

Servants, scoundrels, and hitchhikers: current understanding of the complex interactions between crayfish and their ectosymbiotic worms (Branchiobdellida)

Authors: Skelton, James, Farrell, Kaitlin J., Creed, Robert P., Williams, Bronwyn W., Ames, Catlin, et al.

Source: Freshwater Science, 32(4) : 1345-1357

Published By: Society for Freshwater Science

URL: <https://doi.org/10.1899/12-198.1>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Servants, scoundrels, and hitchhikers: current understanding of the complex interactions between crayfish and their ectosymbiotic worms (Branchiobdellida)

James Skelton^{1,6}, Kaitlin J. Farrell^{2,7}, Robert P. Creed^{3,8},
Bronwyn W. Williams^{4,9}, Catlin Ames^{5,10}, Brian S. Helms^{5,11},
James Stoekel^{5,12}, AND Bryan L. Brown^{1,13}

¹ Department of Biological Sciences, Virginia Polytechnic Institute and State University,
2125 Derring Hall, Blacksburg, Virginia 24061 USA

² Odum School of Ecology, University of Georgia, 140 East Green Street, Athens, Georgia 30602 USA

³ Department of Biology, Appalachian State University, 575 Rivers Street, Boone,
North Carolina 28608 USA

⁴ Department of Zoology, Southern Illinois University, 1125 Lincoln Drive, Carbondale,
Illinois 62901 USA

⁵ Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, Alabama 36849 USA

Abstract. Astacoidean crayfishes serve as hosts to obligate ectosymbiotic annelids called branchiobdellidans. Branchiobdellidans can act either as mutualistic cleaners or as ectoparasites and can have strong effects on crayfish growth and survivorship. This potentially vital aspect of crayfish biology has gone largely unexplored until recently. We reviewed the current state of knowledge regarding this symbiosis and examined factors that contribute to variability in the effects of branchiobdellidans on crayfish. We show that branchiobdellidans affect crayfish in various ways depending on branchiobdellidan species, abundance, and ecological context. We also discuss evidence for regulatory controls that crayfish exert over their symbionts and symbiont–host preferences. Last, we evaluate the utility and challenges of using the crayfish–branchiobdellidan association as a model system for ecological and evolutionary research and point to promising areas for future study. Further investigations of the complex interactions between crayfish and their ectosymbionts will greatly advance the field of crayfish biology and offer many exciting opportunities for the study of symbioses.

Key words: symbiosis, mutualisms, cleaning symbiosis, context dependence, partner choice, partner regulation.

Crayfish are well known inhabitants of streams, rivers, lakes, and ponds, and have drawn the attention of many aquatic biologists for several important reasons. Crayfish are keystone consumers that have strong effects on plant and animal community structure

through direct and indirect trophic interactions (Hart 1992, Creed 1994, Momot 1995, Charlebois and Lamberti 1996, Parkyn et al. 1997). Many species are ecosystem engineers that entrain sediments while feeding and excavating burrows and alter in-stream organic-matter processing by shredding coarse particulate organic matter (Parkyn et al. 1997, Statzner et al. 2000, Creed and Reed 2004, Usio and Townsend 2004, Zhang et al. 2004, Brown and Lawson 2010), particularly highly recalcitrant material, such as *Rhododendron* sp. (Huryn and Wallace 1987, Schofield et al. 2001). Moreover, several invasive species of crayfish threaten biodiversity in ecosystems around the globe (Gherardi 2007), and many more crayfish species are threatened by extinction from multiple anthropogenic factors

⁶ E-mail addresses: skelto3@vt.edu

⁷ kfarrell@uga.edu

⁸ creedrp@appstate.edu

⁹ bwwilliams@siu.edu

¹⁰ catlin.ames@gmail.com

¹¹ helmsbs@auburn.edu

¹² jas0018@auburn.edu

¹³ stonefly@vt.edu

(Taylor et al. 2007). Thus, crayfish have been the focus of a large body of ecological and organismal research.

An underappreciated aspect of crayfish biology is that the crayfish body is not simply a single organism, but rather a complex consortium of microbial and metazoan taxa. The crayfish exoskeleton can host a wide diversity of organisms including bacteria, clusters of stalked ciliates, sessile rotifers, annelid worms, flatworms, and other crustaceans (review by Edgerton et al. 2002). Many of these organisms may be only incidental associates, but others are obligate ectosymbionts (Hobbs et al. 1967, Gelder and Rowe 1988, Gelder 2010). Branchiobdellidans, members of an order of clitellate annelids, are obligate ectosymbionts primarily of astacoidean crayfishes. This association is common across much of the Holarctic, including the Euro-Mediterranean region, East Asia, and North and Central America (Gelder 1999b, Fard and Gelder 2011). Historically, branchiobdellidans were considered ectocommensals that had no significant effects on their hosts (e.g., McManus 1960, Young 1966, Bishop 1968), although some were described as parasites with negative host effects (Holt 1963, Hobbs et al. 1967, Grabda and Wierzbicka 1969). More recent empirical studies have shown that crayfish and branchiobdellidans maintain a complex and variable association that potentially benefits both the host and its symbiotic worms (Brown et al. 2002, 2012, Brown and Creed 2004, Lee et al. 2009).

Branchiobdellidans benefit from their associations with crayfish in a variety of ways. They graze on material, such as diatoms, bacteria, and protozoans, that accumulates on their host's exoskeleton or in the host gill chamber (Jennings and Gelder 1979, Gale and Proctor 2009), and a small number of species may consume host tissue (Grabda and Wierzbicka 1969). Most branchiobdellidans obtain their nutrition from material acquired on the exoskeleton of the host, but individuals also consume algae and metazoans from nearby substrate (Jennings and Gelder 1979, Gelder 2010) and, consequently, can survive long periods without a host, at least in vitro (Penn 1959, Young 1966). Despite their ability to survive separated from their host, branchiobdellidans are generally considered obligate ectosymbionts because, to the best of our knowledge, they reproduce only on a live crustacean (Young 1966, Gelder 2010; see Woodhead 1950 for a possible exception), and therefore, are reproductively dependent on their hosts. Current knowledge of branchiobdellidan reproductive biology is summarized in Gelder (2010). Only one account has been published of a possibly "free-living" branchiobdellidan, but it seems probable that this observation was of worms that were separated from their hosts before collection (Holt 1973a).

Historically, the effects of branchiobdellidans on their hosts received little attention, and claims of commensal or parasitic interactions were largely speculative (e.g., McManus 1960, Young 1966, Hobbs et al. 1967, Hobbs and Lodge 2010). Recent experimental work has demonstrated that branchiobdellidans may exert both positive and negative effects on their hosts and, in some cases, affect crayfish growth and survivorship (Brown et al. 2002, 2012, Lee et al. 2009). Therefore, this complex interaction is a potentially important aspect of crayfish biology. Because of the influential role that crayfish play in aquatic systems, branchiobdellidans may have indirect effects on local communities and ecosystem processes via their direct effects on crayfish. Field and laboratory experiments have provided methods for manipulative experiments using the crayfish–branchiobdellidan association and have demonstrated utility of this symbiosis for addressing more general ecological and evolutionary questions (Brown et al. 2002, 2012, Brown and Creed 2004, Lee et al. 2009). Thus, the objectives of our contribution are to: 1) bring attention to the crayfish–branchiobdellidan association as an important yet understudied facet of crayfish biology and ecology and 2) illustrate the utility of the crayfish–branchiobdellidan association as a model system for addressing general ecological and evolutionary questions. We summarize the current state of knowledge of the crayfish–branchiobdellidan association and briefly discuss on-going research efforts and promising future directions for work using the crayfish–branchiobdellidan association as a model system for testing theory concerning the ecology, evolution, and maintenance of symbiotic interactions.

Pairwise Interactions between Crayfish and Branchiobdellidans

Early researchers studying branchiobdellidans assumed most species were commensals of crayfish (e.g., Goodnight 1941, McManus 1960, Young 1966, Bishop 1968), though some species were thought to be parasites largely because of their propensity for inhabiting the host's gill chamber (Holt 1963, Hobbs et al. 1967). In the first attempt to demonstrate parasitism in branchiobdellidans, Grabda and Wierzbicka (1969) used radioactive tracers to confirm that one gill-chamber-inhabiting species (*Branchiobdella hexadonta*) consumed tissues or hemolymph from their host. More recent observational studies have revealed significant correlations between the presence/abundance of gill-chamber-inhabiting branchiobdellidans and the number of visible scars (melanization) on the hosts' gills (Quaglio et al. 2006, Rosewarne et al. 2012).

Such studies suggest that some branchiobdellidan taxa may consume host tissues and can cause visible damage, but they do not necessarily demonstrate that the interaction is parasitic based on net outcomes for the species involved (*sensu* Bronstein 1994). Moreover, these studies did not account for other effects of branchiobdellidans on their host, including possible positive effects that could effectively offset the negative effects of parasitism.

Experiments designed to assess the net effect of branchiobdellidans on aspects of host fitness have produced conclusions ranging from net-positive to net-negative outcomes. The branchiobdellidan *Cambarincola fallax* did not affect the growth, molting frequency, or stamina of *Orconectes rusticus* during a 115-d laboratory experiment, and these results led the author to conclude that the association between these taxa is commensal (Keller 1992). Conversely, a similar experiment demonstrated significant positive effects of the branchiobdellidan *Cambarincola ingens* on growth rate and survival of the host crayfish *Cambarus chasmodactylus* (Brown et al. 2002). The positive effects of *C. ingens* on their hosts were attributed to a cleaning service because *C. ingens* removed potentially fouling organic materials that had collected in the host's gill chamber (Brown et al. 2002). Consequently, Brown et al. (2002) concluded that the association between *C. ingens* and *C. chasmodactylus* is probably a cleaning-symbiosis mutualism similar to the long known and familiar associations among tropical coral reef fishes (e.g., Limbaugh 1961, Trivers 1971, Losey 1972, Losey and Margules 1974, Grutter 1999). Together, the results of Keller (1992) and Brown et al. (2002) suggest that the effects of branchiobdellidans on their hosts may vary among hosts or branchiobdellidan species.

The net effects of branchiobdellidans on a crayfish also may depend on the abundance or density of branchiobdellidans on an individual host. In field experiments involving 2 single-species pairs of crayfish and branchiobdellidans in 2 different watersheds, the net effect shifted from positive to negative with increasing initial branchiobdellidan densities (Brown et al. 2012). Individual crayfish (*C. chasmodactylus* and *Cambarus chaugaensis*) that were exposed to low to intermediate branchiobdellidan densities (*C. ingens* and *Xironodrilus* sp., respectively) had significantly higher growth rates than crayfish with no branchiobdellidans (Brown et al. 2012). However, crayfish that were exposed to high densities of branchiobdellidans consistently showed reduced growth rates compared to no-worm controls (Brown et al. 2012; Fig. 1). During these experiments, branchiobdellidan density and the number of scars on crayfish gills were

strongly positively correlated (Brown et al. 2012), suggesting the interaction shifted from mutualism to parasitism at high branchiobdellidan densities as the negative effects of increasing gill damage outweighed the positive effects of cleaning (Brown et al. 2012). Branchiobdellidan densities vary considerably among locations and branchiobdellidan species. In the experiments mentioned above, 12 worms/host was considered high because it was greater than the typical densities observed for those species at those locations. However, crayfish in some populations regularly host hundreds of branchiobdellidans (BWW, JS, personal observation).

In addition to species-specific and demographic variability, the outcomes of symbiotic interactions are often influenced by the biotic and abiotic factors that form the environmental context of the interaction (Bronstein 1994, Thompson 2005). Recent experimental evidence suggests that context probably is an important determinant of the net effect of branchiobdellidans on their hosts. In laboratory experiments, the Korean crayfish (*Cambaroides similis*) experienced increased growth in the presence of multiple species of branchiobdellidans when conditions favored the growth of microbial biofilms (Lee et al. 2009). However, under conditions that reduced microbial growth, the same suite of worms had no measurable effect on host growth (Lee et al. 2009). These results are congruent with conclusions of Brown et al. (2002) that the positive effects of branchiobdellidans on their host result from removal of epibiotic material and demonstrate the importance of context in determining the net outcome for the host.

Spatiotemporal Variability in Branchiobdellidan Assemblages

Species composition, abundance, and diversity of branchiobdellidans vary across spatial and temporal scales. Approximately 150 species representing 21 genera have been described from North and Central America, Europe, and eastern Asia (Gelder et al. 2002), but taxonomic diversity is unevenly distributed across these regions. Surveys and synopses have encompassed broad geographic areas of North and Central America including the Canadian Prairie Provinces (Williams et al. 2009), Mesoamerica (Holt 1973b), and the west coast of North America (Holt 1974, 1977, 1981) and more limited areas including New England (Gelder et al. 2001), the Mountain Lake region of northwestern Virginia (Hobbs et al. 1967), and Great Smoky Mountains National Park (Gelder and Williams 2011). Each of these studies provides a glimpse of the natural variability in branchiobdellidan

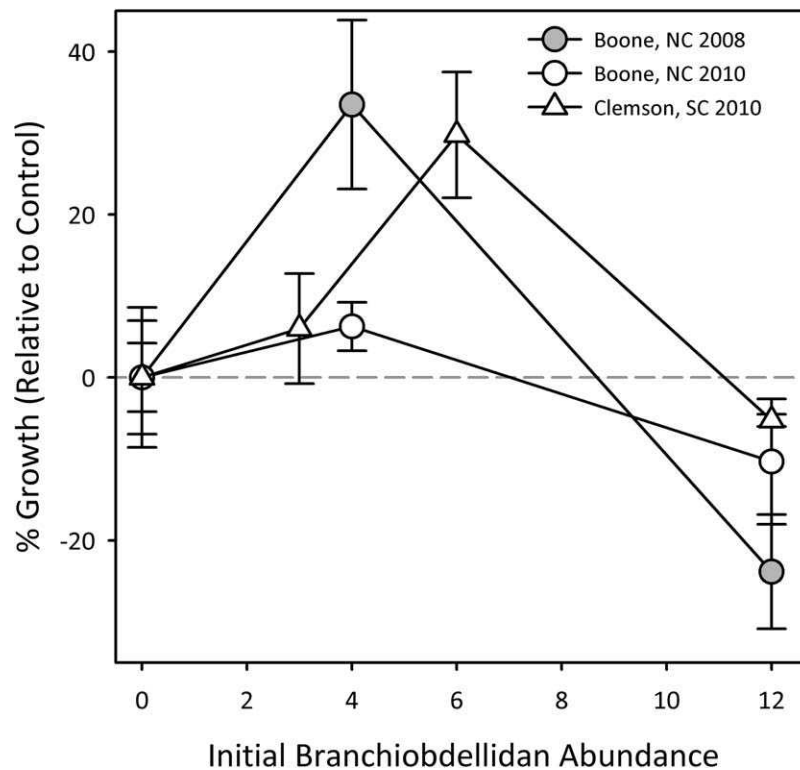


FIG. 1. Results of 3 in situ experiments assessing the effect of variable initial branchiobdellidan density (worms/host) on crayfish growth. Two experiments were done on the South Fork of the New River near Boone, North Carolina (NC), USA, with the crayfish *Cambarus chasmodactylus* and the branchiobdellidan *Cambarincola ingens* over 71 d during the summer 2008 and 103 d during the summer 2010. A 3rd experiment was done over 81 d during summer 2010 in Walldrop Stone Creek near Clemson, South Carolina (SC), USA, with the crayfish *Cambarus chaugaensis* and branchiobdellidans of the genus *Xironodrilus* (open triangles). Plots show total % growth relativized to controls (no worms) vs initial branchiobdellidan density (worm treatments). For each treatment, total growth was relativized by subtracting the mean total growth of the control group in each experiment. In all 3 experiments, low to intermediate initial branchiobdellidan densities had significant positive effects, but high densities marginally reduced growth (Brown et al. 2012).

species composition across a wide range of spatial extent. However, branchiobdellidan diversity and abundance across space, time, and environmental conditions are poorly understood. Authors of early ecological studies described how branchiobdellidan abundance varied as a function of season (Young 1966, Koepp and Schlueter 1977), water temperature, and O₂ tension (Berry and Holt 1959), but the roles of environment, space, and time are only now being explored in combination (DeWitt et al. 2013). Branchiobdellidan diversity is either unknown or underestimated in many regions because the life history of individual species and their sensitivity to environmental conditions are rarely assessed quantitatively. Measuring how branchiobdellidans vary spatiotemporally and across environmental conditions is an important step to elucidating implications of branchiobdellidan diversity for crayfish populations.

Branchiobdellidans display spatial patterns in microhabitat selection on their hosts' bodies. Some

species, including the wide-spread *Bdellodrilus illuminatus*, are found almost exclusively within the crayfish gill chamber (Holt 1963, Hobbs et al. 1967, Gelder and Williams 2011). Others, such as most species of the genera *Xironogiton*, *Ankyrodrilus*, and *Xironodrilus*, show preferences for the chelae and anterior walking legs and share a dorsoventrally flattened morphology that probably is an adaptation to shear stresses experienced at these locations (Fig. 2; Hobbs et al. 1967, Gelder and Williams 2011). Other species are less specific in their microhabitat selection and are found attached at many locations on their hosts (see Gelder and Williams 2011 for descriptions of the microhabitat selection of several wide-spread and localized species). Researchers who have examined the effects of branchiobdellidans on their hosts have focused mainly on species that inhabit the gill chamber and adjacent areas (Brown et al. 2002, 2012, Quaglio et al. 2006, Rosewarne et al. 2012), and the potential importance of other species is generally

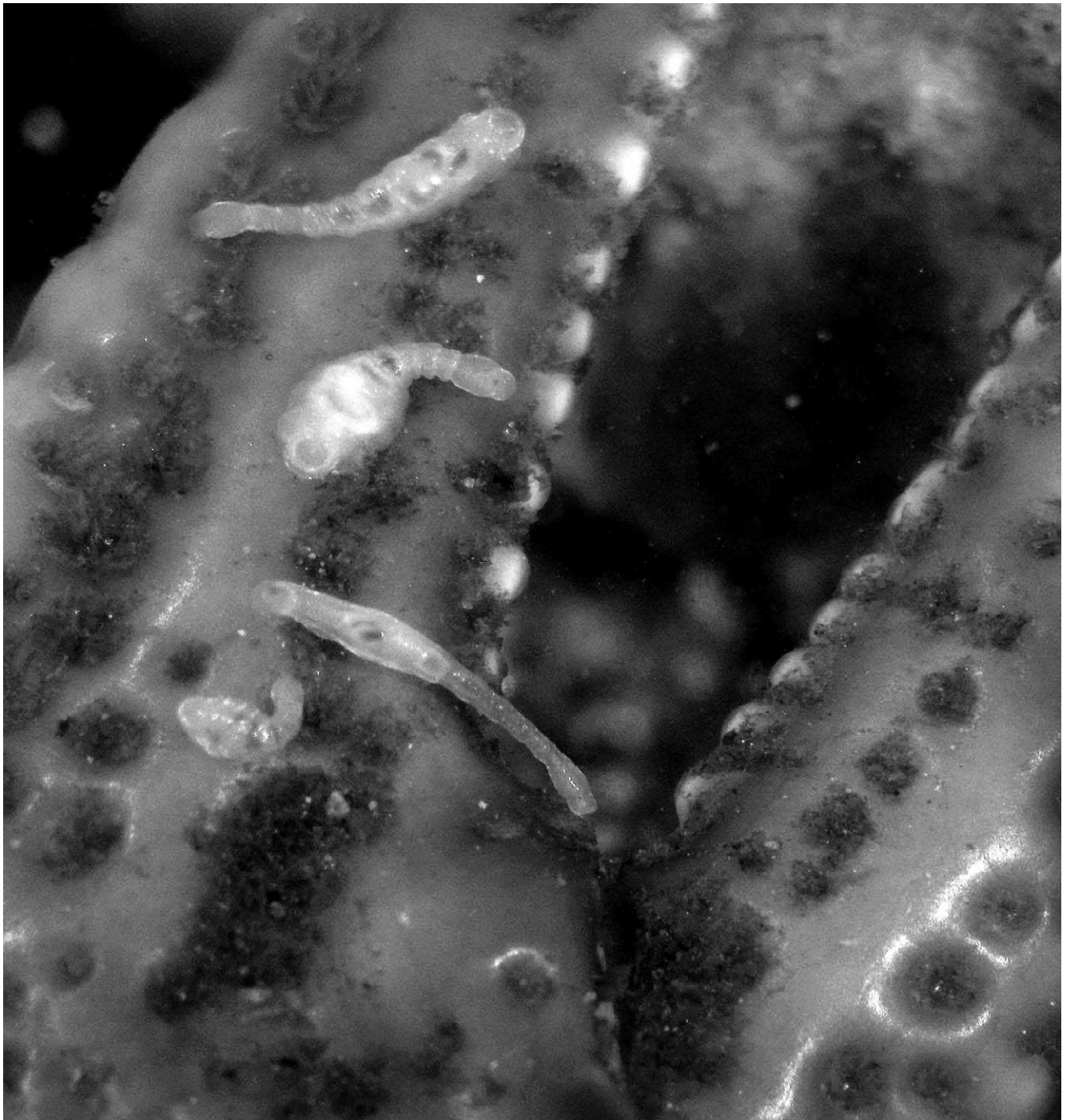


FIG. 2. The branchiobdellidan *Xironogiton instabilis* on the chela of the crayfish *Cambarus bartonii* from Little Stoney Creek, northwestern Virginia, USA. *Xironogiton* displays a dorsoventrally flattened morphology typical of branchiobdellidan species that specialize on crayfish chelae (photo credit: JS).

unknown. The evolutionary and potential coevolutionary forces that have shaped microhabitat selection in branchiobdellidans and the implications of microhabitat selection for the host remain an unexplored opportunity for future research.

Host Specificity, Preference, and Regulation

Studies of the biogeography of branchiobdellidans and their hosts indicate that branchiobdellidans are not strictly host specific. More than twice as many

described species of crayfish exist in North America (~382; Crandall and Buhay 2008) as there are branchiobdellidan species world-wide (~150; Gelder et al. 2002), although many undiscovered or undescribed cryptic branchiobdellidan species certainly exist (Williams et al. 2013). Moreover, a single branchiobdellidan species may be found on multiple host species at a site, and a single crayfish may host several species of branchiobdellidans (e.g., Hobbs et al. 1967, Gelder 1999b, Gelder and Williams 2011). The geographic ranges of branchiobdellidans do not typically reflect the distribution of any particular host species (Holt 1969, Gelder 1999b, Gelder and Williams 2011; but see Füreder et al. 2009), and some species have spread to new host species or, along with their host, successfully invaded novel environments (Gelder 1999a, Gelder et al. 2002, 2012, Quaglio et al. 2006). Branchiobdellidans clearly are not host species-specific *sensu stricto*, but evidence exists that species in this association are not perfectly interchangeable.

Recent observational and experimental work suggests that crayfish species vary in their suitability as branchiobdellidan hosts and that some branchiobdellidans prefer particular host species (Brown and Creed 2004, Gelder and Williams 2011, Farrell et al. in press). The crayfish *C. chasmodactylus* and *Orconectes cristavarius* co-occur in the South Fork of the New River in western North Carolina. The branchiobdellidan *C. ingens* occurs with much greater frequency and abundance on *C. chasmodactylus* than on *O. cristavarius*, even after accounting for differences in host size and despite the greater abundance of *O. cristavarius* (Brown and Creed 2004). In a host-choice experiment, *C. ingens* was 2.5× more likely to colonize *C. chasmodactylus* when given a choice between the 2 host species (Brown and Creed 2004).

Differences in the abundance of *C. ingens* on *C. chasmodactylus* and *O. cristavarius* may reflect more than just the preferences of the worms. Crayfish are covered with mechanoreceptors that enable them to detect the presence and location of branchiobdellidans on their exoskeletons (Gelder 2010). Recent experimental work suggests that crayfish use grooming behaviors to actively regulate branchiobdellidan density and that regulation intensity may vary with crayfish species and local environmental conditions (Thomas et al. 2013, Farrell et al., in press). When branchiobdellidan densities on *C. chasmodactylus* were experimentally manipulated to levels ~2× those typically observed in situ, the crayfish rapidly groomed branchiobdellidans from their exoskeletons using the fingers and dactyls of their anterior walking legs (Farrell et al. in press). Moreover, not all worms were removed and the densities of branchiobdellidans

remaining were similar to the in situ densities that yielded a net positive outcome for *C. chasmodactylus* in previous studies (Brown et al. 2002, 2012). Conversely, introduction of just 1 worm initiated strong and directed grooming behaviors in *O. cristavarius* (Farrell et al. in press), a result suggesting that this species is intolerant of *C. ingens* and that potentially explains *C. ingens* preference for *C. chasmodactylus* as a host. A disparity between *Cambarus* and *Orconectes* as branchiobdellidan hosts also was observed by Gelder and Williams (2011), who noted that branchiobdellidan richness in Great Smoky Mountains National Park was typically greater on *Cambarus* than on *Orconectes*. Thus, for a particular branchiobdellidan species, not all crayfish species are equally suitable hosts.

Crayfish may adjust regulatory grooming behaviors to match environmental context and concomitant changes in the potential benefits of being cleaned or costs of being parasitized. In environments with low levels of epibiotic fouling, crayfish probably experience less benefit from being cleaned and could actually be more susceptible to parasitism if limited epibiotic resources cause a compensatory shift in branchiobdellidan feeding behavior toward increased parasitism (Brown et al. 2012, Thomas et al. 2013). In a laboratory experiment, Thomas et al. (2013) demonstrated that regulatory grooming of *C. chasmodactylus* changed with conditions that promoted either high or low levels of epibiotic fouling of the crayfish exoskeleton and gills. They observed a higher rate of branchiobdellidan removal by *C. chasmodactylus* under low-fouling conditions, suggesting that crayfish modify their worm loads to match changes in the potential costs and benefits of symbiosis.

Branchiobdellidan Taxonomy and Phylogenetics

The crayfish–branchiobdellidan association presents many opportunities for advancing our knowledge of crayfish biology and may provide a useful model system for testing ecological and evolutionary theory, but recent and ongoing shifts in our understanding of branchiobdellidan taxonomy and phylogenetics pose potential challenges to researchers working with this system. Early understanding of branchiobdellidan phylogeny was based primarily on intuition (Holt 1968, 1986) and reflected taxonomy at the time. Later attempts at phylogenetic reconstruction using morphological characters (Gelder and Brinkhurst 1990), spermatological characters (Cardini et al. 2000, Cardini and Ferraguti 2004), and molecular markers (Gelder and Siddall 2001) resulted in low resolution and low node support among most examined taxa. A recent molecular phylogenetic

study focused on phylogenetic relationships among North American branchiobdellidans recovered strong support for clustering that is not fully consistent with current taxonomy (Williams et al. 2013). These results provide a base from which to examine the relative importance of morphological characters currently used to designate taxonomic and systematic rankings within the Branchiobdellida. However, molecular data suggest that in some cases phylogenetically disparate species exhibit a high degree of morphological similarity (Williams et al. 2013). As a result, accurate identification of branchiobdellidans requires care and expertise. Much of the recent work on branchiobdellidan evolution and ecology has taken advantage of live microscopy techniques (described in Gelder 2010) that enhance examination of subtle morphological differences among species. Efforts are currently underway to create a comprehensive interactive on-line key to facilitate identification of branchiobdellidans with live and fixed specimens to make studying them more tractable for biologists with limited familiarity with branchiobdellidan taxonomy (BWW, unpublished data).

Utility of the Crayfish–Branchiobdellidan Symbiosis as a Model System

The evolution of mutualistic interactions has fascinated theoreticians since Darwin (1859). Early researchers realized that the evolution of these associations had to be explained in terms of individual fitness (Hamilton 1964a, b), and researchers using purely theoretical approaches have demonstrated quantitatively that mutualisms may be evolutionarily stable strategies (e.g., Trivers 1971, Axelrod and Hamilton 1981, Nowak and May 1992, Noë and Hammerstein 1994, Doebeli and Knowlton 1998, Nowak and Sigmund 1998). Theoretical approaches are essential for explaining the evolution of species interactions in terms of individual fitness, but they necessarily simplify organisms and their interactions. Such simplifications prevent assessments of many important and multifaceted details that stem from organismal diversity (Herre et al. 1999). Therefore, empiricists must verify theory by testing specific hypotheses about the mechanisms that shape real symbiotic interactions.

One of the best known and most fruitful model systems for studying symbiotic interactions among animals is the cleaning symbioses among tropical coral reef fishes. Multiple cleaner species provide a service to clients by feeding on ectoparasites from the clients bodies. This interaction may be mutualistic because the client is relieved of detrimental parasites

and the cleaner receives a meal (Limbaugh 1961). However, participants also may enhance individual fitness in other, nonmutual ways. First, clients may increase their benefit by consuming cleaners after they have performed their cleaning service (Trivers 1971), but cleaners are rarely consumed even when entering the mouths of clients they clean. Second, cleaners could increase their benefit by consuming client tissues in addition to ectoparasites, and cleaners may actually prefer client tissues (Grutter and Bshary 2003). Indeed, cleaner fish may effectively become parasitic when ectoparasite abundances are low (Losey 1972, Cheney and Cote 2005), a situation that demonstrates the influence of local context on the outcome of the interaction.

Empirical work on cleaning symbioses among tropical fishes has elucidated multiple mechanistic explanations for their evolutionary stability. Clients can influence cleaner behavior through punishment (Bshary and Grutter 2002, Grutter and Bshary 2003) and can minimize parasitic attacks by selectively interacting with cooperators (Noë and Hammerstein 1994, Bshary and Noë 2003). Moreover, clients can limit the temptation for cleaners to defect by regulating the duration of cleaning interactions (Johnstone 2002). These empirical explorations of specific aspects of cleaning interactions and their underlying mechanisms demonstrate that mutualisms may be very tenuous and complicated associations affected by local biotic and abiotic contexts and maintained by numerous complex feedbacks between and among partners.

The association between crayfish and branchiobdellidans offers an opportunity to corroborate and expand on the theoretical and empirical advances gained from studying other mutualistic interactions. Like in coral reef systems, experimental work with crayfish and branchiobdellidans has demonstrated variable (Lee et al. 2009, Brown et al. 2012) and context-dependent (Lee et al. 2009) effects of cleaners on their hosts, and complex species-specific control behaviors that may limit overexploitation (Farrell et al. in press). Differences between crayfish–branchiobdellidan systems and tropical cleaner–fish systems provide an opportunity to test the generality of theory derived largely from coral reef work and to evaluate further necessary constraints for the evolution of cleaning-symbiosis mutualisms.

We present 5 reasons for using the crayfish–branchiobdellidan association as a model system for empirical symbiosis research: 1) *Distribution*. The crayfish–branchiobdellidan association is common throughout many regions of the Holarctic, making it an accessible system to researchers in several coun-

tries. 2) *Ease of experimental manipulations*. The number of branchiobdellidans on individual crayfish can be easily manipulated to establish specific initial worm densities. Methods for these manipulations can be found in Brown et al. (2002) and have been validated by multiple published studies (Brown and Creed 2004, Lee et al. 2009, Brown et al. 2012). 3) *Ease of husbandry*. Both crayfish and branchiobdellidans can be maintained in the laboratory with minimal equipment. Successful experiments have been conducted in small aerated plastic or glass bowls or aquaria (Keller 1992, Brown et al. 2002, Brown and Creed 2004, Lee et al. 2009). Moreover, branchiobdellidans can be stored without a host for long periods in shallow dishes of stream water with no apparent ill effects (Woodhead 1950, Penn 1959, Young 1966). 4) *Amenability of field experimental methods*. Flow-through stream enclosures have been used in field experiments to determine symbiont effects in the context of natural systems (Fig. 3; Brown et al. 2012). To the best of our knowledge, branchiobdellidans transfer only through direct contact between hosts, so maintaining experimentally established densities is a straightforward process of keeping enclosed crayfish separated from naturally occurring crayfish using either fine-mesh (McManus 1960) or enclosures featuring double walls (Brown et al. 2012). 5) *Nonintrusive data collection*. Several experiments conducted thus far on crayfish and their branchiobdellidan associates have used nondestructive repeated-measures data collection to assess crayfish growth and epibiont population dynamics (Brown et al. 2002, 2012, Lee et al. 2009, Thomas et al. 2013). Repeated-measures data collection increases statistical power for assessing treatment effects and allows assessment of changes in effects over time (Potvin et al. 1990). We acknowledge that nonintrusive data collection may not be an option for all species because of wide variation in size, micro-habitat, and visibility of branchiobdellidans.

Continuing and Future Work

Our perception of symbioses has changed from that of interactions with exclusively positive or negative outcomes to a more realistic perspective of interactions with variable outcomes (Ewald 1987, Bronstein 1994). Moreover, appreciation is growing among ecologists of the importance of both positive and negative outcomes in structuring natural communities and modulating ecosystem processes (Bertness and Callaway 1994, Callaway 1995, Bertness and Leonard 1997, Bruno et al. 2003). Crayfish are highly influential in aquatic systems as ecosystem engineers and keystone species (Creed 1994, Creed and Reed

2004, Usio and Townsend 2004, Brown and Lawson 2010). Ongoing research is being done to explore the variable effects of branchiobdellidans on crayfish and the indirect effects that this association may have on aquatic communities and ecosystems. This body of work combines recent theoretical advances in our understanding of symbioses to illustrate how variability in species interactions can lead to variable effects that radiate beyond the focal species throughout natural communities and ecosystems.

Mutualists can have the potential to act as parasites, but coevolved controls can prevent overexploitation that leads to parasitism and potentially could stabilize mutualisms through evolutionary time (Trivers 1971, Axelrod and Hamilton 1981, Bull and Rice 1991, Pellmyr and Huth 1994, Johnstone 2002, Bshary and Bronstein 2011, Kiers et al. 2011). Ongoing work is being done to examine behavioral responses of crayfish to branchiobdellidans under multiple contexts and to document age- and species-specific host responses that may resist overexploitation by epibionts and maximize the life-long net benefit of symbiosis. This work is improving our understanding of how positive interactions are maintained by describing how control mechanisms and changes in ecological context and species ontogeny permit mutually beneficial interactions over a broad range of conditions and developmental stages.

The relationship between branchiobdellidans and crayfish has been studied primarily in surface-water environments, but most crayfish have the ability to burrow and may spend part or all of their life cycle underground (Hobbs 1981). Branchiobdellidans are associated with some burrowing crayfishes (e.g., *Cambarus carolinus*, *Cambarus diogenes*, and *Procambarus clarkii*; Hobbs et al. 1967, Holt and Opell 1993), but the extent of the associations under these conditions is poorly understood, and important basic questions about burrowing crayfish–branchiobdellidan symbioses remain unanswered. The physicochemical disparity between fully aquatic and semiterrestrial environments offers a valuable opportunity to investigate how costs and benefits associated with cleaning symbioses vary among hosts with different life-history strategies or among hosts that move between vastly different habitats within a lifetime. Ongoing research in replicate burrowing chambers (Stoeckel et al. 2011) is beginning to address questions pertaining to burrowing crayfish and their symbionts.

Research has provided a foundation for understanding of the ecology and evolution of the crayfish–branchiobdellidan association, but what we know is dwarfed by what we do not know. Systematic sampling in understudied or unsampled areas is



FIG. 3. Flow-through enclosure/exclosures used in field experiments to test for effects of branchiobdellid worms on host crayfish growth (Brown et al. 2012). Flow-through design maintains natural stream conditions while double-walled barriers prevent exchange of branchiobdellidans between enclosed and free-living crayfish (photo credit: BLB).

needed to increase our knowledge of branchiobdellidan diversity and distribution and to inform studies assessing evolutionary and ecological patterns. In addition, work evaluating concordance among different data sets (e.g. morphological, molecular), and thus, species concepts, is needed to infer the evolutionary history of the Branchiobdellida. Basic life-history characteristics and the physiological constraints of most branchiobdellidan species remain undescribed.

The effects that branchiobdellidans have on crayfish have been elucidated for only a handful of species, and the implications of variability in local branchiobdellidan diversity and interspecific interactions among branchiobdellidans are still unknown. Exploring how ecological and evolutionary forces have shaped patterns in branchiobdellidan diversity and the consequences of those patterns for crayfish and surrounding communities is an exciting and promising avenue for

future research. Furthermore, understanding how anthropogenic disturbances, such as species introductions and habitat alteration, alter the symbiotic interaction between crayfish and branchiobdellidans will help inform efforts to protect biodiversity and ecosystem integrity in the many places that crayfish live.

Acknowledgements

Previous versions of this manuscript were greatly improved by thoughtful and insightful comments from Zachary Loughman and 2 anonymous referees. This is contribution 692 to the Auburn University Museum of Natural History.

Literature Cited

- AXELROD, R., AND W. D. HAMILTON. 1981. The evolution of cooperation. *Science* 211:1390–1396.
- BERRY, J. W., AND P. C. HOLT. 1959. Reactions of two species of Branchiobdellidae (Oligochaeta) to high temperatures and low oxygen tensions. Virginia Agricultural Experiment Station, Virginia Polytechnic Institute Bulletin 141:3–11.
- BERTNESS, M. D., AND R. CALLAWAY. 1994. Positive interactions in communities. *Trends in Ecology and Evolution* 9: 191–193.
- BERTNESS, M. D., AND G. H. LEONARD. 1997. The role of positive interactions in communities: lessons from intertidal habitats. *Ecology* 78:1976–1989.
- BISHOP, J. E. 1968. An ecological study of the branchiobdellid commensals (Annelida: Branchiobdellidae) of some mid-western Ontario crayfish. *Canadian Journal of Zoology* 46:835–843.
- BRONSTEIN, J. L. 1994. Conditional outcomes in mutualistic interactions. *Trends in Ecology and Evolution* 9: 214–217.
- 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 (Berlin)* 132:250–255.
- BROWN, B. L., R. P. CREED, J. SKELTON, M. 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 (Berlin)* 170:199–207.
- BROWN, B. L., AND R. L. LAWSON. 2010. Habitat heterogeneity and activity of an omnivorous ecosystem engineer control stream community dynamics. *Ecology* 91: 1799–1810.
- BRUNO, J. F., J. J. STACHOWICZ, AND M. D. BERTNESS. 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution* 18:119–125.
- BSHARY, R., AND J. L. BRONSTEIN. 2011. A general scheme to predict partner control mechanisms in pairwise cooperative interactions between unrelated individuals. *Ethology* 117:271–283.
- BSHARY, R., AND A. S. GRUTTER. 2002. Asymmetric cheating opportunities and partner control in a cleaner fish mutualism. *Animal Behaviour* 63:547.
- BSHARY, R., AND R. NOË. 2003. The ubiquitous influence of partner choice on the dynamics of cleaner fish–client reef fish interactions. Pages 167–184 in P. Hammerstein (editor). *Genetic and cultural evolution of cooperation*. MIT Press, Cambridge, Massachusetts.
- BULL, J., AND W. RICE. 1991. Distinguishing mechanisms for the evolution of co-operation. *Journal of Theoretical Biology* 149:63–74.
- CALLAWAY, R. M. 1995. Positive interactions among plants. *Botanical Review* 61:306–349.
- CARDINI, A., AND M. FERRAGUTI. 2004. The phylogeny of Branchiobdellida (Annelida, Clitellata) assessed by sperm characters. *Zoologischer Anzeiger* 243:37–46.
- CARDINI, A., M. FERRAGUTI, AND S. R. GELDER. 2000. A phylogenetic assessment of the branchiobdellidan family Branchiobdellidae (Annelida, Clitellata) using spermatological and somatic characters. *Zoologica Scripta* 29:347–366.
- CHARLEBOIS, P. M., AND G. A. LAMBERTI. 1996. Invading crayfish in a Michigan stream: direct and indirect effects on periphyton and macroinvertebrates. *Journal of the North American Benthological Society* 15:551–563.
- CHENEY, K. L., AND I. M. COTE. 2005. Mutualism or parasitism? The variable outcome of cleaning symbioses. *Biology Letters* 1:162–165.
- CRANDALL, K. A., AND J. E. BUHAY. 2008. Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae – Decapoda) in freshwater. *Hydrobiologia* 595:295–301.
- CREED, R. P. 1994. Direct and indirect effects of crayfish grazing in a stream community. *Ecology* 75:2091–2103.
- 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.
- DARWIN, C. 1859. *On the origins of species by means of natural selection*. Murray, London, UK.
- DEWITT, P. D., B. W. WILLIAMS, Z. Q. LU, A. N. FARD, AND S. R. GELDER. 2013. Effects of environmental and host physical characteristics on an aquatic symbiont. *Limnologia* 43: 151–156.
- DOEBELI, M., AND N. KNOWLTON. 1998. The evolution of interspecific mutualisms. *Proceedings of the National Academy of Sciences of the United States of America* 95: 8676–8680.
- EDGERTON, B. F., L. H. EVANS, F. J. STEPHENS, AND R. M. OVERSTREET. 2002. Synopsis of freshwater crayfish diseases and commensal organisms. *Aquaculture* 206:57–135.
- EWALD, P. W. 1987. Transmission modes and evolution of the parasitism–mutualism continuum. *Annals of the New York Academy of Sciences* 503:295–306.
- FARD, A. N., AND S. R. GELDER. 2011. First report of *Branchiobdella kozarovi* Subchev, 1978 (Annelida: Clitellata) in Iran, and its distribution in the Eastern Euro-

- Mediterranean subregion. *Acta Zoologica Bulgarica* 63: 105–108.
- FARRELL, K. J., R. P. CREED, AND B. L. BROWN. Preventing overexploitation in a mutualism: partner control in the crayfish–branchiobdellid symbiosis. *Oecologia* (Berlin). (in press).
- FÜREDER, L., M. SUMMERER, AND A. BRANDSTÄTTER. 2009. Phylogeny and species composition of five European species of *Branchiobdella* (Annelida: Clitellata: Branchiobdellida) reflect the biogeographic history of three endangered crayfish species. *Journal of Zoology* 279: 164–172.
- GALE, K. S. P., AND H. C. PROCTOR. 2009. Diets of two congeneric species of crayfish worm (Annelida: Clitellata: Branchiobdellidae) from western Canada. *Canadian Journal of Zoology* 89:289–296.
- GELDER, S. R. 1999a. Distribution of native and exotic branchiobdellidans (Annelida: Clitellata) on their respective crayfish hosts in northern Italy, with the first record of native *Branchiobdella* species on an exotic North American crayfish. *Journal of Limnology* 58: 20–24.
- GELDER, S. R. 1999b. 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. 2010. Branchiobdellida, section III. Pages 402–410 in J. H. Thorp and A. P. Covich (editors). *Ecology and classification of North American freshwater invertebrates*. Academic Press, London, UK.
- GELDER, S. R., AND R. BRINKHURST. 1990. An assessment of the phylogeny of the Branchiobdellida (Annelida: Clitellata), using PAUP. *Canadian Journal of Zoology* 68: 1318–1326.
- GELDER, S. R., H. C. CARTER, AND D. N. LAUSIER. 2001. Distribution of crayfish worms or branchiobdellidans (Annelida: Clitellata) in New England. *Northeastern Naturalist* 8:79–92.
- 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.
- GELDER, S. R., J.-F. PARPET, AND F. QUAGLIO. 2012. First report of two North American branchiobdellidans (Annelida: Clitellata) or crayfish worms on signal crayfish in Europe with a discussion of similar introductions into Japan. *Annales de Limnologie – International Journal of Limnology* 48:315–322.
- GELDER, S. R., AND J. P. ROWE. 1988. Light microscopical and cytochemical study on the adhesive and epidermal gland cell secretions of the branchiobdellid (Annelida: Clitellata). *Canadian Journal of Zoology* 66:2057–2064.
- GELDER, S. R., AND M. E. SIDDALL. 2001. Phylogenetic assessment of the Branchiobdellidae (Annelida, Clitellata) using 18S rDNA, mitochondrial cytochrome c oxidase subunit I and morphological characters. *Zoologica Scripta* 30:215–222.
- GELDER, S. R., AND B. W. WILLIAMS. 2011. First distributional study of branchiobdellida (Annelida: Clitellata) in the Great Smoky Mountains National Park, North Carolina and Tennessee, USA, with a redescription of *Cambarincola holostomus* Hoffman, 1963. *Southeastern Naturalist* 10:211–220.
- GHERRARDI, F. 2007. Understanding the impact of invasive crayfish. Pages 507–542 in F. Gherardi (editor). *Biological invaders in inland waters: profiles, distribution, and threats*. Springer, Dordrecht, The Netherlands.
- GOODNIGHT, C. J. 1941. The Branchiobdellidae (Oligochaeta) of Florida. *Transactions of the American Microscopical Society* 60:69–74.
- 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.
- GRUTTER, A. S. 1999. Cleaner fish really do clean. *Nature* 398: 672–673.
- GRUTTER, A. S., AND R. BSHARY. 2003. Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions. *Proceedings of the Royal Society of London Series B: Biological Sciences* 270:S242–S244.
- HAMILTON, W. D. 1964a. The genetical evolution of social behaviour. I. *Journal of Theoretical Biology* 7:1–16.
- HAMILTON, W. D. 1964b. The genetical evolution of social behaviour. II. *Journal of Theoretical Biology* 7:17–52.
- HART, D. D. 1992. Community organization in streams: the importance of species interactions, physical factors, and chance. *Oecologia* (Berlin) 91:220–228.
- HERRE, E. A., N. KNOWLTON, U. G. MUELLER, AND S. A. REHNER. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution* 14:49–53.
- HOBBS, H. H. 1981. The crayfishes of Georgia. *Smithsonian Contributions to Zoology* 318:1–549.
- HOBBS, H. H. J., 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.
- HOBBS, H. H. J., AND D. M. LODGE. 2010. Decapoda. Pages 901–967 in J. H. Thorp and A. P. Covich (editors). *Ecology and classification of the North American freshwater invertebrates*. Academic Press, London, UK.
- HOLT, P. C. 1963. A new branchiobdellid (Branchiobdellidae: *Cambarincola*). *Journal of the Tennessee Academy of Science* 38:97–100.
- HOLT, P. C. 1968. New genera and species of branchiobdellid worms (Annelida: Clitellata). *Proceedings of the Biological Society of Washington* 81:291–318.
- HOLT, P. C. 1969. The relationships of the branchiobdellid fauna of the southern Appalachians. The distributional history of the biota of the southern Appalachians, Part I: Invertebrates. Virginia Polytechnic Institute, Blacksburg. *Research Division Monograph* 1:191–219.
- HOLT, P. C. 1973a. A free-living branchiobdellid (Annelida: Clitellata)? *Transactions of the American Microscopical Society* 92:152–153.

- HOLT, P. C. 1973b. A summary of the branchiobdellid (Annelida: Clitellata) fauna of Mesoamerica. Smithsonian Institution Press, Washington, DC.
- HOLT, P. C. 1974. An emendation of the genus *Triannulata* Goodnight, 1940, with the assignment of *Triannulata montana* to *Cambarincola* Ellis 1912 (Clitellata: Branchiobdellida). Proceedings of the Biological Society of Washington 87:57–72.
- HOLT, P. 1977. An emendation of genus *Sathodrilus* Holt 1968 (Annelida: Branchiobdellida), with the description of four new species from the Pacific drainage of North America. Proceedings of the Biological Society of Washington 90:116–131.
- HOLT, P. C. 1981. A resume of the members of the genus *Cambarincola* (Annelida: Branchiobdellida) from the Pacific drainage of the United States. Proceedings of the Biological Society of Washington 94:675–695.
- HOLT, P. C. 1986. Newly established families of the order Branchiobdellida (Annelida, Clitellata) with a synopsis of the genera. Proceedings of the Biological Society of Washington 99:676–702.
- HOLT, P. C., AND B. D. OPELL. 1993. A checklist of and illustrated key to the genera and species of the Central and North American Cambarincolidae (Clitellata: Branchiobdellida). Proceedings of the Biological Society of Washington 106:251–295.
- HURYN, A. D., AND J. B. WALLACE. 1987. Production and litter processing by crayfish in an Appalachian mountain stream. Freshwater Biology 18:277–286.
- JENNINGS, J., AND S. GELDER. 1979. Gut structure, feeding and digestion in the branchiobdellid oligochaete *Cambarincola macrodonta* Ellis 1912, an ectosymbiote of the freshwater crayfish *Procambarus clarkii*. Biological Bulletin 156:300–314.
- JOHNSTONE, R. A. 2002. From parasitism to mutualism: partner control in asymmetric interactions. Ecology Letters 5:634–639.
- KELLER, T. A. 1992. The effect of the branchiobdellid annelid *Cambarincola fallax* on the growth rate and condition of the crayfish *Orconectes rusticus*. Journal of Freshwater Ecology 7:165–171.
- KIERS, E. T., M. DUHAMEL, Y. BEESSETY, J. A. MENSAH, O. FRANKEN, E. VERBRUGGEN, C. R. FELLBAUM, G. A. KOWALCHUK, M. M. HART, AND A. BAGO. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333: 880–882.
- KOEPP, S., AND E. A. SCHLUETER. 1977. The ecology of a branchiobdellid (Annelida: Oligochaeta) from a Texas pond. Texas Journal of Science 28:285–294.
- LEE, J. H., T. W. KIM, AND J. C. CHOE. 2009. Commensalism or mutualism: conditional outcomes in a Branchiobdellidae crayfish symbiosis. Oecologia (Berlin) 159:217–224.
- LIMBAUGH, C. 1961. Cleaning symbiosis. Scientific American 205:42–49.
- LOSEY, G. S. 1972. The ecological importance of cleaning symbiosis. Copeia 1972:820–833.
- LOSEY, G. S., AND L. MARGULES. 1974. Cleaning symbiosis provides a positive reinforcer for fish. Science 184: 179–180.
- MCMANUS, L. R. 1960. Some ecological studies of the Branchiobdellidae (Oligochaeta). Transactions of the American Microscopical Society 79:420–428.
- MOMOT, W. T. 1995. Redefining the role of crayfish in aquatic ecosystems. Reviews in Fisheries Science 3:33–63.
- NOË, R., AND P. HAMMERSTEIN. 1994. Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behavioral Ecology and Sociobiology 35:1–11.
- NOWAK, M. A., AND R. M. MAY. 1992. Evolutionary games and spatial chaos. Nature 359:826–829.
- NOWAK, M. A., AND K. SIGMUND. 1998. Evolution of indirect reciprocity by image scoring. Nature 393:573–577.
- PARKYN, S. M., C. F. RABENI, AND K. J. COLLIER. 1997. Effects of crayfish (*Paranephrops planifrons*: Parastacidae) on in-stream processes and benthic faunas: a density manipulation experiment. New Zealand Journal of Marine and Freshwater Research 31:685–692.
- PELLMYR, O., AND C. J. HUTH. 1994. Evolutionary stability in the mutualism between yuccas and yucca moths. Nature 372:257–260.
- PENN, G. H. 1959. Survival of branchiobdellid annelids without a crayfish host. Ecology 40:515–516.
- POTVIN, C., M. J. LECHOWICZ, AND S. TARDIF. 1990. The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. Ecology 71:1389–1400.
- QUAGLIO, F., C. MOROLLI, R. GALUPPI, C. BONOLI, F. MARCER, L. NOBILE, G. DE LUISE, AND M. P. TAPIERI. 2006. Preliminary investigations of disease-causing organisms in the white-clawed crayfish *Austropotamobius pallipes* complex from streams of Northern Italy. Bulletin Francais de la Pêche et de la Pisciculture 380:1271–1290.
- ROSEWARNE, P., R. MORTIMER, AND A. DUNN. 2012. Branchiobdellidan infestation on endangered white-clawed crayfish (*Austropotamobius pallipes*) in the UK. Parasitology 1: 1–7.
- SCHOFIELD, K. A., C. M. PRINGLE, J. L. MEYER, AND A. B. SUTHERLAND. 2001. The importance of crayfish in the breakdown of rhododendron leaf litter. Freshwater Biology 46:1191–1204.
- STATZNER, B., E. FIEVET, J. Y. CHAMPAGNE, R. MOREL, AND E. HEROUIN. 2000. Crayfish as geomorphic agents and ecosystem engineers: biological behavior affects sand and gravel erosion in experimental streams. Limnology and Oceanography 45:1030–1040.
- STOECKEL, J. A., B. S. HELMS, AND E. CASH. 2011. Evaluation of a crayfish burrowing chamber design with simulated groundwater flow. Journal of Crustacean Biology 31: 50–58.
- TAYLOR, C. A., G. A. SCHUSTER, J. E. COOPER, R. J. DiSTEFANO, A. G. EVERSOLE, P. HAMR, H. H. HOBBS, 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 32:372–389.
- THOMAS, M. J., R. P. CREED, AND B. L. BROWN. 2013. The effects of environmental context and initial density on symbi-

- ont populations in a cleaning symbiosis. *Freshwater Science* 32:000–000.
- THOMPSON, J. N. 2005. *The geographic mosaic of coevolution*. University of Chicago Press, Chicago, Illinois.
- TRIVERS, R. L. 1971. The evolution of reciprocal altruism. *Quarterly Review of Biology* 46:35–57.
- USIO, N., AND C. R. TOWNSEND. 2004. Roles of crayfish: consequences of predation and bioturbation for stream invertebrates. *Ecology* 85:807–822.
- WILLIAMS, B. W., S. R. GELDER, AND H. PROCTOR. 2009. Distribution and first reports of Branchiobdellida (Annelida: Clitellata) on crayfish in the Prairie Provinces of Canada. *Western North American Naturalist* 69:119–124.
- WILLIAMS, B. W., S. R. GELDER, H. C. PROCTOR, AND D. W. COLTMAN. 2013. Molecular phylogeny of North American Branchiobdellida (Annelida: Clitellata). *Molecular Phylogenetics and Evolution* 66:30–42.
- WOODHEAD, A. E. 1950. Life history cycle of the giant kidney worm, *Diectophyma renale* (Nematoda), of man and many other mammals. *Transactions of the American Microscopical Society* 69:21–46.
- YOUNG, W. 1966. Ecological studies of the Branchiobdellidae (Oligochaeta). *Ecology* 47:571–578.
- ZHANG, Y., J. S. RICHARDSON, AND J. N. NEGISHI. 2004. Detritus processing, ecosystem engineering and benthic diversity: a test of predator–omnivore interference. *Journal of Animal Ecology* 73:756–766.

Received: 6 December 2012

Accepted: 30 July 2013