COMMUNITY-LEVEL RESPONSE TO HABITAT STRUCTURE MANIPULATIONS: AN EXPERIMENTAL CASE STUDY IN A TROPICAL ECOSYSTEM

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ABSTRACT

Across the globe, environmental change is resulting in novel ecosystems that have altered habitat structure and functioning. Research is needed to understand how changes in habitat structure in these new ecosystems impact community interactions, especially when these manipulations are being proposed to reduce invasive species. We conducted an experiment in Hawaii to determine how changes in habitat structure, represented by leaf litter and understory vegetation, affect the abundance of an invasive generalist predator, the coqui frog (*Eleutherodactylus coqui*) and its potential prey (invertebrates). This study consisted of four treatments: two vegetation treatments (50% and 100% removal of vegetation with diameter at breast height <5 cm) and two leaf litter treatments (50% and 100% removal). Removal of 50% of habitat structure, either vegetation or leaf litter, was not sufficient to produce long-term changes in coqui or invertebrate densities. Only full removal of habitat structure resulted in reduced densities of coqui after four months. The abundance of leaf litter invertebrates and invertebrates flying close to the forest floor was

higher in the 100% vegetation removal treatment compared to leaf litter removal treatments, and the abundance of foliage invertebrates was higher in the 100% leaf litter removal treatment compared to vegetation removal treatments. Invertebrate responses were complicated because they not only responded to the loss of habitat but also the reduction of coquis in treatments. Coquis in treatments moved to microhabitats that contained increased prey. Treatments appeared to impact coquis by removing structure needed for diurnal retreats, breeding and foraging. In summary, both the 100% removal of leaf litter or vegetation can reduce coqui densities in relative small (20 m x 20 m areas), even when surrounded by intact, invaded forest. This study provides greater understanding of the impact of habitat structure manipulation, a typical management employed to control an invasive frog, in a novel ecosystem.

KEYWORDS: Invasive Species; *Eleutherodactylus coqui*; Habitat Structure; Invertebrate Prey; Hawaii; Novel Ecosystems

1. INTRODUCTION

Many ecosystems are rapidly being transformed into new, non-historical configurations, with different species and species abundances (Hobbs et al. 2006). With an increase in these novel ecosystems comes an increasing need to understand how to manage them (Hobbs et al., 2006; Seastedt et al., 2008). The introduction of invasive species is one example of how novel ecosystems are formed (Seastedt et al., 2008), and habitat manipulation is a common restoration strategy to control invasives. However, habitat manipulation can affect community dynamics, such as predator-prey relationships, in unpredictable ways, especially in novel ecosystems (Hawlena et al., 2010; Salo et al., 2010). Thus, more tests of species responses to restoration

techniques in novel ecosystems are needed to determine when management outcomes may be different than those expected from historical counterparts.

As an example, experimental manipulation of habitat structure in a variety of systems has found mixed effects on predators and prey (Pianka, 1973; Orth et al., 1984; Henden et al., 2011). In some studies, generalist predators are more affected by prey availability than habitat complexity (Birkhofer et al., 2008), while in other studies the opposite pattern is found (Halaj et al., 1998, 2000; Birkhofer et al., 2007). Still other studies have found that both habitat and prey can affect predators (Mathews et al., 2004). Similar patterns have been found for prey species, with both predators and habitat determining prey abundance (Halaj and Wise, 2002; Buskirk, 2005). Thus, in novel ecosystems where predator and prey composition has changed, interactions among species, and thus the outcome of restoration efforts, may also change.

One location where novel ecosystems are especially common is Hawaii, where nearly all native ecosystems below 500 m in elevation have been altered or destroyed by centuries of agriculture and development (Mueller-Dombois and Fosberg, 1998). Various forms of direct and indirect control are used in Hawaii to manage undesirable non-native species, including the introduced frog, *Eleutherodactylus coqui* (hereafter the coqui; Beard et al., 2009). The coqui is a small, terrestrial frog native to Puerto Rico that was introduced to Hawaii in the late 1980s via the horticultural trade (Kraus et al., 1999). Since its introduction, its range has increased and it can be found in some parts of the Island of Hawaii with densities up to two times its highest density in Puerto Rico (Woolbright et al., 2006; Beard et al., 2008).

In Hawaii as much as $4 million has been spent annually to control this species, although budgets have declined as success has been elusive, especially on the Island of Hawaii (Beard and Pitt, 2012). Control efforts have largely focused on chemical control, but in some areas,

mechanical control has been used successfully, especially in combination with chemical control. Specifically, on Kauai the coqui has been all but eliminated with vegetation and litter removal from a 7-ha area that was also treated with citric acid. Because coquis are so dependent upon both litter and vegetation for habitat, mechanical control has focused on reducing these two aspects of their habitat. Leaf litter often provides diurnal retreats and breeding sites for coquis (Stewart and Pough, 1983; Townsend and Stewart, 1986). In fact, coquis densities are highest when the amount of litter on the forest floor is highest (Woolbright 1991). At night coquis are primarily found on vegetation, which they use to attract mates, such as for calling sites (Stewart and Pough, 1983; Townsend and Stewart, 1986; Townsend, 1989), and there is often a positive relationship between the amount of vegetation structure in the understory and coqui density (Beard et al. 2003; Beard et al., 2008). Therefore, a reduction in these two habitat types is expected to reduce coqui density (Beard and Pitt, 2005; Beard et al., 2009).

However, coqui density is also positively related to invertebrate densities (Woolbright, 1989; Beard, 2001; Beard et al. 2008). In areas where coqui density is high, they consume up to an estimated 690,000 prey items per hectare per night (Beard et al., 2008). They are opportunistic foragers and can change dominant prey depending on availability (Stewart and Woolbright, 1996; Beard, 2007). In Hawaii, appear mostly to forage in the leaf litter (Beard, 2007; Choi and Beard, 2012). In Puerto Rico, they mostly forage upon foliage invertebrates (Stewart and Woolbright, 1996). Coquis in Hawaii also consume foliage invertebrates (Beard, 2007); sampling methods may limit our ability to detect reductions in these prey following invasion (Choi and Beard, 2012). Altering habitat structure may not only directly affect potential coqui prey, which we anticipate would affect coqui density, but also microclimate, such as humidity and temperature, which in turn, could affect the invertebrate community (Richardson et

al., 2000; Vargas et al., 2001; Sayer, 2006; Nakamura et al., 2009; Robson et al., 2009) and amount of suitable coqui habitat (Woolbright, 1991; Pounds and Crump, 1994).

The objective of this study was to determine the effect of habitat structure manipulation, more specifically leaf litter and understory vegetation removal, on both predator (coqui) and potential prey (invertebrate) abundances in a novel, altered ecosystem in Hawaii. Because coquis use leaf litter primarily to breed and vegetation primarily to find mates and both have been shown to be positively related with density, we expected that reductions in both would reduce density. Because coquis forage in both the leaf litter and on foliage, we expected removals of these habitats would also remove prey, which would then indirectly reduce coquis density. To determine the relative importance of these interactions, we examined both direct and indirect linkages. We further examined potential mechanisms affecting predator and prey availability by determining the effect of habitat manipulations on microclimate and habitat usage by the predator.

2. MATERIALS AND METHODS

2.1. Study site

We conducted research in the Nanawale Forest Reserve (19°28' N, 154°54' W; elevation 230 m), in the southeast region of the island of Hawaii. Mean annual precipitation is 300-400 cm, with peak rainfall occurring between November and April (Giambelluca et al., 1986). Mean annual temperature is 23˚C (Nullet and Sanderson, 1993). Because there is little seasonal variation, we expected little variation in coqui or invertebrate abundances during the experiment (Price, 1983). The substrate is rough a'a lava flow, approximately 400 years old (Wolfe and Morris, 1996). The reserve has extremely high coqui densities, estimated to be up to 91,000

coqui/ha, which is two to three times mean densities observed in the native range (Woolbright et al., 2006; Beard et al., 2008). Dominant overstory trees include: non-natives *Psidium cattleianum* Sabine, *Falcataria moluccana* (Miquel) Barneby and Grimes, and *Cecropia obtusifolia* Bertol. Dominant understory includes: native *Cibotium* spp., and non-native *Melastoma candidum* D. Don and *Clidemia hirta* (L.) D. Don. (Tuttle et al., 2009). Mean percent canopy cover in our study site was 99%. As a result of the influx of non-native under- and overstory vegetation in this forest reserve, endemic *Metrosideros polymorpha* and *Cibotium* sp. no longer dominate the vegetation and the ecosystem has moved into a novel, non-historic composition.

2.2. Experimental design

We used a completely randomized design consisting of five, 20 m x 20 m experimental plots, four treatments and a control plot, in four replicate locations (blocks), for a total of 20 plots. Coquis are highly territorial, remain within a few meters of their retreat sites, and will remain within 20 m x 20 m areas over many years (Woolbright, 1985; 2005). The four treatments consisted of two vegetation treatments [50% and 100% removal of vegetation with diameter at breast height (DBH) < 5 cm] and two leaf litter treatments (50% and 100% removal). Plots were at least 15 m apart, and blocks were from 500 m to 950 m apart. We took pre-treatment measurements in January and February 2010. We imposed treatments at the beginning of the study (February to March), two months later (April to May) and then one month later (June), for a total of three treatment applications (hereafter referred to as treatment applications 1, 2, and 3). After initial treatment, the two subsequent treatment applications were used as maintenance treatments due to the high litterfall and vegetation re-growth in this area.

Each plot was divided into sixteen, 5 m x 5 m subplots. For the 50% leaf litter removal treatment, we removed litter from eight of those subplots, resulting in a checkerboard pattern of removal. Removing litter in this way allowed us to maintain treatments in subsequent treatment applications. For the 100% removal treatment, we removed litter from all 16 subplots. We removed litter by hand. All of the litter removed from each of the 5 m x 5 m subplots was weighed. We developed wet to dry weight conversions using four, 3 kg subsamples from each leaf litter removal plot, dried in a drying oven at 50º C until constant weight (wet-dry weight conversion, $R^2 = 0.81$).

For the 50% vegetation removal treatment, we removed all vegetation with DBH <5 cm from eight subplots, resulting in a checkerboard pattern of removal. For the 100% vegetation removal treatment, we removed all vegetation with DBH <5 cm from all 16 subplots. We removed vegetation using machetes and hand pulling. We weighed all vegetation removed from four 5 m x 5 m subplots in each of the vegetation removal plots, and estimated the total amount removed per plot. We developed a wet to dry weight conversion using four, 3 kg subsamples from each vegetation removal plot, dried at 50º C until constant weight (wet-dry weigh conversion, $R^2 = 0.61$). Photographs of subplots pre- and post-treatment can be seen in Figure 1.

<Insert Figure 1>

2.3. Coqui density

We estimated coqui density using standard line transect distance sampling (Buckland et al., 2005). We divided our plots into four, 5 m x 20 m sections or transects. Two researchers walked slowly through each of the four, 5 m x 20 m transects for 30 minutes with headlamps, surveying for all coquis in each transect. We recorded all coquis (both seen and/or heard), distance from observer, height off the forest floor to the nearest 0.1 m, and type of structure used

by the coqui (leaves > 1m off the forest floor; forest floor (including soil, rocks, downed vegetation); trees; leaves < 1 m off the forest floor (mostly forbs, grass, fern). We completed each block in two consecutive nights, and we completed all four blocks in eight-night periods. We began surveying at 1900 hour and it took 1 hour per plot. We conducted distance sampling prior to treatment application one, and then two days following the last day of treatment application following treatment applications 1, 2, and 3.

2.4. Invertebrates

All invertebrate sampling occurred once pre-treatment and immediately following treatment applications 2 and 3. Four samples were collected from each plot during each sampling period, one from each of the 5 m x 20 m transects. To make sure invertebrate samples were representative of treatments, in the 50% removal treatments half the samples were collected in 5 m x 5 m subplots where removals had occurred and half were collected in subplots where removals had not occurred.

After sundown, we collected leaf litter invertebrates by collecting leaf litter from four 0.5 m x 0.5 m areas in each plot. Leaf litter was placed in Berlese-Tullgren funnels within 2 hours of collection to extract the invertebrates, which were then stored in 70% ethanol for later quantification. We collected flying invertebrates from four 10 cm x 18 cm sticky traps placed vertically 0.75 m off the forest floor in each plot (as in Beard et al., 2003). Sticky traps were left in the plots for one week, wrapped in plastic wrap, and stored in the freezer for later quantification. We collected foliage invertebrates using vacuum sampling. A modified hand-held vacuum (Black and Decker, Towson, MD, USA) was run for 90 seconds along all vegetation in a 1 m x 1 m area from 0.5 m off the forest floor to 2 m, in a slow steady pace. In

the 100% removal treatment, we ran the vacuum for 90 seconds in the air or along any vegetation that was not removed (i.e., DBH >5 cm). In the 50% removal treatment, half of the samples were collected by running the vacuum in the air, and the other half of the samples were collected by running the vacuum along vegetation for 90 seconds. Collected invertebrates were placed in 70% ethanol for later quantification.

2.5. Environmental variables

We measured temperature and relative humidity using HOBOs (Pro Series H08-032-08, Onset Computer Corporation, Pocasset, MA), placed in each plot within one block for 4 days to 2 weeks at a time, taking readings once every minute, and then were rotated to the next block to take measurements throughout the length of the experiment. In each plot, we took temperature and relative humidity readings for a total of 37 to 46 days including pre- and post-treatment measures. For our analyses, we selected 3 days of readings for each plot within a location and time period (i.e., pre-treatment and after each treatment application), because this was the maximum number of days that could be selected consistently across treatment applications. The actual days selected were those closest to when the treatments were conducted and as close as possible to when data was collected in the other blocks. We placed the HOBOs on the forest floor, because both subadult and adult coquis can be found at this height at different times (Beard et al., 2003; Beard, 2007).

2.6. Statistical analyses

To test for treatment effects (i.e., differences in biomass and leaf litter removed), we conducted a two-way factorial analysis of variance (mixed model ANOVA) with location and location x treatment interaction as the random variables.

We analyzed distance sampling data with program DISTANCE (Buckland et al., 2005; Thomas et al., 2006), whereby we used the perpendicular distance from the transect line to the recorded coqui to calculate a probability density function that models the decreased likelihood of observing animals with increasing distance from the transect line. This function is then used to correct the counts and estimate the density of coquis and the associated 95% confidence interval. Data were fit to key detection functions (half-normal or hazard-rate) and a cosine series expansion, which provided a better fit to the data than other functions [based on Akaike Information Criterion (AIC), Windows, SAS Institute, Cary, North Carolina].

To assess the effects of treatment and treatment application on coqui density, we conducted a two-way factorial analysis of covariance (ANCOVA), with pre-treatment measures as the covariate, location and the location x treatment interaction as the random variables. We also assessed whether there were any pre-treatment differences among treatments for all variables measured using one-way ANOVAs. These tests revealed that pre-treatment there were no significant differences among treatment plots, and thus are not presented. Even though we found no differences pre-treatment, we included pre-treatment measures as a covariate in our post-treatment tests to account for any pre-treatment variability. In these tests, pre-treatment was only significant in temperature and humidity data.

We used the ANCOVA model structure to assess the effects of treatment and treatment application on density of coquis and on our other response variables: invertebrates collected from leaf litter, sticky trap, and vacuum sampling, diurnal (600 h to 1859 h) and nocturnal (i.e., 1900 h to 559 h) temperature and relative humidity, and height of coquis off the forest floor. We used mean height used by the coquis pre-treatment and after each treatment application.

We also performed a single sample chi-square test with a Bonferroni adjustment to assess if there were differences in vegetation types (leaves >1m, leaves <1m, forest floor, trees) used by coquis after treatment.

To meet the assumptions of normality and homogeneity of variance for these tests, we cube-root transformed coqui density, and leaf litter, sticky trap, and vacuum collected invertebrates. All ANOVAs and ANCOVAs were conducted using SAS v 9.2 for Windows (SAS Institute, Cary, North Carolina). ANOVAs were conducted using PROC MIXED, and ANCOVAs were conducted using PROC GLIMMIX (Appendix A). We followed these analyses with Holm's step-down Bonferroni *post hoc* multiple comparisons. We considered $P<0.05$ significant for all statistical analyses.

3. RESULTS

3.1. Treatment effects

Overall, after 6 months we removed a total of 2,253 kg dry weight from our plots over three treatment applications. We removed a total of 696 kg dry weight from the 100% leaf litter treatment, 425 kg dry weight from the 50% leaf litter removal treatment, 915 kg dry weight from the 100% vegetation removal treatment, and 217 kg dry weight from the 50% vegetation removal treatment. The amount of vegetation removed varied by treatment ($F_{4,42}=4.28$, $P=0.0054$) and treatment application ($F_{2,42}=10.67$, $P=0.0002$), with the most material removed from the 100% vegetation removal, followed by the 100% leaf litter removal, the 50% leaf litter removal, and the 50% vegetation removal. Most vegetation was removed during the first treatment application, with less being removed in treatment applications 2 and 3 (Figure 2).

<Insert Figure 2>

3.2. Coqui density

There was an overall interaction between treatment application and treatments ($F_{8,34.92}$=2.87, P=0.01). After treatment application 3, the 100% leaf litter removal and the 100% vegetation removal treatments had lower coqui densities than control plots; densities were much higher after this treatment application overall (Figure 3).

<Insert Figure 3>

3.3. Invertebrates

We found an effect of treatment application and treatment on invertebrates collected from leaf litter ($F_{1,143.1}$=7.69, P=0.006 and $F_{4,143.2}$=5.19, P=0.0006, respectively). The number of invertebrates collected from leaf litter was higher in the 100% vegetation removal treatment than in the other treatments, though not different than the control (Figure 4). Overall, more invertebrates were found in samples after treatment application 2 than after treatment application 3 (Figure 5). To determine if the increase in invertebrates was not simply due to an increase in leaf litter produced by the vegetation removal treatment, we ran an ANOVA on the leaf litter biomass removed from plots to extract leaf litter invertebrates. We found no effect of treatment on leaf litter mass removed ($F_{4,14.5}$=0.59, P=0.6743), suggesting that an increase in litterfall rate did not drive the difference in the number of leaf litter invertebrates collected in this treatment.

<Insert Figure 4>

<Insert Figure 5>

The number of invertebrates collected from sticky traps was higher in the 100% vegetation removal treatment than the other treatments and controls ($F_{4,11.7}$=9.87 P=0.001; Figure 6).

<Insert Figure 6>

We found an interaction between treatment application and treatments on invertebrates collected from vacuum sampling ($F_{4,133.7}$=4.08, P=0.004). Both the 50% and 100% vegetation removal treatments had fewer invertebrates following treatment application 2, but only the 100% vegetation removal treatment still had fewer invertebrates following treatment application 3 (Figure 7).

<Insert Figure 7>

3.4. Habitat usage by coquis

We found an effect of treatment application on coqui height off the forest floor ($F_{2,39}$=4.15 P=0.0231). Height off the forest floor was higher after treatment application 2 than after treatment application 3 (Figure 8). Coquis were found on different vegetation types post-treatment application periods 1, 2, and 3 ($F_{437,12}$=42.883, P<0.0001; $F_{515,12}$=74.943, P<0.0001; $F_{435,12}$=79.960, P<0.0001). For example, there were more coquis were found on the forest floor in the 100% vegetation removal treatments than in leaf litter removal treatments or controls (Figure 9, A, B, and C). There were more coquis found on trees in the 100% leaf litter removal treatment than the 100% vegetation removal treatment after treatment applications 1 and 3.

<Insert Figure 8>
<Insert Figure 9>

3.5. Effect of treatment on temperature and humidity

Temperature and humidity were affected by treatment application and daytime versus nighttime measures ($F_{2,64.8}$=50.94, P<0.0001; $F_{2,62.22}$=60.81, P<0.0001, respectively), but not by treatment ($F_{4,11.69}$=1.28, P=0.33) and there was no interaction between treatment and treatment application ($F_{8,67.68}$=0.66, P=0.73) . Treatment application 3 had the highest overall temperature during the daytime, while nighttime temperatures stayed relatively constant over time (Figure 10). The same pattern was observed with the humidity measurements (Figure 11).

<Insert Figure 10>
<Insert Figure 11>

4. DISCUSSION

Mechanical management of coquis in Hawaii has focused on removing both understory vegetation and litter (Beard and Pitt 2005). The results of this study show that coqui abundance will be reduced by 100% understory vegetation removal or 100% leaf litter removal because coqui densities were lower in these treatments than in control plots after repeated application, but not with 50% removal. Our results show that we can measure reductions in coqui densities with these manipulations in relatively small, 20 m x 20 m plots, even when the manipulations are surrounded by intact, invaded forest. Our results suggest that in the case of not wanting to remove understory, it is possible to remove litter and have a similar effect on coquis. In addition, it implies that these manipulations on small properties in the face of surrounding invasion can attain lower frog densities.

The response of coquis alone to 100% understory and leaf litter removal does not indicate whether it was the loss of breeding habitat, calling habitat, or foraging habitat that caused the reduction in frogs. To try to understand these interactions, we also measured the response of potential prey to these treatments. We found more leaf litter invertebrates in the 100% understory removal treatment than in all other treatments, and close to more than in the controls ($P = 0.078$). Similarly, we found more flying invertebrates right above the forest floor in the 100% understory-removal treatment than in any other treatment. As expected, foliage invertebrates did not show a similar increase with the 100% understory removal treatments, but rather showed a decrease in these treatments compared to controls. We may have observed an increase in leaf litter and flying invertebrates in the 100% understory removal treatment because of the reduction in predation pressure by coquis in this treatment. In addition, it would not be

surprising if in treatments where vegetation was removed, some invertebrates moved into the litter. We also consistently found a higher number of foliage invertebrates in the 100% leaf litter removal treatment than either vegetation removal treatment. We may have observed an increase in foliage invertebrates in the 100% leaf litter removal retreatment because there was a reduction in predation pressure by coquis in this treatment but also because, where leaf litter was removed, invertebrates may have moved from the forest floor onto the foliage. Because invertebrates increased in the remaining microhabitats after treatments were in place, this supports the idea that prey abundance generally increases with coqui removal (Sin et al., 2008; Choi and Beard, 2012).

Changes in microhabitats used by coquis after treatments suggest that they were moving to areas with higher prey availability. In the 100% vegetation removal treatment, coquis that remained in the treatment were more often found at lower heights (leaves < 1 m) and on the forest floor compared with control plots. This is not unexpected based on the lack of understory substrate remaining in the treatment, but also in light of the decrease in potential prey on understory vegetation and increase in potential prey in the leaf litter and flying invertebrate close to the forest floor. In the leaf litter removal treatments, coquis that remained in the treatments were more often found in the trees, where there was also greater prey availability than in the vegetation removal plots. Because there were no differences in temperature or humidity with treatment or during at night (when coquis are active) throughout the experiment, we expect these shifts in microhabitat use were more driven by changes in habitat and prey availability than by changes in microclimate.

We further investigated our results to separate the effects of habitat loss and lower prey availability on coqui densities in treatments. Because we observed that coqui densities were

lower in the 100% vegetation removal treatment, and that densities of leaf litter and flying invertebrates were higher in this treatment, the reduction in coqui densities does not appear to result from lower prey availability, especially because coquis in Hawaii typically consume a large proportion of leaf litter invertebrates (Beard, 2007; Choi and Beard, 2012). Furthermore, the observed lower coqui densities in the 100% leaf litter removal treatment occurred where there were more foliage invertebrates, again suggesting that it was habitat loss over loss of prey driving the pattern. While it is very likely that both the loss of habitat and prey contributed to coqui reductions, because with the loss of habitat there were increases in invertebrates in other microhabitats where the generalist predator can forage, and this did not counterbalance coqui reductions, the reductions appear primarily to be due to loss of needed habitat or structure.

It is well known that coqui abundance in its native habitat is driven by the amount of habitat (structure) in the environment available for diurnal retreat and breeding habitat (Stewart and Pough, 1983; Woolbright, 1991). It has been suggested that lava substrate in Hawaii may serve this purpose (Woolbright et al., 2006; Beard et al., 2008; Tuttle et al., 2009). While lava substrate may provide diurnal and breeding habitat in some areas of Hawaii, our results suggest that leaf litter and vegetation also provide important structure that is needed to maintain coqui densities. Because of the positive relationship between understory vegetation structure and coquis observed in previous studies (Beard, 2001; Beard et al, 2008), it is perhaps not surprising that understory vegetation removal would also lower coqui densities. Our results highlight the importance of both leaf litter and understory vegetation structure for coquis.

When considering the above findings, it is important to notice that coqui density was only affected after treatment application 3. In this treatment application period, there were more coquis found across all treatments. While we are unable to say for certain why this might be the

case, there are several potential explanations. The density sampling done following treatment application 3 was done at the end of June to early July, when ambient temperatures and humidity were consistently higher in all of our plots. Higher temperatures and humidity in Puerto Rico have been shown to result in greater coqui activity (Townsend and Stewart, 1994) and differences in density (Stewart, 1995). Thus, this response likely indicates more activity and not necessarily more coquis over time. It is likely that with the higher number of coquis observed in plots after treatment application 3, we were better able to capture treatment effects statistically. We cannot determine from our data if these effects were present in the earlier treatments or whether we observed some type of cumulative or threshold response by the third treatment application. It is also interesting to note that the total number of leaf litter invertebrates decreased in treatment application 3, perhaps in response to the increased number of active coqui.

In addition to adding to a growing body of literature of empirical studies examining the effect of habitat modification on community structure in novel ecosystems (Seastedt et al., 2008), these findings are particularly important in Hawaii where management suggestions for coquis include vegetation and litter removal (Beard and Pitt, 2005; Beard et al., 2009). Vegetation removal may not only be effective at reducing coqui, but may also alter invertebrate communities in this novel ecosystem (Seastedt et al., 2008). While we actually saw an *increase* in invertebrates where coquis densities were reduced, it is unknown if the increase was in endemic or introduced invertebrate species; but we know these sites are dominated by non-native invertebrates (Tuttle et al. 2009). Furthermore, because coquis have been found to change invertebrate communities and increase nutrient cycling rates, with possible benefits to non-native plants (Sin et al., 2008; Choi and Beard 2012), efforts to remove coquis may or may not move

the ecosystem to a more desirable state. The most successful management strategy will include recognizing the impacts of coquis on the ecosystem as well as the changes that may result from managing the coqui in the ecosystem.

The novel ecosystem approach to managing invasive species has not been documented much in the literature. Applying this approach may result in more efficient and cost-effective long-term management with increased ability to detect or respond to undesirable direct or indirect consequences (Seastedt et al., 2008; Hobbs et al., 2006). In our case, while we might have expected that coqui responses to habitat manipulations in a novel ecosystem may be different from those in the native range, based on differences in dominant prey and preferred microhabitats between the ranges (Beard 2007), we did not observe a response to habitat manipulation different from we would expect in Puerto Rico. Given that habitat structure manipulations are currently being suggested in Hawaii as a way to reduce coquis (Beard and Pitt 2005), it is important for public management campaigners to recognize that this management is likely to affect the abundance of coquis as well as the prey community. Given the high litterfall rate and vegetative re-growth seen in our forested plots, managers need to weigh the feasibility of vegetation and leaf litter removal over the long-term on individual properties and may, instead, recommend maintaining the area immediately surrounding the property owners' living space to be kept free of vegetation and leaf-litter.

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Figure Legends

Fig. 1. Photograph examples of plots pre- and post-treatment.

Fig. 2. Mean aboveground biomass removed (kg) (±1 SE) during each treatment application. Letters indicate significant differences by treatment application ($P<0.05$). Treatment Application 1 = Feb 19 to Mar 4, Treatment Application 2 = Apr 28 to May 12, and Treatment 3 Application = June 8 to June 22.

Fig. 3. Mean density of coqui (#/ m^2) (±1 SE) post-treatment application. Letters indicate significant differences by treatment within each treatment application from post-hoc Holm's step-down Bonferroni ($P<0.05$).

Fig. 4. Mean total number of invertebrates collected from leaf litter sampling (±1 SE) by treatment. Letters indicate significant differences from post-hoc Holm's step-down Bonferroni ($P<0.05$).

Fig. 5. Mean total number of invertebrates collected from leaf litter sampling (±1 SE) by treatment application. Letters indicate significant differences from post-hoc Holm's step-down Bonferroni ($P<0.05$).

Fig. 6. Mean total number of invertebrates collected from sticky trap sampling (±1 SE) by treatment. Letters indicate significant differences from post-hoc Holm's step-down Bonferroni ($P<0.05$).

Fig. 7. Mean total number of invertebrates collected from vacuum sampling (±1 SE) by treatment application. Letters indicate significant differences from post-hoc Holm's step-down Bonferroni ($P<0.05$).

Fig. 8. Mean differences in coqui height above forest floor (m) (±1 SE) by treatment application. Letters indicate significant differences from post-hoc Holm's step-down Bonferroni ($P<0.05$).

Fig. 9 A-C. Observed counts of coquis found on each vegetation type in each treatment plot, after the three treatment applications, labeled A, B, and C (N = 437; N = 515; N = 435 total coquis observed, respectively). Letters indicate significant differences for a vegetation type across treatment ($P<0.05$).

Fig. 10. Mean comparisons (±1 SE) for differences in temperature (ºC) by treatment applications and daytime versus nighttime. Letters indicate significant differences from post-hoc Holm's step-down Bonferroni ($P<0.05$).

Fig. 11. Mean comparisons (±1 SE) for differences in humidity level (g/m^3) by treatment applications and daytime versus nighttime. Letters indicate significant differences from post-hoc Holm's step-down Bonferroni ($P<0.05$).

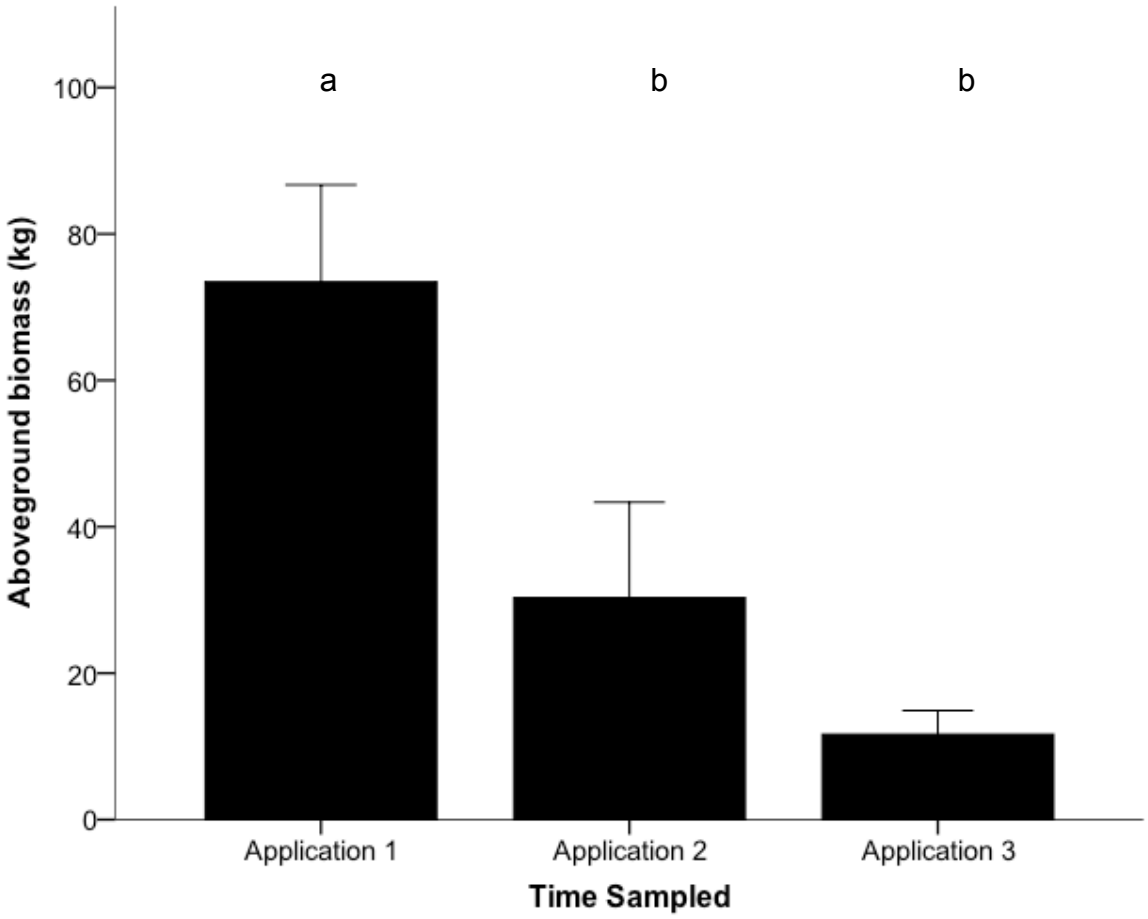
a
b
b
100
80
60
40
20
0
Aboveground biomass (kg)
Application 1
Application 2
Application 3
Time Sampled

Fig. 2

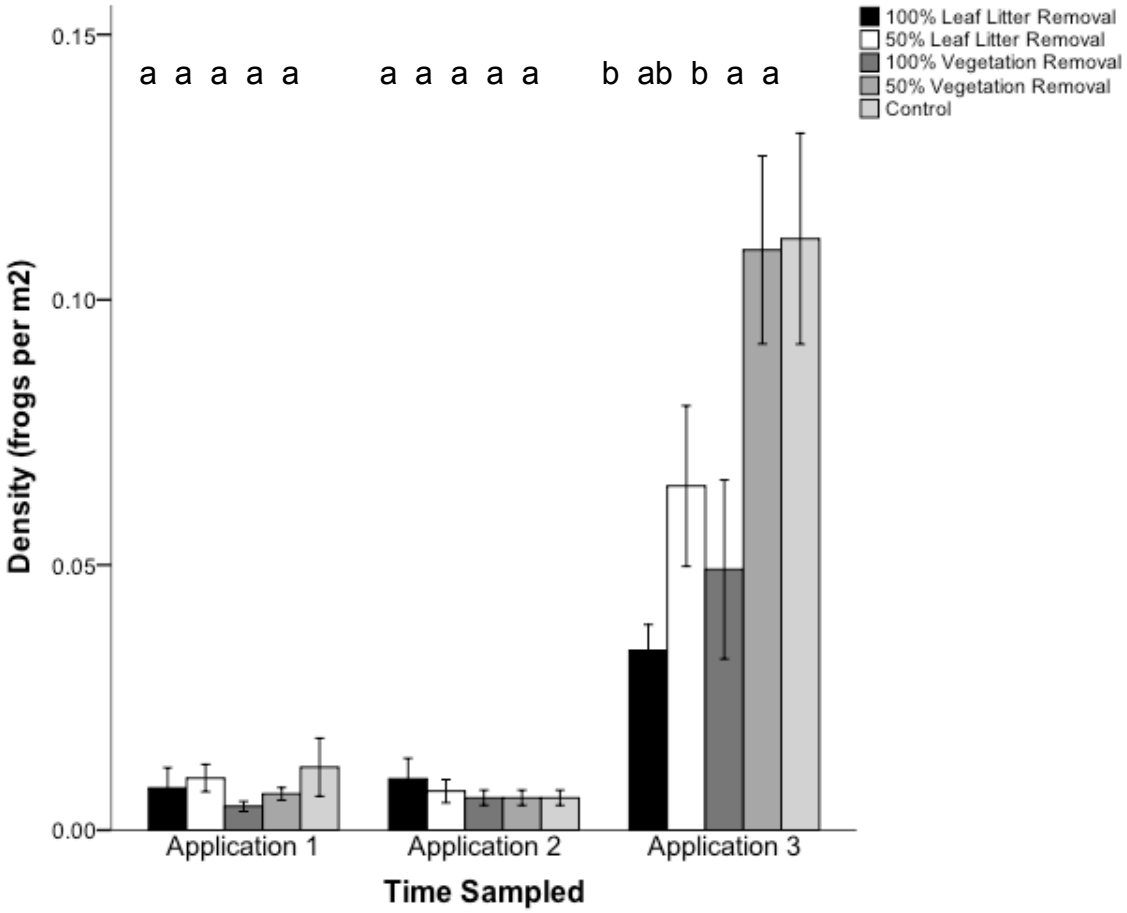
100% Leaf Litter Removal
50% Leaf Litter Removal
100% Vegetation Removal
50% Vegetation Removal
Control
a a a a a
a a a a a
b ab b a a
0.15
0.10
0.05
0.00
Density (frogs per m2)
Application 1
Application 2
Application 3
Time Sampled

Fig. 3

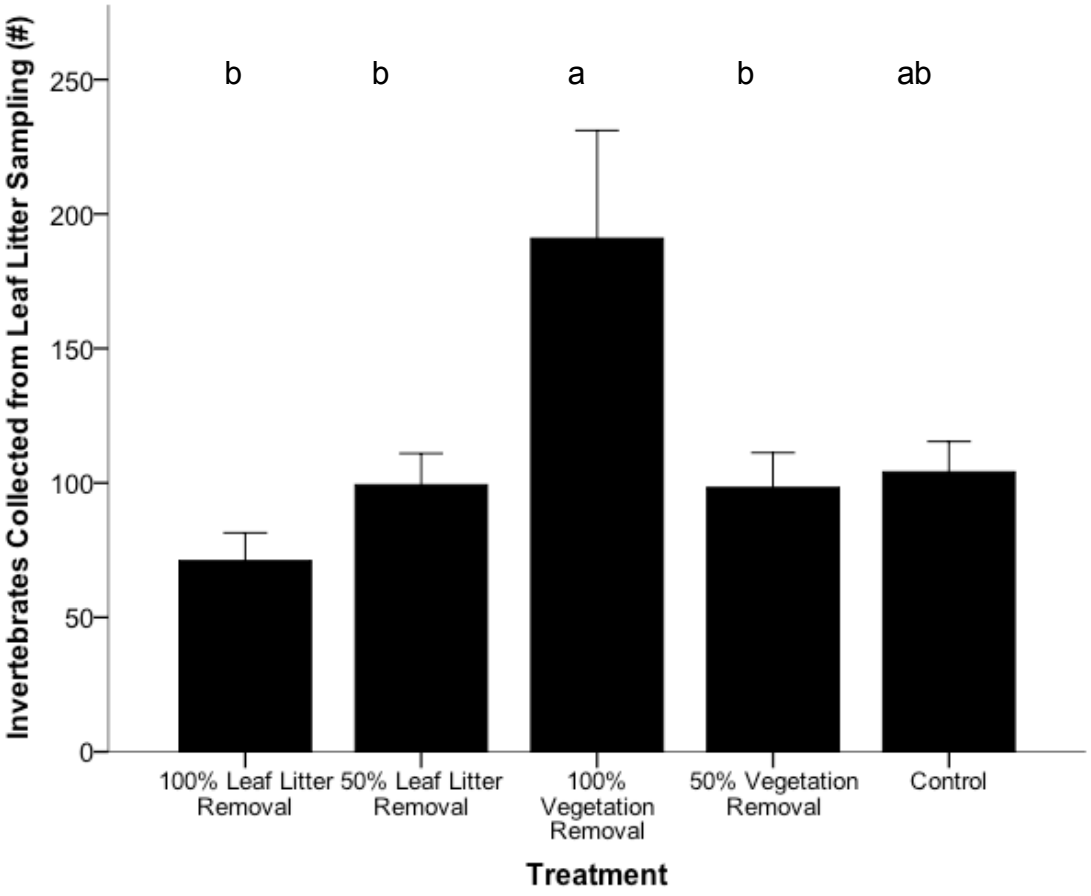
Invertebrates Collected from Leaf Litter Sampling (#)
250
200
150
100
50
0
b
b
a
b
ab
100% Leaf Litter Removal
50% Leaf Litter Removal
100% Vegetation Removal
50% Vegetation Removal
Control
Treatment

Fig. 4

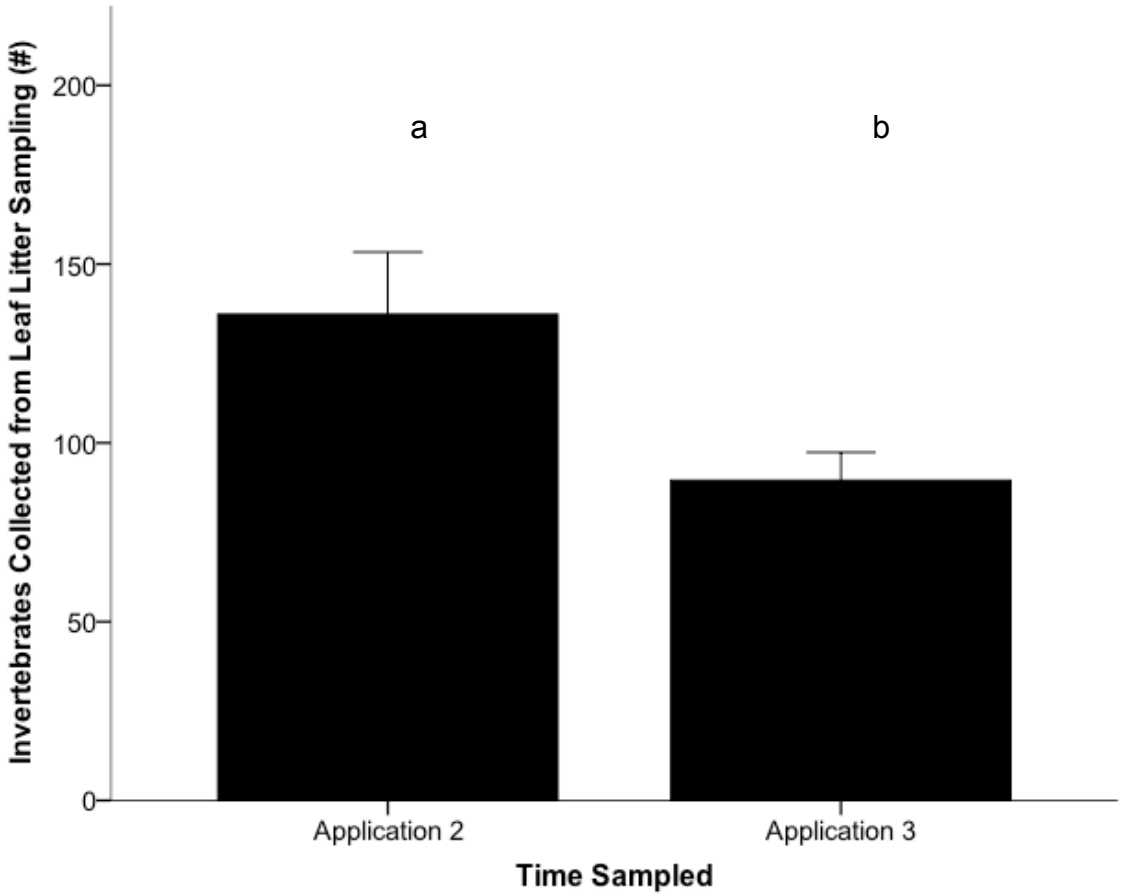
Invertebrates Collected from Leaf Litter Sampling (#)
200
150
100
50
0
a
b
Application 2
Application 3
Time Sampled

Fig. 5

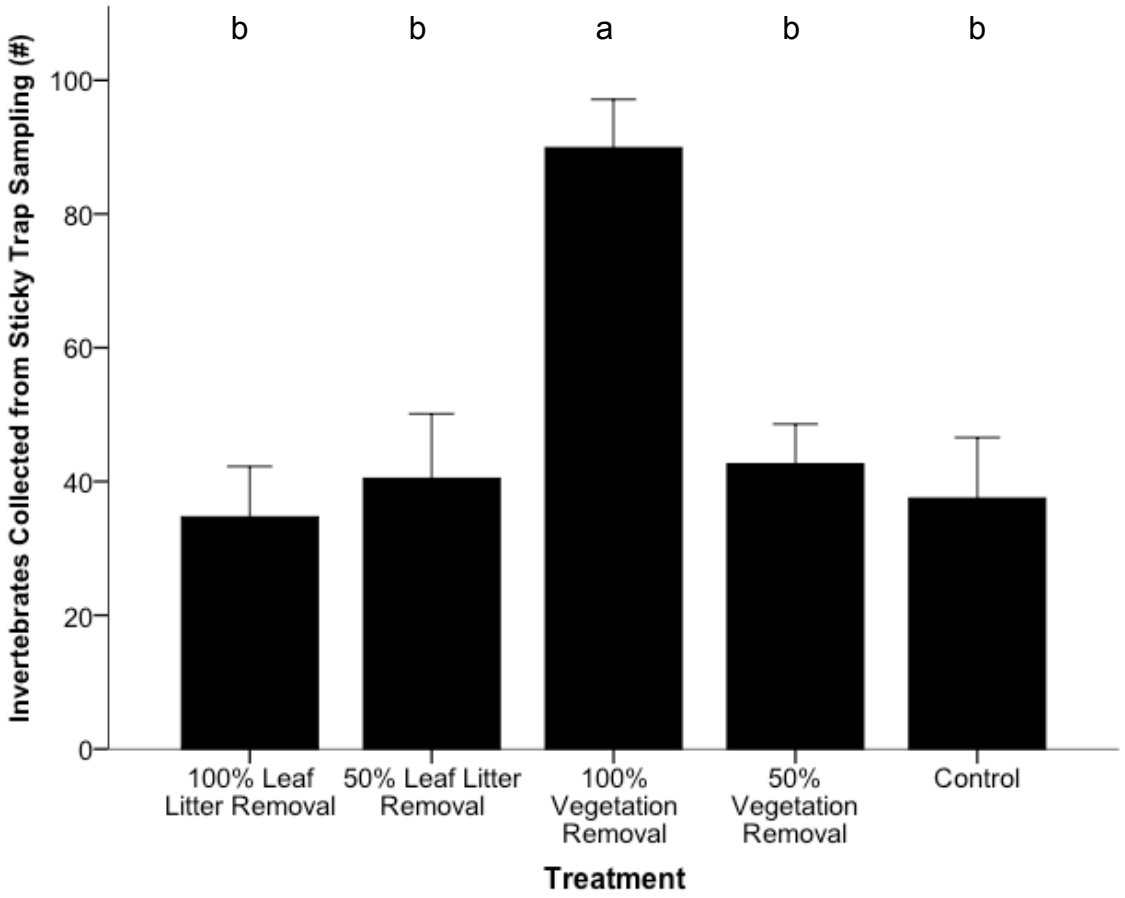
b
b
a
b
b
Invertebrates Collected from Sticky Trap Sampling (#)
100
80
60
40
20
0
100% Leaf Litter Removal
50% Leaf Litter Removal
100% Vegetation Removal
50% Vegetation Removal
Control
Treatment

Fig. 6

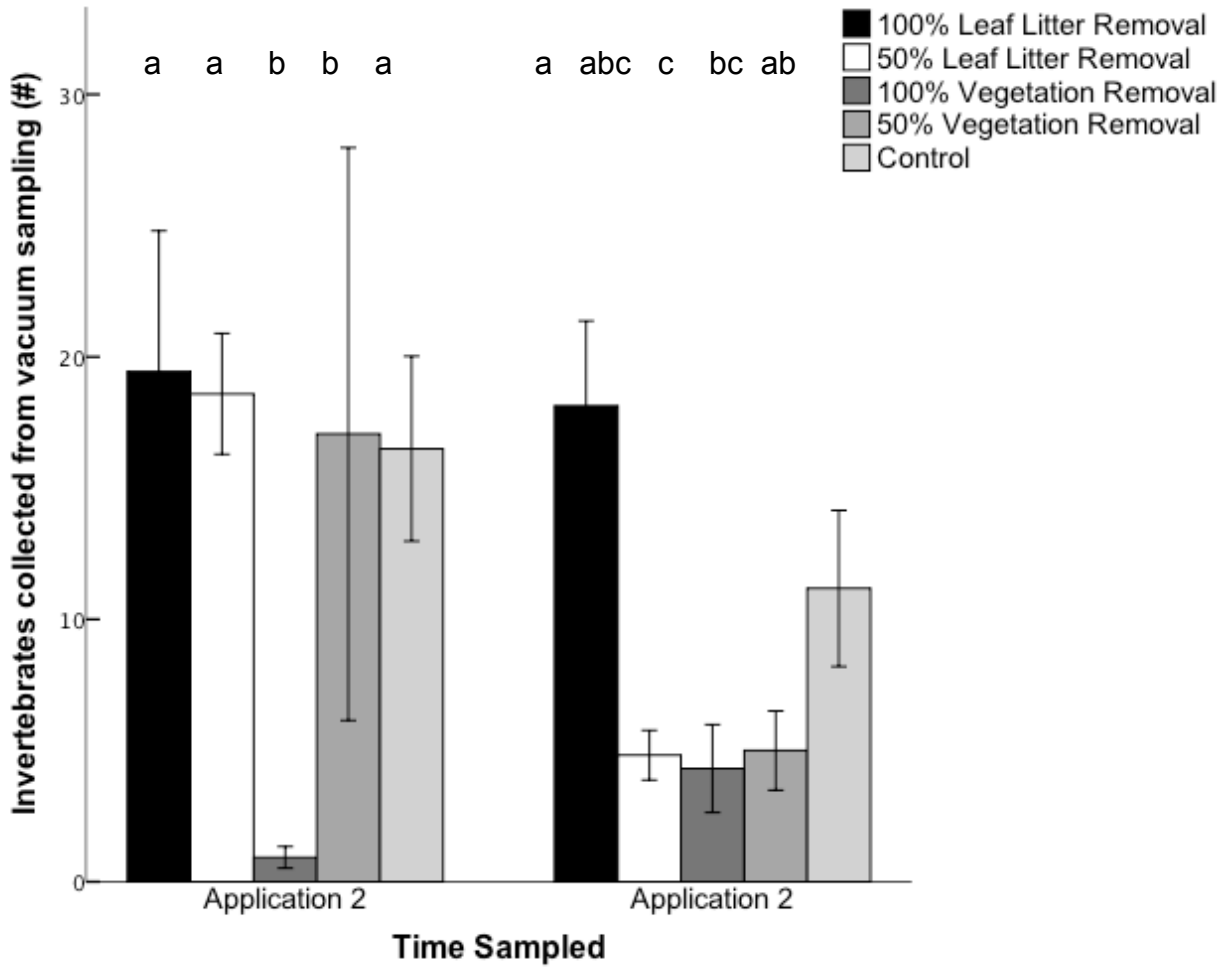
a a b b a
a abc c bc ab
100% Leaf Litter Removal
50% Leaf Litter Removal
100% Vegetation Removal
50% Vegetation Removal
Control
Invertebrates collected from vacuum sampling (#)
30
20
10
0
Application 2
Application 2
Time Sampled

Fig. 7

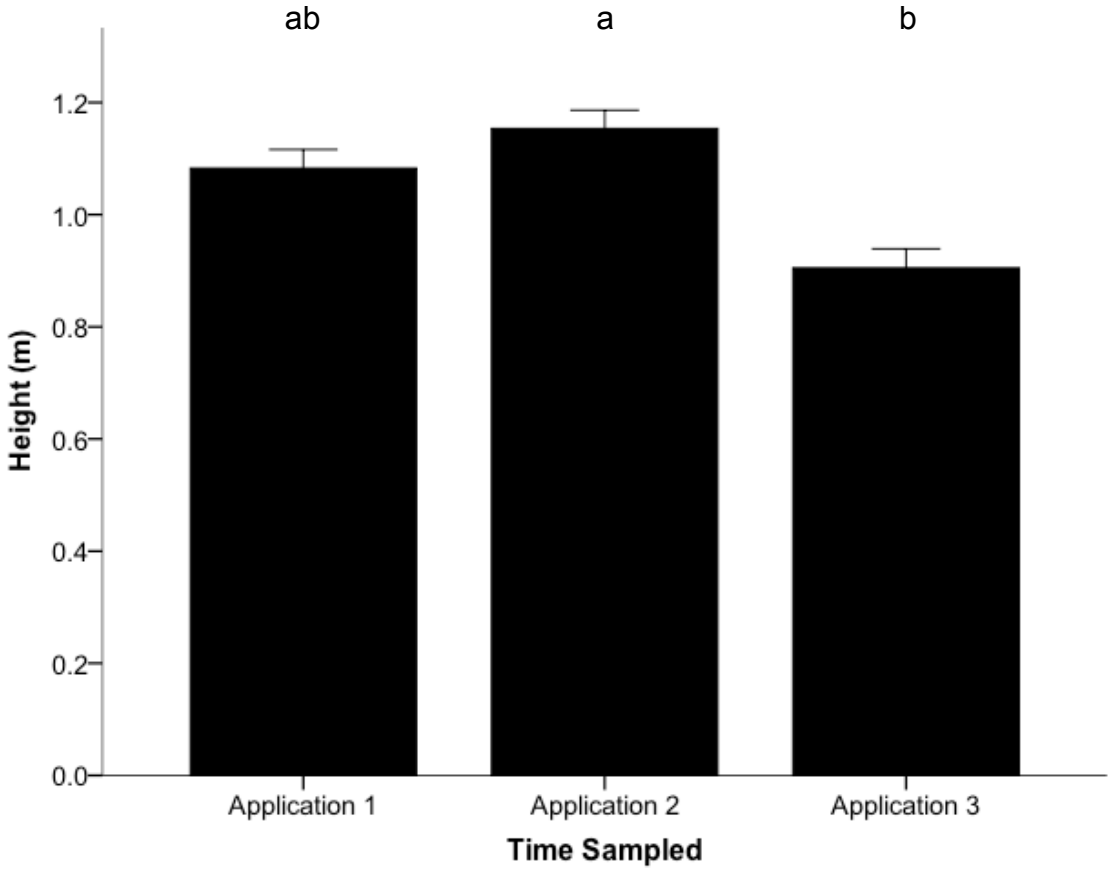
ab
a
b
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Height (m)
Application 1
Application 2
Application 3
Time Sampled

Fig. 8

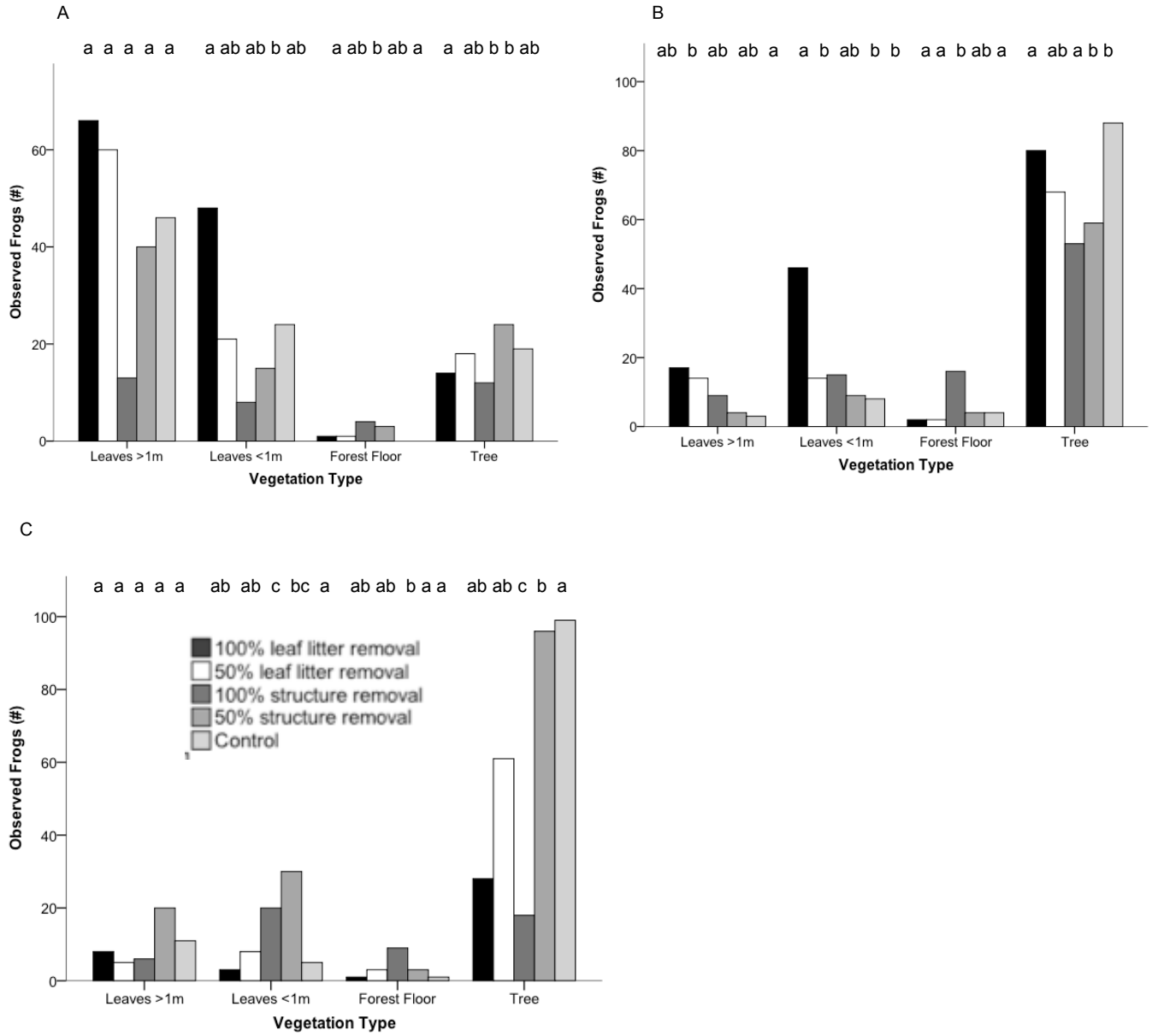
A
a a a a a a ab ab b ab a ab b ab a a ab b b ab
Observed Frogs (#)
0
20
40
60
Leaves >1m
Leaves <1m
Forest Floor
Tree
Vegetation Type
B
ab b ab ab a a b ab b b a a b ab a a ab a b b
Observed Frogs (#)
0
20
40
60
80
100
Leaves >1m
Leaves <1m
Forest Floor
Tree
Vegetation Type
C
a a a a a ab ab c bc a ab ab b a a ab ab c b a
100% leaf litter removal
50% leaf litter removal
100% structure removal
50% structure removal
Control
Observed Frogs (#)
0
20
40
60
80
100
Leaves >1m
Leaves <1m
Forest Floor
Tree
Vegetation Type

Fig. 9

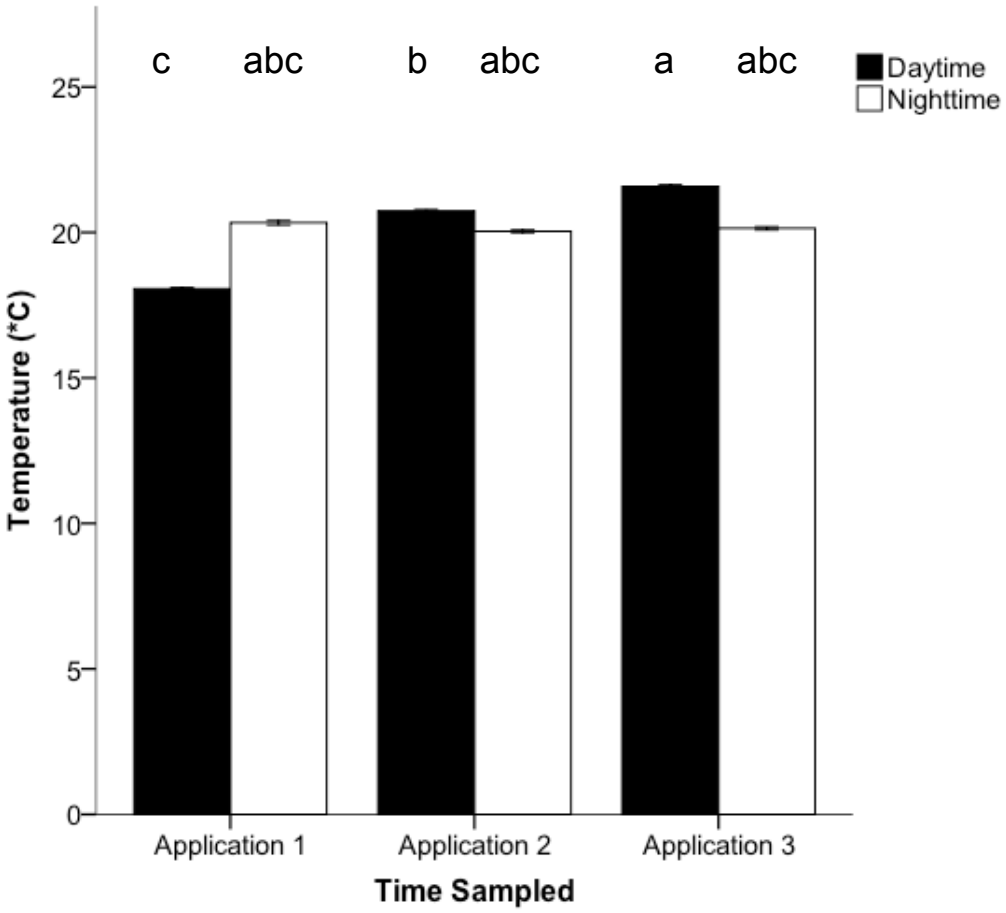
c
abc
b
abc
a
abc
Daytime
Nighttime
25
20
15
10
5
0
Temperature (*C)
Application 1
Application 2
Application 3
Time Sampled

Fig. 10

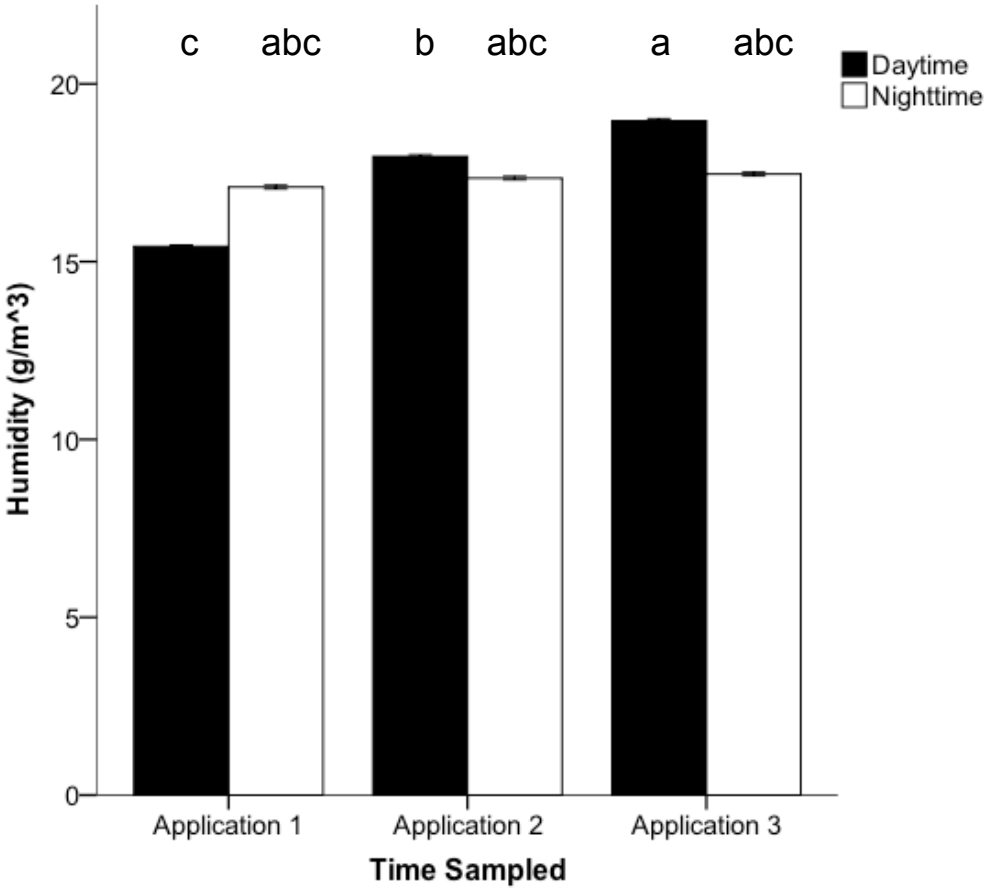
c
abc
b
abc
a
abc
Daytime
Nighttime
20
15
10
5
0
Humidity (g/m^3)
Application 1
Application 2
Application 3
Time Sampled

Fig. 11