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Coqui Frog Invasions Change Invertebrate Communities in Hawaii

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Abstract The Puerto Rican coqui frog (*Eleutherodactylus coqui*) invaded Hawaii in the late 1980s. Because the coqui reaches high densities and consumes large quantities of invertebrates, it was hypothesized to change invertebrate communities where it invades. Previous research found that coquis can change invertebrate communities, but these studies used highly manipulative, small-scale experiments. The objective of this research was to determine whether coquis create community-level changes in invertebrate communities at the landscape scale. We collected leaf litter, flying, and foliage invertebrates on both sides of 15 coqui invasion fronts across the island of Hawaii. Multivariate analyses show that coquis are associated with changes in leaf-litter communities, primarily reductions in Acari, but are not associated with overall changes in flying or foliage communities. Across sites, coquis reduced the total number of leaf-litter invertebrates by 27%, specifically by reducing Acari by 36%. Across sites, coquis increased flying Diptera by 19%. Changes were greater where coqui densities were higher. We suggest that coquis changed leaf-litter communities primarily through direct predation, but that they increased Diptera through the addition of frog carcasses and excrement. Results support previous studies conducted in more controlled settings, but add to our understanding of the invasion by showing that coqui effects on invertebrate communities are measurable at the landscape scale.

Keywords *amphibian, anuran, community impacts,* Eleutherodactylus coqui*, invasive species, non-native species*

Introduction

When species establish outside their native range, they often have complex interactions with and change the native community. Introduced species can change native communities by extirpating (Clavero and Garcia-Berthou 2005; Fritts and Rodda 1998), reducing (Lodge 1993; Porter and Savignano 1990; Sanders et al. 2003), and even increasing native species (Barber et al. 2008; Cohen and Carlton 1998; Roemer et al. 2002). However, community-level changes induced by some non-natives can be difficult to observe, especially if the changes occur to communities that have high spatial and temporal variability, such as invertebrate communities.

Compared to other non-native taxa, the impacts of non-native amphibians, especially on invertebrate communities, are only moderately known. Of 183 known globally introduced amphibians, studies on ecological impacts have been conducted only on a handful of species, primarily cane toads (*Chaunus marinus*), American bullfrogs (*Lithobates catesbeianus*), and African clawed frogs (*Xenopus laevis*) (Kraus 2009). These studies show that non-native amphibians reduce prey (Greenlees et al. 2006; Lafferty and Page 1997), reduce predators (Catling et al. 1999; Doody et al. 2006; Phillips et al. 2003), and cause cascading effects on other species (Kiesecker and Blaustein 1998). Thus, studies conducted on the effects of invasive amphibians indicate they have community-level impacts.

The Puerto Rican coqui frog (*Eleutherodactylus coqui*) has rapidly colonized and established in Hawaii (Kraus and Campbell 2002). It is especially widespread on the island of Hawaii while it has been greatly controlled on the other islands (Beard et al. 2009). Since its introduction, researchers have proposed that coquis may impact invertebrate communities (Beard and Pitt 2005; Kraus et al. 1999), largely because of

52 their high densities, up to 91,000 frogs/ha (Beard et al. 2008; Woolbright et al. 2006), and
53 ability to consume up to 690,000 prey items/ha/night (Beard et al. 2008). This potential
54 impact is of concern because Hawaiian invertebrates constitute the largest component of
55 Hawaii's biological diversity, with the majority of species occurring as single-island
56 endemics (Howarth and Mull 1992).

57 Previous studies using enclosures found that coquis have the ability to change
58 invertebrate communities in controlled, manipulated settings (Beard et al. 2003; Sin et al.
59 2008; Tuttle et al. 2009). However, the implications of these studies at the landscape
60 level are uncertain. Previous studies suggest that coquis forage primarily on leaf-litter
61 invertebrates, but also on foliage invertebrates, and that leaf-litter invertebrates are the
62 most likely to be reduced (Beard 2007; Sin et al. 2008). Previous work also suggests that
63 coquis may increase some flying invertebrates (Tuttle et al. 2009). Thus, various
64 components of the invertebrate community may be directly or indirectly impacted, and
65 the impacts may be greater where coqui density is higher. Lastly, high abundances of
66 prey may bolster and support higher coqui densities, which may facilitate further invasion
67 (Beard et al. 2008).

68 The objective of this study was to determine whether coqui invasions create
69 community-level changes in invertebrate communities at the landscape scale. We
70 compared invertebrate communities in adjacent invaded and non-invaded plots because
71 this method allowed us to control for environmental variability between plots and to
72 observe the impacts of the invasion in a variety of habitats. We also assessed site-
73 specific diet composition to determine how well invertebrate sampling captured available
74 prey and to better understand the direct and indirect effects of the coquis on invertebrates.

Finally, we investigated how coqui density influences and is affected by invertebrate communities.

Methods

Study sites

Research was conducted at 15 sites along coqui invasion fronts on the island of Hawaii, USA, from May to August 2009 (Fig. 1). Sites were selected to capture a diversity of elevation (range 35 to 912 m), climate, geological history, and vegetation. Mean annual temperatures across study sites ranged from 18 to 23˚C (Nullet and Sanderson 1993), and mean precipitation ranged from 1000 to 6000 mm/year (Price 1983). Volcanic parent material across all sites ranged from 155 to >10,000 years in age (Trusdell et al. 2005). Dominant overstory differed among sites and included *Aleurites moluccana* (site: MK, site abbreviations are in Fig. 1), *Bambusa vulgaris* (GL and MV), *Eucalyptus* sp. (CA, HK, HM, KP and WP), *Macadamia integrifolia* (CC and KE), *Metrosideros polymorpha* (ER, FF, KO, MKB, PP and SA), *Musa* sp. (HL), *Psidium cattleianum* (KU), *Schinus terebinthifolius* (PB), and *Spathodea campanulata* (WM). Dominant understory also differed among sites: *Coffea arabica* (CC, HL and KE), *Dicranopteris linearis* (ER, FF and SA), *Freycinetia arborea* (KO), *Hedychium* sp. (GL, MV and WM), *Melastoma malabathricum* (PP), *Psidium cattleianum* (CA, HM and KU), *Psydrax odorata* (MK), *Psychotria mariniana* (KP), *Schinus terebinthifolius* (MKB and PB), and *Urochloa maxima* (HK and WP).

Site selection

Presence and absence of coquis on each side of the front were determined by listening for 20 min between 1900 and 0200 h, peak hours of calling (Woolbright 1985), for the loud (70 dB at 0.5 m) two-note mating call on three separate nights over a one-week period (Beard and Pitt 2005). Designations were also confirmed during subsequent sampling. No frogs other than coqui were ever seen or heard at any of the sites. A mean distance of 380 m separated plots on either side of the invasion front (57-1000 m). The coqui is known to be very territorial and individuals remain within 20 m x 20 m areas for many years (Woolbright 1985; Woolbright 2005); however, they do move each night and can home up to 100 m (Gonser and Woolbright 1995). Therefore, all data for plots on either side of a front were collected within a 48 hr period to minimize the likelihood of dispersal between plots during our period of observation.

To address our objective, plots on either side of a front needed to differ only by the presence of coqui. To determine whether there were other environmental characteristics that differed between plots on either side of a front, we collected and compared environmental variables (canopy cover, ground cover, stem density, understory density, and dominant canopy and understory composition). All measurements, including invertebrate sampling and frog surveys described below, were taken within 30 m x 30 m plots located on each side of each front.

Of the original 20 sites selected for this study, 15 did not differ ($P > 0.05$) for any of the environmental variables measured and were considered in the rest of the study. Five sites that had significant differences between plots on either side of the front for one or more environmental measurement were removed from further analyses.

Invertebrate sampling

Invertebrates were sampled after 2200 h using three collection methods. All invertebrate samples were collected at the center of the plot and 10.6 m from the center at 45 degrees from the cardinal axes, for a total of five samples for each collection method per plot. Leaf litter was collected from 0.25 m x 0.25 m areas, dried in Berlese-Tullgren funnels, and invertebrates were extracted and stored in 70% ethanol (leaf-litter samples). Flying invertebrates were collected using yellow 10 cm x 18 cm sticky traps (Chevron Ortho, Marysville, OH, USA) hung vertically from the dominant vegetation with a 10 cm side 1 m from the ground and left out for 48 h (sticky trap samples). Finally, invertebrates were collected from trunks and leaves of the dominant vegetation using a modified hand-held vacuum (Black & Decker, Towson, MD, USA) for 30 sec at each point. Vacuumed invertebrates were collected and immediately stored in 70% ethanol (vacuum samples). All invertebrates were later counted and identified to lowest recognizable taxonomic unit (RTU), mostly to scientific order, but in some cases family, using a dissecting microscope.

Coqui survey and sampling

Because we hypothesized that changes in invertebrate communities may be greater where coqui density was higher, we estimated coqui density in each invaded plot using distance-sampling surveys (Buckland et al. 2001; Fogarty and Vilella 2001). Surveys were conducted only on nights for which the relative humidity was greater than 80% to ensure favorable conditions frog activity. Beginning at 1930 h, two researchers surveyed with headlamps one of six adjoining 5-m wide, 30-m long parallel transects, slowly walking and searching for frogs for 45 min. When a frog was either seen or heard, the distance from the observer and height from the forest floor were recorded. At the end of each

146 transect, researchers moved to the next adjoining transect, until the entire plot and total of
147 six transects were surveyed for coquis, for a total time of 270 min per plot.
148 The night following distance sampling, starting at 2000 h, we collected coquis for
149 stomach-content analysis. Two researchers searched for frogs via headlamp each of six
150 (5 m x 30 m) transects for 30 min across the entire plot. Observed frogs were hand-
151 captured and euthanized. In the laboratory, individuals were dissected, and pierced
152 stomachs were stored in vials of 70% ethanol until analysis. Snout-vent length (SVL) for
153 each individual was measured to the nearest 0.1 mm using dial calipers and placed into a
154 stage class (adult or preadult) based on visual inspection of gonads. Later, stomach
155 contents were counted and identified to lowest RTU using a dissecting microscope.
156
157 **Statistical analysis**
158 To determine the effect of frog treatment (coqui vs. non-coqui) and site (15 sites) on
159 invertebrate community composition as a whole, we used permutational multivariate
160 analysis of variance (perMANOVA) (Anderson 2001) with the Adonis function in the
161 Vegan package in R 2.0.1 (Oksanen et al. 2008). Adonis builds a dissimilarity matrix
162 describing the multivariate community and tests for treatment effects by identifying
163 spatial community centroids and calculating the squared distance of dissimilarity. Adonis
164 generates non-parametric ANOVA results by building the null distribution of the test
165 statistic calculated through 1000 data permutations (Oksanen et al. 2008). We evaluated
166 invertebrate community composition using matrices of taxon-abundance data, and
167 constructed distance matrices using a Bray-Curtis index. We analyzed each of the
168 collection methods (leaf litter, sticky traps, vacuum sampling) separately. When results
169 yielded significant frog treatment (coqui vs. non-coqui) and site (15 sites) differences for

the perMANOVA, we conducted principal components analyses (PCA) using the pca function with a covariance matrix in the labdsv library in R 2.0.1 to visualize the results.

To determine the effect of frog treatment (coqui vs. non-coqui) and site (15 sites) on: 1) the abundance of total invertebrates; and 2) the abundance of each taxon comprising more than 5% of each environmental collection, we conducted a two-way factorial ANOVA. We analyzed each of the three invertebrate collection methods separately. For all ANOVAs, we treated treatment and site as fixed factors. Data were modeled using a negative binomial distribution to handle the count data (O'Hara and Kotze 2010).

To estimate coqui densities at each site, we used Program DISTANCE (Thomas et al. 2006), which fits distance sampling data to specific detection functions and evaluates the models using Akaike's Information Criterion (AIC). We fit the data to key detection functions (uniform, half-normal, or hazard-rate) and series expansions (cosine, simple polynomial, or hermite polynomial). Program DISTANCE was unable to estimate site-specific densities for two sites (KP and KU) because of low detectability for frogs. For these sites, we estimated density using the linear relationship between observed frogs and frog densities for the other sites ($R^2 = 0.9602$).

We could not use density as a covariate in the ANOVAs because density and site were confounded factors. Instead, we used site-specific coqui densities to determine the relationship between coquis and invertebrate abundances. More specifically, to investigate the relationship between coqui abundance and differences in invertebrates across invasion fronts, we assumed that the non-coqui sites represented invertebrate communities pre-invasion and analyzed relationships between site-specific coqui densities and the difference in invertebrate abundance between paired coqui and non-

194 coqui sites. We also analyzed the relationship between site-specific coqui densities and
195 total invertebrate abundance in paired non-coqui sites to investigate the relationship
196 between prey availability and coqui abundance. Correlations were analyzed for
197 invertebrate taxa comprising > 5% of environmental collections or coqui diet. Out of all
198 15 sites, one site (CC) had a much higher coqui density than the other sites. We
199 conducted analyses with and without this site, but findings were never different, and
200 therefore all sites were included in the analysis.

201 We also used two-way ANOVAs to investigate differences in dietary taxa
202 between stage classes, for which we treated stage class (adult and preadult) and site as
203 fixed factors, and treated individuals within site as sub-samples. Taxa that comprised >
204 5% of coqui diet were analyzed from eight sites where there were sufficient numbers for
205 comparison of both adults and preadults (i.e. • 5 individuals). Data were modeled using a
206 negative-binomial distribution (O'Hara and Kotze 2010).

207 For this study, we were mostly interested in treatment effects (effects of the
208 coqui) including whether they were consistent or differed across sites. Thus, while we
209 could have analyzed the data using site as a random effect instead of a fixed effect, we
210 chose to use site as a fixed effect because it gave us more insight into coqui impacts
211 (treatment effects) that varied across sites. From a management perspective, the analysis
212 allowed us to highlight taxa that might not always be impacted by the invasion but may
213 be impacted in some areas.

214 In the multivariate and ANOVA analyses, site was significant in 96% of the
215 analyses. This suggests that sites had different invertebrate communities, which was
216 expected because sites were chosen to capture a high degree of landscape variability.
217 Because site was almost always significant, significant site effects are not discussed

unless we observed an interaction with coqui treatment effects. We conducted ANOVAs using PROC GLIMMIX and Spearman correlations using PROC CORR in SAS v. 9.1.3 for Windows (SAS Institute 2006). We considered tests significant when $P < 0.05$ and presented values where appropriate.

Results

Invertebrate communities

We collected and identified a total of 21,382 invertebrates from the 15 coqui sites and 28,184 invertebrates from the 15 non-coqui sites. Of the collected invertebrates, leaf-litter samples made up 90.4%, sticky-trap samples made up 7.4%, and vacuum samples made up 2.2% of the total (Fig. 2). Samples across all collection types and treatments consisted primarily of Acari (50.6%), Collembola (21.1%), Hymenoptera (7.6%), and Isopoda (5.7%).

Multivariate analyses on leaf-litter invertebrate communities showed that coqui changed the composition of leaf-litter invertebrates; however, these changes varied by site (Table 1). PCAs on leaf-litter invertebrate communities showed that sites with frogs had fewer Acari, but effects on Collembola and Hymenoptera seemed to vary by site (Fig. 3). Overall, coqui sites had 26.9% fewer total leaf-litter invertebrates (treatment, $F_{1,120} = 10.89$, $P = 0.0013$). Taxon-specific ANOVAs supported the PCA ouput and showed that coqui sites also had 36.0% fewer leaf-litter Acari, but changes in leaf-litter Collembola, Hymenoptera, and Isopoda varied by site (Fig. 4a).

Multivariate analyses on flying invertebrate communities showed that coqui impacts on flying-invertebrate composition varied across sites (Table 1). ANOVAs supported this result and showed that coquis affected total flying-invertebrate abundance

at some sites (Fig 2). But taxon-specific ANOVAs showed that coquis increased flying Diptera abundance by 19.0% across sites, while effects on flying Hemiptera and Hymenoptera varied by site (Fig. 4b). Coquis had no effect on Collembola collected on sticky traps.

Multivariate analyses on foliage-invertebrate community composition also showed impacts varied across sites (Table 1). ANOVAs supported this showing that coquis affected total foliage-invertebrate abundance at some sites (Fig. 2), while taxon-specific ANOVAs showed that coquis affected foliage Hymenoptera at some sites, but did not change foliage Acari, Araneae, Collembola, or Diptera (Fig. 4c).

Coqui density and correlations

A total of 988 frogs was observed during distance sampling on all invaded plots (range 25 to 239). Site-specific population densities ranged from 347 to 6,983 frogs/ha.

When we compared coqui density to changes in invertebrate abundance (differences between non-coqui and coqui sites), coqui density was positively related to reductions in leaf litter and foliage Acari ($R^2 = 0.5250$, $P = 0.0445$; $R^2 = 0.5474$, $P = 0.0347$). In contrast, coqui density (from coqui sites) was positively correlated with some invertebrate groups from the non-coqui sites, specifically leaf-litter Araneae ($R^2 = 0.5738$, $P = 0.0253$) as well as flying and foliage Coleoptera abundance ($R^2 = 0.6289$, $P = 0.0120$; $R^2 = 0.5670$, $P = 0.0275$, respectively).

Coqui diet selection

We identified a total of 6,701 prey items from 874 coqui stomachs (range 30 to 122 stomachs per site). Across sites, dominant prey included Hymenoptera (32.80%),

Coleoptera (12.10%), Amphipoda (8.73%), Collembola (7.89%), Acari (7.22%), and Isopoda (5.75%). At eight sites with both preadult and adult frogs, 233 preadult frogs consumed 402.2% more Acari and 213.1% more Collembola than 392 adult frogs, while effects of stage class on Amphipoda, Coleoptera, and Isopoda varied by site (Fig. 5).

Discussion

Across 15 sites on the island of Hawaii, we found that coqui frogs were associated with a reduction in the total number of leaf-litter invertebrates, primarily Acari. While coqui sites had no overall consistent change in foliage-invertebrate communities, Diptera abundances increased in flying communities across sites. Although enclosure experiments previously conducted in Hawaii suggested that coquis may reduce leaf-litter invertebrates and increase flying invertebrates (Sin et al. 2008; Tuttle et al. 2009), these patterns have not previously been measured at the landscape scale. Similar to other invasive amphibians, coquis have the potential to induce measurable changes in invertebrate communities at the landscape scale (Catling et al. 1999).

We expected to see the greatest change in the leaf-litter invertebrate community because coquis primarily consume leaf-litter invertebrates in Hawaii (Beard 2007). The observed reduction of total invertebrates in this community (27%) was largely driven by the reduction in highly abundant Acari by 36%. These results were similar to previously conducted enclosure studies that showed that coqui reduce total leaf-litter invertebrates by 14% (Sin et al. 2008) and microbivore (primarily Acari and Collembola) abundance by 40% (Tuttle et al. 2009).

Based on dietary studies alone, we might not expect the flying-invertebrate community to be greatly impacted by the coqui invasion (Beard 2007). However, we

290 found a 19% increase in Diptera with coquis, which is similar to an enclosure study that
291 found coquis increased Diptera by 27% (Tuttle et al. 2009). Tuttle et al. (2009) suggested
292 that Diptera may increase with coqui because Diptera larvae may feed on readily
293 available frog carcasses and excrement. When we investigated specific Diptera families
294 in our samples, we found that Sciaridae was responsible for the greatest increase in
295 Diptera abundance, and this family is known to feed on carcasses (Perotti et al. 2010).
296 Thus, we propose that coquis increase Diptera by increasing nutrient availability in the
297 system though excrement and carcasses (Beard et al. 2002).

298 We expected coquis to have the potential to change foliage invertebrates (Beard
299 2007); however, we found no consistent directional change in the foliage-invertebrate
300 community composition with coquis. In Puerto Rico, foliage invertebrates are the
301 dominant prey consumed by coquis (Stewart and Woolbright 1996), whereas in Hawaii
302 they have not been found to be as important as leaf-litter invertebrates (Beard 2007). The
303 lower abundance of foliage invertebrates compared to leaf-litter invertebrates (at least
304 based on our sampling), as well as fewer coqui predators in the leaf litter compared to the
305 native range (Beard and Pitt 2005), might explain why the frogs consume more
306 invertebrates in the leaf litter as opposed to the foliage in Hawaii. In addition, it is also
307 possible that sites with younger a'a and pahoehoe lava substrates may provide an
308 abundance of subterranean diurnal retreat sites and breeding spots, increasing the amount
309 of time frogs spend on the ground foraging in the leaf litter.

310 Another potential explanation might be attributed to collection biases resulting
311 from our invertebrate sampling techniques. Our methods were chosen to sample
312 invertebrates available to foraging coqui and other taxa that may experience indirect
313 change due to the coqui. However, some invertebrate taxa that were prominent in coqui

diets were underrepresented in environmental collections. For example, some large-bodied and highly mobile cockroaches (Blattodea) and grasshoppers (Orthoptera) were frequently found in adult stomachs (62 and 48 items, respectively), but were not common in environmental samples. Because these taxa should have been collected on the foliage, this suggests that portions of foliage-invertebrate communities might have been impacted by coquis but were not measured in this study.

We found positive correlations between coqui density and reductions of leaf-litter Acari. This reduction is likely attributed to direct predation, especially because Acari are prominent prey items in preadult diets. Preadult to adult ratios in Hawaii are estimated at 2.5:1 (±1.7 SD) (Beard et al. 2008), and it has been shown that preadults consume more, smaller prey items than adults (primarily Acari, Collembola) (Beard 2007). Because prey size is positively correlated with body size (Woolbright and Stewart 1987) and smaller prey items compose a large percentage of preadult diets, preadult frogs are likely affecting smaller-bodied taxa like Acari and Collembola, while adults are more likely affecting larger invertebrates like Amphipoda and Coleoptera. In addition, we found positive correlations between coqui density and the relative abundance of prominent, large-bodied prey taxa in the environment (Araneae, Coleoptera), which suggests that greater large-bodied prey abundance may support higher densities of coquis (Beard et al. 2008).

Findings from our dietary analysis concur with the same six prominent taxonomic orders found in a previous dietary study conducted in Hawaii (Beard 2007): Acari, Amphipoda, Isopoda, Coleoptera, Collembola, and Araneae. These taxa were not only common in the coqui diet but were also common in our invertebrate-community samples, which suggests that coqui prey selection reflects prey availability. Although dietary

studies are temporal snapshots of consumed prey items, this dietary analysis was conducted across a number of different environments and habitats, which further supports that these taxa are both the dominant prey and abundant invertebrates in Hawaii.

It is reasonable to assume that different habitats result in different invertebrate communities and prey availability to coquis. This discussion focused on the effects that were consistent across sites, but several taxa had significant treatment and site interactions. For example, Collembola, Hymenoptera, and Isopoda in the leaf litter, and Hymenoptera in the foliage, were primarily reduced by coqui at sites. However, these results were inconsistent, and at some sites these taxa increased with coqui. In contrast, flying Hemiptera and Hymenoptera, similar to the response in Diptera, increased with coqui at most sites, but at some sites these taxa showed the opposite pattern. Unfortunately, we could not identify any environmental or biological factor that might have driven these site-level responses. But, the result is important because it suggests that some coqui effects are not uniform but vary among sites. Furthermore, we can use these results to identify orders (such as Collembola) containing endemic species that may be impacted by coquis at some sites. When considering the community-level impacts of coqui, it is important to also consider the effects on these other taxa that had site-specific responses.

One concern with these types of observational studies is that measured effects may not be a result of the invasive species but some other environmental factor that is associated with the invasive species. To account for this possibility, we used strict requirements regarding what sites could be included in our study, and the invaded and un-invaded sites could not differ in any environment parameter that we measured. This prevented us from including all of our original sites in the analysis. Although we cannot

362 rule out the possibility that there were other factors contributing to the results, the fact
363 that these results support both other dietary and enclosure studies suggests the changes
364 that we observed can be attributed to the coqui.

365 Because we measured impacts of the coqui at sites along the invasion front, these
366 results may represent short-term or only partial impacts of the invasion due to the recent
367 nature of the invasion and potential lag-time responses by the invertebrate community
368 (Krushelnycky and Gillespie 2010). Alternatively, densities of invaders could be higher
369 as they invade new areas before they deplete resources, and short-term effects might be
370 greater than long-term effects (Morrison 2002). Over time, a predator-prey dynamic
371 equilibrium may be reached, possibly different than what is found at the invasion front
372 (Buckley et al. 2005). In addition, because sites were not sampled over time, there could
373 be unobserved seasonal differences in invertebrate communities and coqui activity.

374 In conclusion, our findings show that coqui frogs change invertebrate
375 communities at 15 sites in Hawaii, and especially reduce highly abundant prey taxa, such
376 as Acari. We observed some consistent changes in invertebrate communities, such as
377 reductions in Acari and increases in Diptera, that will likely occur in most invaded sites.
378 We found some other results that were only present at some sites, such as changes in
379 Collembola, Hymenoptera, and Isopoda. These results are more daunting because it is
380 difficult to know at what sites these changes might be present. We suggest that future
381 research monitor the temporal response by invertebrate communities to the coqui
382 invasion at these sites over time. Because coquis have measurable effects on invertebrate
383 communities, this should be taken into consideration as control measures are evaluated.
384

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502

503

504 **Table 1** Results from perMANOVA multivariate analysis of variance comparing
505 the effects of treatment (coqui vs. no coqui) and site on invertebrate community
506 abundance analyzed by taxa. Asterisk (*) indicates test significance ($P < 0.05$)

		Taxa	
Model	DF	R^2	Pr(>F)
Leaf litter treatment	1	0.0114	0.017*
Leaf litter site	14	0.4017	<0.001*
Leaf litter treatment*site	14	0.0897	0.002*
Flying treatment	1	0.0069	0.100
Flying site	14	0.4037	<0.001*
Flying treatment*site	14	0.0989	<0.001*
Foliage treatment	1	0.0089	0.117
Foliage site	14	0.2412	<0.001*
Foliage treatment*site	14	0.1317	<0.001*

507

Figure Legends

Fig. 1 Fifteen coqui sampling sites in the present study on the island of Hawaii. Site abbreviations are Captain Cook (CC); Eden Roc (ER); Fern Forest (FF); Glenwood (GL); Honokaa (HK); Holualoa (HL); Hamakua (HM); Kaloko (KO); Kalopa (KP); Kulani (KU); Manuka (MK); Manuka B (MKB); Paradise Park (PP); Saddle Road (SA); and Waikaumalo (WK). Gray lines indicate state routes

Fig. 2 Mean numbers of invertebrates (± SE) in three invertebrate communities with and without coqui frogs (n = 15). Significant treatment results are marked with (*), and significant treatment-site interactions are marked with (†) ($P < 0.05$)

Fig. 3 Principal component analysis of leaf-litter invertebrate taxa at sites with and without coquis (n = 15 sites). Total variance explained by each axis and two most important contributing taxa in parentheses

Fig. 4 Mean numbers of invertebrate (± SE) taxa comprising more than 5% of each collection method at sites with and without coqui frogs by: (**a**) leaf-litter, (**b**) flying-, and (**c**) foliage-invertebrate communities (n = 15). Significant treatment results are marked with (*), and significant treatment-site interactions are marked with (†) ($P < 0.05$)

Fig. 5 Mean numbers of prey consumed (± 1 SE) by adult and preadult frogs (n = 8 sites). Significant treatment results are marked with (*), and significant treatment-site interactions are marked with (†) ($P < 0.05$)