

The rules for symbiont community assembly change along a mutualism–parasitism continuum

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Summary

1. Symbiont community assembly is driven by host–symbiont and symbiont–symbiont interactions. The effects that symbionts exert on their hosts are often context-dependent, and existing theoretical frameworks of symbiont community assembly do not consider the implications of variable outcomes to assembly processes.

2. We hypothesized that symbiont–symbiont interactions become increasingly important along a parasitism/mutualism continuum because; (i) negative outcomes favour host resistance which in turn reduces symbiont colonization and subsequently reduce symbiont–symbiont interactions, whereas (ii) positive host outcomes favour tolerance and consequently higher symbiont colonization rates, leading to stronger interactions among symbionts. We found support for this hypothesis in the cleaning symbiosis between crayfish and ectosymbiotic branchiobdellidan worms.

3. The symbiosis between crayfish and their worms can shift from parasitism/commensalism to mutualism as crayfish age. Here, field surveys identified changes in worm density, diversity and composition that were concomitant to changing symbiosis outcomes. We conducted several laboratory experiments and behavioural assays to relate patterns from the field to their likely causal processes.

4. Young crayfish typically hosted only two relatively small worm species. Older crayfish hosted two additional larger species. In laboratory experiments, young crayfish exhibited a directed grooming response to all worm species, but were unable to remove small species. Conversely, adult crayfish did not exhibit grooming responses to any worm species. Relaxed grooming allowed the colonization of large worm species and initiated symbiont–symbiont intraguild predation that reduced the abundance and altered the behaviour of small worm species. Thus, the dominant processes of symbiont community assembly shifted from host resistance to symbiont–symbiont interactions through host ontogeny and a concomitant transition towards mutualism.

5. This work shows that host resistance can have a prevailing influence over symbiont community assembly when symbiosis is disadvantageous to the host. However, when symbiosis is advantageous and resistance is relaxed, symbiont colonization rate and consequently abundance and diversity increases and interactions among symbionts become increasingly important to symbiont community assembly.

Key-words: cleaning symbiosis, crayfish, mutualisms, ontogeny, parasite, parasite ecology, succession, symbiosis

Introduction

Contrary to traditional typological classification systems, it is now generally recognized that symbioses lie on a

dynamic continuum with parasitism at one end and mutualism at the other (Ewald 1987; Bronstein 1994; Leung & Poulin 2008). This realization unites theorists and empiricists working from opposite ends of the symbiosis continuum because research from parasite systems can inform studies of mutualisms and vice versa (Thompson 1988;

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Leung & Poulin 2008). The symbiosis continuum also provides a conceptual framework that incorporates the possibility that specific pairs of species, or perhaps even specific pairs of individuals, may shift between parasitism and mutualism in response to changing contexts of the interaction (Bronstein 1994; Leung & Poulin 2008; Brown *et al.* 2012). For instance, the costs and benefits of symbiosis may change through an individual's ontogeny, yielding positive and negative outcomes at different life stages (Palmer *et al.* 2010; Yang & Rudolf 2010; Yule, Miller & Rudgers 2013).

Because the outcomes of symbiosis can change with context, selection may favour context-dependent resistance to symbiont colonization. When symbiosis offers a fitness advantage, selection favours hosts that allow or actively facilitate colonization of symbionts (e.g. Van Rhijn & Vanderleyden 1995; Nyholm *et al.* 2000), and consequently colonization is typically not restricted by host defences. Conversely, when symbiosis is detrimental (i.e. parasitism), selection can lead to co-evolutionary arms races between hosts which try to resist colonization and parasites which try to circumvent host resistance (Van Valen 1974; Ebert & Hamilton 1996). Thus, when symbiosis decreases host fitness, host defences often limit colonization of symbionts to all but a few taxa or strains which are specially adapted to evade the defences of a particular host (Holmes & Price 1986; Ulrich, Sadd & Schmid-Hempel 2011), and when symbiosis increases host fitness, those barriers to colonization are removed. While these direct effects on individual symbiont taxa are relatively straightforward, the influence of hosts on symbiont colonization rates may lead to more complex indirect influences over the ecological processes that shape diverse within-host communities of symbionts, a level of ecological organization termed the *symbiont infracommunity* (sensu Bush *et al.* 1997).

General ecological theory predicts that higher patch colonization rates result in higher local diversity, more niche overlap among species and consequently stronger interspecific interactions (Cornell & Lawton 1992). By extension of general theory, we hypothesized that ecological contexts that yield negative effects of symbiosis on host fitness will lead to relatively low infracommunity diversity and weak within-host-symbiont interactions because adaptive host resistance restricts symbiont colonization. Conversely, contexts which yield positive host outcomes will lead to more diverse symbiont infracommunities and strong interactions among symbionts because colonization is not resisted, but perhaps is encouraged by the host. We tested our hypothesis in an intensive field survey and a series of laboratory experiments involving the cleaning symbiosis between crayfish and a multi-species assemblage of ectosymbiotic annelid worms (Annelida: Branchiobdellida).

Crayfish throughout Europe, North America and Asia frequently host multi-species assemblages of obligate ectosymbiotic worms (Gelder 2010). Branchiobdellidans

have no free-living life stage, and available studies suggest that they require a host for reproduction (Creed *et al.* 2015). These worms sometimes provide a beneficial cleaning service to their hosts. Accumulations of sediments and biofilms, particularly on respiratory surfaces, are an often major challenge to crayfish physiology (Bauer 1998). By feeding on potentially harmful epibiotic accumulations such as bacterial flocs and sediments that may interfere with respiratory processes and ammonia excretion, branchiobdellidans may increase crayfish growth and survivorship under certain contexts (Brown, Creed & Dobson 2002; Lee, Kim & Choe 2009). Cleaning symbiosis mutualisms between branchiobdellidans and crayfish are a widespread phenomenon and a generally important facet of crayfish biology. Evidence of this generality comes from multiple studies of many disparate crayfish and branchiobdellidan species in North America and Asia that have experimentally demonstrated a positive effect of branchiobdellidans on the growth and survivorship of their hosts (Brown, Creed & Dobson 2002; Lee, Kim & Choe 2009; Brown *et al.* 2012; Skelton *et al.* 2013; Thomas 2014; Ames, Helms & Stoeckel 2015).

Although branchiobdellidans may provide benefits to their hosts, they also levy costs. For example, branchiobdellidans may consume host tissues (e.g. Grabda & Wierzbicka 1969; Brown *et al.* 2012; Rosewarne, Mortimer & Dunn 2012; Rosewarne *et al.* 2014). As a consequence of the simultaneous costs and benefits of symbiosis, the net outcome of symbiosis for crayfish is context-dependent as ecological context shifts the balance of the costs and benefits, similar to many other well-studied mutualistic associations (e.g. Thompson & Pellmyr 1992; Johnson, Graham & Smith 1997; Johnstone & Bshary 2002; Wulff 2008; Palmer *et al.* 2010; Yang & Rudolf 2010; Yule, Miller & Rudgers 2013). The net effect of branchiobdellidans on their host changes with the rate of sediment and biofilm accumulation (Lee, Kim & Choe 2009; Thomas, Creed & Brown 2013; Ames, Helms & Stoeckel 2015), symbiont density (Brown *et al.* 2012) and host age (Skelton, Creed & Brown 2014; Thomas 2014). Thus, an individual crayfish could maximize its lifelong benefit of symbiosis by actively modulating its interactions with branchiobdellidans.

In response to changing outcomes of symbiosis, crayfish use adaptive grooming responses to resist symbiont colonization when symbiosis is not likely to be beneficial. For example, young crayfish are not likely to benefit from the cleaning services of their symbionts because they grow rapidly and moult very frequently (St John 1976), and moulting effectively relieves crayfish of all epibiotic accumulations (Bauer 1998). Thus, for a young crayfish, symbiosis with branchiobdellidans is not likely to be very beneficial, whereas adult crayfish are more likely to benefit from symbiosis because they moult infrequently (Skelton, Creed & Brown 2014; Thomas 2014). Concomitant to changes in the outcome of symbiosis, crayfish display an ontogenetic shift in resistance to symbiont coloniza-

tion; young hosts use grooming behaviours to rapidly remove and often consume colonizing worms, whereas adult crayfish remove their worms much less frequently (Farrell, Creed & Brown 2014; Skelton, Creed & Brown 2014). Other evidence suggests that crayfish also adjust their resistance to symbiont colonization in response to the rate of sediment and biofilm accumulation and symbiont density (Thomas, Creed & Brown 2013).

Based on our hypothesis, we predicted: (i) Successful colonization of branchiobdellidans on young crayfish will be limited to relatively few taxa that are less susceptible to host resistance, and consequently, interspecific symbiont interactions on young crayfish will be weak. (ii) Colonization of older crayfish will not be limited by host resistance, leading to more diverse symbiont infracommunities and stronger interspecific symbiont interactions. To test our predictions, we conducted a field survey of the crayfish *Cambarus sciotensis* (Rhoades, 1944) and their ectosymbionts to identify patterns of symbiont diversity and composition through host ontogeny. We then used laboratory behavioural assays to demonstrate an ontogenetic shift in host response to the colonization of multiple branchiobdellidan species. We experimentally assessed the effects of host age and host response on the successful colonization of each symbiont species. We also experimentally examined interspecific interactions among symbiont species typical of young and old hosts. Congruent with our hypothesis, we found that the factors controlling symbiont infracommunity structure changed as the interaction between crayfish and their worms move along the symbiosis continuum towards mutualism. Specifically, we observed that symbiont colonization rates, within-host diversity, and consequently, interspecific interactions increased as crayfish grow, the benefits of symbiosis increase and host resistance is relaxed.

Materials and methods

FIELD SURVEY

Our study was conducted in the Mountain Lake region of southwest Virginia. This region supports populations of several native crayfish and at least 12 branchiobdellidan species (Hobbs, Holt & Walton 1967), including some of the species previously shown to have mutualistic associations (Brown *et al.* 2012; Thomas 2014). Individual crayfish in this area host as many as several hundred branchiobdellidans, belonging to eight or more species and several genera (Hobbs, Holt & Walton 1967; Gelder & Williams 2011; Skelton *et al.* 2013). We collected crayfish and their symbionts from a third-order tributary of the New River near Newport Virginia, USA (37.301939, -80.487472; lat/long in DDD) during February 2012. Crayfish were collected by hand, dip net and seine. Crayfish identification followed the keys of Hobbs, Holt & Walton (1967), and branchiobdellidan identification followed the keys of Hobbs, Holt & Walton (1967) and Holt & Opell (1993). All crayfish were sexed and measured by mass and carapace length (distance in mm from acumen of rostrum to dorsal posterior edge of carapace; CL).

HOST RESISTANCE STUDY

We examined age-specific host resistance to colonization of four branchiobdellidan species present at the study site. For each worm species, we tested 24 crayfish (96 in total; $size_{min} = 12.11$ mm CL, $size_{max} = 43.42$ mm CL). Crayfish were tested in eight groups of 12 individuals throughout the summer of 2012 in a randomized testing sequence. Individual crayfish and worms were not used more than once. We used a stratified sampling design to maximize the evenness of host size within each test group. Animals were collected from the field the day before observation. On the day of collection, worms belonging to four species [*Cambarincola fallax* (Hoffman, 1963), *C. ingens* (Hoffman 1963), *Pterodrilus allicornis* (Moore 1895) and *Ankyrodrilus koronaeus* (Holt 1965)] to be used in behavioural tests were removed from the host and stored in stream water at 5 °C. All remaining worms and their eggs were killed by 5-min immersion in 10% MgCl₂ hexahydrate solution which kills all worm and worm eggs, but has no noticeable effect on crayfish (Brown, Creed & Dobson 2002). Crayfish were placed individually in circular (20 cm diameter) transparent plastic dishes filled with 1 L of stream water at 21 °C. Dishes with crayfish were kept in the dark testing area undisturbed for 12 h before testing. All observations were made under dim red light to reduce visibility of the observer to the crayfish. Each test consisted of a 3-min baseline observation to ensure that individuals were not displaying signs of sickness or stress, followed by inoculation of a single worm placed on the dorsal aspect of the host carapace within the areola, followed by a 5-min observation of host behaviour and quantification of directed grooming responses. Directed grooming responses were defined as using one or more walking legs to scratch and/or grab at the inoculated worm or its immediate vicinity (~1 worm body length). Similar methodology has been successfully used in previous work to examine host-specific differences in grooming responses and branchiobdellidan abundance (Farrell, Creed & Brown 2014). Results of a preliminary test conducted by two observers were congruent among observers; a single observer conducted subsequent tests.

PERSISTENCE EXPERIMENTS

We conducted a series of laboratory experiments to determine the effects of age-related variation in host resistance on the successful colonization of all four branchiobdellidan species. We measured symbiont persistence on a new host over a 1-week period as a proxy for colonization ability. Crayfish from 14.4 to 43.60 mm CL were selected from the field to create parallel host size gradients for each treatment. All worms present on these crayfish were killed by 5-min immersion in 10% MgCl₂ hexahydrate solution. Each experiment included 24 crayfish that were split into two treatment groups: (i) crayfish with ablated dactyls on both chelate walking legs 2 and 3 and (ii) a reference group with intact dactyls. Dactyl ablation is the removal of the dactyl from the claw on a leg and reduces the effectiveness of crayfish grooming in removing ectosymbionts and therefore provides a control group for detecting host grooming effects on symbiont persistence. This treatment does not completely prevent the removal of ectosymbionts by their host, but does reduce the efficacy of grooming and thus provides evidence of the grooming effect when compared to un-ablated controls (Skelton, Creed & Brown 2014). We have used this treatment in previous experi-

ments and have observed no obvious effects on crayfish behaviour or survival (Skelton, Creed & Brown 2014). After immersion in $MgCl_2$ and dactyl ablation, crayfish were placed individually in 40 L experimental chambers and given a week to acclimate and recover. We then applied six worms of each species (*C. fallax*, *C. ingens*, *P. alaicornis* and *A. koronaeus*) to the dorsal aspect of the carapace on each crayfish. Worm species were tested individually to avoid confounding effects of interspecific interactions on worm persistence. After 8 days, we sacrificed all animals in 70% ethanol and enumerated the remaining worms.

SYMBIONT INTERACTION EXPERIMENT

We used methods similar to the persistence experiments described above to assess symbiont–symbiont interactions across host ontogeny by testing for numerical and functional responses (*sensu* Sousa 1994) among symbiont species typical of young and old hosts. This experiment included two symbiont species identified during the field study as numerically dominant on young hosts (early taxa; *C. fallax* and *P. alaicornis*), and one species typically found only on older hosts (late taxon; *C. ingens*). Worm species were applied separately and in mixed culture combinations at species-specific densities typical to field observations (15 mature individuals for *C. fallax* and *P. alaicornis*, and six mature individuals for *C. ingens*). Interspecific numerical responses were assessed by comparing symbiont survival, and functional responses were assessed by comparing worm microhabitat use over 40 days as a function of the presence/absence of other symbiont species in an incomplete factorial design. Data were collected non-destructively as repeated measures at 6, 24 and 40 days. Three species combinations were used to assess symbiont–symbiont interactions of species typical to young hosts; *C. fallax* only, *P. alaicornis* only and *C. fallax* with *P. alaicornis*. To test for interactions among species typically found on older hosts, we combined *C. fallax* with *C. ingens*, *P. alaicornis* with *C. ingens* and a combination of all three species. We had 24 experimental units and eight replicates for each species' response.

DATA ANALYSIS

We identified ontogenetic patterns of symbiont communities from survey data by examining changes in total symbiont abundance, symbiont infracommunity diversity (Simpson's Index) and species relative abundances across host CL. Carapace length was used instead of age because it is readily measurable, it provides a reasonable proxy of age and it is more reliable than mass for estimating the age of temperate crayfish (Belchier *et al.* 1998). We used generalized additive models (GAM; mgcv package for R v 1.7-11) to regress each measure of symbiont community structure against host CL. We chose GAM over other analytical techniques because when provided with large sample sizes, it can detect complex nonlinear responses over a gradient. Because we predicted a sudden change in infracommunity assembly processes coincident with relaxed host grooming, we used piecewise regression and principal coordinates ordination to look at threshold responses in the slope of the relationship between infracommunity composition and host ontogeny. We conducted a principle coordinates ordination (pcoa; ape package for R v 3.0-1) based on a relativized Bray–Curtis dissimilarity matrix of symbiont infra-communities and used piecewise regression to look for a host size (CL) at which the relationship between host CL and the first two principal coordinates of infracommunity

composition changed significantly (piecewise linear function; SiZer package for R v 0.1-4) (Toms & Lesperance 2003).

To determine the effects of symbiont species identity and host age on directed grooming responses, we used binomial GAMs to model the probability of a 'directed grooming' response as a function of crayfish size (CL; mm) and the categorical factor worm species (with interaction term). We used a binary response variable defined as the presence or absence of a directed grooming response during the 5-min response observation rather than count data of the behavioural responses to avoid violations of independence among responses. In the persistence experiments, we assessed species-specific effects of the age-specific host grooming response on symbiont persistence by using generalized linear models (glm function; stats package for R v 2.13.1) to model the probability of each inoculated worm persisting on the host through the 8-day trial as a function of host size and amputation treatment (with interaction term) and a binomial distribution. Here, we used GLM instead of GAM because practical limitations of experimentation limited our sampling effort and GLM is more robust to small sample sizes (WisZ *et al.* 2008).

Finally, to assess numerical responses of branchiobdellidans to symbiont–symbiont interactions, we used a generalized linear mixed-model (GLMM) for each symbiont species (lmer function; lme4 package for R v 0.999375-42) to model the proportion of individuals of each worm persisting over the 40-days experiment as a function of time and the presence/absence of other species, assuming a binomial distribution and including individual crayfish as a random effect with random intercept and slope. We used a mixed-model formulation to account for random variation in the probability of individual worm persistence among individual hosts. Functional responses of *C. fallax* and *P. alaicornis* to each other and to *C. ingens* were assessed visually by non-metric multidimensional scaling ordination (NMDS; metaMDS function; vegan package for R v 2.0-0) of a Jaccard distance matrix of the multivariate response of number of individuals of the focal species at each attachment site. We tested for statistical significance using permutational multivariate analysis of variance (PERMANOVA; adonis function; vegan package for R v 2.0-0; Jaccard distance, 9999 permutations) to test for effects of the presence/absence of other species on the distribution of the proportions of individuals across all attachment sites with time as a covariate. For all GAMs, GLMs and GLMMs, predictor variables were determined *a priori* based on hypotheses specific to each study. Validation of error distributions and default basis dimensions was assessed visually by scatterplots comparing fitted values to raw data, predicted values and residuals, histograms of residuals and Q-Q plots (Zuur *et al.* 2009). In cases of excessive over-dispersion, 'quasi' distribution families were used to estimate the over-dispersion parameter from the data.

Results

FIELD SURVEY

We observed consistent changes in symbiont abundance, diversity and composition across host ontogeny. We recovered 3533 branchiobdellidans belonging to four species and three genera from 86 *Cambarus sciotensis* (Table 1). Total symbiont abundance increased asymptotically with host size (GAM; $F = 534.7$, $P < 0.0001$, $edf = 8.724$, $n = 86$, $k = 9$, $adj. R^2 = 0.27$). There was a

rapid increase in total symbiont abundance in hosts from ~15 to ~30 mm CL, but no obvious trend beyond ~30 mm CL (Fig. 1a). Symbiont diversity also increased asymptotically with host CL (GAM; $F = 31.01$, $P < 0.0001$, $\text{edf} = 2.6$, $n = 86$, $k = 9$, $\text{adj. } R^2 = 0.54$; Fig. 1b). We also identified a distinct shift in symbiont community composition at 26.88 mm CL for principal coordinates (PCo) 1 and 2. The slope of the relationship between PCo axis 1 and host size changed from -0.040 to -0.002 at 26.88 mm CL. The slope of the relationship between PCo axis 2 and host size changed from -0.003 to 0.013 at 26.88 mm CL. Thus, there was a threshold change in the relationship between crayfish size and community composition that occurred at *c.* 27 mm CL.

All four symbiont species showed significant changes in their respective relative abundances through host ontogeny (Fig. 2). *Cambarincola fallax* was the most abundant species on crayfish smaller than the infracommunity composition threshold value of 26.88 mm CL (shown as vertical dotted reference lines in Fig. 2), and the relative abundance of *C. fallax* declined with host size (GAM; $X^2 = 6.959$, $P < 0.0001$, $n = 86$, $\text{adj. } R^2 = 0.731$). The relative abundance of *P. alcornis* showed a hump-shaped response to host CL, peaking at ~30 mm, just beyond the composition threshold size of 26.88 mm (GLM; $X^2 = 7.322$, $P < 0.0001$, $n = 86$, $\text{adj. } R^2 = 0.719$). Both *C. ingens* ($X^2 = 37.26$, $P < 0.0001$, $n = 86$, $\text{adj. } R^2 = 0.428$) and *A. koronaeus* ($X^2 = 156.1$, $P < 0.001$, $n = 86$, $\text{adj. } R^2 = 0.665$) were generally absent on crayfish smaller than the composition threshold size, but increased in relative abundance thereafter.

HOST RESISTANCE STUDY

Host-directed grooming behaviours decreased significantly with host size (GAM; $X^2 = 21.98$, $P < 0.0001$, $n = 93$, $\text{adj. } R^2 = 0.400$; Fig. 3). There were no significant effects of worm species identity on directed grooming response or significant interactions between worm species and host size; therefore, worm species was dropped as a factor from all models. Large and small crayfish responded differently to the introduction of branchiobdellidans as small crayfish were much more likely to attempt to remove the symbiont than were larger crayfish; 60% (27/45) of crayfish smaller than the 26.88 mm CL displayed a directed grooming response to the introduction of a worm during the 5-min observation period, whereas 8% (4/48) larger than 26.88 mm CL, and 0% (0/29) crayfish larger than 30.03 mm CL displayed a directed grooming response.

PERSISTENCE EXPERIMENT

Symbionts had distinct species-specific variation in their ability to successfully persist on young hosts (Fig. 4). Persistence of *C. fallax* was not significantly affected by either host size or dactyl amputation. *Pterodrilus alcornis* persistence increased with host size, but was unaffected by the dactyl treatment. Persistence of *C. ingens* increased with host size, with dactyl ablation, and there was a significant interaction term. Lastly, persistence of *A. koronaeus* increased with host size, but was unaffected by the dactyl treatment (see Table 2 for full models).

Table 1. Prevalence, mean and maximum intensity, and total number of individuals of four species of branchiobdellidans collected from 86 crayfish during field survey.

Branchiobdellidans	Prevalence (% infested)	Mean intensity (ind/host \pm SD)	Max intensity (ind/host)	Total
<i>Ankyrodrilus koronaeus</i>	60	8.88 \pm 9.29	47	462
<i>Cambarincola fallax</i>	100	18.28 \pm 13.70	67	1572
<i>Cambarincola ingens</i>	62	3.53 \pm 3.08	12	187
<i>Pterodrilus alcornis</i>	88	17.26 \pm 14.90	61	1312

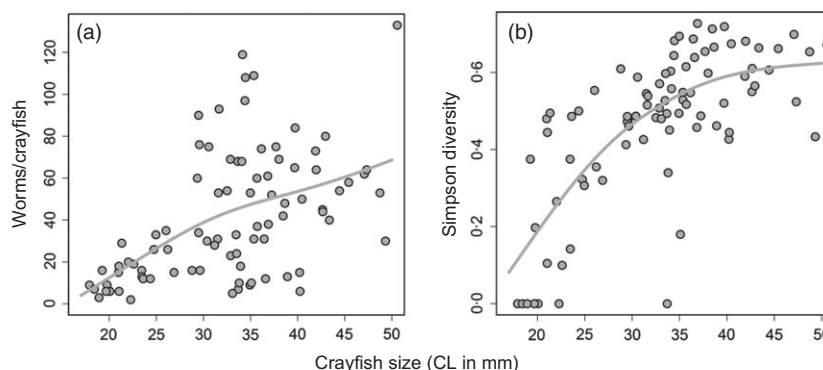


Fig. 1. Positive relationships between crayfish size and (a) total branchiobdellidan symbiont abundance and (b) Simpson diversity per crayfish. Solid grey line represents GAM model fits. Circles are observed data.

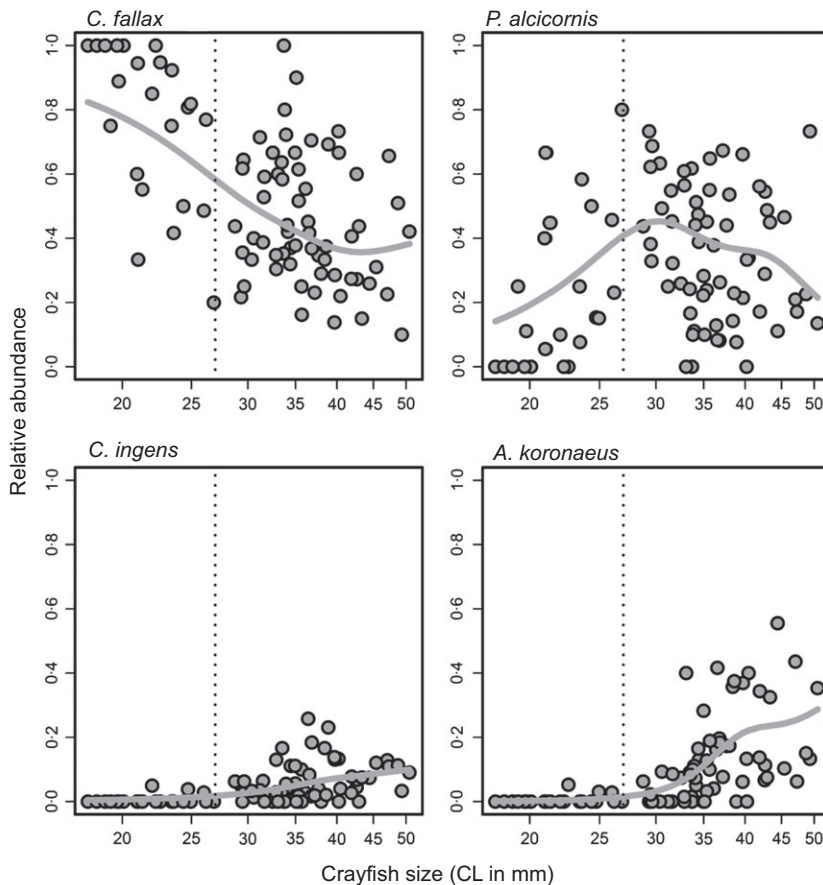


Fig. 2. Relationships between crayfish size and the relative abundances of four co-occurring branchiobdellidan species. Solid line represents GAM model fit of mean relative abundance, and circles represent observed data. Vertical reference line represents the infracommunity composition threshold identified by piecewise regression of PCo axes 1 and 2 at which the slope of the relationship between host size and symbiont community composition changes.

SYMBIONT INTERACTION EXPERIMENT

Of the two species able to persist on young hosts, one (*C. fallax*) had a numerical response to the arrival of *C. ingens*, and the other (*P. alcornis*) displayed a functional response. Persistence of *C. fallax* was reduced by the presence of *C. ingens* and not significantly affected by the presence of *P. alcornis* (Table 3). Persistence of *P. alcornis* was not significantly affected by the presence of *C. ingens* or *C. fallax*. Examination of attachment site use revealed a significant effect of *C. ingens* on the site use of *P. alcornis* (PERMANOVA; $F = 2.282$, $P = 0.049$). Most of this effect was evident along the first NMDS axis, which was strongly correlated with use of the lateral aspect of the ventral abdominal surface ($r = -0.66$), and the pleopods ($r = 0.84$), indicating more frequent attachment of *P. alcornis* to the pleopods and reduced attachment to the ventral abdomen in the presence of *C. ingens*. Site use of *P. alcornis* was not significantly affected by the presence of *C. fallax*, and site use of *C. fallax* was not significantly affected by the presence of *C. ingens* or *P. alcornis*.

Discussion

Hosts may maximize their lifetime fitness by adjusting resistance to context-dependent changes in the costs and

benefits of symbiosis (Yule, Miller & Rudgers 2013; Skelton, Creed & Brown 2014). Yet, the implications of context dependence for symbiont community assembly have not yet been explored. We predicted an increase in symbiont colonization, infracommunity diversity and symbiont–symbiont interactions as crayfish age and the benefits of symbiosis increase. Congruent with these predictions, field survey uncovered a punctuated increase in infracommunity diversity during crayfish ontogeny. Young crayfish (< 27 mm CL) typically hosted only two species of branchiobdellidans, *C. fallax* and *P. alcornis*. However, there was a threshold change in the relationship between crayfish size and symbiont composition that occurred at ~30 mm CL, beyond which the two larger species, *C. ingens* and *A. koronaeus*, first appeared and subsequently became an increasingly larger portion of the symbiont infracommunity. Thus, the observed increase in symbiont species through host ontogeny does not merely reflect increasing numbers of symbionts per host and an associated sampling effect, but instead, it is the result of additional novel taxa that were almost exclusively present on older hosts.

The observed punctuated change in infracommunity structure was tightly coupled with an ontogenetic shift in host resistance to colonizing symbionts. During the resistance experiment, the introduction of a single worm elicited a directed grooming response from the majority of

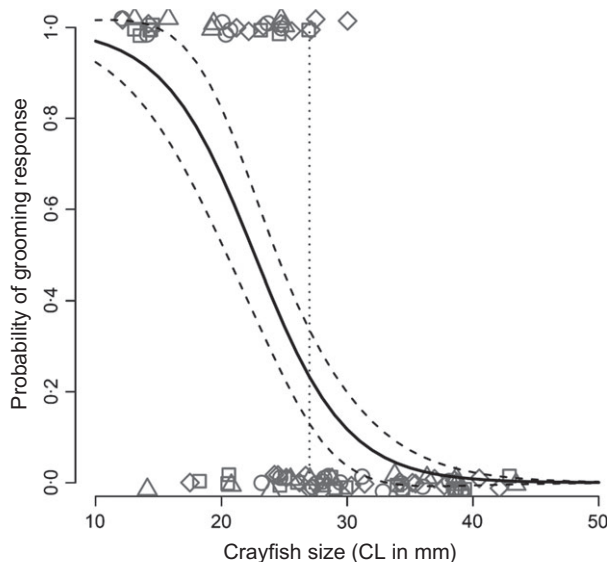


Fig. 3. Decreased host resistance to colonization through host ontogeny shown as probability of host grooming response to the introduction of a single branchiobdellidan during 5-min behavioural tests as a function of host size. Solid line represents GAM model fit, dotted lines represent 95% confidence envelope around grooming probability at a given size. Shapes indicate responses to each worm species where 1 = response, 0 = no response, circles = *Cambarincola fallax*, squares = *Pterodrilus alcornis*, triangles = *C. ingens* and diamonds = *Ankyrodrilus koronaeus*. Symbols were jittered for clarity. Vertical reference line represents the infracommunity composition threshold size identified by piecewise regression of PCo axes 1 and 2 from field survey at which the slope of the relationship between host size and symbiont community composition changes.

young hosts. This result is consistent with previous work which identified the grooming response as an adaptive strategy for avoiding symbiosis during life stages in which symbionts are not likely to be beneficial (Farrell, Creed & Brown 2014; Skelton, Creed & Brown 2014). Young crayfish displayed a consistent grooming response to all worm species, and large crayfish did not respond to any symbiont species, indicating that host resistance in this system is not specific to symbiont species. In contrast to young hosts, grooming responses were rarely on crayfish larger than 27 mm and were not observed in crayfish larger than 31 mm CL. The punctuated changes in symbiont diversity and composition observed in the field and the ontogenetic shift in host resistance demonstrated in the laboratory coincide when *Cambarus sciotensis* reaches *c.* 27–30 mm CL. Thus, the combined results of the host resistance study and field survey provide correlative evidence that an ontogenetic shift in host resistance alters infracommunity assembly dynamics.

We conducted the persistence experiment to confirm that ontogenetic changes in host resistance can cause the changes in symbiont diversity and species composition observed in the field, and to test the mechanisms likely associated with those changes. Based on field observations of worm abundance and the results of our resistance

experiment, we predicted that crayfish grooming was only effective against the colonization of *C. ingens* and *A. koronaeus*. Although young hosts consistently tried to remove all species of symbionts, the persistence experiment showed that some symbiont species were able to avoid removal. *Cambarincola fallax* was able to persist on crayfish of all sizes. This result matches field data showing complete prevalence of *C. fallax* on crayfish of all sizes. Additionally, the persistence experiment demonstrated that *C. ingens* and *A. koronaeus* were unable to persist on young hosts, but were able to persist on older hosts. Again, this result mirrored observations from the field showing few occurrences of these species on small hosts, but high prevalence on larger hosts. Persistence of *P. alcornis* also increased significantly with host size, but unlike later arriving species, a considerable fraction of individuals persisted on young hosts. This result was again consistent to field observations in which the prevalence and relative abundance of *P. alcornis* were intermediate to the early arriving *C. fallax* and the late arrivers, *C. ingens* and *A. koronaeus*. Also consistent with predictions, dactyl ablation increased persistence of *C. ingens* on young hosts and did not affect persistence of either early arriving species, demonstrating that these taxa are less affected by host grooming than *C. ingens*.

Unexpectedly, persistence of the late arriving species, *A. koronaeus*, was not increased by dactyl ablation. However, *A. koronaeus* is unique among the species considered in this study in that it typically attaches to the distal portions of the host's large claws and walking legs (Hobbs, Holt & Walton 1967), whereas the other species typically attach to the ventral aspects of the cephalothorax and abdomen (Hobbs, Holt & Walton 1967; Brown, Creed & Dobson 2002). While attached to the claws and legs, *A. koronaeus* was vulnerable to direct removal by host mouthparts in addition to removal via the fingers and dactyls of the walking legs. Thus, the dactyl ablation treatment was ineffective for *A. koronaeus* because of the preferred attachment sites of this particular species.

One alternative explanation for the absence of larger branchiobdellidans on younger, smaller hosts is that small hosts do not provide sufficient resources for larger branchiobdellidans. However, this alternative hypothesis is not a likely explanation for our experimental results or for patterns observed in the field. In addition to material collected from their host, branchiobdellidans also consume material from nearby non-host substrate and the water column and can survive extended periods without food (Gale & Proctor 2009; Gelder 2010). In fact, we and others (e.g. Woodhead 1950) have maintained stocks of branchiobdellidans in glass dishes for weeks, with no addition of food and no major mortality. Therefore, the great reduction in the number of *C. ingens* and *A. koronaeus* on small hosts at the end of our 1-week persistence experiment cannot be attributable to insufficient resources and was much more likely the result of the host's beha-

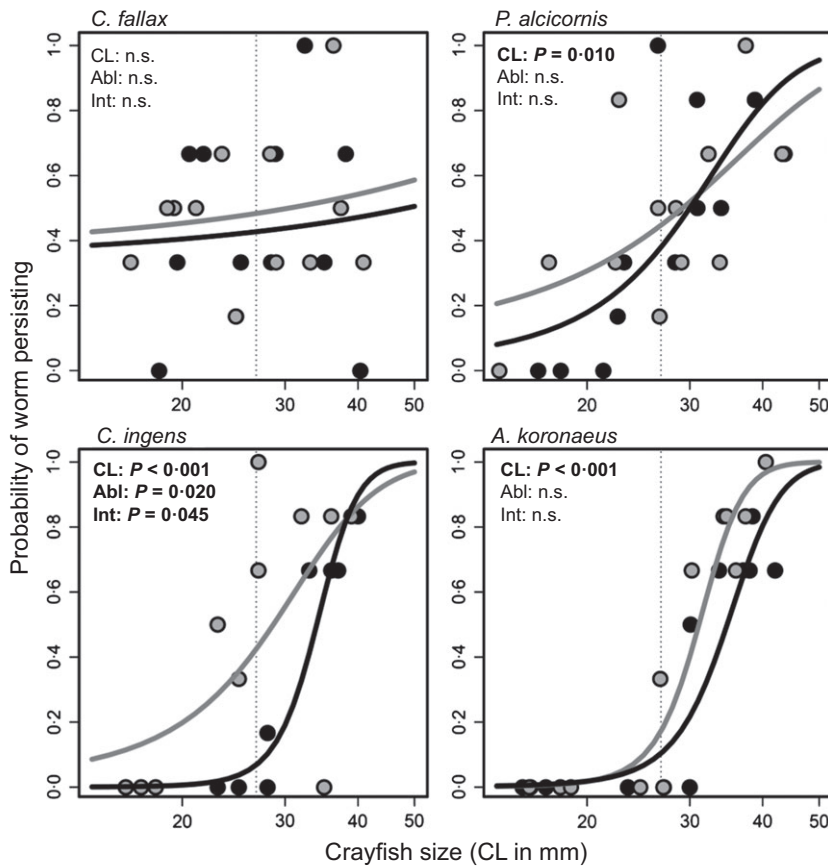


Fig. 4. The effect of host size and dactyl ablation on the persistence of four species of branchiobdellidans. Solid lines represent GLM model fits for dactyl-ablated (grey) and control (black) groups for probability of persistence at a given size. Symbols show proportion of colonists present at the conclusion of the 8-days experiment. Significant effects determined by GLM of host size (CL), ablation treatment (Abl) and their interaction (Int) are highlighted in bold. Vertical reference line represents the infracommunity composition threshold size identified by piecewise regression of PCo axes 1 and 2 at which the slope of the relationship between host size and symbiont community composition changes.

Table 2. GLM analysis of persistence experiments for four branchiobdellidans showing the effects of host size (carapace length) and ablation of host dactyls on the individual probability of worm persistence through an 8-day trial. Pseudo- R^2 for each species model was calculated using McFadden's method for null deviances based on 23 d.f., and residual deviances based on 20 d.f.

Species model	Coefficient	Estimate	SE	<i>z</i>	<i>P</i>	Pseudo- R^2
<i>C. fallax</i>	Intercept	-0.545	0.869	-0.627	0.531	0.020
	Carapace length	0.018	0.031	0.585	0.558	
	Ablation	-0.111	1.299	-0.086	0.932	
	Carapace length × ablation	-0.004	0.045	-0.096	0.924	
<i>P. alvicornis</i>	Intercept	-2.597	0.994	-2.614	0.009	0.387
	Carapace length	0.089	0.034	2.590	0.010	
	Ablation	-1.982	1.552	-1.277	0.201	
	Carapace length × ablation	0.064	0.054	1.186	0.236	
<i>C. ingens</i>	Intercept	-4.634	1.127	-4.113	< 0.001	0.628
	Carapace length	0.162	0.040	4.010	< 0.001	
	Ablation	-7.675	3.292	2.331	0.019	
	Carapace length × ablation	0.200	0.099	2.005	0.044	
<i>A. koronaeus</i>	Intercept	-11.296	2.553	-4.424	< 0.001	0.819
	Carapace length	0.363	0.081	4.510	< 0.001	
	Ablation	1.914	3.444	0.556	0.578	
	Carapace length × ablation	-0.093	0.105	-0.892	0.372	

vioural response documented in our host resistance assay and previous studies (Jones & Lester 1996; Farrell, Creed & Brown 2014; Skelton, Creed & Brown 2014). Because grooming in the small crayfish of the un-ablated groups essentially reduced symbiont infestation intensities to zero in a time shorter than the time it would require to starve or otherwise remove branchiobdellidans by resource limi-

tation, we argue that grooming is the most likely force behind the patterns of species-specific symbiont frequencies and intensity observed across host ontogeny in the field. For similar reasons, variation in intermoult period cannot account for the observed patterns because rapid effects of grooming response would supersede the time needed for complete moult cycles to occur.

Table 3. Results of GLMMs from symbiont interaction experiment showing the effects of the presence/absence of additional species (with interaction) and time on persistence of focal species (first column)

Symbiont spp	Parameter	Estimate	SE	<i>P</i>
<i>C. fallax</i>	Intercept	0.445	0.318	0.162
	<i>C. ingens</i>	-0.838	0.374	0.025*
	<i>P. alaicornis</i>	-0.491	0.364	0.178
	Day	-0.041	0.007	< 0.001**
	<i>C. ingens</i> × <i>P. alaicornis</i>	0.616	0.536	0.251
<i>P. alaicornis</i>	Intercept	-0.914	0.291	0.001*
	<i>C. ingens</i>	0.127	0.362	0.726
	<i>C. fallax</i>	0.371	0.355	0.296
	Day	-0.053	0.009	< 0.001**
	<i>C. ingens</i> × <i>C. fallax</i>	-0.432	0.509	0.396

*Significance at $P < 0.05$, **significance at $P < 0.001$.

The effects of grooming observed here are consistent with previous work on other crayfish ectosymbionts populations. For example, differences in grooming responses between crayfish species have been shown to explain difference in the frequency and intensity of *C. ingens* infestation on co-occurring crayfish species (Farrell, Creed & Brown 2014). Also, field survey and laboratory experiments showed age-specific grooming of the crayfish *Cambarus bartonii* limits colonization of the branchiobdellidan *X. appalachius* to mainly adults, though some *X. appalachius* may persist on resistant hosts by occupying microhabitats that are inaccessible to grooming efforts (Skelton, Creed & Brown 2014). Additionally, work conducted on an unrelated but functionally similar group of ectosymbiotic flatworms (Platyhelminthes: Temnocephalida) identified crayfish grooming behaviour as the most important factor for determining symbiont population size (Jones & Lester 1996). Thus, crayfish grooming appears to be a ubiquitously important component of crayfish ectosymbiont ecology and is therefore likely to influence evolution of symbiont life-history strategies.

Symbiont body size could explain the disparity in the effectiveness of host resistance among symbiont species and may reflect a trade-off in life-history strategies among symbiont species. In this study, both early arriving symbiont species were relatively small; the maximum total length (preserved) of *C. fallax* is ~4 mm and that of *P. alaicornis* is ~3 mm. Conversely, both species that were unable to colonize young hosts are considerably larger, up to ~10 mm. Indeed, the authors have personally experienced the difficulty of removing these smaller taxa with fine forceps and the relative ease of removing the larger taxa. Being small may offer *C. fallax* and *P. alaicornis* the ability to exploit a greater portion of the host population. However, being large may offer *C. ingens* and *A. koronaeus* the ability to exploit a larger range of food resources, including other branchiobdellidans. Moreover, given the observed negative impacts of *C. ingens* on *C. fallax*, a

body size trade-off that permits *C. fallax* to colonize small hosts that are inaccessible to *C. ingens*, could be essential to the coexistence of these species, similar to familiar competition-colonization trade-offs (e.g. Levins & Culver 1971; Yu & Wilson 2001).

Parasitologists have long recognized the potential importance of symbiont-symbiont interactions in structuring infracommunities (Sousa 1994; Poulin 2007; Johnson & Buller 2010; Ulrich & Schmid-Hempel 2012), and several authors have attempted to formalize a conceptual framework for understanding the circumstances that lead to strong interactions among parasitic symbionts (Holmes 1973; Rohde 1979; Holmes & Price 1986; Esch, Bush & Aho 1990). A summary of this work described parasite infracommunities as lying on a continuum with 'isolationist communities', which are composed of weakly interacting or non-interacting symbiont species, at one end. At the other end of the continuum are 'interactive communities' which are composed of more strongly interacting symbiont species (Sousa 1994). Symbiont communities are predicted to be interactive when colonization rates are relatively high and within-host communities approach saturation. Isolationist communities are predicted when colonization is low and within-host communities do not approach saturation (Holmes & Price 1986; Bush *et al.* 1997).

Does the relaxation of host resistance lead to more interactive symbiont infracommunities? Based on general ecological theory, we predicted that the removal of host resistance as a species filter would result in the successful colonization of more symbiont taxa, and consequently an increased influence of species interactions within the infracommunity. Although increased diversity alone may suggest a more interactive symbiont infracommunity, species interactions do not necessarily increase with the addition of species, and experimental evidence is typically needed to verify the strength of symbiont interactions (Bush *et al.* 1997). Sousa (1994) identified two 'smoking guns' indicative of strong interspecific interactions within infracommunities: first numerical responses (abundance of one species is altered by presence/abundance of another) and secondly functional responses (i.e. behaviour of one species changes in response to another). In our symbiont interaction experiment, we found no evidence of numerical or functional responses among the species that commonly co-occur on small *Cambarus sciotensis*, suggesting that the infracommunities of small *Cambarus sciotensis* are representative of the isolationist infracommunities described by others (Holmes & Price 1986). However, we identified numerical and functional responses of interspecific interactions among branchiobdellidans that co-occur on large hosts. Infection intensity of *C. fallax* was significantly decreased by the presence of *C. ingens*. Alternatively, the number of *P. alaicornis* surviving the experiment was unaffected by *C. ingens*, but attachment site use of *P. alaicornis* was significantly altered. Both *P. alaicornis* and *C. ingens* commonly attach to the ventral surfaces of the host abdomen (Hobbs, Holt & Walton 1967;

Brown, Creed & Dobson 2002). *P. alaicornis* moved in response to *C. ingens* from the ventral surface of the host abdomen to the pleopods (which extend from the abdomen), demonstrating a functional response of *P. alaicornis* to *C. ingens*. Intraguild predation and cannibalism have been observed in other branchiobdellidan species (Gale & Proctor 2009) and in *C. ingens* (Thomas 2014), and the responses we observed were likely the result of direct and indirect intraguild predation effects of the large *C. ingens* on the much smaller *C. fallax* and *P. alaicornis*. Thus, our results are congruent with the prediction that relaxation of host resistance leads to increased symbiont colonization, diversity and consequently stronger symbiont–symbiont interactions. Extrapolation of our results suggests that when all else is held equal, symbiont–symbiont interactions are likely to have a stronger influence over the assembly of infracommunities of mutualistic symbionts than infracommunities of parasites because of the direct and indirect modulating effects of host resistance.

Our results provide support for emerging conceptual frameworks that go beyond traditional pairwise conceptualizations of symbioses to emphasize the importance of within-host species interactions to symbiont ecology and evolution. In addition, we show that the context of multi-species symbioses determines the dominant processes that shape patterns of symbiont diversity when hosts modulate resistance to colonization to match underlying costs and benefits of symbiosis. Because context-dependent outcomes and ontogenetic shifts are typical of many symbioses, the hypotheses advanced in this study are generally applicable to multi-species symbiotic systems.

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Data accessibility

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.cq983> (Skelton 2016).

References

- Ames, C.W., Helms, B.S. & Stoeckel, J.A. (2015) Habitat mediates the outcome of a cleaning symbiosis for a facultatively burrowing crayfish. *Freshwater Biology*, **60**, 989–999.
- Bauer, R.T. (1998) Gill-cleaning mechanisms of the crayfish *Procambarus clarkii* (Astacidea: Cambaridae): experimental testing of setobranch function. *Invertebrate Biology*, **117**, 129–143.
- Belchier, M., Edsman, L., Sheehy, M.R.J. & Shelton, P.M.J. (1998) Estimating age and growth in long-lived temperate freshwater crayfish using lipofuscin. *Freshwater Biology*, **39**, 439–446.
- Bronstein, J.L. (1994) Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution*, **9**, 214–217.
- Brown, B., Creed, R.P. & Dobson, W.E. (2002) Branchiobdellid annelids and their crayfish hosts: are they engaged in a cleaning symbiosis? *Oecologia*, **132**, 250–255.
- Brown, B.L., Creed, R.P., Skelton, J., Rollins, M.A. & Farrell, K.J. (2012) The fine line between mutualism and parasitism: complex effects in a cleaning symbiosis demonstrated by multiple field experiments. *Oecologia*, **170**, 199–207.
- Bush, A.O., Lafferty, K.D., Lotz, J.M. & Shostak, A.W. (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, **83**, 575–583.
- Cornell, H.V. & Lawton, J.H. (1992) Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. *Journal of Animal Ecology*, **61**, 1–12.
- Creed, R.P., Lomonaco, J.D., Thomas, M.J., Meeks, A. & Brown, B.L. (2015) Reproductive dependence of a branchiobdellidan annelid on its crayfish host: confirmation of a mutualism. *Crustaceana*, **88**, 385–396.
- Ebert, D. & Hamilton, W.D. (1996) Sex against virulence: the coevolution of parasitic diseases. *Trends in Ecology & Evolution*, **11**, 79–82.
- Esch, G.W., Bush, A.O. & Aho, J.M. (eds) (1990) *Parasite Communities: Patterns and Processes*. Chapman and Hall, London, UK.
- Ewald, P.W. (1987) Transmission modes and evolution of the parasitism–mutualism continuum. *Annals of the New York Academy of Sciences*, **503**, 295–306.
- Farrell, K.J., Creed, R.P. & Brown, B.L. (2014) Preventing overexploitation in a mutualism: partner regulation in the crayfish–branchiobdellid symbiosis. *Oecologia*, **174**, 501–510.
- Gale, K.S.P. & Proctor, H.C. (2009) Diets of two congeneric species of crayfish worm (Annelida: Clitellata: Branchiobdellidae) from western Canada. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **89**, 289–296.
- Gelder, S.R. (2010) Branchiobdellida, section III. *Ecology and Classification of North American Freshwater Invertebrates* (ed. J.H.T.A.A.P. Covich), pp. 402–410. Academic Press, London, UK.
- Gelder, S.R. & Williams, B.W. (2011) First Distributional Study of Branchiobdellida (Annelida: Clitellata) in the Great Smoky Mountains National Park, North Carolina and Tennessee, USA, with a redescription of *Cambaricola holostomus* Hoffman, 1963. *Southeastern Naturalist*, **10**, 211–220.
- Grabda, E. & Wierzbicka, J. (1969) The problem of parasitism of the species of the genus *Branchiobdella* Odier, 1823. *Polskie Archiwum Hydrobiologii*, **16**, 93–104.
- Hobbs, H.H.J., Holt, P.C. & Walton, M. (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.
- Holmes, J.C. (1973) Site selection by parasitic helminths: interspecific interactions, site segregation, and their importance to the development of helminth communities. *Canadian Journal of Zoology*, **51**, 333–347.
- Holmes, J.C. & Price, P.W. (1986) Communities of parasites. *Community Ecology: Pattern and Process* (eds J. Kikkawa & D.J. Anderson), pp. 187–213. Blackwell Science Publications, Melbourne, Australia.
- Holt, P. & Opell, B. (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.
- Johnson, P.T.J. & Buller, I.D. (2010) Parasite competition hidden by correlated coinfection: using surveys and experiments to understand parasite interactions. *Ecology*, **92**, 535–541.
- Johnson, N.C., Graham, J.H. & Smith, F.A. (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist*, **135**, 575–586.
- Johnstone, R.A. & Bshary, R. (2002) From parasitism to mutualism: partner control in asymmetric interactions. *Ecology Letters*, **5**, 634–639.
- Jones, T.C. & Lester, R.J.G. (1996) Factors influencing populations of the ectosymbiont *Diceratocephala boschmai* (Platyhelminthes; Temnocephalida), on the redclaw crayfish *Cherax quadricarinatus* maintained under laboratory conditions. *Aquaculture*, **143**, 233–243.
- Lee, J.H., Kim, T.W. & Choe, J.C. (2009) Commensalism or mutualism: conditional outcomes in a Branchiobdellidae crayfish symbiosis. *Oecologia*, **159**, 217–224.
- Leung, T. & Poulin, R. (2008) Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. *Vie et Milieu*, **58**, 107.
- Levins, R. & Culver, D. (1971) Regional coexistence of species and competition between rare species. *Proceedings of the National Academy of Sciences*, **68**, 1246–1248.

- Nyholm, S.V., Stabb, E.V., Ruby, E.G. & McFall-Ngai, M.J. (2000) Establishment of an animal–bacterial association: recruiting symbiotic vibrios from the environment. *Proceedings of the National Academy of Sciences*, **97**, 10231–10235.
- Palmer, T.M., Doak, D.F., Stanton, M.L., Bronstein, J.L., Kiers, E.T., Young, T.P. *et al.* (2010) Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. *Proceedings of the National Academy of Sciences*, **107**, 17234–17239.
- Poulin, R. (2007) Are there general laws in parasite ecology? *Parasitology*, **134**, 763–776.
- Rohde, K. (1979) A critical evaluation of intrinsic and extrinsic factors responsible for niche restriction in parasites. *The American Naturalist*, **114**, 648–671.
- Rosewarne, P., Mortimer, R. & Dunn, A. (2012) Branchiobdellidan infestation on endangered white-clawed crayfish (*Austropotamobius pallipes*) in the UK. *Parasitology*, **1**, 1–7.
- Rosewarne, P., Svendsen, J., Mortimer, R.G. & Dunn, A. (2014) Muddied waters: suspended sediment impacts on gill structure and aerobic scope in an endangered native and an invasive freshwater crayfish. *Hydrobiologia*, **722**, 61–74.
- Skelton, J. (2016) Data from: The rules for symbiont community assembly change along a mutualism–parasitism continuum. Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.cq983>.
- Skelton, J., Creed, R.P. & Brown, B.L. (2014) Ontogenetic shift in host tolerance controls initiation of a cleaning symbiosis. *Oikos*, **123**, 677–686.
- Skelton, J., Farrell, K.J., Creed, R.P., Williams, B.W., Ames, C., Helms, B.S. *et al.* (2013) Servants, scoundrels, and hitchhikers: current understanding of the complex interactions between crayfish and their ectosymbiotic worms (Branchiobdellida). *Freshwater Science*, **32**, 1345–1357.
- Sousa, W.P. (1994) Patterns and processes in communities of helminth parasites. *Trends in Ecology & Evolution*, **9**, 52–57.
- St John, F.L. (1976) Growth rate, life span and molting cycle of the crayfish *Orconectes sanborni*. *The Ohio Journal of Science*, **76**, 73–77.
- Thomas, M.J. (2014) Ontogenetic Shifts and Symbiont Succession in a Freshwater Cleaning Symbiosis Mutualism. Masters Masters Thesis, Appalachian State University, Boone, NC, USA.
- Thomas, M.J., Creed, R.P. & Brown, B.L. (2013) The effects of environmental context and initial density on symbiont populations in a freshwater cleaning symbiosis. *Freshwater Science*, **32**, 1358–1366.
- Thompson, J.N. (1988) Variation in interspecific interactions. *Annual Review of Ecology and Systematics*, **19**, 65–87.
- Thompson, J.N. & Pellmyr, O. (1992) Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. *Ecology*, **73**, 1780–1791.
- Toms, J.D. & Lesperance, M.L. (2003) Piecewise regression: a tool for identifying ecological thresholds. *Ecology*, **84**, 2034–2041.
- Ulrich, Y., Sadd, B.M. & Schmid-Hempel, P. (2011) Strain filtering and transmission of a mixed infection in a social insect. *Journal of Evolutionary Biology*, **24**, 354–362.
- Ulrich, Y. & Schmid-Hempel, P. (2012) Host modulation of parasite competition in multiple infections. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2982–2989.
- Van Rhijn, P. & Vanderleyden, J. (1995) The Rhizobium–plant symbiosis. *Microbiological Reviews*, **59**, 124–142.
- Van Valen, L. (1974) Molecular evolution as predicted by natural selection. *Journal of Molecular Evolution*, **3**, 89–101.
- Wisz, M.S., Hijmans, R.J., Li, J., Peterson, A.T., Graham, C.H., Guisan, A. *et al.* (2008) Effects of sample size on the performance of species distribution models. *Diversity and Distributions*, **14**, 763–773.
- Woodhead, A.E. (1950) Life history cycle of the giant kidney worm, *Diocotophyia renale* (Nematoda), of man and many other mammals. *Transactions of the American Microscopical Society*, **69**, 21–46.
- Wulff, J.L. (2008) Life history differences among coral reef sponges promote mutualism or exploitation of mutualism by influencing partner fidelity feedback. *The American Naturalist*, **171**, 597–609.
- Yang, L.H. & Rudolf, V. (2010) Phenology, ontogeny and the effects of climate change on the timing of species interactions. *Ecology Letters*, **13**, 1–10.
- Yu, D.W. & Wilson, H.B. (2001) The competition–colonization trade-off is dead; long live the competition–colonization trade-off. *The American Naturalist*, **158**, 49–63.
- Yule, K.M., Miller, T.E.X. & Rudgers, J.A. (2013) Costs, benefits, and loss of vertically transmitted symbionts affect host population dynamics. *Oikos*, **122**, 1512–1520.
- Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer Science and Business Media, New York, NY, USA.

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