Oecologia
DOI 10.1007/s00442-012-2280-5

COMMUNITY ECOLOGY - ORIGINAL RESEARCH

# The fine line between mutualism and parasitism: complex effects in a cleaning symbiosis demonstrated by multiple field experiments

**Bryan L. Brown · Robert P. Creed · James Skelton · Mark A. Rollins · Kaitlin J. Farrell**

Received: 2 August 2011 / Accepted: 3 February 2012


**Abstract** Ecological theory and observational evidence suggest that symbiotic interactions such as cleaning symbioses can shift from mutualism to parasitism. However, field experimental evidence documenting these shifts has never been reported for a cleaning symbiosis. Here, we demonstrate shifts in a freshwater cleaning symbiosis in a system involving crayfish and branchiobdellid annelids. Branchiobdellids have been shown to benefit their hosts under some conditions by cleaning material from host crayfish's gill filaments. The system is uniquely suited as an experimental model for symbiosis due to ease of manipulation and ubiquity of the organisms. In three field experiments, we manipulated densities of worms on host crayfish and measured host growth in field enclosures. In all cases, the experiments revealed shifts from mutualism to parasitism: host crayfish growth was highest at intermediate densities of branchiobdellid symbionts, while high symbiont densities led to growth that was lower or not significantly different from 0-worm controls. Growth responses were consistent even though the three experiments involved different crayfish and worm species and were performed at different locations. Results also closely conformed to a previous laboratory experiment using the same system. The mechanism for these shifts appears to be that branchiobdellids switched from cleaning host gills at intermediate densities of worms to consuming host gill tissue at high densities. These outcomes clearly demonstrate shifts along a symbiosis continuum with the maximum benefits to the host at intermediate symbiont densities. At high symbiont densities, benefits to the host disappear, and there is some evidence for a weak parasitism. These are the first field experimental results to demonstrate such shifts in a cleaning symbiosis.

**Keywords** Symbiosis · Mutualism · Parasitism · Context dependence · Cleaning behavior

Communicated by Steven Kohler.

B. L. Brown · J. Skelton
Department of Forestry and Natural Resources, Clemson University, Clemson, SC 29634, USA
e-mail: skelto3@vt.edu

*Present Address:*
B. L. Brown (✉) · J. Skelton
Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA
e-mail: stonefly@vt.edu

R. P. Creed · M. A. Rollins · K. J. Farrell
Department of Biology, Appalachian State University, Boone, NC 28608, USA
e-mail: creedrp@appstate.edu

M. A. Rollins
e-mail: rollinsma@appstate.edu

K. J. Farrell
e-mail: farrellkj@appstate.edu

## Introduction

The majority of ecological interactions (e.g., predation, herbivory, parasitism, and commensalism) benefit only one species. Mutualisms are distinct in that they benefit both organisms involved in the interaction. But is there really a fundamental difference between mutualisms and the exploitative interactions listed above, or do these various interactions simply lie on an exploitation continuum from predation/parasitism to mutualism (Ewald 1987)? If mutualisms lie on such a continuum, then slight changes in the behavior or density of one partner in a mutualistic pair, or

Published online: 19 February 2012

changes in the environmental context within which the interaction occurs, could shift the outcome from being mutually beneficial to neutral or even negative for one or both partners.

Cleaning symbioses are typically mutualistic interactions in which one species—the cleaner—removes ectoparasites, necrotic tissue, or fouling organisms from another species—the client (Limbaugh 1961; Losey 1979; Poulin and Grutter 1996; Côté 2000). However, recent research on the classic coral reef cleaning symbioses suggests that the nature of the interaction between cleaners and clients can shift from mutualism to parasitism with changing environmental context, specifically the relative abundance of ectoparasites on the clients (Cheney and Côté 2005). Therefore, these mutualisms clearly seem to lie on an exploitation continuum. Aside from the coral reef cleaning symbioses, one of the few cleaning symbioses to be studied experimentally is the interaction between crayfish and branchiobdellid worms (Annelida: Branchiobdellidae) (Brown et al. 2002; Brown and Creed 2004; Lee et al. 2009).

Branchiobdellids are ectosymbionts associated with crayfish throughout the holarctic region (Fig. 1; Gelder 1999). Previous experiments that manipulated branchiobdellid abundance revealed positive effects of the worms on crayfish growth and reduced crayfish mortality with increasing worm densities (Brown et al. 2002; Lee et al. 2009). Branchiobdellids appear to have a positive effect on crayfish by removing epibionts and debris from the gill epithelia (Jennings and Gelder 1979), presumably increasing rates of gas exchange and ammonia excretion (Brown et al. 2002). Although crayfish have inherent mechanisms for reducing gill fouling by particulate matter in the form of setobranch setae, the setobranchs are ineffective at removing attached epibionts (e.g., bacteria, protozoa) from the gill epithelia between molts (Bauer 1998). However, these fouling agents are common prey of branchiobdellid worms (Holt 1973b; Jennings and Gelder 1979), and their consumption by the worms is believed to be the mechanism through which branchiobdellids can benefit their hosts (Brown et al. 2002).

Branchiobdellids also strongly benefit from their relationship with crayfish. They are rarely found unassociated with a crustacean host, and such occurrences are likely the result of worms simply becoming dislodged (but see Holt 1973a for a possible exception). Benefits of the symbiosis for the worms include habitat, food in the form of epibiotic and particulate matter on the host crayfish exoskeleton (Jennings and Gelder 1979; Gale and Proctor 2011), and reproduction (Young 1966). In fact, there is strong evidence suggesting that successful reproduction of branchiobdellids only occurs on a live crustacean host (Fig. 1; Young 1966; Creed et al., unpublished data; but see Woodhead 1950 for a possible exception).

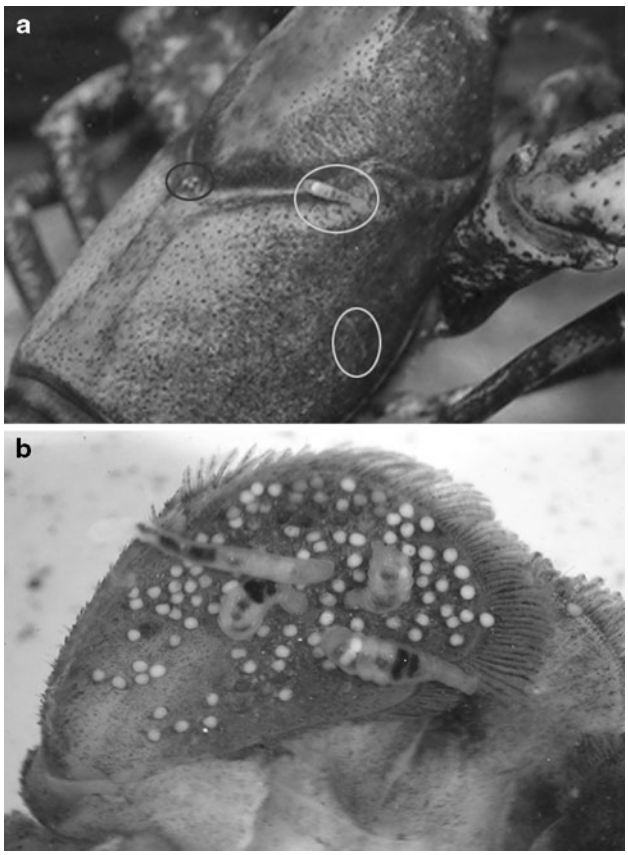


**Fig. 1** Branchiobdellid worms on crayfish. **a** Two branchiobdellids (*Cambarincola ingens*) attached on the dorsal and lateral carapace (*white circles*) of the crayfish *Cambarus chasmodactylus*. The *darker circle* indicates a small cluster of branchiobdellid eggs. **b** An aggregation of branchiobdellids of the genus *Xironodrilus* on the uropod (part of the tail) of the crayfish *Cambarus chaugaensis*. Small white branchiobdellid eggs are visible on uropod and can also been seen through the body wall of the adult worms prior to deposition

Positive effects of the association for both worms and crayfish support the conclusion that the relationship between crayfish and branchiobdellids is a mutualism (Brown et al. 2002; Lee et al. 2009), though the interaction may be a commensalism (positive effect for worms, no effect on crayfish) when gill fouling rates of the host are low (Lee et al. 2009). However, while previous results showing mutualistic effects are compelling, both studies were conducted in laboratory settings. Clearly, the crayfish–branchiobdellid interaction required evaluation in a field setting to ascertain whether positive effects of branchiobdellids occur when the crayfish are in their natural environment. Additionally, these previous studies used branchiobdellid densities that were low to medium, based on field conditions, and therefore provide little information regarding potential effects across a broader range of symbiont densities. Because the densities of branchiobdellid worms on crayfish hosts are quickly and easily manipulated

(Brown et al. 2002; Lee et al. 2009), the crayfish–branchiobdellid system provides an excellent model of symbiosis that can be used to examine symbiont effects over a wide range of densities using both laboratory and field experimentation.

We evaluated the effect of branchiobdellid worms on crayfish growth in three field experiments. Two of the experiments were conducted in the South Fork of the New River near Boone, NC, USA, and another was conducted in Clemson, SC, USA. Based on the results of our prior laboratory experiment (Brown et al. 2002), we hypothesized that crayfish growth would increase with increasing symbiont density, though we reasoned that benefits would most likely reach an asymptote at high worm densities. We used two locations and two species of both crayfish and worms to address not only whether the results of our prior laboratory experiment would be repeated in field experiments but also whether the interaction was consistent across locations and across species of crayfish and branchiobdellids.

## Materials and methods

We performed three separate experiments to investigate the effect of branchiobdellid ectosymbionts on host crayfish growth under field conditions. The three experiments had very similar methodologies. We therefore describe the methods for the first of these experiments in detail (conducted in Boone, NC, USA, in 2008), and then describe the methods for the subsequent two experiments primarily based on their differences from the Boone 2008 experiment.

### Boone 2008 experiment

We manipulated densities of the branchiobdellid annelid *Cambarincola ingens* on the crayfish *Cambarus chasmodactylus* to examine the effects on host growth under field conditions in the South Fork of the New River in Boone, NC, USA. Our prior work with the crayfish–branchiobdellid system established methods for manipulating worm densities on crayfish for use in experiments (Brown et al. 2002; Brown and Creed 2004). Branchiobdellids can be easily coaxed unharmed from their hosts, using fine-tipped laboratory probes, and reintroduced to crayfish at desired densities. A 5-min bath of 10% $MgCl_2$ solution also kills attached branchiobdellids and their eggs even if they cannot be visually detected. The $MgCl_2$ bath may produce a temporary anaesthesis in crayfish, but they do not appear to be damaged by the treatment based on our previous experience and on a prior experiment designed to detect an effect of the $MgCl_2$ for these purposes (Brown et al. 2002).

We collected crayfish from the South Fork of the New River in Boone, NC, USA, and they were randomly assigned to one of the three treatments: 0, 4, or 12 large worms (hereafter abbreviated, e.g., 0w) where large $\approx$6–10 mm length. A density of four large worms is commonly observed on *C. chasmodactylus* in the New River, NC (R.P.C. and B.L.B., personal observation) and comparable to densities used in previous experiments (Brown et al. 2002). While worm numbers on a large *C. chasmodactylus* can reach 30–40 (Brown and Creed 2004), most are small worms. Our treatment of 12 large *C. ingens* therefore represents a density that is rare but not unattainable in field conditions for *C. chasmodactylus* in the size range used in the experiment. It has also been suggested that worm densities may exceed normal densities on crayfish that are in poor health (Quaglio et al. 2006). Large *C. ingens* are most commonly found on the ventral surfaces of crayfish (between the walking legs and on the abdomen), particularly on the first abdominal segment and along the bottom lateral margin of the host carapace. However, *C. ingens* are relatively mobile and can be found on nearly all parts of their hosts' exoskeleton (Brown and Creed 2004). Crayfish used in the experiment ranged in carapace length (CL) from 25 to 37 mm with initial blotted wet mass (BWM) between 5.31 and 17.13 g.

To examine the effects of branchiobdellids on their hosts under realistic field conditions, we used an enclosure/exclosure methodology. The enclosures (1 m $\times$ 0.5 m $\times$ 0.5 m) consisted of a welded aluminum frame with solid aluminum sides and bottoms, with upstream and downstream ends of double-walls of 12-mm wire mesh separated by a gap of at least 10 cm. The double walls of mesh insured that enclosed crayfish did not acquire additional branchiobdellids through contact with free-living crayfish, since direct physical contact is the only known means of dispersal for branchiobdellids between crayfish hosts (Young 1966). Enclosures also featured a debris-shedding prow designed to deflect larger debris and reduce accumulation of finer debris such as leaves. Enclosures were anchored into the substratum by driving rebar stakes through slotted brackets welded to the sides of the enclosures. Enclosures contained a mixture of scrubbed cobbles and washed gravel. Initial water depth and current velocity inside enclosures were similar (mean $\pm$ 1SE water depth 20.1 $\pm$ 3.9 cm, current velocity 9.6 $\pm$ 3.1 cm/s). Crayfish were randomly allocated to treatments and then assigned to individual enclosures in a randomized block design with four replicate blocks. Blocks were rows of enclosures oriented perpendicular to stream flow, with one cage from each treatment in each row. Crayfish were inoculated with worms and placed in the enclosures on 23 July 2008. Crayfish were measured on three dates (4 and 25 September and 2 October) to determine survival and change in BWM over a 71-day period. We also measured water temperature using a data logger. Water temperature ranged from 16 to 24°C with daily

variation of 4–6°C during the first 7 weeks of the experiment, then declined sharply in the last 2 weeks (range 14–18°C). The outer mesh of the cages was cleaned two times daily, and the inner mesh was cleaned every other day to maintain flow through the enclosures.

At the end of the experiment, crayfish were sacrificed and their gills were examined to determine whether branchiobdellids had damaged their gills. To standardize our counts, we counted any obvious lesions on the filaments of the fifth gill on the right side of the crayfish.

Due to a severe flood about 1 month into the experiment, 4 of the 12 enclosures (2 each from the 4 and 12w treatments) were displaced downstream. The crayfish in all of the tumbled cages survived, but were damaged (lost legs, chelae, etc.) and so were omitted from the analysis. There was also a lost replicate in the 0w treatment due to crayfish mortality.

Change in percent BWM over the course of the experiment was analyzed using a mixed models repeated measures analysis of variance (RMANOVA), using the lme function in the nlme package of R Statistical Software (R Development Core Team 2011). We used the fit.contrast function in the labdsv package of R to perform contrasts comparing treatments on the final sampling date.

## Boone 2010 experiment

The Boone 2010 experiment used largely the same methods as the 2008 experiment with only a few differences. First, we limited the range of crayfish sizes in the experiment in order to increase our ability to detect changes in crayfish growth (range BWM 9.18–13.43 g, range CL 31–33 mm). However, low densities of appropriately-sized *C. chasmodactylus* in the South Fork in 2010 necessitated collection from a nearby site in the Middle Fork, approximately 2.5 km from the 2008 collection site. The experiment had a similar randomized block design to 2008, but had six replicates of each treatment rather than four. The duration of the experiment was also longer (103 days), beginning on June 23 with four measurements of crayfish growth on 22 July, 13 and 23 September, and 5 October. While there were no extreme high flow events during the experiment, 2010 was generally a wetter year leading to higher flows in the New River [mean (±1SE) initial depth and current velocity inside enclosures 41.7 ± 0.80 cm and 17.75 ± 0.68 cm/s, respectively]. Water temperatures ranged from 18 to 24°C in late June through August, then from 16 to 22°C until late September, and dropped to 12°C during the last week of the experiment. There was also increased general mortality during the 2010 experiment with losses of two 0w, three 4w, and four 12w treatments replicates. We also analyzed the number of gill scars on crayfish at the end of the experiment. However, in 2010, we counted scars present on all gill filaments for gills associated with the walking legs ($n = 10$ gills per crayfish) rather than on a single gill.

## Clemson 2010 experiment

There were some major differences between the Clemson 2010 experiment and either of the two Boone experiments. The experiment was conducted in Waldrop Stone Creek in the Clemson Experimental Forest, Clemson, SC. Waldrop Stone is a smaller stream than the South Fork of the New River ($\approx$2 m wetted width in W.S. compared to $\approx$8 m in S.F.N.R.) and is located in a more heavily forested area. We also conducted the experiment using different species of both crayfish and branchiobdellid. We used the crayfish *Cambarus chaugaensis*, which is the most common stream-dwelling crayfish in the area and a congener of *C. chasmodactylus* used in the Boone experiments. Branchiobdellids used in the experiment were primarily species in the genus *Xironodrilus*. *C. chaugaensis* is host to multiple species of branchiobdellids, and it is not unusual to find crayfish hosting >150 worms (B.L.B. and J.S., personal observation). However, our use of "large" branchiobdellids most likely ensured that *Xironodrilus* was the only genus involved in the experiment, since *Xironodrilus* is significantly larger than other local taxa. Large *Xironodrilus* (3–6 mm) were typically found attached to the lateral margins of the carapace, the basal portion of the pereiopods, and the ventral aspect of the uropods. Enclosure design also differed slightly from the Boone experiments. Interior dimensions were the same as with the Boone enclosures and the Clemson cages also had double-walled mesh, but were wood-framed and did not have the debris-shedding prow, though the prow was largely unnecessary due to the smaller stream size. Debris was cleaned from the front of the cages twice weekly.

The experiment also differed in some of the specifics of experimental design. The experiment began with six replicates of four treatments (compared to the three treatments in the Boone experiments). For the Clemson experiment, we used two intermediate worm densities of three and six worms in conjunction with the control (0w) and 12w treatments used in the Boone experiments. Surveys of Waldrop Stone Creek provided estimates of 5.2 ± 2.4 (mean ± SD) medium and large *Xironodrilus* sp. on crayfish approximately the same size as those used in the experiment. As in the Boone experiments, the two intermediate densities are commonly observed for crayfish of the size used in the experiment (range CL 23.9–26.5 mm, range BWM 4.2–6.45 g) while the 12 large-worm treatment would be considered a high, but not improbable, density of large worms. We lost seven experimental units during the experiment: one 0w, two 3w, four 6w, and two 12w. Duration of the

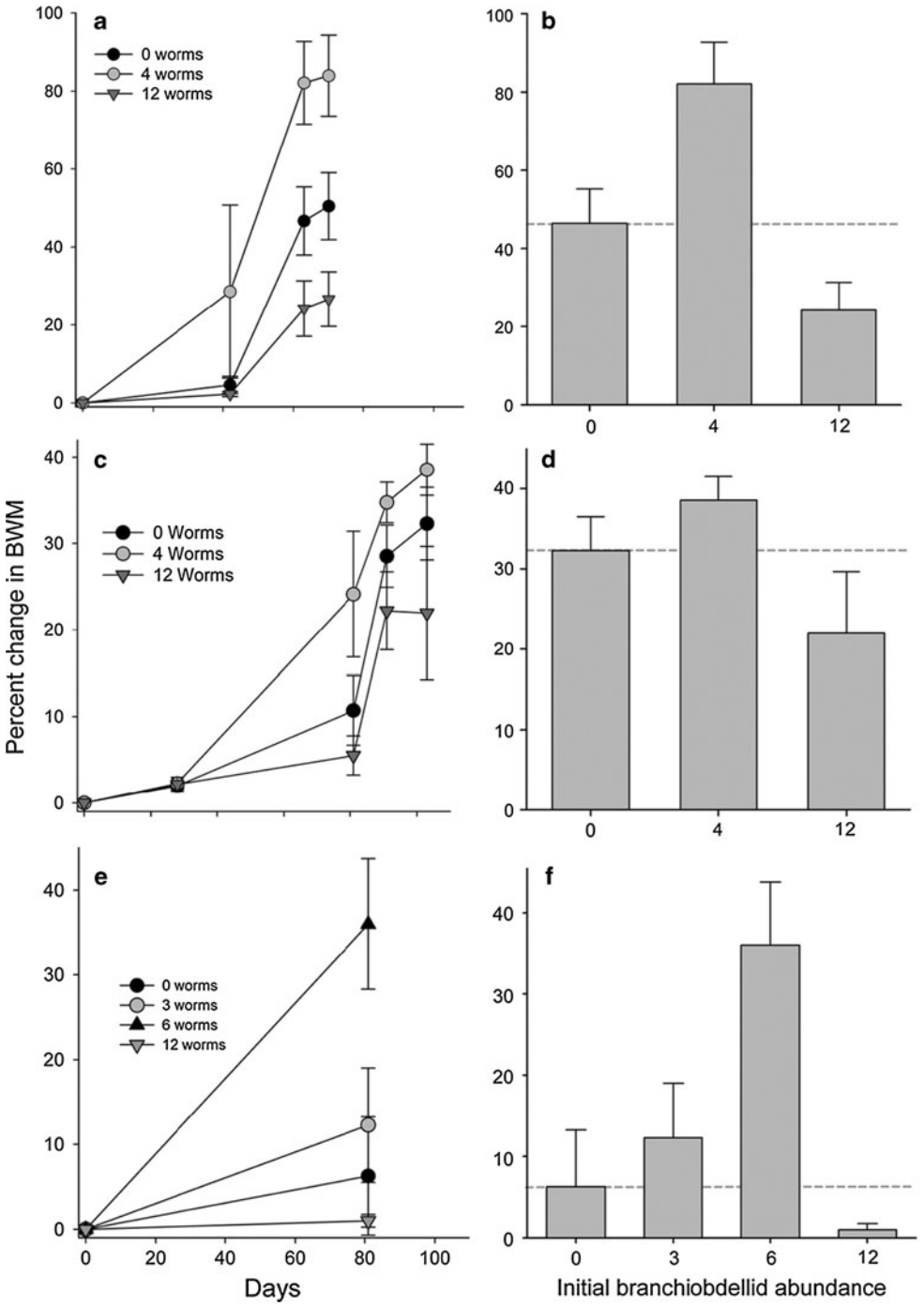


**Fig. 2** Crayfish growth in three experiments that manipulated branchiobdellid abundance in stream enclosures. **a**, **b** Boone 2008: a 71-day experiment conducted in Boone, NC, USA, with the crayfish *Cambarus chasmodactylus* and the branchiobdellid *Cambarincola ingens*; **c**, **d** Boone 2010: a 103-day experiment, also in Boone, with the same two species; **e**, **f** Clemson 2010: an 81-day experiment in Clemson, SC, USA, with the crayfish *Cambarus chaugaensis* and branchiobdellids of the genus *Xironodrilus*. **a**, **c**, **e** Time series growth is measured as percent change in blotted wet mass, BWM, mean ± 1SE. In all three experiments, there were significant effects of both the branchiobdellid treatment and time based on RMANOVA. **b**, **d**, **f** Final blotted wet mass growth is measured as change in BWM (g) based on mass on final day of experiment, mean + 1SE. *Dotted lines* indicate mean growth by crayfish in the control (0w treatment)

experiment was 81 days with only one measure of crayfish growth at the end of the experiment.

## Results

Branchiobdellids had a significant influence on crayfish growth in all three experiments. In the Boone 2008 experiment, there was a significant overall effect of the branchiobdellid treatment ($F_{2,3} = 11.3$, $p = 0.040$) and a significant treatment × time interaction on crayfish growth (Fig. 2a). Crayfish in the 4-worm (w) treatment exhibited the highest growth. Crayfish in the 0 and 12w treatments exhibited increases in mass, but they were not as large as in the four-worm treatment. The major divergence in BWM among the three treatments occurred between days 42 and 62; during this period, all crayfish molted. The relationship between final percent change in crayfish BWM and initial

worm number was unimodal, further illustrating the strong positive effect of the 4w treatment on crayfish growth relative to the other two treatments (Fig. 2b). According to linear contrasts, the 4w treatment was significantly different from either the 0 or 12w treatments (4 vs. 0w: $t = -2.67$, $p = 0.05$; 4 vs. 12w: $t = -4.17$, $p = 0.01$). Additionally, growth of crayfish in the 12w treatment was lower than crayfish in the 0w, suggesting that at high densities branchiobdellids might have an adverse effect on crayfish growth, though the 12w treatment was not significantly lower than the control based on a linear contrast (0 vs. 12w: $t = -1.90$, $p = 0.13$).

In post-experiment crayfish dissections, we found lesions on the gills of crayfish in the 12w treatment ($7.5 \pm 1.5$ mean $\pm$ 1SE) but no lesions were present on gills of crayfish from the other two treatments. We also found that the number of large worms decreased during the experiment and were only found on the 4w treatment crayfish (average of $1 \pm 1$). However, small worms (likely the result of reproduction in the 4 and 12w treatments) were present in similar relative abundances to the initial worm densities 0w $= 0.33 \pm 0.33$, 4w $= 4 \pm 1$, and 12w $= 6 \pm 2$.

Results of the 2010 experiment conducted in Boone were very similar to the 2008 experiment (Fig. 2c). Once again, there was a significant overall effect of the branchiobdellid treatment on crayfish growth (from RMANOVA, branchiobdellid treatment effect $F_{2,7} = 7.81$, $p = 0.017$). Additionally, the highest growth occurred at intermediate branchiobdellid densities, while the lowest growth occurred in the highest branchiobdellid densities as in 2008. Although the outcome of the 2010 experiment was qualitatively identical to the 2008 experiment, results were quantitatively weaker and there was only a marginally significant difference between the 4 and 12w treatments on the last sampling date (Fig. 2d; 4 vs. 12w: $t = -2.12$, $p = 0.07$). Earlier in the experiment, immediately following the molts of the majority of the crayfish, there was a significant difference between the 4 and 12w ($t = -2.48$, $p = 0.04$) and a marginally significant difference between the 0 and 4w ($t = -2.00$, $p = 0.08$) treatments. Growth was generally lower in the 2010 experiment than in 2008, despite the longer length of the experiment. There was a highly significant relationship between the branchiobdellid treatment and gill scarring in the 2010 experiment, with increased branchiobdellid density producing increased gill scarring on the crayfish host (Fig. 3). As with the Boone 2008 experiment, the number of large worms decreased through the course of the experiment in the 4 and 12w treatments (4w $= 3 \pm 0.84$, 12w $= 2 \pm 0.71$), and reproduction resulted in additional small worms (total worms 4w $= 4.4 \pm 1.17$, 12w $= 5.25 \pm 2.7$). There was also one colonization event by a single large worm on a 0w crayfish.

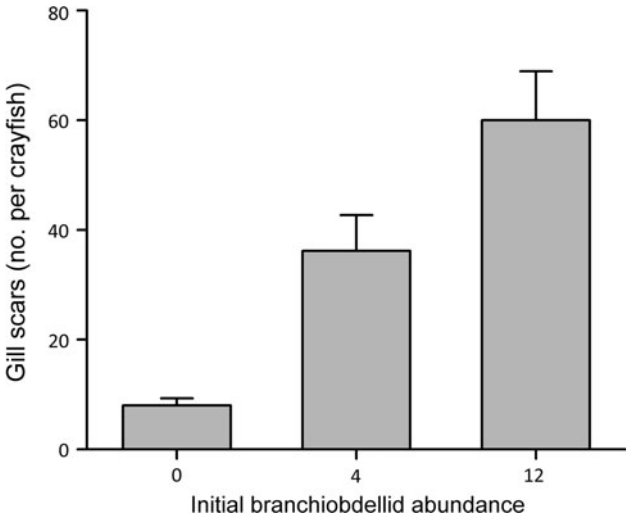


**Fig. 3** The amount of gill scarring (mean $\pm$ 1SE) on crayfish as a function of branchiobdellid treatment in the 2010 experiment conducted in Boone, NC, USA. There was a highly significant effect of the branchiobdellid treatment on the number of gill scars based on one-way ANOVA ($F_{2,14} = 32.1$, $p < 0.0001$)

The Clemson 2010 experiment produced results that were remarkably comparable to the two experiments conducted in Boone (Fig. 2e). There was a significant overall effect of the branchiobdellid treatment on crayfish growth (one-way ANOVA, $F_{3,11} = 3.94$; $p = 0.039$), and the distribution of treatment effects was identical to the Boone experiments, with intermediate densities of branchiobdellids producing the highest growth of host crayfish and higher densities producing reduced growth rates (Fig. 2f), with significant differences between several treatments on the final date according to linear contrasts (0 vs. 6w: $t = -2.91$, $p = 0.01$; 3 vs. 6w: $t = -3.06$, $p = 0.01$; 6 vs. 12w: $t = 3.31$, $p = 0.007$). As in the two Boone experiments, the number of large worms declined over the course of the experiment with final counts of large worms by treatment as 3w $= 0.75 \pm 0.48$, 6w $= 0$, 12w $= 1 \pm 0.41$. There was also a single contamination of a 0w treatment crayfish by a single large worm. While it is likely that all crayfish molted during the 81-day experiment, we did not have visual confirmation of all molts.

## Discussion

Branchiobdellids significantly affected the growth of their crayfish hosts in all three experiments. However, contrary to our predictions, the benefits of branchiobdellids to crayfish hosts did not continuously increase (or increase asymptotically) with branchiobdellid density. In all three experiments, the effect of the worms on crayfish growth followed a unimodal pattern with maximum host benefit

occurring at intermediate worm densities, but with the highest worm densities producing slightly lower growth than the control (zero worm treatment). This reduced growth at the highest worm density may possibly indicate a weak parasitism, though the differences between the highest worm treatments and the controls were not statistically significant according to contrasts. Additionally, in all three experiments, the worm density that produced maximum benefit to crayfish (as measured by growth relative to controls) was remarkably consistent with our prior laboratory experiment examining the same relationship (Brown et al. 2002). In fact, polynomial fits to the final growth data for each of the three experiments (Fig. 2b, d, f) has a maximum very close to six worms, which was the worm density that produced the maximum benefit in our laboratory experiment (Brown et al. 2002). Furthermore, the relationship was consistent across years (2008 and 2010), across locations 140 km apart (Boone, NC, and Clemson, SC), and across two species of both host crayfish and branchiobdellid worm. From these results, it seems clear that (1) the crayfish–branchiobdellid symbiosis can shift from a mutualism to a parasitism depending on symbiont density, and (2) that the relationship is similar for more than one species pair of branchiobdellid and host crayfish.

How can this shift from mutualism to parasitism be explained mechanistically? At low to moderate densities, the worms appear to effectively clean debris and epibionts (e.g., bacteria, protozoa) from the crayfish gills without damaging them (Jennings and Gelder 1979), increasing gas exchange and ammonia excretion (Brown et al. 2002). However, at higher densities, such as 12 large worms, resources such as detritus and epibionts on the exoskeleton and gills may become limiting for the worms. The lesions observed on the gills of the crayfish in the 12-worm treatment (in Boone 2008), and the strong positive relationship between branchiobdellid density and number of lesions (Boone 2010; Fig. 3), suggests that large worms turned to feeding directly on the crayfish, ingesting gill tissue and/or hemolymph (=blood) when other resources became limiting. The lesions we observed on the crayfish gills are similar to those reportedly made by parasitic species of branchiobdellids (e.g., Quaglio et al. 2006). Moreover, the fact that branchiobdellids were mutualists at intermediate densities but parasites at high densities appears to reconcile conflicting statements in the literature about the branchiobdellid–crayfish association. These worms have been reported to be mutualists, commensals, or facultative parasites (Goodnight 1940; Brown et al. 2002; Quaglio et al. 2006; Lee et al. 2009). Our experimental results demonstrate that branchiobdellids can be either mutualists or parasites, and that the outcome appears dependent upon worm density.

Is it fair to claim a shift from mutualism to parasitism if the decreased growth in the highest branchiobdellid treatments was not significantly lower than growth in the controls according to contrasts? Several pieces of evidence point to parasitism by branchiobdellids despite the lack of statistical significance. First, the means of the 12w treatment were lower than the control means in all three experiments, even though we lacked the statistical power to discriminate at an error rate of 0.05 (two of the three experiments had $p \leq 0.15$). This lack of power was due to mortality in our field experiments, but was not related to any particular treatment. Second, it is clear that branchiobdellids are damaging the gills of their hosts and that there is the potential for the damage to be even more extensive than what we measured. While our study focused on large branchiobdellids, large worms comprise only a fraction of the total worms on a crayfish. Twelve worms may represent a high density of large worms, but total worm density can exceed 30 on *C. chasmodactylus* (Brown and Creed 2004), and frequently exceeds 150 on *C. chaugaensis* (B.L.B. and J.S., personal observation). Even though larger worms likely inflict more per capita damage, small worms also damage gills (Quaglio et al. 2006), so it is highly likely that the negative effects we measured were relatively modest compared to what could potentially occur.

Even though all three field experiments were similar in terms of their outcomes and those outcomes were similar to our prior laboratory experiment, there were some important differences in results. First, growth rates were higher in all field experiments when compared to our laboratory experiment. In the laboratory experiment (Brown et al. 2002), growth rates of all treatments were less than 10% in a 60-day experiment for an approximate %change in BWM/day of 0.17%. That rate is considerably lower than per day maximum growth rates (i.e., growth in the intermediate branchiobdellid treatment) in any of the three field experiments (Boone 2008 = 1.13%/day, Boone 2010 = 0.39%/day, Clemson 2010 = 0.44%/day). However, it is also obvious that the Boone 2008 experiment produced considerably higher growth rates than either of the 2010 experiments. There are multiple potential explanations for the difference in growth rates between Boone 2008 and 2010, including a longer duration of experiment in 2010 (i.e., averaging growth over a longer period) and different sources of crayfish between the 2 years. It is also likely that the reduced growth observed in the 4w treatment in 2010 is related to the increase in gill scarring. This result suggests that there may have been differences in resource levels on the crayfish which resulted in some gill feeding by worms in this treatment, which could have affected growth. However, we cannot offer a definitive explanation based on our data.

Mortality decreased replication in all three experiments. Such mortality is common in field experiments and can

result from many often unidentifiable factors. In the case of our experiments, the causes of mortality were not immediately obvious. Importantly, in none of the three experiments was mortality specifically related to treatment. Despite the decreased replication in individual experiments, the fact that three different experiments produced nearly identical results, and that these results closely mimicked those of our previous laboratory experiment, make our results extremely robust when taken together. Moreover, the results were consistent regardless of the differences incorporated into the studies, i.e., differences in location, species, and year. We therefore suggest that, when viewed together, our results constitute strong evidence of shifts from mutualism to parasitism in the crayfish–branchiobdellid symbiosis.

Final worm numbers differed from the number originally applied, particularly in the 12-worm treatment. However, changes in worm number during the experiment do not preclude the effectiveness of the branchiobdellid manipulation. There are several reasons to expect declines in large worms based on natural processes. Large worm losses may have been the result of natural senescence, i.e., mature worms may only live 1 or 2 months, and thus some had died by the end of the experiments. Additionally, some form of partner control may exist in which the crayfish or even the worms themselves maintain the density of large worms on a host. Some worms may have been accidentally dislodged, particularly during high flow events that may scour the crayfish as in the Boone 2008 experiment. Under normal field conditions, multiple generations of worms constituting multiple size classes are usually present on a crayfish, and thus large worms would be replaced with some regularity. However, since we chemically removed all branchiobdellids and eggs at the beginning of the experiments, smaller size classes were not available to replace lost large worms except through reproduction. A second important point with regard to the efficacy of our worm manipulation is that there was very little contamination of the 0-worm treatment (only two worms across all three experiments) that served as controls. Finally, the mechanisms through which branchiobdellids benefit their hosts (i.e., gill cleaning) and harm their hosts (i.e., feeding on gill tissue and hemolymph) are expected to have cumulative rather than simply acute effects, as evidenced by the relationship between branchiobdellid treatment and the level of gill scarring (Fig. 3).

That branchiobdellids functioned as mutualists at low densities but weak parasites at high densities demonstrates that cleaning symbioses do not have fixed outcomes under all conditions. Instead, cleaning symbiosis mutualisms may shift to commensalisms or even parasitisms as a function of cleaner density or behavior (Grutter and Bshary 2003) as well as environmental conditions (e.g., fouling rate of gills or ectoparasite abundance) (Cheney and Côté 2005; Lee et al. 2009). There is also a strong possibility that that one or both of the species involved may be exerting control over the interaction to some degree, as has been demonstrated in coral reef and yucca–yucca moth systems (Pellmyr and Huth 1994; Bshary et al. 2008). This regulation could simply be a product of branchiobdellid population dynamics or limitation by food resources on a host crayfish. Crayfish themselves may also take a more active role in the regulation of branchiobdellids through grooming behavior, and they have even been observed to use their walking legs to remove branchiobdellids (B.L.B., R.P.C., J.S., K.F., personal observation). However, whether such grooming can regulate branchiobdellid densities is unknown, and it should be noted that branchiobdellids frequently occur on areas of a host that are easily groomed, including on antennae and mouthparts, but host crayfish are apparently either tolerant of the worms or unable to sense them (McManus 1960; Bishop 1968; Brown and Creed 2004).

Our results demonstrate that cleaners can affect client growth in a natural environment, an effect that has not been demonstrated experimentally for any cleaner–client symbiosis (Poulin and Grutter 1996; Cheney and Côté 2003). We have also shown that the outcomes of cleaning symbioses are not fixed and can be a function of symbiont density. We can likely learn much more about cleaning symbioses by studying such interactions in systems other than the classic coral reef systems, specifically in other invertebrate–invertebrate associations in which the cleaner inhabits or is able to access the clients' gill chambers. It is likely that many such associations exist in marine and freshwater environments given the diversity of invertebrates in these systems. As we have demonstrated, these types of invertebrate systems are amenable to experimentation as cleaner presence and density can be easily manipulated. Increased study of such systems may help us understand how these fascinating associations evolved and are maintained, as well as helping us to understand how such cleaning interactions influence the communities and ecosystems within which the mutualists reside.

**Acknowledgments** We are grateful to Katie Greene, Brett Tornwall, Laura Ammons, Josh Lomonaco, David Young, Adam Speake, Ryan Hassler, Travis Knott, Chelsae Weithman, Patty Whitener, John Brunt, Jon Boroughs, Tim Torongeau, Matt Sanderson, and Brandon Carpenter for help with the experiments. We thank everyone at Boone Ironworks for help in designing and building the cages. Funding for this research was provided by the National Science Foundation (DEB-0949823 to R.P.C. and DEB-0949780 to B.L.B.), a Research Development Award to R.P.C. from the Graduate School at Appalachian State University, and by funds from Clemson University's University Research Grant Committee to B.L.B. Discussions with Jeff Butts and comments on previous versions on the manuscript by Robert Baldwin, Mathew Turnbull, Julie Ryan and two anonymous reviewers were helpful and greatly appreciated.

## References


Bauer RT (1998) Gill-cleaning mechanisms of the crayfish *Procambarus clarkii* (astacidea: cambaridae): experimental testing of setobranch function. Invertebr Biol 117:129–143

Bishop JE (1968) An ecological study of the branchiobdellid commensals of some mid-western Ontario crayfish. Can J Zool 46:835–843

Brown BL, Creed RP (2004) Host preference by an aquatic ectosymbiotic annelid on 2 sympatric species of host crayfishes. J North Am Benthol Soc 23:90–100

Brown BL, Creed RP, Dobson WE (2002) Branchiobdellid annelids and their crayfish hosts: are they engaged in a cleaning symbiosis? Oecologia 132:250–255

Bshary R, Grutter AS, Willener AST, Leimar O (2008) Pairs of cooperating cleaner fish provide better service quality than singletons. Nature 455:964–966

Cheney KL, Côté IM (2003) The ultimate effect of being cleaned: does ectoparasite removal have reproductive consequences for damselfish clients? Behav Ecol 14:892–896

Cheney KL, Côté IM (2005) Mutualism or parasitism? The variable outcome of cleaning symbioses. Biol Lett 1:162–165

Côté IM (2000) Evolution and ecology of cleaning symbioses in the sea. Oceanogr Mar Biol Annu Rev 38:311–355

Ewald PW (1987) Transmission modes and evolution of the parasitism–mutualism continuum. Ann NY Acad Sci 503:295–306

Gale KSP, Proctor HC (2011) Diets of two congeneric species of crayfish worm (annelida:clitellata:branchiobdellidae) from western Canada. Can J Zool 89:289–296

Gelder SR (1999) Zoogeography of branchiobdellidans (annelida) and temnocephalidans (platyhelminthes) ectosymbiotic on freshwater crustaceans, and their reactions to one another in vitro. Hydrobiologia 406:21–31

Goodnight CJ (1940) The branchiobdellidae of North American crayfishes. Ill Biol Monogr 17:5–75

Grutter AS, Bshary R (2003) Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions. Proc R Soc Lond B 270:S242–S244

Holt PC (1973a) A free-living branchiobdellid (annelida: clitellata)? Trans Am Micros Soc 92:152–153

Holt PC (1973b) A summary of the branchiobdellid (annelida: clitellata) fauna of Mesoamerica. Smithson Contrib Zool 142:1–40

Jennings JB, Gelder SR (1979) Gut structure, feeding and digestion in the branchiobdellid oligochaete *Cambarincola macrodonta* Ellis 1912, an ectosymbiote of the freshwater crayfish *Procambarus clarkii*. Biol Bull 156:300–314

Lee J, Kim T, Choe J (2009) Commensalism or mutualism: conditional outcomes in a branchiobdellid–crayfish symbiosis. Oecologia 159:217–224

Limbaugh C (1961) Cleaning symbiosis. Sci Am 205:42–49

Losey GS (1979) Fish cleaning symbiosis: proximate causes of host behavior. Anim Behav 27:669–685

McManus LR (1960) Some ecological studies of the branchiobdellidae. Trans Am Microsc Soc 79:420–428

Pellmyr O, Huth CJ (1994) Evolutionary stability of mutualism between yuccas and yucca moths. Nature 372:257–260

Poulin R, Grutter AS (1996) Cleaning symbioses: proximate and adaptive explanations. Bioscience 46:512–517

Quaglio F et al (2006) Preliminary investigations of disease-causing organisms in the white-clawed crayfish *Austropotamobius pallipes* complex from streams of northern Italy. Bull Fr Pêche Piscic 380–381:1271–1290

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Woodhead AE (1950) Life history cycle of the giant kidney worm, *Dioctophyma renale* (nematoda), of man and many other mammals. Trans Am Microsc Soc 69:21–46

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