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Strong effects of a mutualism on freshwater community structure

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Abstract. Numerous mutualisms have been described from terrestrial and marine communities and many of these mutualisms have significant effects on community structure and function. In contrast, there are far fewer examples of mutualisms from freshwater habitats and there is no evidence that any mutualism has community-wide or ecosystem-level consequences. Northern hemisphere crayfish are host to a variety of ectosymbiotic worms called branchiobdellidans. The association between some of these "crayfish worms" and their hosts is a mutualism. The outcome of the association is context dependent and can be influenced by host size, symbiont number, and the environment. Here we document in two experiments that the mutualism between crayfish and these worms alters the effect of crayfish on stream community structure and sediment deposition, an important ecosystem variable. We enclosed crayfish stocked with 0 worms and intermediate (3–6) and high worm densities (12) in cages in streams in Boone, North Carolina and Clemson, South Carolina, United States. At both locations, there was a negative relationship between initial worm density and final macroinvertebrate abundance. There was a significant effect of worm treatment on macroinvertebrate community structure in both the Boone and Clemson experiments. In Boone, there were effects on both overall macroinvertebrate abundance and community composition, whereas in Clemson, changes to community structure were primarily driven by changes in total abundance. There was a negative relationship between benthic sediment volume and initial worm density in both experiments, primarily later in the experiments, though these effects were influenced by sediment deposition rates. Our results are the first to demonstrate strong effects of a mutualism on freshwater communities. Both members of this mutualism are found throughout the northern hemisphere, so similar impacts may occur in many other waterways. Given that various species in addition to crayfish function as keystone species and ecosystem engineers in freshwater systems throughout the world, mutualisms involving these strongly interacting species may be as important to the structure and functioning of freshwater systems as comparable mutualisms in marine and terrestrial systems.

Key words: *bioturbation; crayfish; ecosystem engineer; keystone species; streams; symbiosis.*

Introduction

Numerous mutualisms have been described and experimentally evaluated in terrestrial and marine systems, including plant–pollinator mutualisms, various protective mutualisms (e.g., ant–plant, coral–crustacean), resource-exchange mutualisms (e.g., plant–mycorrhizae, coral–zooxanthellae), and cleaning symbioses (Hay et al. 2004, Bronstein et al. 2006, Hoeksema et al. 2010, Trager et al. 2010). In contrast, very few mutualisms have been documented from freshwater habitats (Dodds and Whiles 2010, Holomuzki et al. 2010). Similarly, although the significance of a number of terrestrial and marine mutualisms for community structure has also been demonstrated (Hay et al. 2004, Pringle and Gordon 2013), no such evidence exists for freshwater systems (Dodds and Whiles 2010, Holomuzki et al. 2010). Dodds and Whiles (2010) suggest that one explanation for this pattern is that freshwater systems have been in existence for a much shorter period of time relative to many marine and terrestrial communities and thus there has not been sufficient time for mutualisms to evolve. Alternatively, they also suggest, along with Holomuzki et al. (2010) and Silknetter et al. (2020), that freshwater mutualisms and their impacts on freshwater systems could have simply been overlooked.

Many vertebrate and invertebrate consumers can exert strong direct and indirect effects on freshwater communities (McPeek 1990, Creed 1994, Wellborn et al. 1996, Creed and Reed 2004, Creed 2006). The mechanisms underlying these strong effects include consumption of dominant competitors or predators in

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lower trophic levels (Brooks and Dodson 1965, McPeek 1990, Wellborn et al. 1996). They also involve consumption of dominant algal and macrophyte species, which may also lead to shifts in community structure (Lamberti and Resh 1983, Power et al. 1985, Creed 1994, 2000). Other taxa exert strong effects on freshwater communities via nonconsumptive, ecosystem engineering effects (Flecker 1996, Crowl et al. 2001, Creed and Reed 2004, Usio and Townsend 2004, Vaughn et al. 2008, Creed et al. 2010). These effects include habitat modification, altering sediment deposition, and changing the flow of resources (Jones et al. 1994). If the effects of strongly interacting species such as keystone species and ecosystem engineers can be significantly modulated by associated symbionts, then these symbionts may indirectly influence freshwater community structure.

Crayfish are keystone species (sensu Paine 1969) and ecosystem engineers in many freshwater communities, influencing the abundance of other species through consumption, habitat alteration, and bioturbation (Creed 1994, Statzner et al. 2003, Creed and Reed 2004, Usio and Townsend 2004, Albertson and Daniels 2018). They have strong consumptive effects on algae, vascular macrophytes, and macroinvertebrates (Lodge and Lorman 1987, Creed 1994, Usio and Townsend 2004). Their strong direct effects may lead to strong indirect effects on other members of their communities (Creed 1994). They are also important detritivores, especially in summer months, and can modulate the flow of fine particulate organic matter to other consumers (Creed and Reed 2004). They redistribute bottom sediments of lakes and streams (Statzner et al. 2003, Creed and Reed 2004, Usio and Townsend 2004) and even have significant effects on the sediment budget of entire watersheds (Rice et al. 2016).

Many Holarctic crayfish are hosts for ectosymbiotic, crayfish worms (Annelida; Branchiobdellida; Gelder 1999, Skelton et al. 2013, 2016, Thomas et al. 2016). These symbionts spend their entire lives on their crayfish hosts and will only successfully reproduce on a live crayfish (Young 1966, Creed et al. 2015, but see James et al. 2017). Succession of crayfish worms has been documented with smaller worms colonizing smaller crayfish only to be replaced by larger worms on larger hosts (Skelton et al. 2016, Thomas et al. 2016). Worm numbers are regulated by their hosts and through intraguild predation (Farrell et al. 2014, Skelton et al. 2014, Thomas et al. 2016). Some of these worms significantly improve crayfish survival and growth (Brown et al. 2002, 2012, Lee et al. 2009, Thomas et al. 2016) by cleaning the crayfish's gills, which may affect gas exchange and ammonia excretion, although this effect appears to be context dependent (Lee et al. 2009). Given the strong effect some of these worms can have on their hosts, and that crayfish can have strong effects on aquatic ecosystems, it is likely that the impacts of many crayfish species on freshwater communities are modulated by these symbionts.

We conducted two field experiments to evaluate if the effects of crayfish on stream communities were influenced by their branchiobdellidan symbionts. We hypothesized, given the effects of worms on crayfish survival and growth observed in previous lab and field experiments (Brown et al. 2002, 2012, Lee et al. 2009, Thomas et al. 2016), that the impact of the worms on their hosts would be transmitted to the rest of the community through effects on crayfish consumption and bioturbation.

Methods

Environmental context influences many ecological interactions, including mutualisms, so we evaluated the effects of the crayfish–branchiobdellidan symbiosis on macroinvertebrate communities in two different sized streams. The Boone experiment was conducted in a section of the South Fork of the New River, which is a fourth-order river, in the Greenway for the town of Boone, North Carolina (36°21′12.2″ N, 81°65′30.3″ W). The river is 8–15 m wide, has a largely open canopy and a diverse fish community (~25 spp.; Helms and Creed 2005, Fortino and Creed 2007, Brown et al. 2012). In contrast, Waldrop Stone Creek is a second-order stream that is approximately 2 m wide, flows through a heavily forested area in the Clemson Experimental Forest (34°44′28.4″ N, 82°49′13.9″ W) near Clemson, South Carolina, and has a less diverse fish community (~2 spp.; Brown et al. 2012). Host and symbiont identities along with symbiont density are also sources of context dependence (Silknetter et al. 2020) so we used two different crayfish hosts, two different species of branchiobdellidan, and we varied symbiont density. Thus, we were able to assess how differences in both environmental and host–symbiont context influenced the outcome of this symbiosis on these communities. Other aspects of the experiments were similar, for example, area of enclosures, initial worm densities to make results comparable.

Boone experiment

In this experiment, we placed crayfish with 0, 4, or 12 large (≥6 mm long), branchiobdellidan worms (0-, 4- and 12-worm treatments) inside enclosures (1.0 m long × 0.5 m wide × 0.5 m high, internal dimensions) in the New River in the summer of 2010. A fourth treatment (no-crayfish [NC]) served as a control for crayfish effects on macroinvertebrates and sediments. The experimental design was a randomized complete block design with the 24 enclosures placed in six rows with four treatments per row.

Crayfish ([*Cambarus chasmodactylus*], total carapace length [CL] 31–33 mm; initial blotted wet mass [BWM] 9.18–13.43 g) were collected from a nearby section of the Middle Fork of the New River. Worms (*Cambarincola ingens*) and their cocoons (each with one egg) were removed manually and with a 10% $MgCl_2$ hexahydrate

bath (Brown et al. 2002). Crayfish were randomly assigned to one of the three crayfish treatments and those to receive worms were restocked accordingly. One crayfish was stocked per enclosure with a resulting density of 2 crayfish/m^2. In this portion of the New River, the mean density of *C. chasmodactylus* is approximately 0.5–1.0 m^{-2}, though we have recorded densities as high as 3 m^{-2} (Helms and Creed 2005). A density of four large worms is commonly observed on *C. chasmodactylus* of this size in the New River, and the mean (±1 SE) number of large worms on the crayfish at the time of collection was 4.06 (±0.1).

The enclosures had welded aluminum frames, solid sides and bottoms, and were held in place with four pieces of rebar driven into the substrate (see also Skelton et al. 2013). Upstream and downstream ends had two walls of 12-mm wire mesh separated by a gap of at least 10 cm to ensure that enclosed crayfish did not come into contact with free-living crayfish and acquire more worms, which are transmitted through direct contact. Enclosure tops had aluminum frames with large panels of 12-mm mesh. Tops were hinged to allow ready access to baskets and crayfish inside and could be locked. Enclosures contained a layer of washed stream gravel ~6–8 cm deep. Two wire baskets (30 × 30 cm with 5-cm-high walls, 6-mm mesh) containing 6–7 large cobbles and a slate tile (10 × 10 cm), the latter to serve as a standardized substrate for surficial sediment accumulation, were placed on top of the gravel in each enclosure along with additional cobbles to provide shelter for the crayfish. Initial water depth and current velocity inside cages were similar (mean [±1 SE] water depth 41.7 ± 0.8 cm; current velocity 17.8 ± 0.7 cm/s). Current velocities inside enclosures were ~72% of that in the stream channel adjacent to enclosures. Water temperature was monitored hourly with an Onset Pro-v monitor. During the study, water temperatures ranged from 16° to 26°C. The experiment began on 23 June 2010; crayfish were added to enclosures 1 d after the enclosures were placed in the river.

A single basket was sampled from each enclosure on each of two dates (22 July and 30 August 2010) during the experiment to determine macroinvertebrate abundance and the volume of accumulated sediment. On 22 July (29 d), we randomly selected one basket for sampling; the remaining basket was sampled on 30 August (68 d). On each sampling date, the slate tile was carefully removed under water to a sealable plastic bag placed immediately downstream for determination of surficial sediment deposition. Next, we transferred the basket to a Surber sampler-shaped sampling device lined with a piece of removable 250-μm mesh placed immediately downstream. The contents of the sampler and the mesh were then placed into a plastic dish pan. The cobbles were scrubbed using a soft plastic brush. Cleaned baskets and cobbles were returned to the cages, but not sampled again. Basket contents were preserved in 70% ethanol. Basket contents were later passed through 1-mm and 250-μm sieves, and we determined the volume of these two particulate fractions in a graduated cylinder. Material retained on the 1-mm sieve was considered coarse particulate matter (CPM) and the material retained on the 250-μm sieve was considered fine particulate matter (FPM). Both fractions were a mixture of inorganic sediments and organic material and were primarily measures of interstitial sediment deposition. When the baskets were set up, they did not contain any pieces of gravel (1–2-cm diameter). When sampling the baskets, we also enumerated the number of pieces of gravel they contained, as this served as an additional indicator of crayfish bioturbation.

All macroinvertebrates were identified to family from the CPM and FPM. We subsampled the FPM and, based on these subsamples, estimated macroinvertebrate abundance for each FPM fraction. We then combined macroinvertebrate counts from the two sediment fractions to determine total macroinvertebrate abundance in the baskets.

Clemson experiment

This experiment was also conducted during the summer of 2010. We installed 30 flow-through enclosures in Walldrop Stone Creek. Enclosures were constructed from 3.8-cm wood boards and 6-mm wire mesh. Enclosures (1.0 m long × 0.5 m wide × 0.5 m high, internal dimensions) had hinged and locking top panels for access. The side walls consisted of two layers of wire mesh, separated by 3.8 cm that prevented contact and symbiont exchange between experimental and free-living crayfish. Enclosures were placed individually along the thalweg of the creek, separated by at least 3 m, and held in place with four pieces of rebar driven into the substrate. We placed six 25 × 20 cm mesh baskets in each enclosure, which together covered most of the bottom of the enclosure. Baskets were then filled with gravel and several cobbles from the stream.

The experimental design was a completely randomized design with six replicates for each of five treatments; a single crayfish with 0, 3, 6, or 12 branchiobdellidans (referred to as 0W, 3W, 6W, and 12W), and a no-crayfish control (NC). All crayfish used in the experiment were *Cambarus chaugaensis*, and the branchiobdellidans were *Xironodrilus appalachius*. Crayfish were medium-size adults (range CL 23.9–26.5 mm). Preliminary surveys revealed a mean density of 5.2 ± 2.4 (mean ± SD) large worms per crayfish. Thus our two intermediate worm density treatments (3W and 6W) represented typical densities, and 12W represented a high, but realistic density. Crayfish were added to enclosures 1 d after the enclosures were placed in the river.

The experiment started on 19 June 2010. We sampled benthic macroinvertebrates and sediments on five sampling dates during the 95-d experiment: 14 July, 27 July, 8 August, 30 August, 22 September. For each enclosure on each sampling date, a randomly selected basket was carefully lifted from the enclosure and placed into a

clean plastic tub. Basket substrates were scrubbed with soft plastic brushes to remove macroinvertebrates. Gravel and cobbles were then placed back in the basket, which was returned to the enclosure but not sampled again. Sediment and macroinvertebrates were then transferred to sealable plastic bags and stored at 5°C until processing. Macroinvertebrates were removed from samples under a microscope, preserved in 70% ethanol and identified to the lowest practical taxonomic level using Merritt et al. (2008). Sediment volume was measured by displacement of water in a large graduated cylinder. Sediment here also refers to a combination of organic particles and inorganic sediments.

Data analysis

Data analyses for the two experiments were similar to facilitate comparison of results. The biggest difference was that the Clemson experiment was conducted as a 5-point time series, and the Boone experiment was sampled on two dates. Additionally, in both experiments, one replicate of the no-crayfish treatment was lost during a flood, so all analyses were adjusted accordingly.

For the Boone experiment, sediment that accumulated on the surface of the tiles was considered a measurement of surficial sediment; these data were analyzed with a general linear model (GLM) ANOVA. There were three metrics of interstitial basket sediments in this experiment—CPM and FPM volume (ml) as well as gravel counts—so these data were first analyzed together using a MANOVA followed by GLM ANOVAs for individual metrics. The following outliers were identified in the August samples and removed using Dixon's test (Sokal and Rohlf 1981): Three outliers were removed from the tile sediment data so n for the treatments are as follows: NC = 4, 0W = 6, 4W = 5, 12W = 5; one outlier was removed from the 12W treatment for CPM. As a result, df for the F values was as follows: FPM and gravel—3,14; CPM—3,13; tiles—3,11; no outliers were identified in the July samples. The analysis for the gravel data was run on log10-transformed data to homogenize the variances; untransformed values are presented in Table 1. For the Clemson experiment, sediment data were analyzed using repeated-measures analysis of variance (RMANOVA) with branchiobdellidan treatment and time as within-subjects factors and experimental unit as the repeated measure. We also employed linear contrasts for sediment volume on the final two sampling dates in order to isolate the following effects: (1) no-crayfish vs. crayfish (NC vs. all other treatments), and (2) crayfish with no symbionts vs. crayfish with symbionts (0W vs. 3W + 6W + 12W).

Total macroinvertebrate abundance and taxon richness from the baskets were analyzed with general linear models (GLMs). In the Boone experiment, we analyzed total invertebrate abundance for the two sample dates using a two-way GLM ANOVA; the two factors were row and treatment. In the Clemson experiment, we used the R package nlme and the function *lme( )* to calculate a general linear mixed model (GLMM with a Gaussian error distribution) for the whole time series, with branchiobdellidan treatment and time as fixed effects, and with experimental unit as the random effect. For an examination of the last sampling date in the Clemson experiment, we used one-way ANOVA. In both experiments, we also examined the relationship between total basket invertebrates and sediment volume using linear regression.

In both experiments, differences in macroinvertebrate taxonomic composition across treatments were visualized using nonmetric multidimensional scaling (NMDS) with both the Bray-Curtis (B-C) distance metric and the Morisita-Horn (M-H) index. We used two metrics in tandem, because B-C captures both changes in relative abundances and in overall abundance, and M-H is density invariant and primarily detects differences in relative abundances irrespective of changes in total abundance (Jost et al. 2011). Both were applied using the *metaMDS ( )* function in the *vegan* package (Oksanen et al. 2016) of the R programming environment (version 3.5.2, R Development Core Team 2016). We used a scree plot of Stress vs. dimensionality to assess the appropriate number of axes in our solution. Correlations among total invertebrate abundance and the NMDS axes were also calculated. We used the *adonis( )* function in R's vegan package to perform permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to assess differences among treatments in the two studies. PERMANOVAs were conducted using both the B-C and M-H metrics and 999 matrix permutations. There was no significant row effect in the multivariate analyses of the Boone data so it was dropped from the analysis.

Table 1. Correlations of the six top-ranked taxa with ordination scores on the two ordination axes for the two experiments using the Bray-Curtis distance metric.

Rank	Boone experiment variable	r	Clemson experiment variable	r
NMDS axis 1				
1	Chironomidae	0.856	Chironomidae	−0.925
2	Elmidae	0.735	*Cordulegaster*	−0.566
3	*Corbicula*	0.732	Ceratopogonidae	−0.505
4	Oligochaeta	0.659	*Hexatoma*	−0.376
5	Gomphidae	0.624	*Hetaerina*	−0.322
6	Snails	0.600	Sphaeriidae	−0.321
NMDS axis 2				
1	Calopterygidae	0.679	Ceratopononidae	0.623
2	Polycentropidae	0.659	*Nigronia*	−0.501
3	Heptageniidae	0.654	Elmidae	−0.474
4	Ephemerellidae	0.549	Sphaeriidae	0.419
5	*Corbicula*	−0.426	*Paraleptophlebia*	0.401
6	Baetidae	0.347	*Stenelmis*	−0.401

Note: Boone data are from 30 August 2010 and Clemson data are from 22 September 2010.

Results

Boone experiment

There was a significant effect of treatment on total invertebrates on the final sample date, 30 August 2010 (Fig. 1A). Baskets in the 0W treatment had the highest macroinvertebrate abundances. The NC and 4W treatments had lower, and similar, abundances, and the lowest abundances were in the 12W treatment. There was no significant treatment effect on taxon richness ($F_{3,14}$ = 1.87, P = 0.181). There were significant effects of worm treatment on invertebrate community structure on 30 August 2010 for the NMDS with the B-C metric (Fig. 2A). We decided on a two-dimensional solution for this NMDS, which had a Stress of 0.048, suggesting that the ordination was an excellent representation of the dissimilarity structure. There was clear separation of treatments along Axis 1. Further, the 4W treatment was separated from the others on Axis 2, and the accompanying PERMANOVA indicated significant differences in community composition ($F_{3,18}$ = 2.14, P = 0.036). Several taxa, including chironomid midges, elmid beetles, the clam *Corbicula*, oligochaets, gomphid dragonflies, and snails, exhibited strong, positive correlations with Axis 1 (Table 1). Calopterygid damselflies, polycentropid caddisflies, and three families of mayflies were positively correlated with Axis 2, and *Corbicula* was negatively correlated with it. Total invertebrate abundance also exhibited a strong, positive correlation (r = 0.885) with Axis 1. The NMDS with the M-H index (Fig. 2B) was marginally significant ($F_{3,18}$ = 4.44, P = 0.060) and had a stress of 0.071. As in the previous analysis, there was some separation of treatments along Axis 1. There was greater separation on Axis 2. Crayfish treatments with worms, especially the 12W treatment, showed less overlap with the NC and 0W treatments on Axis 2. Chironomids and elmids exhibited the strongest correlations with Axis 1. Calopterygids, heptageniids, polycentropids, and ephemerellids were positively correlated with Axis 2, whereas *Corbicula* and chironomids were negatively correlated with it (Appendix S1: Table S1). Total invertebrate abundance exhibited a weaker correlation with Axis 1 in this analysis (r = 0.430).

There was a significant effect of branchiobdellidan treatment on crayfish bioturbation on 30 August (Table 2). The most sediment was recovered from tiles in the 0W treatment, intermediate amounts from the NC and 4W treatments, and the least amount from tiles in the 12W treatment. Similar patterns were found for basket sediment metrics (CPM, FPM, and gravel). The MANOVA for the three basket variables was significant for treatment (Wilks' lambda $F_{9,26}$ = 2.661, P = 0.025) and there was no significant effect of the row in which cages were located. The highest values for CPM and FPM were in the NC and 0W treatments, the 4W treatment was intermediate and the least was recovered from the 12W treatment (Table 2). In contrast, gravel

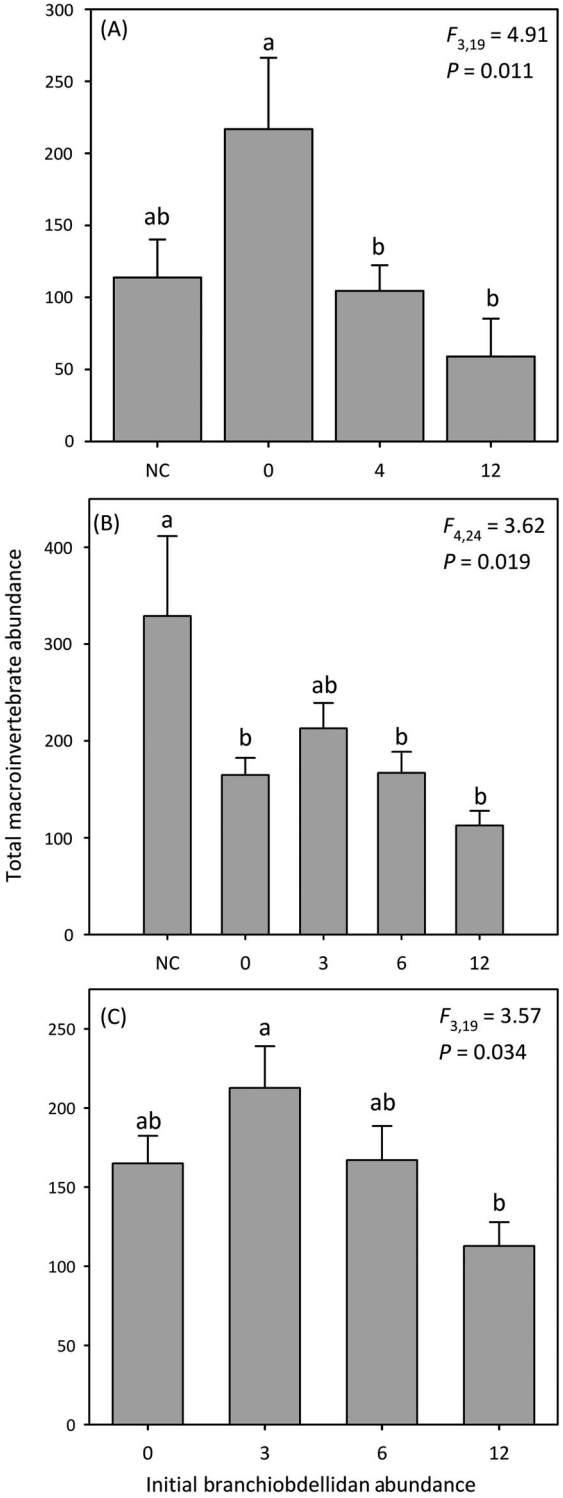

FIG. 1. Total invertebrate abundance by treatment for the two experiments on the final date of each experiment. (A) Boone experiment—30 August 2010. (B) Clemson experiment—22 September 2010. (C) Total invertebrate abundance from the Clemson experiment with only the branchiobdellidan treatments. Means (±1 SE) are shown. Letters above bars indicate the results of a post hoc Tukey honestly significant difference test.

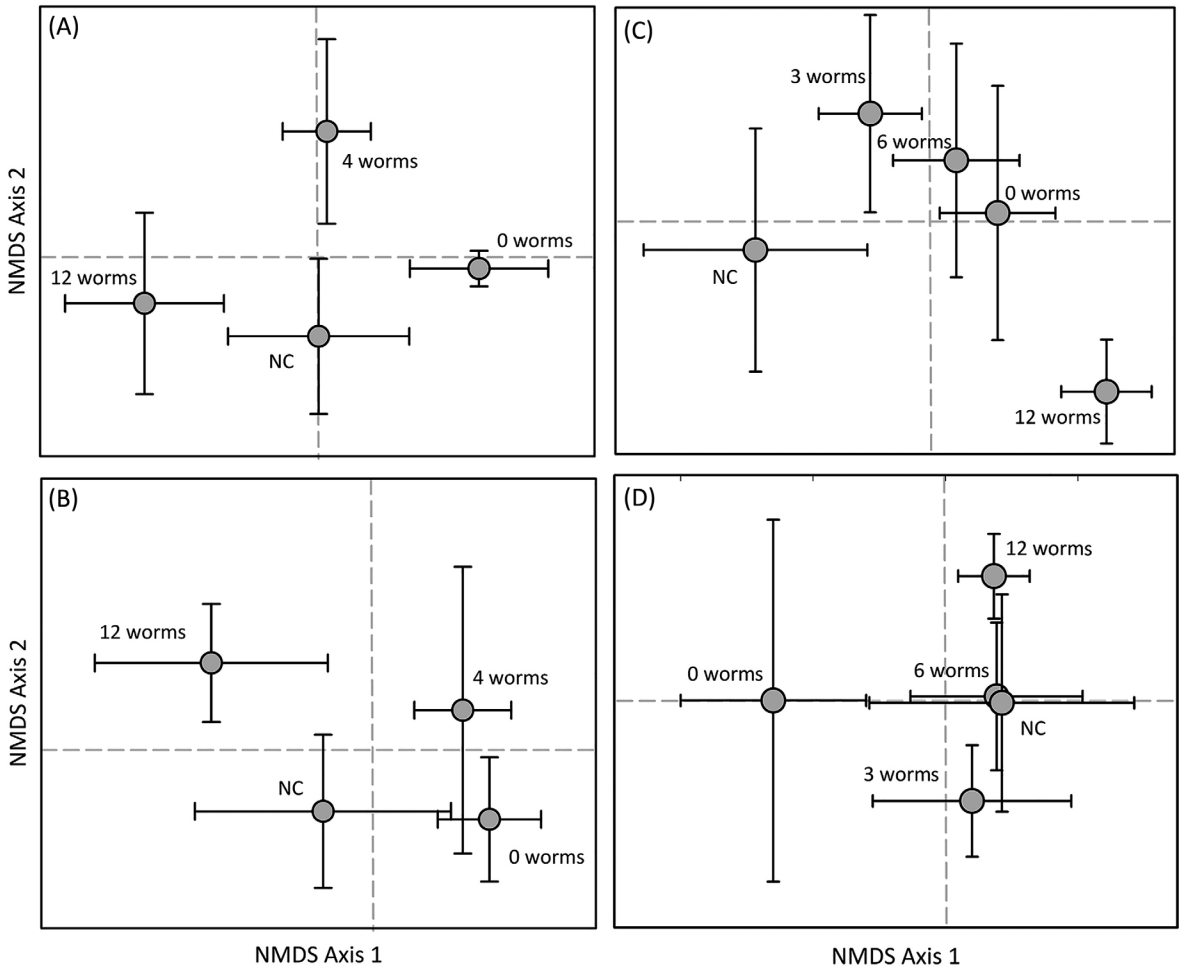

FIG. 2. Nonmetric multidimensional scaling (NMDS) ordinations for the two experiments. (A) Ordination using the Bray-Curtis metric for all taxa recovered from the baskets on 30 August 2010 in the Boone experiment. Two-dimensional stress = 0.048. PERMANOVA results: $F_{3,18}$ = 2.14,P = 0.036. (B) Ordination using the Morisita-Horn metric for all taxa recovered from the baskets on 30 August 2010 in the Boone experiment. Two-dimensional stress = 0.071. PERMANOVA results: $F_{3,18}$ = 4.44, P = 0.060. (C) Ordination using the Bray-Curtis metric for all taxa recovered from baskets on 22 September 2010 in the Clemson experiment. Two-dimensional stress = 0.067. PERMANOVA results: $F_{4,24}$ = 2.24, P = 0.032. (D) Ordination using the Morisita-Horn metric for all taxa recovered from baskets on 22 September 2010 in the Clemson experiment. Two-dimensional stress = 0.081. PERMANOVA results: $F_{4,24}$ = 1.13, P = 0.402. Means (±1 SD) are shown.

abundance showed the opposite pattern with no gravel in the NC baskets and increasing gravel abundance with increasing initial worm abundance (Table 2). Significant univariate effects were observed for CPM and gravel but not FPM. The regression for total macroinvertebrates on tile sediment volume was positive and significant ($F_{1,18}$ = 39.91, P < 0.0001, r^2 = 0.689, slope = 1.236). Regressions of total invertebrate abundance on CPM and FPM volume were also positive and significant (CPM: $F_{1,20}$ = 70.32, P < 0.0001, r^2 = 0.779, slope = 0.4856; FPM: $F_{1,21}$ = 13.86, P = 0.001, r^2 = 0.398, slope = 0.0631). We also regressed total invertebrate abundance on total basket sediments (CPM + FPM) to provide an analysis comparable to the South Carolina results (Fig. 3A). This regression was also significant ($F_{1,21}$ = 18.48, P < 0.0001, r^2 = 0.468). The two treatments in which crayfish had worms were closer to the origin, that is, lower sediment volumes and lower total invertebrate abundance, whereas the 0W and NC treatments covered a broader range. A similar pattern of treatment distribution was observed in the regressions for total invertebrates on CPM and FPM individually.

Crayfish growth was highest in the 4W treatment, and worm numbers in the 12W treatment had decreased on the crayfish by the end of the experiment (Table 3). One 0W crayfish had picked up a worm. There were no significant treatment effects for total macroinvertebrates, community composition, or sediment volume on 22 July 2010 (Appendix S1: Fig. S1).

TABLE 2. Results of the individual ANOVAs for tile sediment volume as well as coarse particulate matter (CPM), fine particulate matter (FPM), and gravel from the baskets (Boone experiment).

	Treatment					
Variable	NC	0W	4W	12W	*F*	*P*
Tile sediment	48.0^{AB} (26.2)	107.3^{A} (33.2)	34.4^{AB} (11.8)	7.4^{B} (1.3)	3.92	**0.040**
CPM	196.8^{AB} (64.1)	319.3^{A} (90.9)	125.8^{AB} (34.6)	76.6^{B} (11.3)	3.64	**0.042**
FPM	1,392.4 (539.9)	1,612.2 (372.3)	975.7 (350.4)	632.0 (182.7)	1.48	0.264
Gravel	0.0^{B} (0.0)	4.0^{AB} (2.6)	7.5^{AB} (2.9)	11.2^{A} (4.5)	3.65	**0.037**

Notes: *P* values in bold are significant results from the individual ANOVAs. Shown are means (±1 SE). All values for sediment volume for tiles, CPM and FPM are in milliliters. Values for gravel are for number of pieces of gravel. The *F* and *P* values shown are for the individual ANOVAs. Treatment means were compared using a Tukey's test; means with the same letter are not significantly different. Treatment refers to the no-crayfish treatment (NC) and the three worm treatments (0 worms [0W], 4 worms [4W], and 12 worms [12W]).

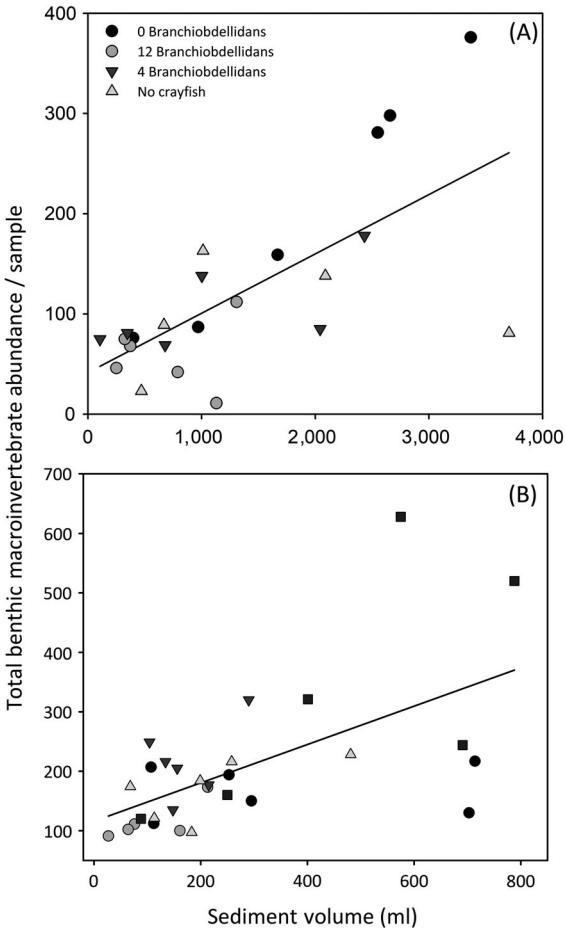

FIG. 3. Regressions of total invertebrate (TI) abundance on total sediment volume (TSV) for the final dates for each of the two experiments. (A) Boone experiment—30 August 2010. The regression was significant ($F_{1,21}$ = 18.48, P < 0.0001) and r^2 = 0.468. The equation for the line was TI = 41.46 + 0.05923 TSV (ml). TSV here includes both coarse particulate matter and fine particulate matter. (B) Clemson experiment—22 September 2010. The regression was significant ($F_{1,27}$ = 14.78, P = 0.0007) and r^2 = 0.350. The equation for the line was TI = 116.02 + 0.322 TSV (ml).

Clemson experiment

There was a significant effect of treatment on macroinvertebrate abundance for the final sampling date of the experiment ($F_{4,24}$ = 3.62, P = 0.019; Fig. 1B). The highest abundance was in the NC treatment and the lowest abundance occurred in the 12W treatment, which had over 60% fewer macroinvertebrates than the NC treatment. The 0W, 3W, and 6W treatments were all intermediate to those extremes, with invertebrate abundances around 50–60% of that observed in the NC treatment group. When the effects of branchiobdellidan treatment were analyzed without the NC treatment, abundance in the 12W treatment level was significantly lower than that in the 3W treatment, with 0W and 6W being intermediate (Fig. 1C). There were also significant effects of branchiobdellidan treatment on macroinvertebrate abundance when examined as a whole time series of five samples (Appendix S2: Fig. S1). These effects were not apparent through Day 48 of the experiment but emerged on Days 72 and 95 (branchiobdellidan treatment, $F_{4,25}$ = 2.87, P = 0.044). Taxon richness followed a similar trend, but there were no significant differences among treatment groups ($F_{4,24}$ = 1.37, P = 0.28).

There were differences in macroinvertebrate community composition on the final day of this experiment for the NMDS with the B-C metric, primarily driven by the NC and 12W groups diverging significantly from the 0W, 3W, and 6W groups (Fig. 2C). The ordination solution was very good with a Stress of 0.067, and PERMANOVA indicated that these treatment groups had diverged significantly ($F_{4,24}$ = 2.24, P = 0.032). Several taxa, including two dipteran taxa (Chironomidae, Ceratopogonidae), and dragonfly larvae in the genus *Cordulegaster* were negatively correlated with Axis 1 (Table 1). Total invertebrate abundance was also negatively correlated (r = −0.938) with Axis 1. Correlations with Axis 2 were weaker; the strongest correlations were with Ceratopogonidae and megalopterans of the genus *Nigronia*. The NMDS with the M-H metric (Fig. 2D) had a stress of 0.081 but was not significant

TABLE 3. Crayfish growth (percent change in mass) and final worm numbers (total number per crayfish) for the two experiments. Growth data for the Boone experiment are from 13 September (Day 81); worm data are from 3 October (Day 103). Growth and worm data for the Clemson experiment are from 8 September (Day 81). Means (±1 SE) are presented in the table.†

	Treatment				
	0 worms	3 worms	4 worms	6 worms	12 worms
Boone experiment					
Growth (%)	10.7 (4.0)		24.1 (7.3)		5.5 (2.3)
Final worm number	0.17 (0.17)		4.40 (1.17)		5.25 (2.70)
Clemson experiment					
Growth	6.3 (7.0)	12.3 (6.8)		36.0 (7.7)	1.0 (0.8)
Final worm number	0.98 (0.58)	1.33 (0.32)		3.5 (0.34)	4.83 (1.45)

†For additional discussion of these results see Brown et al. (2012).

(PERMANOVA $F_{4,24} = 1.13$, $P = 0.402$). Interestingly, the 0W treatment separated out from all the other treatments on Axis 1 but there was no separation of the treatments on Axis 2. The strongest correlations with Axis 1 were with chironomids, *Cordulegaster*, the stonefly *Tallaperla* and the mayfly *Danella*. The strongest correlations with Axis 2 were with ceratopogonid and tanypodinae midges, the mayfly *Paraleptophlebia* and *Nigronia* (Appendix S2: Table S1). Total invertebrate abundance exhibited a weaker correlation with Axis 1 in the analysis with the M-H index ($r = 0.532$). There were also strong indications that branchiobdellidan symbionts affected temporal community dynamics (Appendix S2: Fig. S2). The temporal trajectories of macroinvertebrate communities over the 95 d of the experiment differed in only subtle ways in the NC, 0W, 3W, and 6W groups, but the 12W group had a completely different dynamic throughout the course of the experiment; beginning with the first sampling date, the 12W communities differed both in terms of composition on a single date, and in terms of overall temporal trajectory.

There were significant effects of symbiont treatment on sediment volume on Days 72 and 95 (Fig. 4). On Day 72, an ANOVA of sediment volume across treatments was marginally significant ($F_{4,25} = 2.39$, $P = 0.078$), and the linear contrast comparing all crayfish treatments vs. the no-crayfish treatment showed that the no-crayfish treatment had significantly higher sediment volume ($t = 2.99$, $P = 0.0061$). On Day 95, one-way ANOVA did not detect differences among treatments ($F_{4,25} = 1.93$, $P = 0.14$) but the symbiont vs. no-symbiont contrast revealed significantly higher sediment volume in the no-symbiont group ($t = -2.63$, $P = 0.014$). There were no significant effects of the symbiont treatment group on sediment accumulation across the full time series (Fig. 4; worm treatment $F_{4,25} = 1.62$, $P = 0.20$; treatment × time interaction $F_{4,145} = 0.63$, $P = 0.64$).

As in the Boone experiment, there was a positive linear relationship between sediment volume and total invertebrate abundance (Fig. 3B; $F_{1,27} = 14.78$, $r^2 = 0.35$, $P = 0.0007$). There was a similar pattern of

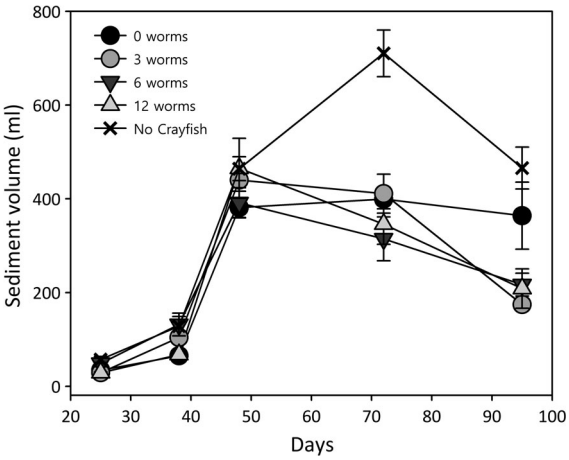

FIG. 4. Time series for sediment data from the South Carolina experiment. Means (±1 SE) are shown.

treatment groups in this regression with the three symbiont treatments (3W, 6W, 12W) all clustered in the lower left region of the figure—that is, low sediment and low macroinvertebrates—and the NC and 0W groups spread across the range of this relationship.

Crayfish growth was highest in the 6W treatment; worm numbers had declined on crayfish by the end of the experiment (Table 3). One 0W crayfish had picked up a worm.

DISCUSSION

Here we report the first evidence that a mutualism involving two freshwater taxa strongly influenced freshwater community structure and the accumulation of inorganic sediment and organic matter. Our data suggest that the impacts of the crayfish on community structure were a combination of consumptive and nonconsumptive (e.g., bioturbation) effects, and that crayfish impacts were modulated by their symbiotic branchiobdellidans. These effects were observed in two experiments despite differences in host and symbiont species as well as in the two locations that led to differences in

macroinvertebrate abundances and in the amount of sediment accumulation.

Branchiobdellidan presence and density influenced the effect of the crayfish on macroinvertebrate abundance and community structure. Crayfish with moderate worm densities grew faster than the 0W controls and those stocked with high worm densities (see also Brown et al. 2012). These differences in growth likely underlie some of the effects on macroinvertebrates reported here. The faster growth rate of crayfish with moderate worm loads, that is, mutualistic effect of symbionts, suggests that these crayfish had a strong impact on macroinvertebrates because of increased consumption relative to control crayfish. This inference is supported by the reduced abundance of macroinvertebrates in both experiments in treatments in which crayfish were stocked with moderate worm numbers compared to the 0W controls. Increased consumption would also result in increased crayfish movement as they searched for macroinvertebrate prey, which would account for the reduced sediment volumes by the end of the two experiments. Macroinvertebrate abundances and sediment volumes were the lowest in the 12W treatment in both experiments, possibly because of negative effects of high worm densities on their hosts early in the experiment. At the 12W density, the worms extensively damaged the crayfish gills which could have reduced crayfish energetic reserves (Brown et al. 2012). Crayfish actively remove worms when they exceed mutualistic levels (Farrell et al. 2014, Skelton et al. 2014, Creed and Brown 2018) and they did so in these experiments. As the experiment progressed and worm numbers declined, the damaged gills healed. The lower sediment volumes and macroinvertebrate abundances in the 12W treatment suggest that these crayfish were most likely trying to increase their energetic reserves prior to the fall molt.

Crayfish affected sediment accumulation in the two systems, although the effect was more straightforward in the Boone experiment. In Boone, crayfish significantly reduced sediment accumulation on the surface of the tiles and interstitial CPM and FPM later in the experiment. The positive regressions between tile sediment, CPM and FPM volume, and macroinvertebrate abundance suggests that a significant proportion of the impact of crayfish on macroinvertebrates may have also been due to bioturbation effects as macroinvertebrate numbers were lower when sediment was reduced. In the Clemson experiment, a storm beginning around Day 42 of the experiment resulted in high levels of sediment deposition, increasing sediment volume by about 400% over a 10-d period (Fig. 4). Following this event, there was divergence among treatment groups. On the first sampling date after the storm, the presence of crayfish had a very strong effect on sediment volume. That effect decreased over the next 2 wk but did not disappear. During that same period, an effect of symbionts emerged, with symbiont treatments having significantly lower sediment volumes than treatment groups without symbionts. On the last sampling date, there was also a strong relationship between macroinvertebrate abundances and sediment volume, with a clear tendency for the three branchiobdellidan treatments (3W, 6W, 12W) to have lower sediment volumes and lower macroinvertebrate abundances.

The relationship between macroinvertebrate abundance and sediment levels has been observed in previous studies of crayfish effects. Creed and Reed (2004) reported that chironomids that were too small to be captured and consumed by crayfish still declined in the presence of crayfish; they suggested that this decline was due to increased bioturbation by crayfish and that these smaller chironomids declined because there was less sediment for them to inhabit. Helms and Creed (2005) reported a similar positive relationship between chironomids and sediment volume. Usio and Townsend (2004) conducted an experiment designed specifically to separate macroinvertebrate responses to crayfish bioturbation from consumption and found that both mechanisms were important in explaining community responses. Our results, like those of Usio and Townsend (2004), point to both consumption and bioturbation being important mechanisms through which crayfish can affect macroinvertebrate abundance and community structure, and both of these mechanisms were also affected by branchiobdellidan symbionts.

The effect of this mutualism on macroinvertebrate community structure was detectable in both studies with the NMDS with B-C metric despite differences in habitat characteristics, species composition, and hydrology. Results of the second NMDS with the M-H metric were mixed. There was a marginally significant effect for the Boone experiment, but the effect was lost for the Clemson experiment. When the NMDS results are considered in conjunction with the total invertebrate results, it appears that there was an effect on both macroinvertebrate abundance and community composition in the Boone experiment. In the Clemson experiment, the lack of a significant effect with M-H index suggests that the B-C results largely reflected a change in macroinvertebrate abundance with treatment. This conclusion is supported by the much lower correlations for total invertebrate abundance with Axis 1 observed in both experiments when we used the M-H metric. Although changes in abundance are an important community metric, it is interesting that the compositional effect disappeared in the Clemson results. One factor that might have contributed to this difference was the flood that occurred at the Clemson site approximately 1 month before the end of the experiment. There may not have been enough time for a compositional signal to develop. This conclusion is supported by the results of the Boone experiment in which it took 2 months for the compositional changes to occur. Our results suggest that this mutualism could influence freshwater communities throughout the Holarctic region where crayfish and branchiobdellidans are present. However, in systems

with higher frequency and unpredictability of disturbances such as floods, the impact of this mutualism will likely decline, as will the impact of other biotic interactions (Poff and Ward 1989, Creed 2006).

Although our study provides the first evidence of a strong effect of a mutualism on freshwater community structure, there is growing evidence that other positive interactions may play important roles in some freshwater communities (Silknetter et al. 2020). For example, bluehead chub (*Nocomis leptocephalus*) are a key species in many southeastern U.S. streams. These fish build large stone nests that are utilized by many other fish species, primarily other leuciscids, as a spawning site (Johnston 1994, Peoples and Frimpong 2016). The presence and abundance of these nest associates in a stream are directly related to the presence of bluehead chub. At least two of these nest associates have beneficial effects on bluehead chub reproduction (Peoples and Frimpong 2016, Silknetter et al. 2019). If other associates have positive effects on bluehead chubs, and other chubs in the genus *Nocomis*, then this mutualism could also have important effects on stream community structure.

Our primary goal was to assess the effect of the mutualism between crayfish and their worms on community structure. However, our 12W treatment provided us the opportunity to assess how the effect of crayfish could change if worms reached a parasitic density, even if only temporarily. Crayfish stocked with high densities of worms (12W treatment) gained less mass than controls (Brown et al. 2012). These 12W crayfish also affected macroinvertebrate community structure and reduced sediment volume. The effects of crayfish in our experiments were because of changes in crayfish behavior and not changes in crayfish density. Similarly, Reisinger and Lodge (2016) found that when crayfish were infected with a parasite, their effect on their community was altered, again because of a change in behavior. Other studies have found strong impacts of parasitic symbioses on benthic invertebrate communities. The microsporidian parasite *Cougerdella* infects and kills a dominant, grazing caddisfly (*Glossosoma*; Kohler and Wiley 1997). The loss of *Glossosoma* from Michigan streams resulted in dramatic changes in community structure (Kohler and Wiley 1997). When these results are considered in conjunction with ours, it seems clear that parasitic symbionts can exert strong effects in freshwater systems via their impact on ecologically important hosts. The impacts observed by Kohler and Wiley (1997) were due to declines in *Glossosoma* density, whereas in our study and that of Reisinger and Lodge (2016) they were the result of changes in crayfish consumption and bioturbation rates. Given the strong effects observed by us, Reisinger and Lodge (2016), and Kohler and Wiley (1997), impacts of other parasitic symbionts on their hosts may be a fruitful area for future research in freshwater ecology. Some branchiobdellidans appear to be strictly parasitic, so they could have strong impacts on freshwater systems when they weaken their crayfish hosts. Further, although potential community and ecosystem effects of introduced crayfish have garnered substantial interest (Lodge and Lorman 1987, Lodge et al. 1994) the introduction of their associated symbionts should also be of concern, because these symbionts may affect behavior or density of native hosts. The introduction of crayfish plague to Eurasia is a clear example of this phenomenon (Holdich et al. 2009).

In marine and terrestrial communities, the strong indirect effects of symbionts on their communities are generally via their impacts on hosts that are persistent, foundation species (sensu Dayton 1972) such as corals and plants. These foundation species provide habitat and resources for other members of their communities, and their loss can result in large changes in species diversity. Although there are taxa (e.g., macrophytes and macroalgae) that may act as foundation species in freshwater systems, there do not appear to be comparable, persistent foundation species with the possible exception of freshwater mussels and the beds they create (Vaughn et al. 2008). Further, there is no evidence at present of any mutualisms involving freshwater foundation species. As a result, the probability that symbioses involving foundation species affect community structure in freshwater systems seems low. Rather, it seems likely that strong indirect effects of symbionts on freshwater communities will occur when they affect mobile taxa that can function as keystone species or ecosystem engineers. Crayfish fill both of those roles (Creed 1994, Creed and Reed 2004, Usio and Townsend 2004), as do other crustaceans and various fish species (Power et al. 1985, Flecker 1996, Crowl et al. 2001), so it is likely that it will be through these species that symbiont effects may be exerted. Thus, freshwater communities may not differ from marine and terrestrial systems with respect to the important roles that mutualisms may play in these communities. The difference may lie in the type of host species involved and the mechanisms by which these hosts influence their communities.

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Literature Cited

Albertson, L. K., and M. D. Daniels. 2018. Crayfish ecosystem engineering effects on riverbed disturbance and topography are mediated by size and behavior. Freshwater Science 37:836–844.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32–46.

Bronstein, J. L., R. Alaracon, and M. Geber. 2006. The evolution of plant–insect mutualisms. New Phytologist 172:412–428.

Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150:28–35.

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. R. 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.

Creed, R. P. 1994. Direct and indirect effects of crayfish grazing in a stream community. Ecology 75:2091–2103.

Creed, R.P., Jr. 2000. Is there a new keystone species in North American lakes and rivers? Oikos 91:405–408.

Creed, R. P. 2006. Predator transitions in stream communities: a model and evidence from field studies. Journal of the North American Benthological Society 25:533–544.

Creed, R. P., and B. L. Brown. 2018. Multiple mechanisms can stabilize a freshwater mutualism. Freshwater Science 37:760–768.

Creed, R. P., J. Lomonaco, M. J. Thomas, A. Meeks, and B. L. Brown. 2015. Reproductive dependence of a branchiobdellidan worm on its crayfish host: confirmation of a mutualism. Crustaceana 88:385–396.

Creed, R. P., and J. M. Reed. 2004. Ecosystem engineering by crayfish in a headwater stream community. Journal of the North American Benthological Society 23:224–236.

Creed, R. P., A. Taylor, and J. R. Pflaum. 2010. Bioturbation by a dominant detritivore in a headwater stream: litter excavation and effects on community structure. Oikos 119:1870–1876.

Crowl, T. A., W. H. McDowell, A. P. Covich, and S. L. Johnson. 2001. Freshwater shrimp effects on detrital processing and nutrients in a tropical headwater stream. Ecology 82:775–783.

Dayton, P. 1972. Toward an understanding of community resilience and the potential effects of enrichments to the benthos at McMurdo Sound, Antarctica. Pages 81–96 *in* B. C. Parker, editor. Proceedings of the Colloquium on Conservation Problems in Antarctica. Allen Press. Lawrence, Kansas, USA.

Dodds, W. K., and M. R. Whiles. 2010. Freshwater ecology. Second edition. Academic Press, San Diego, California, USA.

Farrell, K. J., R. P. Creed, and B. L. Brown. 2014. Preventing overexploitation in a mutualism: partner regulation in the crayfish-branchiobdellid symbiosis. Oecologia 174:501–510.

Flecker, A. S. 1996. Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology 77:1845–1854.

Fortino, K., and R.P. Creed, Jr. 2007. Abiotic factors, competition or predation: what determines the distribution of young crayfish in a watershed?. Hydrobiologia 575:301–314.

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.

Hay, M. E., J. D. Parker, D. E. Burkepile, C. C. Caudill, A. E. Wilson, Z. P. Hallinan, and A. D. Chequer. 2004. Mutualisms and aquatic community structure: the enemy of my enemy is my friend. Annual Review of Ecology, Evolution and Systematics 35:175–197.

Helms, B. S., and R. P. Creed. 2005. The effects of 2 coexisting crayfish on an Appalachian river community. Journal of the North American Benthological Sociey 24:113–122.

Hoeksema, J. D., et al. 2010. A meta-analysis of context dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13:394–407.

Holdich, D. M., J. D. Reynolds, C. Souty-Grosset, and P. J. Sibley. 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. Knowledge and Management of Aquatic Ecosystems 394–395:11.

Holomuzki, J. R., J. W. Feminella, and M. E. Power. 2010. Biotic interactions in freshwater benthic habitats. Journal of the North American Benthological Society 29:220–244.

James, J., K. E. Davidson, R. Hunt, and J. Cable. 2017. Assessing the invasion potential of non-native branchiobdellidans: experimental studies of survival, reproduction and competition. Knowledge and Management of Aquatic Ecosystems 418:35.

Johnston, C. E. 1994. Nest association in fishes: evidence for mutualism. Behavioral Ecology and Sociobiology 35:379–383.

Jones, C. G., J. H. Lawton, and M. Shachak. 1994. Organisms as ecosystem engineers. Oikos 69:373–386.

Jost, L., A. Chao, and R. L. Chazdon. 2011. Compositional similarity and beta diversity. Pages 66–83 *in* A. Magurran and B. J. McGill, editors. Biological diversity: frontiers in measurement and assessment. Oxford University Press, Oxford, UK.

Kohler, S. L., and M. J. Wiley. 1997. Pathogen outbreaks reveal large-scale effects of competition in stream communities. Ecology 78:2164–2176.

Lamberti, G. A., and V. H. Resh. 1983. Stream periphyton and insect herbivores: an experimental study of grazing by a caddisfly population. Ecology 64:1124–1135.

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.

Lodge, D. M., M. W. Kershner, J. E. Aloi, and A. P. Covich. 1994. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. Ecology 75:1265–1281.

Lodge, D. M., and J. G. Lorman. 1987. Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. Canadian Journal of Fisheries and Aquatic Science 44:591–597.

McPeek, M. A. 1990. Determination of species composition in the *Enallagma* damselfly assemblages of permanent lakes. Ecology 71:1714–1726.

Merritt, R. W., K. W. Cummins, and M. Berg. 2008. An introduction to the aquatic insects of North America. Fourth edition. Kendall-Hunt Publishing Co., Dubuque, Iowa, USA.

Oksanen, J., et al. 2016. Vegan: community ecology package, R package version 2.4-0. https://cran.r-project.org/package=vegan

Paine, R. T. 1969. A note on trophic complexity and community stability. American Naturalist 103:91–93.

Peoples, B. K., and E. A. Frimpong. 2016. Context-dependent outcomes in a reproductive mutualism between two freshwater fish species. Ecology and Evolution 6:1214–1223.

Poff, N. L., and J. V. Ward. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. Canadian Journal of Fisheries and Aquatic Sciences 46:1805–1818.

Power, M. E., W. J. Matthews, and A. J. Stewart. 1985. Grazing minnows, piscivorous bass, and stream algae: dynamics of a strong interaction. Ecology 66:1448–1456.

Pringle, E. G., and D. M. Gordon. 2013. Protection mutualisms and the community: geographic variation in an ant–plant symbiosis and the consequences for herbivores. Sociobiology 60:242–251.

R Development Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org

Reisinger, L. S., and D. M. Lodge. 2016. Parasites alter freshwater communities in mesocosms by modifying invasive crayfish behavior. Ecology 97:1497–1506.

Rice, S. P., M. F. Johnson, K. Mathers, J. Reeds, and C. Extence. 2016. The importance of biotic entrainment for base flow fluvial sediment transport. Journal of Geophysical Research: Earth Surface 121:890–906.

Silknetter, S., R. P. Creed, B. L. Brown, E. Frimpong, J. Skelton, and B. K. Peoples. 2020. Positive biotic interactions in freshwaters: a review and research directive. Freshwater Biology 65:811–832.

Silknetter, S., Y. Kanno, K. L. Kanapeckas Metris, E. Cushman, T. L. Darden, and B. K. Peoples. 2019. Mutualism or parasitism: Partner abundance affects host fitness in a fish reproductive interaction. Freshwater Biology 64:175–182.

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., S. Doak, M. Leonard, R. P. Creed, and B. L. Brown. 2016. The rules for symbiont community assembly change along a mutualism–parasitism continuum. Journal of Animal Ecology 85:843–853.

Skelton, J., K. J. Farrell, R. P. Creed, B. W. Williams, C. Ames, B. S. Helms, J. Stoeckel, and B. L. Brown. 2013. Servants, scoundrels, and hitchhikers: our current understanding of the complex interactions between crayfish and their ectosymbiotic worms (Branchiobdellida). Freshwater Science 32:1345–1357.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. Second edition. W.H. Freeman, New York, New York, USA.

Statzner, B., O. Peltret, and S. Tomanova. 2003. Crayfish as geomorphic agents and ecosystem engineers: effect of a biomass gradient on baseflow and flood-induced transport of gravel and sand in experimental streams. Freshwater Biology 48:147–163.

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

Trager, M. D., S. Bhotika, J. A. Hostetler, G. V. Andrade, M. A. Rodriguez-Cabal, C. S. McKeon, C. W. Osenberg, and B. M. Bolker. 2010. Benefits for plants in ant–plant protective mutualisms: a meta-analysis. PLoS One 5:e14308.

Usio, N., and C. R. Townsend. 2004. Roles of crayfish: consequences of predation and bioturbation for stream invertebrates. Ecology 85:807–822.

Vaughn, C. C., S. J. Nichols, and D. E. Spooner. 2008. Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society 27:409–423.

Wellborn, G. A., D. K. Skelly, and E. E. Werner. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27:337–363.

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

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