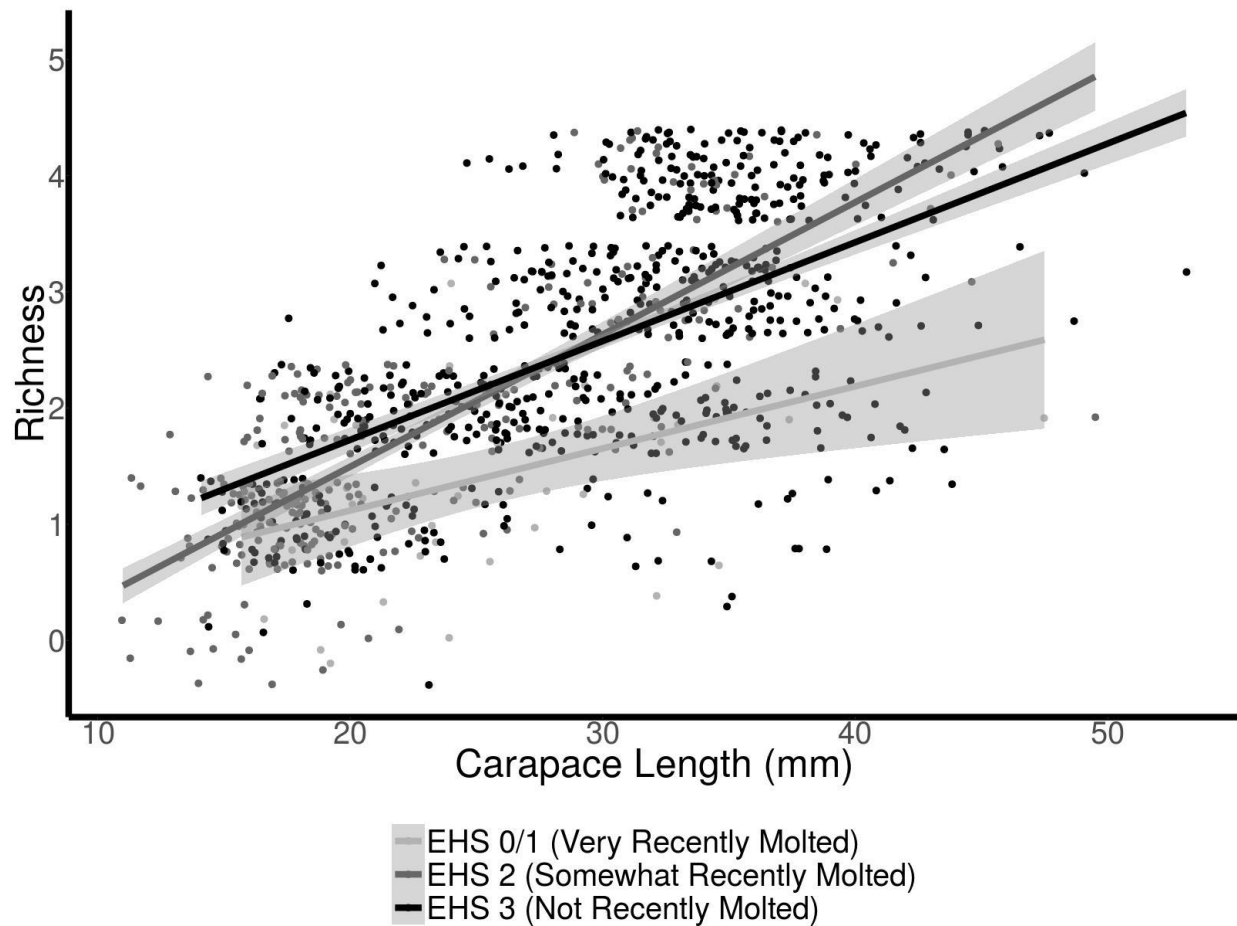


FIGURE 3.5

Linear fits to branchiobdellidan worm richness as a function of carapace length, separated by carapace hardness. Crayfish with a carapace hardness of EHS 0/1 (crayfish that have molted very recently) have a branchiobdellidan worm community with lower richness than other crayfish. Smaller crayfish with a carapace hardness of EHS 2 (crayfish that have molted somewhat recently) have a branchiobdellidan worm community with a lower richness than smaller crayfish with a carapace hardness of EHS 3 (crayfish that have not molted recently), but large crayfish with a carapace hardness of EHS 2 have branchiobdellidan worm communities with a higher richness than large crayfish with a carapace hardness of EHS 3.

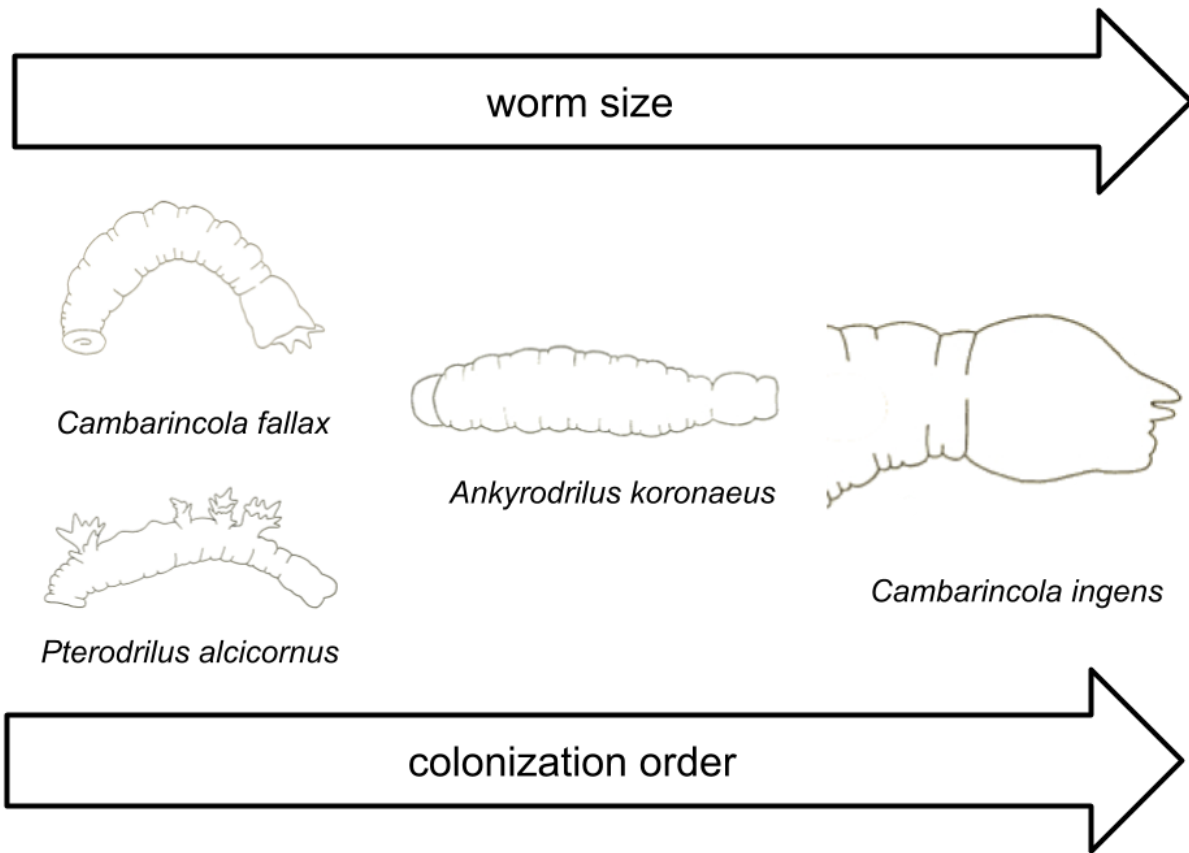


FIGURE 3.6

The size and approximate colonization order of the branchiobdellidan worms living on *Cambarus appalachiensis* at Sinking Creek. *Cambarincola fallax* and *Pterodrilus alcicornus* are smaller worms that quickly colonize small crayfish and recently molted crayfish, while *Ankyrodrilus koronaeus* and *Cambarincola ingens* are larger worms that persist on larger crayfish that have not molted recently. The drawings of the worms are adapted from those done by Horton H. Hobbs Jr., Perry C. Holt, and Margaret Walton (Hobbs Jr. et al. 1967).

## REFERENCES

- Aiken, D. E., and S. L. Waddy. 1992. The growth process in crayfish. *Reviews in Aquatic Sciences* 6:335–381.
- Ames, C. W., B. S. Helms, and J. A. Stoeckel. 2015. Habitat mediates the outcome of a cleaning symbiosis for a facultatively burrowing crayfish. *Freshwater Biology* 60:989–999.
- Anderson, M. J., K. E. Ellingsen, and B. H. McArdle. 2006. Multivariate dispersion as a measure of beta diversity. *Ecology Letters* 9:683–693.
- Bell, S. 2018. Mountain Lake revisited: impacts of invasion on native symbiotic systems. Virginia Polytechnic Institute and State University, Blacksburg, VA, USA.
- Brodie, J. F., C. E. Aslan, H. S. Rogers, K. H. Redford, J. L. Maron, J. L. Bronstein, and C. R. Groves. 2014. Secondary extinctions of biodiversity. *Trends in Ecology & Evolution* 29:664–672.
- Brown, B. L., and R. P. Creed. 2004. Host preference by an aquatic ectosymbiotic annelid on 2 sympatric species of host crayfishes. *Journal of the North American Benthological Society* 23:90–100.
- Brown, B. L., R. P. Creed, and W. E. Dobson. 2002. Branchiobdellid annelids and their crayfish hosts: are they engaged in a cleaning symbiosis? *Oecologia* 132:250–255.
- Brown, B. L., R. P. Creed, J. Skelton, M. A. Rollins, and K. J. Farrell. 2012. The fine line between mutualism and parasitism: complex effects in a cleaning symbiosis demonstrated by multiple field experiments. *Oecologia* 170:199–207.
- Castillo-Escrivà, A., A. Mestre, J. S. Monrós, and F. Mesquita-Joanes. 2013. Population dynamics of an epibiont Ostracoda on the invasive red swamp crayfish *Procambarus clarkii* in a western Mediterranean wetland. *Hydrobiologia* 714:217–228.

- Chase, J. M. 2007. Drought mediates the importance of stochastic community assembly. *Proceedings of the National Academy of Sciences* 104:17430–17434.
- Christian, N., B. K. Whitaker, and K. Clay. 2015. Microbiomes: unifying animal and plant systems through the lens of community ecology theory. *Frontiers in Microbiology* 6.
- Colwell, R. K., R. R. Dunn, and N. C. Harris. 2012. Coextinction and persistence of dependent species in a changing world. *Annual Review of Ecology, Evolution, and Systematics* 43:183–203.
- Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. *Science* 199:1302–1310.
- Corey, S. 1990. Life history of *Cambarus robustus* Girard in the Eramosa-Speed River system of Southwestern Ontario, Canada (Decapoda, Astacidea). *Crustaceana* 59:225–230.
- Costello, E. K., K. Stagaman, L. Dethlefsen, B. J. M. Bohannan, and D. A. Relman. 2012. Toward an understanding of the human microbiome. *Science* 336:1255–1262.
- Creed, R. P. 1994. Direct and indirect effects of crayfish grazing in a stream community. *Ecology* 75:2091–2103.
- De Roos, A. M., T. Schellekens, T. Van Kooten, and L. Persson. 2008. Stage-specific predator species help each other to persist while competing for a single prey. *Proceedings of the National Academy of Sciences* 105:13930–13935.
- Dennard, S., J. T. Peterson, and E. S. Hawthorne. 2009. Life history and ecology of *Cambarus halli* (Hobbs). *Southeastern Naturalist* 8:479–494.
- Dimijian, G. G. 2000. Evolving together: the biology of symbiosis, part 1. *Baylor University Medical Center Proceedings* 13:217a – 226.
- Fabricius, J. C. 1798. *Supplementum entomologiae systematicae*. Proft et Storch, Hafniae (Copenhagen), Denmark.
- Fitzpatrick, J. F. 1995. Horton H. Hobbs, Jr. (29 March 1914 - 22 March 1994). *Journal of Crustacean Biology* 15:797–799.

- Flinders, C. A., and D. D. Magoulick. 2005. Distribution, habitat use and life history of stream-dwelling crayfish in the Spring River drainage of Arkansas and Missouri with a focus on the imperiled Mammoth Spring crayfish (*Orconectes marchandi*). *The American Midland Naturalist* 154:358–374.
- Gelder, S. R. 1996. A review of the taxonomic nomenclature and a checklist of the species of the Branchiobdellae (Annelida: Clitellata). *Proceedings of the Biological Society of Washington* 109:653–663.
- Gelder, S. R. 1999. Zoogeography of branchiobdellidans (Annelida) and temnocephalidans (Platyhelminthes) ectosymbiotic on freshwater crustaceans, and their reactions to one another in vitro. *Hydrobiologia* 406:21–31.
- Gelder, S. R. 2001. In memoriam - Professor Perry C. Holt, PhD (1910-1999). *Hydrobiologia* 463:xxi–xxiii.
- Gelder, S. R., N. L. Gagnon, and K. Nelson. 2002. Taxonomic considerations and distribution of the Branchiobdellida (Annelida: Clitellata) on the North American continent. *Northeastern Naturalist* 9:451–468.
- Govedich, F. R., B. A. Bain, W. E. Moser, S. R. Gelder, R. W. Davies, and R. O. Brinkhurst. 2010. Chapter 12 - Annelida (Clitellata): Oligochaeta, Branchiobdellida, Hirudinida, and Acanthobdellida. Pages 385–436 in J. H. Thorp and A. P. Covich, editors. *Ecology and Classification of North American Freshwater Invertebrates*. Third edition. Academic Press, San Diego.
- Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27:857–871.
- Grabda, E., and J. Wierzbicka. 1969. The problem of parasitism of the species of the genus *Branchiobdella* Odier, 1823. *Polskie Archiwum Hydrobiologii* 16:93–104.
- Grinnell, J. 1917. The niche-relationships of the California Thrasher. *Auk* 34:427–433.

- Hamr, P., and M. Berrill. 1985. The life histories of north-temperate populations of the crayfish *Cambarus robustus* and *Cambarus bartoni*. *Canadian Journal of Zoology* 63:2313–2322.
- Hobbs Jr., H. H., P. C. Holt, and M. Walton. 1967. The crayfishes and their epizootic ostracod and branchiobdellid associates of the Mountain Lake, Virginia, region. *Proceedings of the United States National Museum* 123:1–84.
- Hoffman, R. L. 1963. A revision of the North American annelid worms of the genus *Cambarincola* (Oligochaeta: Branchiobdellidae). *Proceedings of the United States National Museum* 114:271–371.
- Holt, P. C. 1954. A new branchiobdellid of the genus *Cambarincola* (Oligochaeta, Branchiobdellidae) from Virginia. *Virginia Journal of Science* 30:27–31.
- Holt, P. C. 1965. On *Ankyrodrilus*, a new genus of branchiobdellid worms (Annelida). *Virginia Journal of Science* 16:9–21.
- Hubbell, S. P. 2001. *The unified neutral theory of biodiversity and biogeography*. Princeton University Press, Princeton, NJ, USA.
- Johnson, P. T. J., J. C. de Roode, and A. Fenton. 2015. Why infectious disease research needs community ecology. *Science* 349:1259504.
- Jones, D. R., and A. G. Eversole. 2011. Life history characteristics of the Elk River crayfish. *Journal of Crustacean Biology* 31:647–652.
- Karlson, R. H., and L. E. Hurd. 1993. Disturbance, coral reef communities, and changing ecological paradigms. *Coral Reefs* 12:117–125.
- Ke, P.-J., and T. Nakazawa. 2018. Ontogenetic antagonism-mutualism coupling: perspectives on resilience of stage-structured communities. *Oikos* 127:353–363.
- Koepp, S. J. 1975. Effects of host ecdysis on population structure of the epizootic branchiobdellid *Cambarincola vitrea*. *Science of Biology Journal* 1:39–42.

- Larson, E. R., and D. D. Magoulick. 2011. Life-history notes on *Cambarus hubbsi* Creaser (Hubbs crayfish) from the South Fork Spring River, Arkansas. *Southeastern Naturalist* 10:121–132.
- Lee, J. H., T. W. Kim, and J. C. Choe. 2009. Commensalism or mutualism: conditional outcomes in a branchiobdellid–crayfish symbiosis. *Oecologia* 159:217–224.
- Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7:601–613.
- Lenth, R., H. Singmann, J. Love, P. Buerkner, and M. Herve. 2019. emmeans: Estimated marginal means, aka least-squares means. R.
- Logue, J. B., N. Mouquet, H. Peter, and H. Hillebrand. 2011. Empirical approaches to metacommunities: a review and comparison with theory. *Trends in Ecology and Evolution* 26:482–491.
- Loughman, Z. J., T. P. Simon, and S. A. Welsh. 2009. West Virginia crayfishes (Decapoda: Cambaridae): observations on distribution, natural history, and conservation. *Northeastern Naturalist* 16:225–238.
- Loughman, Z. J., S. A. Welsh, and R. F. Thoma. 2017. *Cambarus* (C.) *appalachiensis*, a new species of crayfish (Decapoda: Cambaridae) from the New River Basin of Virginia and West Virginia, USA. *Zootaxa* 4243:432–454.
- MacArthur, R. H. 1958. Population ecology of some warblers of northeastern coniferous forests. *Ecology* 39:599–619.
- May, R. M., and M. A. Nowak. 1994. Superinfection, metapopulation dynamics, and the evolution of diversity. *Journal of Theoretical Biology* 170:95–114.
- McManus, L. 1960. Some ecological studies of the Branchiobdellidae (Oligochaeta). *Transactions of the American Microscopical Society* 79:420–428.



- Mihaljevic, J. R. 2012. Linking metacommunity theory and symbiont evolutionary ecology. *Trends in Ecology & Evolution* 27:323–329.
- Miller, T. E. X., and V. H. W. Rudolf. 2011. Thinking inside the box: community-level consequences of stage-structured populations. *Trends in Ecology & Evolution* 26:457–466.
- Moore, J. P. 1893. On some leech-like parasites of American crayfishes. *Proceedings of the Academy of Natural Sciences of Philadelphia* 45:419–428.
- Moore, J. P. 1894. *Pterodrilus*, a remarkable discodrilid. *Proceedings of the Academy of Natural Sciences of Philadelphia* 46:449–454.
- Moore, M. J., R. J. DiStefano, and E. R. Larson. 2013. An assessment of life-history studies for USA and Canadian crayfishes: identifying biases and knowledge gaps to improve conservation and management. *Freshwater Science* 32:1276–1287.
- Nakazawa, T. 2011. Ontogenetic niche shift, food-web coupling, and alternative stable states. *Theoretical Ecology* 4:479–494.
- Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlenn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2018. *vegan: Community ecology package*. R.
- Penn, G. H. 1959. Survival of branchiobdellid annelids without A crawfish host. *Ecology* 40:514–515.
- Poff, N. L., and J. D. Allan. 1995. Functional organization of stream fish assemblages in relation to hydrological variability. *Ecology* 76:606–627.
- Prins, R. 1968. Comparative ecology of the crayfishes *Orconectes rusticus rusticus* and *Cambarus tenebrosus* in Doe Run, Meade County, Kentucky. *International Review of Hydrobiology* 53:667–714.
- R Core Team. 2018. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

- Reynolds, J. D. 2002. Growth and reproduction. Pages 152–191 *Biology of Freshwater Crayfish*. Wiley-Blackwell, Hoboken, NJ, USA.
- Rhoades, R. 1944. Further studies on distribution and taxonomy of Ohio crayfishes, and the description of a new subspecies. *The Ohio Journal of Science* 44:95–99.
- Richman, N. I., M. Bohm, S. B. Adams, F. Alvarez, E. A. Bergey, J. J. S. Bunn, Q. Burnham, J. Cordeiro, J. Coughran, K. A. Crandall, K. L. Dawkins, R. J. DiStefano, N. E. Doran, L. Edsman, A. G. Eversole, L. Fureder, J. M. Furse, F. Gherardi, P. Hamr, D. M. Holdich, P. Horwitz, K. Johnston, C. M. Jones, J. P. G. Jones, R. L. Jones, T. G. Jones, T. Kawai, S. Lawler, M. Lopez-Mejia, R. M. Miller, C. Pedraza-Lara, J. D. Reynolds, A. M. M. Richardson, M. B. Schultz, G. A. Schuster, P. J. Sibley, C. Souty-Grosset, C. A. Taylor, R. F. Thoma, J. Walls, T. S. Walsh, and B. Collen. 2015. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society B: Biological Sciences* 370:20140060–20140060.
- Roell, M. J., and D. J. Orth. 1992. Production of three crayfish populations in the New River of West Virginia, USA. *Hydrobiologia* 228:185–194.
- Rominger, A. J., K. R. Goodman, J. Y. Lim, E. E. Armstrong, L. E. Becking, G. M. Bennett, M. S. Brewer, D. D. Cotoras, C. P. Ewing, J. Harte, N. D. Martinez, P. M. O'Grady, D. M. Percy, D. K. Price, G. K. Roderick, K. L. Shaw, F. S. Valdovinos, D. S. Gruner, and R. G. Gillespie. 2016. Community assembly on isolated islands: macroecology meets evolution: community assembly on isolated islands. *Global Ecology and Biogeography* 25:769–780.
- Rosenberger, A. E., C. W. Ames, J. T. Westhoff, and R. J. DiStefano. 2016. Life history of the vulnerable endemic crayfish *Cambarus (Erebicambarus) maculatus* Hobbs and Pflieger, 1988 (Decapoda: Astacoidea: Cambaridae) in Missouri, USA. *Journal of Crustacean Biology* 36:615–627.

- Seabloom, E. W., E. T. Borer, K. Gross, A. E. Kendig, C. Lacroix, C. E. Mitchell, E. A. Mordecai, and A. G. Power. 2015. The community ecology of pathogens: coinfection, coexistence and community composition. *Ecology Letters* 18:401–415.
- Skelton, J., R. P. Creed, and B. L. Brown. 2014. Ontogenetic shift in host tolerance controls initiation of a cleaning symbiosis. *Oikos* 123:677–686.
- Skelton, J., R. P. Creed, L. Landler, K. Geyer, and B. L. Brown. 2016a. Geographic patterns of crayfish symbiont diversity persist over half a century despite seasonal fluctuations. *Freshwater Crayfish* 22:9–18.
- Skelton, J., S. Doak, M. Leonard, R. P. Creed, and B. L. Brown. 2016b. The rules for symbiont community assembly change along a mutualism-parasitism continuum. *Journal of Animal Ecology* 85:843–853.
- Smart Jr., G. C. 1962. The life history of the crayfish *Cambarus longulus longulus*. *The American Midland Naturalist* 68:83–94.
- Soliveres, S., L. DeSoto, F. T. Maestre, and J. M. Olano. 2010. Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. *Perspectives in Plant Ecology, Evolution and Systematics* 12:227–234.
- Taylor, C. A. 2000. Systematic studies of the *Orconectes juvenilis* complex (Decapoda: Cambaridae), with descriptions of two new species. *Journal of Crustacean Biology* 20:132–152.
- Taylor, C. A., G. A. Schuster, J. E. Cooper, R. J. DiStefano, A. G. Eversole, P. Hamr, H. H. Hobbs III, H. W. Robison, C. E. Skelton, and R. F. Thoma. 2007. A reassessment of the conservation status of crayfishes of the United States and Canada after 10+ years of increased awareness. *Fisheries* 32:270–277.
- Thomas, M. J., R. P. Creed, and B. L. Brown. 2013. The effects of environmental context and initial density on symbiont populations in a freshwater cleaning symbiosis. *Freshwater Science* 32:1358–1366.

Thomas, M. J., R. P. Creed, J. Skelton, and B. L. Brown. 2016. Ontogenetic shifts in a freshwater cleaning symbiosis: consequences for hosts and their symbionts. *Ecology* 97:1507–1517.

Tilman, D. 1999. The ecological consequences of changes in biodiversity: a search for general principles. *Ecology* 80:1455–1474.

Young, W. 1966. Ecological studies of the Branchiobdellidae (Oligochaeta). *Ecology* 47:571–578.