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Multiple defender effects: synergistic coral defense by mutualist crustaceans

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Abstract The majority of our understanding of mutualisms comes from studies of pairwise interactions. However, many hosts support mutualist guilds, and interactions among mutualists make the prediction of aggregate effects difficult. Here, we apply a factorial experiment to interactions of 'guard' crustaceans that defend their coral host from seastar predators. Predation was reduced by the presence of mutualists (15% reduction in predation frequency and 45% in volume of coral consumed). The frequency of attacks with both mutualists was lower than with a single species, but it did not differ significantly from the expected frequency of independent effects. In contrast, the combined defensive efficacy of both mutualist species reduced the volume of coral tissue lost by 73%, significantly more than the 38% reduction expected from independent defensive efforts, suggesting the existence of a cooperative synergy in defensive behaviors of 'guard' crustaceans. These emergent 'multiple defender effects' are statistically and ecologically analogous to the emergent concept of 'multiple predator effects' known from the predation literature.

Keywords *Alpheus* · Coral reefs · Exosymbiont · Multiple predator effects · *Trapezia*

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Introduction

It is well known from comparative studies that the efficacy of different species of mutualists can vary significantly within a guild (Little et al. 2004; Mieog et al. 2009). However, these studies rarely examine interactions between mutualists within a guild or quantify the consequences of these interactions on the host (but see Palmer et al. 2003). Mutualist–mutualist interactions are of particular interest in diverse systems such as coral reefs, where a number of species can co-occur on single hosts, creating a complex network of possible higher-order interactions (e.g., cooperative, competitive, or intraguild predatory interactions) that may non-independently modify the benefit a host receives by supporting multiple mutualists. Our study tested for an emergent Multiple Defender Effect from two species of crustacean exosymbionts that co-habit a common scleractinian coral and collectively defend the coral from predators.

Individual species within a guild of mutualists may exhibit differences in the efficacy of the services provided to their host species. This pattern has been observed both within defensive mutualisms, such as ant–plant systems (Palmer and Brody 2007; Palmer et al. 2008), pollination

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syndromes (Holland et al. 2004), and corals (Little et al. 2004; Mieog et al. 2009). Intraguild competition or cooperation among mutualists can lead to reduced or enhanced mutualist efficacy relative to that which is expected though a summing of the independent pairwise effects of single species of mutualists in isolation (Stachowicz and Whitlatch 2005). However, few studies have attempted to go beyond estimating the differences in the effects of individual mutualists, which can be achieved through the factorial manipulation of multiple mutualists. Indeed, a recent meta-analysis by Morris et al. (2007) on independent and interactive effects of natural enemies and mutualists on plant performance found that of the 160 studies published on interactive effects (enemy–enemy, enemy–mutualist, and mutualist–mutualist), only ten (reviewed from five different papers) exist on mutualist–mutualist interactions, while 114 focus on enemy–mutualist dynamics, and 36 focus on enemy–enemy dynamics. There was, however, no consistent effect of intraguild mutualist interactions on partner performance; across the ten mutualist–mutualist studies, the authors found evidence for independent, antagonistic, and synergistic effects of groups of mutualists on hosts.

The study of higher-order interactions that lead to a non-independent response is not new to the field of ecology (Billick and Case 1994). Indeed, the study of intraguild dynamics among predators is now commonplace in a number of systems (Griffen 2006; Vance-Chalcraft et al. 2007). An experimental design and analytical framework for testing higher-order interactions within guilds has been developed in these multiple-predator studies. Tests for multiple-predator effects are often conducted using a factorial manipulation of predators (crossing the presence or absence of predator A with the presence or absence of predator B; Exlöv 2000; Warfe and Barmuta 2004). The expected survival probability of prey in the presence of both predator A and B (P_{A+B}) follows the following multiplicative risk model:

$$\hat{P}_{A+B} = P_{NP}(P_A/P_{NP})(P_B/P_{NP}) \quad (1)$$

where P (A is predator A, B is predator B, and NP is no predator) is the survival probability in the presence of different predator treatments (Sih et al. 1998; Soluk and Collins 1988; Vonesh and Osenberg 2003). Expected survival probability in the presence of two predators assumes a multiplicative risk model because predation events by each predator species are dependent upon predation by the other species (i.e., conditional probability—a single prey item cannot be eaten twice). Because two-factor analysis of variance (ANOVA; the appropriate analysis for a factorial design) assumes an additive model, the multiplicative risk model above can be log transformed to an additive form for analysis:

$$\log(\hat{P}_{A+B}) = \log(P_{NP}) + \log(P_A/P_{NP}) + \log(P_B/P_{NP}) \quad (2)$$

In our study detailed below, we tested for intraguild higher-order interactions among mutualists, a term we define as emergent multiple defender effects (MDEs), the analog of emergent multiple predator effects (MPEs).

We focused on combined effects of mutualistic symbioses in coral reefs. Symbioses on reefs are common and are exemplified by the well-known relationship between scleractinian corals and endosymbiotic algae (zooxanthellae) (Muscatine and Porter 1977; Rowan 1998), a mutualism thought to be the fundamental factor allowing coral reefs to persist in the oligotrophic tropics. Although guilds of different endosymbionts are known to provide variable amounts of support to corals and to respond differently to heat stress (Baker et al. 2004; Fabricius et al. 2004; Rowan 2004), their interactive effects are poorly studied (Rowan 1998). By comparison, exosymbiotic crustaceans have received relatively little attention despite their potential importance for the growth, reproduction, and survival of coral (Glynn 1983; Stachowicz and Hay 1999; Stewart et al. 2006) and the potential large-scale effects of these mutualisms on reef dynamics (Glynn 1987).

In the central Pacific, trapeziid crabs of the genus *Trapezia* and the snapping shrimp *Alpheus lottini* form heterosexual pairs and will not tolerate the presence of other conspecifics (Castro 1978; Huber 1987). Other species of crustacean mutualists, however, may be tolerated and effectively increase the 'defense force' of the coral head. In initial studies which we carried out in the summer of 2006, we observed up to five species of *Trapezia* (*T. flavopunctata*, *T. serenei*, *T. bidentata*, *T. areolata*, and *T. bella*), along with *Alpheus lottini*, occupying a single *Pocillopora* coral colony host at the study site. This 'species stacking' of mutualists may allow the colonization of symbionts of different defensive abilities and create intraguild dynamics that facilitate *Pocillopora* survival (C.S. McKeon, unpublished data). Our study system using two common species of 'guard' crustaceans is a first step toward understanding this diversity and represents a state frequently found in low-diversity lagoonal environments. We conducted a series of experiments and behavioral observations on two crustacean species (*Trapezia serenei* and *Alpheus lottini* 'stripes', hereafter referred to as Trapezia and Alpheus, respectively) to assess how the independent and combined effects of these exosymbionts contribute to defense of a host coral, *Pocillopora* cf. *meandrina* (hereafter Pocillopora) from the coral-eating seastar *Culcita novaeguineae* (hereafter Culcita). Our study was designed to answer the following questions: (1) Do exosymbionts effectively defend the coral? (2) Are the defensive responses of both exosymbionts equal? (3) Does a doubling in exosymbiont

density through the addition of a second species increase the defensive efficacy? (4) Are the effects of multiple defensive exosymbiont species independent, antagonistic (less than predicted under the assumption of independence), or synergistic (better than expected)?

Methods

Study species

All species of the coral genus *Pocillopora* are facultative hosts of the *Trapezia* genus of obligate crustaceans (trapeziid crabs) and the *Alpheus lottini* complex (a species complex of alpheid snapping shrimps). Both Trapezia and Alpheus are most commonly found in heterosexual pairs (Castro 1978; Preston 1971) which aggressively defend their territory from conspecifics (Huber 1987). Glynn (1976) described the defensive reactions of crustacean symbionts to attack of their coral host by corallivorous seastars ('Crown of Thorns' seastar *Acanthaster planci*, hereafter referred to as Acanthaster, and Culcita). While Acanthaster is a well-known corallivore, its population is subject to tremendous variability. Culcita, in comparison, is a less well-known corallivore, but more consistently common in the area, and a persistent threat to Pocillopora corals (Glynn and Krupp 1986; Pratchett et al. 2011). The size and shape of Culcita appear to limit its climbing ability, limiting its prey selection in comparison to Acanthaster (Glynn and Krupp 1986). Work within the system to date has shown the importance of exosymbiotic communities of corals in influencing the feeding of corallivorous seastars (Pratchett and Vytopil 2000; Pratchett 2001) and the possibility of communication among co-occurring symbionts (Lassig 1977; Vannini 1985). The 21 species of Trapezia, along with two or three species of the Alpheus complex, can be considered an interacting guild of coral-defending crustaceans. Potential costs to the coral host are largely unstudied, with evidence of provision of lipids by the coral host to Trapezia documented by Stimson (1990). The extent to which each potential symbiont species can be considered beneficial to its host is unknown, but given the high frequency of co-occurrence of Trapezia and Alpheus (134 of 139 Pocillopora surveyed in the area of this study; C.S. McKeon, personal observation) and the evidence for communication among symbionts, there exists a strong potential for interactive effects between these two groups of mutualists.

Experimental design

Colonies of *Pocillopora* cf. *meandrina* were collected from shallow water habitats near the Richard B. Gump Research Station, Moorea French Polynesia. We selected colonies that were roughly hemispherical in shape and of moderate size (average maximum horizontal circumference 61 cm; $n = 80$). The volumes of corals used in the deterrence trials were $20{,}083 \pm 2{,}035$ cm^3 [mean $\pm$ 95% confidence interval (CI)]. We measured the length, width, and height of each experimental coral colony. Colonies were carefully surveyed for exosymbionts, and all had at least one pair of *Trapezia serenei* and one pair of *Alpheus lottini*. We then removed animals to yield the four treatment groups: (1) no exosymbionts, (2) pair of *Alpheus lottini* only (Alpheus), (3) pair of *Trapezia serenei* only (Trapezia), and (4) pair of *Alpheus lottini* and pair of *Trapezia serenei* ('Alpheus + Trapezia'). Corals were then positioned in 3 m of water in the field to maintain the corals between laboratory trials and maximize survival. No colony or mutualist was used in more than one trial.

We collected *Culcita novaeguineae* from shallow water habitats close to the research station. Although both large and juvenile individuals were found in the area, only medium-sized Culcita [mean diameter 16 ± 1.4 cm (standard deviation, SD); $n = 64$] were used for the experiments in this study. Culcita were housed and fed ad libitum in flow-through seawater tanks for the duration of the study. Food was withheld for 24 h before an individual was used in a trial. Individual Culcita were haphazardly assigned to trials, and no individual was used in consecutive trials.

Deterrence trials

We conducted choice experiments by allowing a Culcita to choose between two Pocillopora colonies in large (approx. 150 L) flow-through aquaria. The coral colonies were placed in opposite corners of the aquarium. A Culcita was placed equidistant from the two Pocillopora at dusk. Ten replicates were made for No Exosymbionts versus Alpheus + Trapezia. The following morning we recorded which of the two corals was eaten by Culcita by examining corals for feeding scars, easily identifiable as the area of the colony where the coral tissue was eaten (i.e., bare white skeleton).

Test for emergent MDEs

We used feeding trials to quantify the efficacy of defense for four exosymbiont treatment groups: Trapezia + Alpheus, Trapezia, Alpheus, and No Exosymbionts. Twenty replicates in total were conducted for each exosymbiont treatment in a temporally blocked design with two replicates in each of ten temporal blocks. Trials were conducted in a large octagonal flow-through seawater tank approximately 0.5 m deep and 2 m across. The tank was

divided into eight equal sections using plastic screening. Each section was provisioned with a seastar refugium constructed from concrete blocks. We placed Pocillopora colonies into the tank from the field during mid- to late-afternoon. To minimize variation driven by search time, a single Culcita was placed directly on top of the coral colony. The following morning, we measured the coral size (length, width, height) and the feeding scars left by the Culcita (length, width, depth). We calculated volume consumed as an ellipsoid ($4/3\pi abc$, where a is half the length, b is half the width, and c is half the depth).

Behavioral observations

In a separate study we used behavioral observation methods based on those of Glynn (1980) to measure the reaction of Pocillopora exosymbionts to Culcita presence at different vertical locations on the colony. A coral colony with both Alpheus and Trapezia exosymbionts was placed on an elevated pedestal within a large seawater aquarium. Culcita was presented to the coral sequentially in two different experimental positions, where Culcita was held against the side or the top of the coral colony, respectively. Each trial alternated starting position and lasted 3 min with a 5-min rest period between treatments. Defensive behaviors were scored within 3 min with actions recorded on a per minute basis for proximity and degree of contact of the exosymbionts with the Culcita. If the Trapezia were viewed by the observer to show any response to the presence of the Culcita (usually adjusting its position to directly below the Culcita while remaining deep within the branches of the coral), it was given a score of 0.25. If the Trapezia advanced further, to within 2 cm of the Culcita, then it was given a score of 0.5 points. One point was added each time the Trapezia attacked by snapping at the body or tube feet of the Culcita, and an additional two points were given if the Trapezia attack resulted in a cut to the Culcita or removal of tube feet. If the Trapezia retreated and then re-engaged, the scoring system was restarted and all bouts were summed. The defensive snap of Alpheus involves a distinctive snapping sound (Glynn 1980), each of which was given a score of 1 point.

Data analysis

Deterrence trials were evaluated statistically using a binomial proportions test (Crawley 2007). In the MDEs experiment, we evaluated three measures: (1) frequency of predation, (2) volume of coral consumed, and (3) the location of feeding scars. Corals that were not attacked were not included in calculations of the volume of coral tissue consumed. Using a generalized linear mixed model (fixed effect: exosymbiont treatment; random effects: temporal block) with a binomial distribution and log link (using the JAGS package for Bayesian analysis: see below for additional details), we evaluated the effects of the different exosymbiont treatments on the frequency of predation with a one-way analysis of deviance (ANODEV). Similarly, a linear mixed model [log transformed ln(x); NLME Package R 2.13.1] with the same parameters was fit and evaluated with a one-way ANOVA to test the effects of exosymbiont treatments on the volume of coral consumed. For models of both frequency and volume, we conducted three orthogonal contrasts to evaluate: (1) the effect of symbionts (No Exosymbionts vs. Alpheus, Trapezia, and Alpheus + Trapezia); (2) the differences in defensive efficacy between Alpheus and Trapezia (Alpheus vs. Trapezia); (3) the difference between one and two symbiont pairs (Alpheus, Trapezia vs. Alpheus + Trapezia). Analysis in the one-factor ANOVA format facilitated orthogonal contrasts, which quantify the symbiont effects, differences between symbionts, and density effects; however, none of these contrasts explicitly test for an emergent MDE.

We assume a multiplicative defense model in calculating the expected defensive efficacy of both exosymbionts because the probability of defense in the presence of a given species of exosymbiont is conditional upon the defense by another exosymbiont. That is, the independent protective effects of two co-occurring exosymbionts on a single coral colony may overlap. This formulation is similar to the geometric decline in survival in the presence of multiple predator species in an MPE model. If the probability of *Culcita* attack in the absence of any exosymbionts is Φ_{NE}, and the expected attack rates with Alpheus alone or Trapezius alone are Φ_A and Φ_T respectively, then the expected attack probability is $\hat{\Phi}_{A+T} = \Phi_{NE}(\Phi_A/\Phi_{NE})(\Phi_T/\Phi_{NE})$. Analyzing the data on the log scale (using a generalized linear model) recovers the property of additivity:

$$\log(\hat{\Phi}_{A+T}) = \log(\Phi_A/\Phi_{NE}) + \log(\Phi_T/\Phi_{NE}) + \log(\Phi_{NE}). \quad (3)$$

Any deviations from this expectation then represent synergy (for negative deviations) or interference (for positive deviations) of the defenders.

The expected volume consumed by Culcita in the presence of both exosymbionts $\hat{\Omega}_{A+T}$ similarly follows a log-additive model:

$$\log(\hat{\Omega}_{A+T}) = \log(\Omega_A/\Omega_{NE}) + \log(\Omega_T/\Omega_{NE}) + \log(\Omega_{NE}) \quad (4)$$

where Ω_{NE} is the volume consumed by Culcita in the absence of exosymbionts and Ω_A and Ω_T are the volumes consumed in the single-exosymbiont case.

We statistically tested for a non-independent MDE using a two-factor ANODEV and ANOVA (Billick and Case 1994; Wooten 1994; Bolker 2008) on the frequency of

predation (binomial model, log link) and log (volume consumed) response variables (main effect of Alpheus, main effect of Trapezia, interaction between Alpheus and Trapezia, and a random effect of temporal block), respectively. An interaction in the two-factor analysis indicates an emergent MDE (i.e., either risk enhancement or risk reduction, presumably driven by exosymbiont interactions). Assumed error distributions, random effects, and fixed effects estimates were the same as in the one-factor models for the frequency of predation and volume response variables, respectively.

The effects of defenders on volume consumed can be estimated using a standard linear mixed model, although the lack of balance (because unequal numbers of individuals were attacked in the different treatments) is a slight complication (we used R's NLME package ver. 3.1-102). In contrast, fitting the equivalent model for the binary predation frequency data was technically challenging. We eventually settled on a Bayesian Markov chain Monte Carlo (MCMC) solution implemented in JAGS (Plummer 2003). We found that the multiplicative (log-link) model fitted the data worse than an analogous model using the standard logit link, because the logit-link model was better able to handle block–treatment combinations with high expected predation rates. However, the qualitative results from the logit-link model did not differ from those of the log-link model, so we opted to use the log-link model, which is more interpretable in terms of MDEs.

We used standard non-informative priors for our Bayesian analysis: normal distributions with mean 0 and variance 1,000 (precision = 0.001) for the fixed effect coefficients and a uniform (0.5) prior on the standard deviation of the among-block variance.

We ran a single chain of 50,000 iterations in JAGS, dropping the first 25,000 iterations as 'burn-in' and subsampling the remaining iterations down to 1,000 samples from the posterior distribution. Trace and autocorrelation plots of the chains were well behaved, suggesting good mixing; the Geweke diagnostic from the coda package, which compares the variance in the first 10% and last 50% of the retained values, did not indicate problems. We quote the 95% quantiles of the posterior distribution as the confidence intervals.

We separately evaluated whether the location of the feeding scars in the MDEs experiment feeding differed between corals with and without symbionts using a Pearson's chi-squared test with Yates continuity correction to test whether the location of feeding scars differed.

We tested whether the defensive behaviors of Trapezia and Alpheus differed between the two experimental positions (top and side) using a multivariate analysis of variance (MANOVA). Data were square root transformed to reduce heterogeneity of variance. We used the statistical programming environment R 2.13.1 for the computation of all statistics (R Development Core Team 2011; http://www.R-project.org). Detailed methodology provided in Supplemental Materials.

Results

Deterrence trials

Corals with exosymbionts were less likely to be attacked than unprotected corals. In 90% of trials, Culcita fed upon undefended Pocillopora colonies, but not defended colonies, when given the choice. The mean volume of coral consumed was lower where exosymbionts were present [with exosymbionts: 2.76 (95% CI 3.10, 17.67 cm^3); without: 184.03 (95% CI 145.48, 680.49 cm^3)].

Emergent MDEs

Feeding trials were used to evaluate three variables: frequency of predation, location of predation, and the amount of tissue consumed. Orthogonal contrasts on predation frequency revealed that the presence of symbionts reduced the predation frequency by 15%, (i.e. to 85% of its original value); the posterior probability that symbionts reduced predation frequency was 0.89. There was less evidence of a difference between Alpheus and Trapezia or between one and two exosymbiont pairs (Table 1; Fig. 1a). Qualitative effects of exosymbiont pairs differed between predation

Table 1 Results of orthogonal contrasts testing the effect of different symbiont treatment combinations on two response variables

Source of variation	Estimate (95% CI)	*df*	*P*
Predation frequency: relative change in attack frequency			
No symbionts versus symbionts	85% (60%, 111%)	NA	NA
Alpheus versus Trapezia	95% (61%, 140%)	NA	NA
Two symbionts versus four symbionts	81% (47%, 125%)	NA	NA
Volume eaten: relative fraction of volume eaten			
No symbionts versus symbionts	55% (46%, 67%)	37	<0.001
Alpheus versus Trapezia	132% (105%, 166%)	37	0.017
Two symbionts versus four symbionts	35% (28%, 43%)	37	<0.001

Relative change in predation frequency (one-way generalized linear mixed model), and (B) relative change in volume eaten (mixed effects one-way analysis of variance) were both determined with Wald *t* tests. Intercept estimates, which represent the frequency of attacks and mean volume consumed in the control treatment (Fig. 1a, b, respectively), are not shown

CI confidence interval, *NA* not available

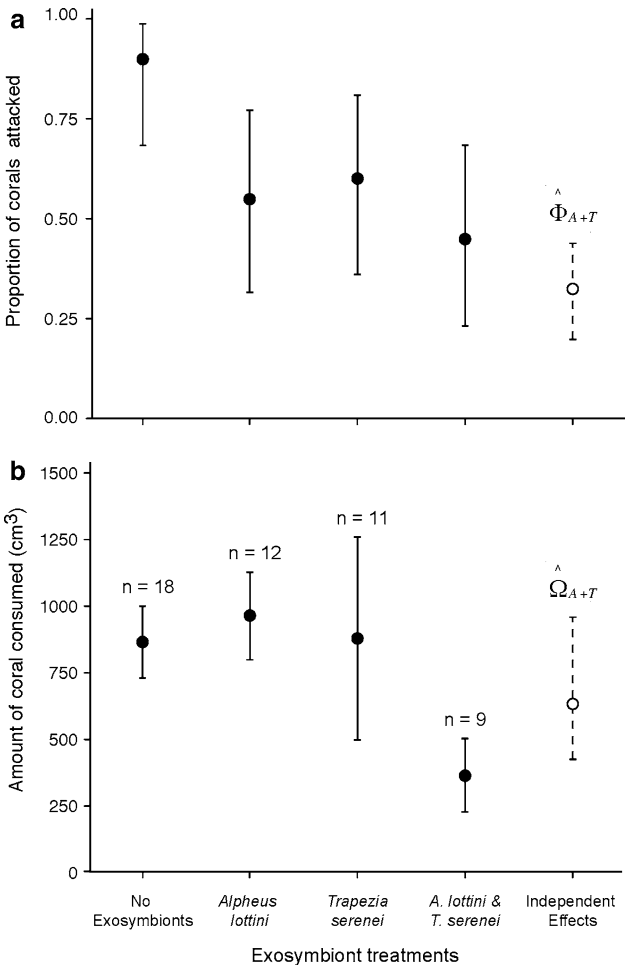

Fig. 1 **a** Effect of exosymbionts on the proportion of corals attacked by the corallivorous seastar *Culcita novaeguineae* ($n = 20$ per treatment). **b** For trials in which predation occurred, the mean volume of coral tissue consumed by *C. novaeguineae*. Expected values ($\hat{\Phi}_{A+T}, \hat{\Omega}_{A+T}$) are calculated using Eqs. 3 and 4; *bars* represent 95% confidence intervals (CIs), derived from an exact binomial test in (**a**) and a normal mode in (**b**). Expected independent effects and associated 95% CI are calculated from a Markov chain Monte Carlo sample of the posterior distribution of the fixed effect parameters

frequency and volume consumed. The volume of coral eaten by Culcita differed significantly between the Alpheus and Trapezia treatments, while the presence of two exosymbiont pairs reduced the volume eaten by 65% compared to the presence of one exosymbiont pair (Table 1; Fig. 1b). Although this reduction is much greater than the analogous estimated 19% reduction in attack probability, the magnitudes of the effects cannot be distinguished statistically.

Evidence for MDEs similarly differed between frequency of predation and volume consumed (Table 2). There was strong evidence of a MDE in volume consumed (57% reduction from the expected difference based on independent defense; $P < 0.001$); in contrast, the best estimate of the MDE on frequency of predation was only a

Table 2 Results tests for multiple defender effects in two response variables

Source of variation	Estimate (95% CI)	*df*	*P*
Predation frequency: relative change in attack frequency			
Alpheus	85% (49%, 114%)	NA	NA
Trapezia	89% (59%, 113%)	NA	NA
Alpheus × Trapezia	94% (49%, 190%)	NA	NA
Volume eaten: relative fraction of volume eaten			
Alpheus	68% (55%, 86%)	37	0.002
Trapezia	91% (73%, 114%)	37	0.39
Alpheus × Trapezia	43% (31%, 61%)	37	<0.001

Results as in Table 1, except for two-way layout

6% reduction, but the confidence interval was wide (51% reduction to 90% increase).

Corals without exosymbionts ($n = 16$) were equally likely to be eaten on the side or top of the coral colony. There was no statistically significant effect of exosymbionts on feeding location (top or side; $n = 28$), despite a trend for corals to be more frequently eaten on the side (21) rather than on the top (7) ($\chi^2 = 1.83$, $P = 0.18$).

Behavioral observations

The position of exposure affected the defensive behavior of exosymbionts ($F_{1,8} = 22.3$; $P < 0.001$). Placement of Culcita on the side of the colony induced significantly lower defensive behavior than placement on the top, for both Trapezia ($F_{1,8} = 33.5$, $P < 0.001$) and Alpheus ($F_{1,8} = 5.8$, $P = 0.04$, Fig. 2). Trapezia responded to the

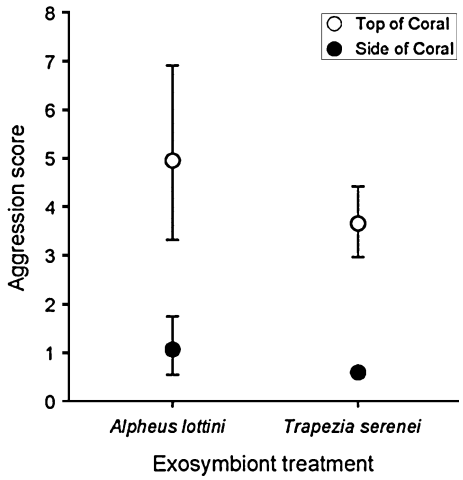

Fig. 2 Aggressive defensive behavior of *Pocillopora* cf. *meandrina* exosymbiont pairs in response to coral predator *C. novaeguineae* presented on either the top (*open circle*) or side (*filled circle*) of the coral head (backtransformed mean ±1 standard error, $n = 5$ for each pairwise combination). The aggression score is a composite of the cumulative aggressive behavior observed over 3 min, which included direct defensive behaviors such as snipping at *C. novaeguineae* tube feet (see "Data analysis" for calculation of aggression score)

presence of Culcita with a trend of increasing aggressive behavior over the 3-min time period, with the majority of pinching and snapping recorded in the last 2 min. Alpheus defensive snapping frequency did not show a particular trend over time, and only once was it observed to make contact with Culcita, snapping at it and removing the tube feet.

Discussion

The effects of Alpheus and Trapezia in reducing predation frequency were independent. However, we found evidence for synergy among exosymbionts in their ability to reduce the volume of coral tissue consumed. This emergent MDE was largely driven by the poor defensive efficacy when either exosymbiont was alone, but increased efficacy in the presence of both exosymbionts (Fig. 1b). These differences in the observation of an MDE depending on the response variable measured suggested that the response of Culcita to exosymbiont defenses may substantially differ between initial interactions and during feeding events. The behavioral data document similar reactions of Alpheus and Trapezia to Culcita threats. With simulated predator presentation, Alpheus and Trapezia began to immediately snip and prod the Culcita. Alone, neither exosymbiont was capable of reducing the volume consumed, but together the two species were able to drastically reduce the volume consumed. While these symbionts were not completely successful in warding off coral predation by Culcita all of the time, aggressive defense of the top and inner branches of the coral may be sufficient to maintain a core of surviving coral polyps to regenerate the colony after predation. Many Pocillopora bear scars of corallivore feeding events, yet have continued to survive and grow. This observation is supported by our behavioral data in which Trapezia and Alpheus more aggressively defended against Culcita attacking from the top of the coral compared to Culcita attacking from the side—where the habitat may be less critical and attacks may be more common.

Study limitations

Our ability to draw inferences about intraguild dynamics is limited by our experimental design: we applied only an additive design (two Alpheus, two Trapezia, two Alpheus + two Trapezia) rather than an additive and substitutive design (two Alpheus, two Trapezia, two Alpheus + two Trapezia, one Alpheus, one Trapezia, one Alpheus + one Trapezia). This limits our ability to differentiate between density and diversity effects (Griffen 2006). Our choice is relevant, however, in the context of the naturally occurring numbers of the experimental organisms. Both Alpheus and Trapezia are most commonly encountered in breeding pairs (Castro 1978; Huber 1987).

A further limitation is our understanding of the system as it performs under field conditions. Our efforts in the lab may not accurately represent conditions in the field. Despite the common co-occurrence of *Trapezia serenei* and *Alpheus lottini* 'stripe', many combinations of at least five species of Trapezia and two species in the *A. lottini* complex can be found in this size class of Pocillopora in the lagoons of Moorea.

The degree to which certain mutualists can be labeled as the 'best defenders' may be dependent on the spatiotemporal scale of observation (i.e., individual species that are highly abundant but only mediocre in their benefit to the host may actually be better than those mutualist species that are highly effective but exhibit recruitment limitation). For example, work in ant–acacia systems (Palmer et al. 2010) has demonstrated the potential value of an abundant, yet inferior mutualist defender when it occurs more commonly within critical life history stages of an individual host tree than a more effective mutualist defender that is also rare. Similar dynamics may operate where coral mutualists share space within a host. Marine organisms are known to have highly variable recruitment in space and time (Gaines and Roughgarden 1985; Roughgarden et al. 1988). Certain species of coral exosymbionts may differ in recruitment and efficacy in coral defense, and this variation in recruitment may alter the frequency of interspecific interactions within the exosymbiont community. Although we cautiously interpret interspecific variation in efficacy between the two mutualists in their benefits we observed, we must acknowledge that these differences as well as the strength of the MDE may vary with host ontogeny, temporal scale of the experiment, and the degree to which species vary in recruitment.

Conclusion

The consideration of intraguild relationships is of general importance in the study of mutualisms (Morris et al. 2007). Interactions between symbionts have the potential to dramatically alter the relationship with the host and may provide insight into why hosts may maintain multiple mutualists despite the potentially negative effects of one individual species within a symbiont guild. We observed differences in the efficacy of defense provided by two coral mutualists. We also describe here how the benefit exosymbionts provide can shift due to synergism between mutualists, suggesting the importance of higher-order interactions in modifying observed benefits and costs of multiple mutualists. Lastly, we found that the positive benefit of intraguild species interactions on mutualisms can depend on the response variable measured.

A number of foundation species depend on diverse suites of mutualists for reproduction, defense, and nutrient acquisition. However, the importance of intraguild species interactions and functional diversity is poorly studied despite the fact that shifts within guilds may fundamentally alter the magnitude of ecosystem services that mutualisms provide (Resetarits 2007). Our study adds evidence to a growing literature suggesting the benefits of 'guard exosymbionts' in maintaining the health of coral reefs and the importance of exosymbiont diversity in enhancing these benefits. Such benefits have broad implications in an ecosystem where the foundation species (corals) provide services such as ecotourism, subsistence fishing, and protection from storm damage.

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Culcita analysis

Ben Bolker

January 25, 2012

1 Introduction

This document demonstrates and describes the analysis for McKeon et al. (2011).

Load required packages:

```
library(MASS)  ## for fractions()
library(emdbook)  ## for as.mcmc.bugs()
library(R2admb)  ## AD Model Builder interface
library(ggplot2)  ## + reshape etc.
library(coefplot2)  ## pretty coefficient comparisons
library(R2jags)  ## + rjags, coda
library(scapeMCMC)  ## prettier MCMC diagnostics
library(nlme)  ## basic mixed models
```

The `glmmADMB` and `lme4` packages will be loaded below.

Package versions used:

```
##   coefplot2     emdbook    epitools     ggplot2    glmmADMB
##       0.1.1       1.3.2       0.5-6       0.8.9     0.7.2.6
##       Hmisc        lme4        MASS        nlme      R2admb
##       3.9-1 0.999375-42     7.3-16     3.1-102       0.7.5
##      R2jags   scapeMCMC
##     0.03-02       1.1-3
```

We used JAGS version 3.2.0.

2 Volume analysis

```
vdata <- read.csv("culcita_volume.csv")
```

Compute derived variables and turn numeric indices into factors:

```
vdata <- transform(vdata,block=factor(block),
                ttt2=factor(ttt2),
                propeaten=predvolume/volume,
                tvol=log(predvolume))
```

2.1 One-way analysis

In this analysis, we use custom contrasts to analyze the effects of symbionts on volume removed (in the subset of the data where a predator attacked).

```
library(nlme)
```

The sensible way to set up custom contrasts is to set up the comparisons you want to make, then invert the matrix, as follows. We want the following contrasts: (0) [intercept] expected from no symbionts, (1) no symb. vs. symbionts, (2) crab v shrimp, (3) pair v two pair.

Here's the "inverse contrast matrix" — each row gives the combination of levels that produces a given parameter. For example, row 2 (no symb. vs. symbiont) says that β_1 will be estimated from $-x_1 + (x_2 + x_3 + x_4)/3$ (the difference between "no symbiont" and the mean of the other three levels):

```
invcontr <- matrix(c(1,0,0,0,
                     -1,1/3,1/3,1/3,
                     0,-1,1,0,
                     0,-1/2,-1/2,1),
                   byrow=TRUE,ncol=4,

dimnames=list(c("none","symbiont","C_vs_S","1_vs_2"),
                     levels(vdata$ttt)))
```

```
fractions(cmat0 <- solve(invcontr))

##                            none symbiont C_vs_S 1_vs_2
## No Symbionts                  1    0        0      0
## Pair of Crabs                 1    1     -1/2   -1/3
## Pair of Shrimp                1    1      1/2   -1/3
## Pairs of Shrimp and Crabs     1    1        0    2/3

cmat <- cmat0[,-1]
contrasts(vdata$ttt2) <- cmat
```

Test that these contrasts are orthogonal:

```
fractions(zapsmall(t(cmat) %*% cmat))

##          symbiont C_vs_S 1_vs_2
## symbiont        3       0      0
## C_vs_S          0     1/2      0
## 1_vs_2          0       0    2/3
```

They are — all the off-diagonal elements are zero. (The intercept is *not* orthogonal to these contrasts — if we computed `t(cmat0) %*% cmat0` it would have non-zero off-diagonal elements — but that's OK [we don't care about the statistical test of the intercept, and in any case that statement is true of the default treatment contrasts in R].)

Analyze with block effects:

```
vol_lmm_1way <- lme(tvol~ttt2,random=~1|block,data=vdata)
(vol_lmm_1way_tab <- summary(vol_lmm_1way)$tTable)

##                Value Std.Error DF t-value   p-value
## (Intercept)   6.7164   0.16055 37  41.833 9.264e-33
## ttt2symbiont -0.5902   0.09319 37  -6.333 2.227e-07
## ttt2C_vs_S    0.2807   0.11257 37   2.494 1.724e-02
## ttt21_vs_2   -1.0623   0.10739 37  -9.892 6.169e-12
```

Compute back-transformed intervals:

```
i1 <- intervals(vol_lmm_1way,which="fixed")
i1$fixed <- exp(i1$fixed)
i1

## Approximate 95% confidence intervals
##
##  Fixed effects:
##                 lower     est.     upper
## (Intercept)  596.4798 825.8034 1143.2931
## ttt2symbiont   0.4589   0.5542    0.6694
## ttt2C_vs_S     1.0540   1.3241    1.6633
## ttt21_vs_2     0.2781   0.3457    0.4297
## attr(,"label")
## [1] "Fixed effects:"
```

2.2 Two-way analysis

```
vol_lmm_2way <- lme(tvol~crab*shrimp,random=~1|block,data=vdata)
round(vol_lmm_2way_tab <- summary(vol_lmm_2way)$tTable,4)
```

```
##                  Value Std.Error DF t-value p-value
## (Intercept)    6.7164    0.1606 37 41.8326  0.0000
## craby         -0.0957    0.1106 37 -0.8654  0.3924
## shrimpy       -0.3764    0.1137 37 -3.3120  0.0021
## craby:shrimpy -0.8262    0.1642 37 -5.0308  0.0000

## compute back-transformed intervals
i2 <- intervals(vol_lmm_2way,which="fixed")
i2$fixed <- exp(i2$fixed)
i2

## Approximate 95% confidence intervals
##
##  Fixed effects:
##                   lower     est.     upper
## (Intercept)   596.4798 825.8034 1143.2931
## craby           0.7262   0.9087    1.1370
## shrimpy         0.5451   0.6863    0.8640
## craby:shrimpy   0.3138   0.4377    0.6105
## attr(,"label")
## [1] "Fixed effects:"
```

Since we are dealing with log-scaled data, we are doing log-contrasts by default (as we want in order to characterize MPEs).

3 Binary data

Now analyze the attacked/not attacked outcomes.

3.1 Preliminaries

```
bdata  <- read.csv("culcitalogreg.csv")
bdata <- transform(bdata,block=factor(block),
                   ttt2=factor(ttt.1),
                   ttt=as.factor(ttt))
```

Use the contrasts we figured out above for the one-way layout.

```
contrasts(bdata$ttt2) <- cmat
```

3.2 GLMs (ignoring blocking)

To make the GLM work we need to specify working starting conditions. (This is a common issue when fitting GLMs with non-standard link functions, such

as the current case which is a binomial model with a log link [the standard or "canonical" link function is the logit].)

Specify -0.4 for the baseline predation ($\exp(-0.4) = 0.67$), 0 for the other effects.

```
b_glm_1way <-
glm(predation~ttt2,family=binomial(link="log"),data=bdata,
                      start=c(-0.4,0,0,0))
summary(b_glm_1way)

##
## Call:
## glm(formula = predation ~ ttt2, family = binomial(link = "log"),
##     data = bdata, start = c(-0.4, 0, 0, 0))
##
## Deviance Residuals:
##    Min      1Q  Median      3Q     Max
## -2.146  -1.093   0.459   1.031   1.264
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)   -0.1054     0.0745   -1.41  0.15749
## ttt2symbiont  -0.5304     0.1435   -3.70  0.00022 ***
## ttt2C_vs_S    -0.0870     0.2725   -0.32  0.74947
## ttt21_vs_2    -0.2442     0.2823   -0.87  0.38700
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 105.850  on 79  degrees of freedom
## Residual deviance:  94.975  on 76  degrees of freedom
## AIC: 103
##
## Number of Fisher Scoring iterations: 4
##

b_glm_2way <-
glm(predation~crab*shrimp,family=binomial(link="log"),data=bdata,
                      start=c(-0.4,0,0,0))
summary(b_glm_2way)

##
## Call:
## glm(formula = predation ~ crab * shrimp, family = binomial(link = "log"),
##     data = bdata, start = c(-0.4, 0, 0, 0))
```

```
##
## Deviance Residuals:
##    Min      1Q  Median      3Q     Max
## -2.146  -1.093   0.459   1.031   1.264
##
## Coefficients:
##                 Estimate Std. Error z value Pr(>|z|)
## (Intercept)      -0.1054     0.0745   -1.41    0.157
## crabC+           -0.4055     0.1972   -2.06    0.040 *
## shrimpS+         -0.4925     0.2156   -2.28    0.022 *
## crabC+:shrimpS+   0.2048     0.3754    0.55    0.585
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 105.850  on 79  degrees of freedom
## Residual deviance:  94.975  on 76  degrees of freedom
## AIC: 103
##
## Number of Fisher Scoring iterations: 4
##
```

3.3 GLMMs: `lme4`

The `glmer` function from the `lme4` package is the most commonly used tools for fitting GLMMs in R. We tried, but failed, to get any version of `glmer` to work in this situation. The stable version ignores user-specified starting conditions (and the default ones don't work), and neither of the two development versions can get through the problem, because at some interim point the model ends up getting a probability value > 1 (since the log link doesn't prevent this) and choking.

(This is what I tried.)

```
detach("package:nlme")  ## nlme and lme4 don't always play nicely
together
library(lme4)
b_glmm_1way <-
glmer(predation~ttt2+(1|block),family=binomial(link="log"),
                    data=bdata,
                    start=list(fixef=coef(b_anal_noblock)-0.3))
```

3.4 GLMs with blocks as fixed effects

We can try fixed-effect models, but as expected it makes the confidence intervals much wider (the following analyses also produce many warning messages about "step size truncated due to divergence" or "step size truncated: out of bounds").

```
b_glmf_1way <-
glm(predation~ttt2+block,family=binomial(link="log"),data=bdata,
                      start=c(-0.4,rep(0,12)))
coef(summary(b_glmf_1way))[1:4,]

##                Estimate Std. Error  z value Pr(>|z|)
## (Intercept)  -2.027e+00   0.932328 -2.17402   0.0297
## ttt2symbiont -6.721e-02   0.050263 -1.33725   0.1811
## ttt2C_vs_S   -2.502e-05   0.002161 -0.01158   0.9908
## ttt21_vs_2   -2.016e-01   0.150772 -1.33716   0.1812

b_glmf_2way <-
glm(predation~crab*shrimp+block,family=binomial(link="log"),data=bdata,
                      start=c(-0.4,rep(0,12)))
coef(summary(b_glmf_2way))[1:4,]

##               Estimate Std. Error    z value Pr(>|z|)
## (Intercept) -2.027e+00  9.323e-01 -2.174e+00   0.0297
## crabC+      -1.590e-09  1.799e-05 -8.839e-05   0.9999
## shrimpS+    -3.952e-05  2.714e-03 -1.456e-02   0.9884
## block2       7.007e-01  1.112e+00  6.304e-01   0.5285
```

3.5 GLMMs: JAGS (1- and 2-way) (Plummer, 2003)

We want to try this in a Bayesian framework instead, where (among other things) the priors can help constrain the estimated values of the random effects. As it turned out, JAGS (Just Another Gibbs Sampler) was the least painful method for fitting the full random-effects model with a log link.

JAGS/BUGS code:

```
model {
 for (i in 1:4) {
     fcoef[i] ~ dnorm(0,0.001)  ## fixed-effect parameters: priors
  }
  sd.b ~ dunif(0,5)             ## prior for block variance
  tau.b <- 1/sd.b^2
   for (i in 1:nblock) {
     reff[i] ~ dnorm(0,tau.b)   ## priors for block random effects
  }
  for (i in 1:nobs) {
    ## linear predictor: design matrix*coeffs + random effects
    eta[i] <- inprod(mf[i,],fcoef)+reff[block[i]]
    p[i] <- exp(eta[i])            ## convert to probabilities
    obs[i] ~ dbern(p[i])          ## Bernoulli response
  }
}
```

This is a fairly standard set of definitions. The only particular points of interest are:

- (line 5) we set a $U(0,5)$ prior on the among-block standard deviation. It would be better to use a prior such as a half-Cauchy that is nearly flat near zero and has a "fat tail" (i.e. a reasonably high probability even for large values); this is suggested by Gelman (Gelman and Hill, 2006; Gelman, 2006), who also points out problems with the traditional weak inverse-gamma priors. Lazy ecologists who don't want to be bothered figuring out how to parameterize a half-t or half-Cauchy distribution often follow the $U()$ approach — but there are at least some hints (`http://errorstatistics.blogspot.com/2011/12/jim-berger-on-jim-berger.html#disqus_thread`) that this is not a good idea either.
- (line 12) we set the model up using a model matrix and compute the inner product of the relevant row with the current value of the coefficients (`inprod(mf[i,],fcoef)`); this is equivalent to specifying something more explicit like `intercept+beta1*shrimp+beta2*crab+beta3*shrimpcrab`. The advantage of the model-matrix approach is that it's more compact and makes it easier to experiment with different model formulations (for example, in this case we can switch between the one-way and two-way layout simply by switching which model matrix we pass to JAGS), but it is admittedly more opaque as well.

Set up 1- and 2-way model matrices for JAGS and ADMB fits:

```
## 1-way design matrix
mmf <- model.matrix(~ttt2,data=bdata)
## 2-way design matrix
mmf2 <- model.matrix(~crab*shrimp,data=bdata)
```

```
library(R2jags)
nblock <- length(levels(bdata$block))
## BUG in current JAGS (3.2.0), do *not* load this module!
## For regular GLMMs (i.e. with a standard link), loading the
## GLM module can significantly speed up running times
## load.module("glm")
```

```
jdat <- list(nobs=nrow(bdata),
                       nblock=nblock,
                       block=as.numeric(bdata$block),
                       obs=bdata$predation,
             mf=matrix(c(mmf),ncol=4))
b_jags_1way <- jags(data=jdat,
                    inits=list(fcoef=coef(b_glm_1way),
```

```
                      reff=rep(0,nblock),sd.b=1,
                      ### set random-number seed
                      .RNG.name="base::Mersenne-Twister",
                      .RNG.seed=77758),
                    n.chains=1,
                    n.iter=2e5,
                    model.file="culcita.bug",
                    parameters=c("fcoef","sd.b"),
                    progress.bar="none")

## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
##    Graph Size: 669
##
## Initializing model
##
```

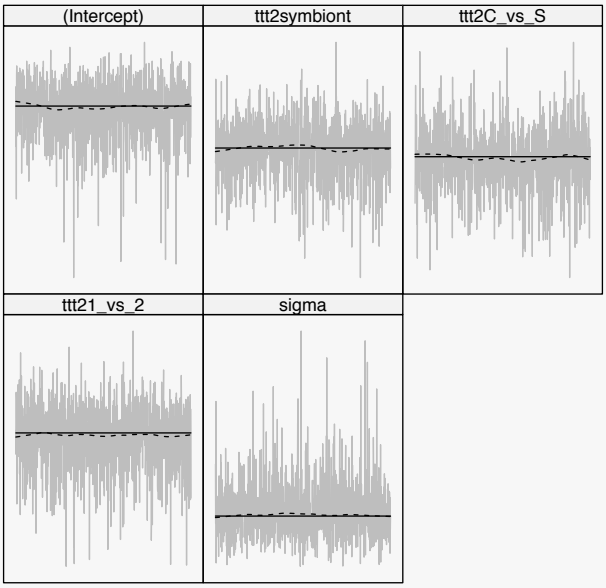
```
## why no effective size??
names_1way <- c(names(coef(b_glm_1way)),"sigma")
ss <- b_jags_1way$BUGSoutput$summary[-1,c(1,2,3,7)]
rownames(ss) <- names_1way
ss

##                   mean     sd    2.5%   97.5%
## (Intercept)  -0.58082 0.2691 -1.1658 -0.1624
## ttt2symbiont -0.17641 0.1415 -0.4868  0.0814
## ttt2C_vs_S   -0.06084 0.2080 -0.5279  0.3475
## ttt21_vs_2   -0.21273 0.2593 -0.7812  0.2118
## sigma         0.54595 0.2901  0.1275  1.2186
```

Back-transform:

```
exp(ss)

##                 mean    sd   2.5%  97.5%
## (Intercept)  0.5594 1.309 0.3117 0.8501
## ttt2symbiont 0.8383 1.152 0.6146 1.0848
## ttt2C_vs_S   0.9410 1.231 0.5898 1.4155
## ttt21_vs_2   0.8084 1.296 0.4579 1.2359
## sigma        1.7262 1.337 1.1360 3.3826
```

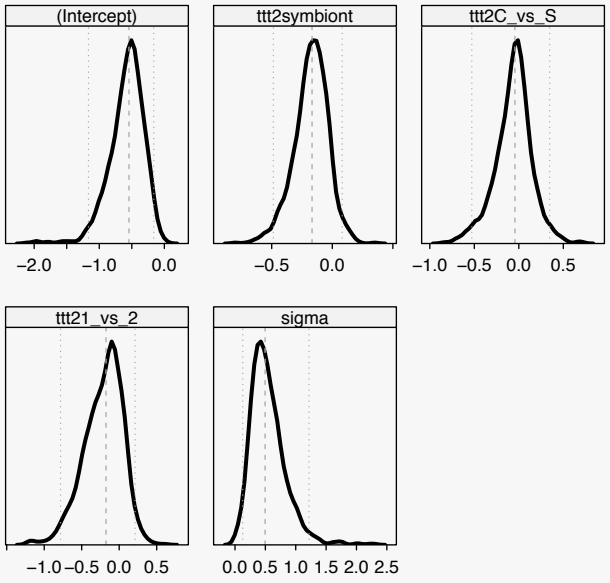
```
library(emdbook)
mm <- as.mcmc.bugs(b_jags_1way$BUGSoutput)
mm <- mm[,-1] ## drop deviance
colnames(mm) <- names_1way
library(scapeMCMC)
```

```
plotTrace(mm)
```



Other reasonable diagnostics:

```
plotDens(mm)  ## density plots
```



Could also do `plotAuto(mm)` (autocorrelation), `plotQuant(mm)` (quantiles (boxplots)), `plotSplom(mm,pch=".")` (Scatter plot matrix).

```
effectiveSize(mm)
```

```
##  (Intercept) ttt2symbiont   ttt2C_vs_S   ttt21_vs_2
##         1097         1000         1000         1000
##        sigma
##         2228
```

```
gg <- geweke.diag(mm)
2*pnorm(abs(gg$z),lower.tail=FALSE)
```

```
##  (Intercept) ttt2symbiont   ttt2C_vs_S   ttt21_vs_2
##       0.2681       0.9101       0.3875       0.8679
##        sigma
##       0.4212
```

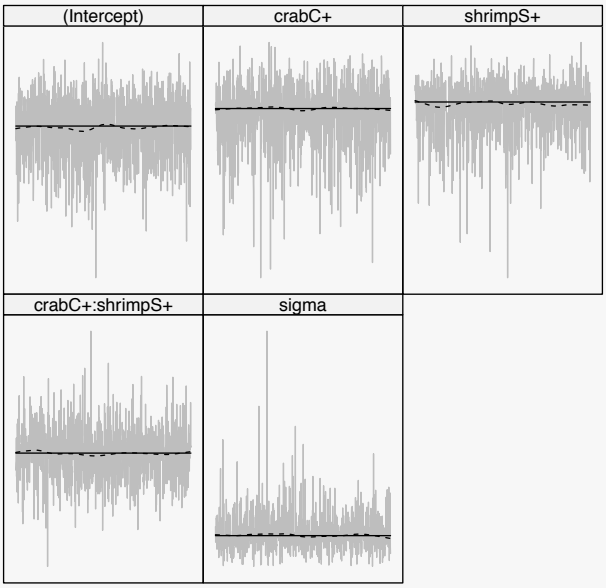
```
colMeans(mm>0) ## Bayesian "p values"
```

```
##  (Intercept) ttt2symbiont   ttt2C_vs_S   ttt21_vs_2
##        0.001        0.074        0.386        0.217
##        sigma
##        1.000
```

```
b_jags_2way <- jags(data=list(nobs=nrow(bdata),
                      nblock=nblock,
                      block=as.numeric(bdata$block),
                      obs=bdata$predation,
                      mf=mmf2),
                    inits=list(fcoef=coef(b_glm_2way),
                      reff=rep(0,nblock),
                      sd.b=1,
                      ### set random-number seed
                      .RNG.name="base::Mersenne-Twister",
                      .RNG.seed=77758),
                    n.chains=1,
                    n.iter=2e5,
                    model.file="culcita.bug",
                    parameters=c("fcoef","sd.b"),
                    progress.bar="none")

## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
##    Graph Size: 669
##
## Initializing model
##
```

```
names_2way <- c(names(coef(b_glm_2way)),"sigma")
mm2 <- as.mcmc.bugs(b_jags_2way$BUGSoutput)
mm2 <- mm2[,-1] ## drop deviance
colnames(mm2) <- names_2way
```

```
plotTrace(mm2)
```




```
effectiveSize(mm2)
```

```
##     (Intercept)          crabC+        shrimpS+
##          1000.0          1000.0          1000.0
## crabC+:shrimpS+           sigma
##           920.4          1000.0
```

```
ss2 <- b_jags_2way$BUGSoutput$summary[-1,c(1,2,3,7)]
rownames(ss2) <- names_2way
ss2
```

```
##                       mean     sd    2.5%   97.5%
## (Intercept)     -0.55000 0.2372 -1.0727 -0.1540
## crabC+          -0.07678 0.1543 -0.4595  0.1572
## shrimpS+        -0.12891 0.1849 -0.5813  0.1449
## crabC+:shrimpS+ -0.11431 0.3180 -0.7420  0.5047
## sigma            0.52626 0.2935  0.0959  1.2094
```

Back-transform:

```
exp(ss2)
```

```
##                    mean    sd   2.5%  97.5%
## (Intercept)     0.5769 1.268 0.3421 0.8573
```

```
## crabC+           0.9261 1.167 0.6316 1.1702
## shrimpS+         0.8791 1.203 0.5592 1.1560
## crabC+:shrimpS+ 0.8920 1.374 0.4762 1.6565
## sigma            1.6926 1.341 1.1007 3.3513
```

3.6 GLMMs: `AD Model Builder` (Fournier et al., 2011)

The next attempt used AD Model Builder.

ADMB definition file:

```
DATA_SECTION
   init_int nobs
   init_int nblocks
   init_int nf      // # fixed effects
   init_matrix fdesign(1,nobs,1,nf) // design matrix for fixed effects
   init_ivector blocks(1,nobs)   // Block index
   init_vector obs(1,nobs)      // Response variable
   init_number bpen
   // number fpen;


PARAMETER_SECTION
   init_vector fcoeffs(1,nf) // fixed-effects param vector
   init_bounded_number sigma(0.001,100,2)
   objective_function_value nll
   random_effects_vector rcoeffs(1,nblocks,2) // (phase 2)

PROCEDURE_SECTION
   nll = 0.0;

   for(int i=1; i<=nobs; i++)
   {
        bern(i, fcoeffs, rcoeffs(blocks(i)), sigma);
   }
   for(int n=1; n<=nblocks; n++)
   {
        rand(rcoeffs(n));
   }
SEPARABLE_FUNCTION void bern(int i, const dvar_vector& fcoeffs, const
    dvariable& r, const dvariable& sigma)

   dvariable fpen=0.0;         // penalty variable
   dvariable eta = fdesign(i)*fcoeffs + r*sigma;
   dvariable p;
   dvariable negeta;

   negeta = -eta;
   negeta = posfun(negeta,0.001,fpen);
   eta = -negeta;
   p = exp(eta);
   nll += bpen*fpen;

   if (obs(i)==0) {
        nll -= log(1-p);
   } else {
        nll -= log(p);
   // nll -= (obs(i)==1 ? log(p) ? log(1-p));
   }
SEPARABLE_FUNCTION void rand(const dvariable& r)
  nll+=0.5*(r*r)+0.5*nblocks*log(2.*M_PI);
  // nll+=0.5*(r*r)+0.5*log(2.*M_PI);

TOP_OF_MAIN_SECTION
```

```
53    gradient_structure::set_MAX_NVAR_OFFSET(764);
```

ADMB preliminaries: set up baseline input, parameter vectors, compile, etc..

```
library(R2admb)
setup_admb("/usr/local/admb")

## [1] "/usr/local/admb"

obs <- bdata$predation
nf <- ncol(mmf)
nobs <- nrow(bdata)
nblocks <- length(levels(bdata$block))
admb_dat <- list(nobs=nobs,
                 nblocks=nblocks,
                 nf=nf,
                 fdesign=mmf,
                 blocks=as.integer(bdata$block),
                 obs=obs,
                 bpen=1e5)
write_dat("culcita",L=admb_dat)
admb_pars <- list(fcoeffs=coef(b_glm_1way),
                  sigma=1,
                  rcoeffs=rep(0.001,nblocks))
write_pin("culcita", L=admb_pars)
compile_admb("culcita", safe=FALSE, re=TRUE, verbose=FALSE)
xargs <- "-shess -noinit -nox -l1 40000000 -nl1 40000000 -ilmn 5
-imaxfn 500 -maxfn 500 "
xargs2 <- "-nox -l1 40000000 -nl1 40000000 -ilmn 5 -imaxfn 500
-maxfn 500 "
```

Utility functions for ADMB fits:

```
cdbg <- 0
cdbg_rpt <- 50
fit_culcita <- function(d,              ## response variable
                        mf=mmf,         ## data frame
                        what="summary",
                        ## return coefs or full model?
                        tpl="culcita",  ## TPL file
                        ## if tpl="culcita2" then linkflag=1 for
log,
                        ##    linkflag=2 for logit link
                        linkflag=1,
                        verbose=FALSE,
                        debug=TRUE,
```

```
                        bpen=1e6,
                        npar=15,      ## number of parameters
                        xargv=1) {
  if (debug) {
    if (cdbg %% cdbg_rpt == 0) {
      cat("*",cdbg,"\n")
    }
    cdbg <<- cdbg+1
  }
  newdat <- admb_dat
  newdat$obs <- d
  newdat$fdesign <- mf
  newdat$nobs <- length(d)
  newdat$bpen <- bpen
  if (tpl=="culcita2") newdat$linkflag <- linkflag
  write_pin(tpl, L=admb_pars)
  write_dat(tpl,L=newdat)
  xx <- if (xargv==1) xargs else xargs2

suppressWarnings(run_admb(tpl,verbose=verbose,extra.args=xargs))
  f <- try(read_admb(tpl),silent=TRUE)
  clean_admb(tpl)
  if (what=="model") return(f)
  if (inherits(f,"try-error")) r <- rep(NA,npar+1)
  else r <- c(coef(f),-logLik(f))
  r
}
```

```
b_admb_1way <-
fit_culcita(bdata$predation,what="model",bpen=1000)

## * 0

b_admb_1way_pen <-
fit_culcita(bdata$predation,what="model",bpen=3e4)
b_admb_2way <-
fit_culcita(bdata$predation,what="model",mf=mmf2,bpen=1000)
```

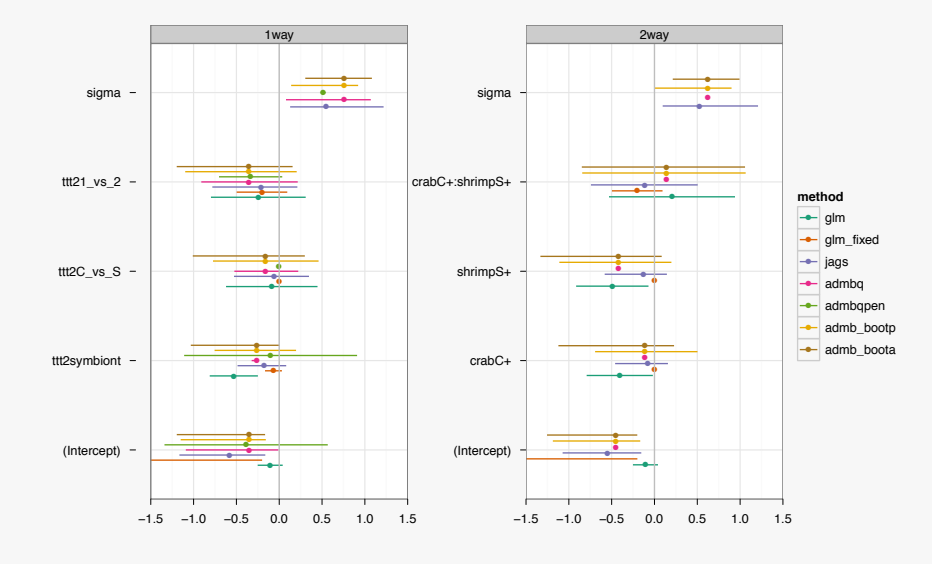
4 Summaries, plots, etc.

In addition to the basic ADMB point estimates calculated as shown above, for which quadratic confidence intervals are available via `confint`, we also computed parametric bootstrap confidence intervals by simulating data from the

model 2000 times and re-computing the confidence intervals. Below we present both the naive (quantile) bootstrap estimates of the confidence intervals and the BCa (adjusted) bootstrap intervals, from the `boot` package. Further details available on request ...

(Grubby code to assemble estimates and confidence intervals for all models suppressed.)

(Plotting code suppressed.)



Key: `glm`=GLM disregarding blocks (pooled estimate); `glm_fixed`=GLM treating blocks as fixed; `jags`=JAGS; `admbq`=ADMB fit, quadratic confidence intervals; `admbqpen`=ADMB fit with stronger boundary penalty; `admb_bootp`=ADMB fit with parametric bootstrap CI; `admb_boota`=ADMB with parametric bootstrap CI, bias-corrected bootstrap intervals.

```
ss <- subset(L.all2,method %in% c("glm","jags"),
             select=c(coef,method,param,est,lo,hi))
rownames(ss) <- seq(nrow(ss))
print(ss,digits=3)
```

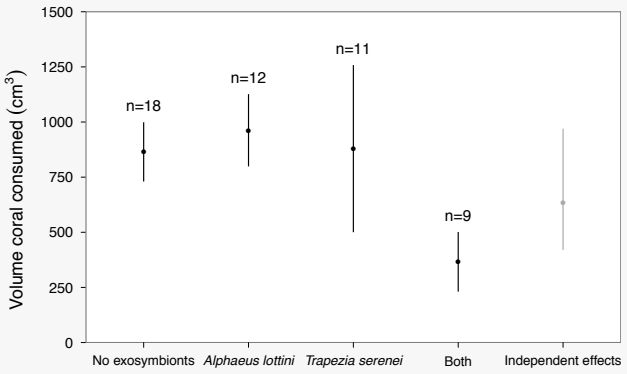
```
##               coef method param     est      lo      hi
## 1      (Intercept)    glm  1way -0.1054 -0.2514  0.0407
## 2     ttt2symbiont    glm  1way -0.5304 -0.8116 -0.2491
## 3       ttt2C_vs_S    glm  1way -0.0870 -0.6211  0.4470
## 4       ttt21_vs_2    glm  1way -0.2442 -0.7974  0.3090
## 5      (Intercept)    glm  2way -0.1054 -0.2514  0.0407
## 6           crabC+    glm  2way -0.4055 -0.7920 -0.0190
## 7         shrimpS+    glm  2way -0.4925 -0.9150 -0.0700
```

```
## 8  crabC+:shrimpS+    glm  2way  0.2048 -0.5309  0.9405
## 9       (Intercept)   jags  1way -0.5808 -1.1658 -0.1624
## 10    ttt2symbiont   jags  1way -0.1764 -0.4868  0.0814
## 11      ttt2C_vs_S   jags  1way -0.0608 -0.5279  0.3475
## 12      ttt21_vs_2   jags  1way -0.2127 -0.7812  0.2118
## 13           sigma   jags  1way  0.5459  0.1275  1.2186
## 14      (Intercept)   jags  2way -0.5500 -1.0727 -0.1540
## 15           crabC+   jags  2way -0.0768 -0.4595  0.1572
## 16         shrimpS+   jags  2way -0.1289 -0.5813  0.1449
## 17 crabC+:shrimpS+   jags  2way -0.1143 -0.7420  0.5047
## 18           sigma   jags  2way  0.5263  0.0959  1.2094
```

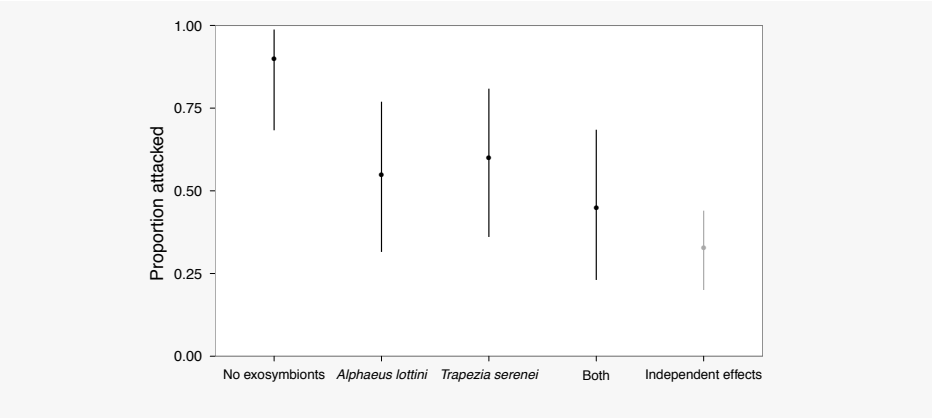
4.1 Figure 1

Derive estimate and confidence intervals for independent estimates:

```
library(lme4)
vol_lmm2_2way <- lmer(tvol~crab*shrimp+(1|block),data=vdata)
vmcmc <- mcmcsamp(vol_lmm2_2way,n=1000)
rr <- rowSums(t(vmcmc@fixef)[,1:3])
mean(rr)

## [1] 6.454

quantile(rr,c(0.025,0.975))

##  2.5% 97.5%
## 6.039 6.877
```



Now do binary equivalent:



References

Fournier, D. A., H. J. Skaug, J. Ancheta, J. Ianelli, A. Magnusson, M. N. Maunder, A. Nielsen, and J. Sibert. 2011. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optimization Methods and Software Pages 1–17. URL `http://www.tandfonline.com/doi/abs/10.1080/10556788.2011.597854`.

Gelman, A. 2006. Prior distributions for variance parameters in hierarchical models. Bayesian Analysis **1**:515–533. URL `http://ba.stat.cmu.edu/journal/2006/vol01/issue03/gelman.pdf`.

Gelman, A. and J. Hill. 2006. Data Analysis Using Regression and Multilevel/Hierarchical Models. Cambridge University Press, Cambridge, England. URL `http://www.stat.columbia.edu/~gelman/arm/`.

McKeon, C. S., A. C. Stier, S. E. McIlroy, and B. M. Bolker. 2011. Multiple defender effects: Synergistic coral defense by mutualist crustaceans. In press, *Oecologia*.

Plummer, M. 2003. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling URL `http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.13.3406`.