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Temporal Foraging Patterns of Nonnative Coqui Frogs (*Eleutherodactylus coqui*) in Hawaii

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ABSTRACT.—**The Puerto Rican Coqui Frog (*Eleutherodactylus coqui*) is a nocturnal, invasive species that was introduced into Hawaii in the 1980s. Because they reach extremely high densities (up to 90,000 frogs/ha), they have the potential to affect invertebrate prey communities. Previously, researchers used frogs collected only at night to characterize their prey. Because Coquis use retreat sites near the forest floor during the day and understory perch sites at night, frogs collected at night might show different amounts and types of prey than would frogs collected in the morning. We analyzed stomach contents of 435 frogs collected in the morning (0300–0600 h) and at night (1900–2200 h) from five sites on the island of Hawaii. Frogs collected in the morning had 1.7 times more prey items and 2.1 times greater prey volume than those collected at night; however, prey composition did not differ between morning- and evening-collected frogs. Across sites, Formicidae (ants) and Amphipoda (amphipods) were the dominant prey, and at least 61.6% of their prey items were nonnative species. Across sites, morning- and evening-collected stomach contents were not different from environmental samples of leaf-litter invertebrates but were different from environmental samples of foliage and flying invertebrates, suggesting that Coquis forage primarily in the leaf litter throughout the night. Previous research that investigated stomach contents of frogs collected only at night greatly underestimated the number and volume of prey items that Coquis consume during the entire foraging period but accurately described their primary prey: nonnative, leaf-litter invertebrates.**

The Puerto Rican Coqui Frog (*Eleutherodactylus coqui*) is an invasive species that was introduced into Hawaii in the 1980s (Kraus et al., 1999). Because it attains extremely high densities, up to 90,000 frogs/ha (Woolbright et al., 2006; Beard et al., 2008), and is an insectivore, ecologists are concerned about how it may alter prey communities (Kraus et al., 1999; Beard and Pitt, 2005), especially considering that the vast majority of endemics in Hawaii are invertebrates (Eldredge and Evenhuis, 2002). Coquis are sit-and-wait predators that use retreat sites during the day, often near the forest floor, and then at night move onto understory perch sites averaging 1 m off the forest floor (Woolbright and Stewart, 1987; Beard, 2007). Using frogs collected in the morning hours in its native range of Puerto Rico, Stewart and Woolbright (1996) determined that Coqui diets consist primarily of foliage invertebrates with some leaf-litter invertebrates. They expected this pattern because the large majority of Coquis use leaf perches at night (Townsend, 1985).

Beard (2007) conducted stomach content analyses in Hawaii and suggested a shift in the primary microhabitat that Coquis use to forage; more specifically, that their prey comes primarily from the leaf litter as opposed to the foliage. One limitation of Beard's (2007) and all previous stomach content analyses conducted in Hawaii on Coqui diets is that they were conducted using frogs collected at night between 1900 h to 2400 h (Tuttle et al., 2009; Choi and Beard, 2012). Frogs collected at night, a few hours after they have emerged from their diurnal retreat sites, might reflect a different prey base than frogs captured after a whole night of foraging (Beard, 2007; Ferreria et al., 2015). Therefore, Beard (2007) hypothesized that stomach content analyses conducted so far in Hawaii may not illustrate the diversity and significance of Coqui diets.

The goal of this research was to develop a better understanding of the feeding habits of Coquis on the island of Hawaii, the only island in Hawaii where Coquis are widespread (Beard and Pitt, 2012). More specifically, we addressed the following three questions: 1) Do Coquis consume a different amount and volume of prey between morning and night? 2) Does diet composition differ between morning and night? 3) If there are temporal changes in diet, what are the conservation implications of these changes?

MATERIALS AND METHODS

We collected frogs at five sites (Akaka Falls State Park, Humane Society, Kaumana Caves State Park, Lava Tree State Park, and Manuka State Park) on the island of Hawaii, USA, from June to July 2014 (Fig. 1). We chose these sites because they have high densities of Coquis (Beard et al., 2008). We collected adult frogs (>25 mm snout–vent length [SVL]) when they first emerge from their diurnal retreat sites (between 1900 and 2200 h) and in the early morning hours (0300–0600 h) when they have finished foraging for the night and are about to return to their diurnal retreat sites. We collected all frogs for one collection period (evening or morning) during one site visit. Because stomach passage time is about 12 h (Woolbright and Stewart, 1987), we expected the evening-collected frogs to reflect foraging during the daytime and up to 3 h after dusk and the morning-collected frogs to reflect foraging during the night and up to 3 h before dusk.

At each site, two researchers searched for frogs within a 50 × 50-m area. We kept hand-captured frogs in individual bags until we euthanized them with a Benzocaine bath at the end of the survey period and placed them in a −20°C freezer. In the laboratory, we measured SVL of each frog with dial calipers to the nearest 0.1 mm. We dissected frogs and assigned them to a sex class based on examination of gonads (male or female). We removed, punctured, and stored stomachs in 70% ethanol until further analysis.

In the laboratory, we identified stomach contents to the lowest identifiable taxonomic unit, typically scientific Order, but in some cases Family (Table 1). For each item, we measured maximum length and width to 0.01 mm using a 10-mm reticle (Magnusson et al., 2003). We calculated volume for each prey item using the formula: $v = 4/3\,\pi \times l/2 \times (w/2)^2$, where l = prey length and w = prey width (Beard, 2007; Vitt et al., 2008). We

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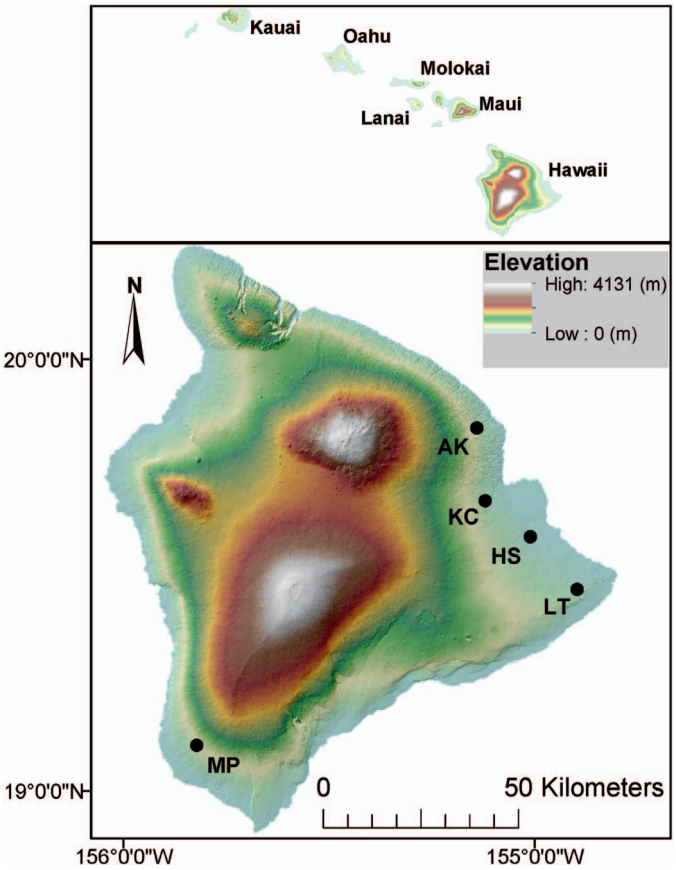

FIG. 1. Location of the five study sites in Hawaii: Akaka Falls State Park (AK), Humane Society (HS), Kaumana Caves State Park (KC), Lava Tree State Park (LT), and Manuka State Park (MP).

determined prey importance (I) for each prey category by calculating: $I = (F\% + N\% + V\%) / 3$, where $F\%$ = percentage of frogs in which the prey item occurred, $N\%$ = numeric percentage, and $V\%$ = volumetric percentage (Beard, 2007; Bonansea and Vaira, 2007; Ferreria et al., 2015). We did not consider detritus, vegetation, and rocks as prey categories but we did count and measure them.

We collected invertebrates at these sites in 2004. We extracted leaf-litter invertebrates from the leaf litter using Tullgren funnels, collected flying invertebrates using a black light, and collected foliage invertebrates using beating traps. Detailed methods used to collect samples, and results, are described in Beard (2007). We used the invertebrate samples here to test whether stomach contents from morning- and evening-collected frogs reflect invertebrates collected in these different microhabitats (i.e., leaf litter, flying, or foliage invertebrates).

Statistical Analysis.—We used a two-way factorial analysis of variance (ANOVA) in a generalized linear mixed model to examine the fixed effect of sex (2 levels) and time of day collected (2 levels) on number of prey items consumed and total volume. We used a split-plot design with whole plots in blocks (sites) and subsamples (individual frogs). Random effects were site, time×site, and time×sex×site. To meet assumptions of normality and heterogeneity for number of prey items, we used a negative binominal distribution and a log-link transformation, but we needed to drop the variance estimate for gender for convergence. For prey volume, we used a normal distribution and fourth-root transformation. We did not include SVL as a covariate for the number of prey model because the variables were not related. We did not include SVL as a covariate in the volume model because only females collected in the morning had a positive relationship between prey volume and SVL (t_{411}= 4.02, $P < 0.0001$). In addition, when SVL was included in the overall model it was not significant and it had no influence on the fixed effects results.

We conducted a nonmetric multidimensional scaling (NMDS) analysis based on a Bray-Curtis dissimilarity matrix to test whether the composition of prey items in the stomach contents varied across collection period (morning vs. evening), sex, and site and used analysis of similarity (ANOSIM) to calculate the

TABLE 1. Frequency of prey items (%), total number of prey items (%), volume of prey items (mm^3; %), and importance (I) of each item in the diet of *Eleutherodactylus coqui* collected from five sites in the island of Hawaii based on the analysis of 238 stomachs collected during the evening period of the study. Asterisks indicate a terrestrial taxon with native representative(s) on the island of Hawaii according to Nishida (2002) and at these sites according to Beard (2007).

Prey category	Frequency (%)	Number (%)	Volume (%)	I
Amphibian				
Eleutherodactylus frogs	1 (0.42)	1 (0.05)	192.60 (1.99)	0.82
Eleutherodactylus eggs	7 (2.94)	36 (1.92)	790.77 (8.16)	4.34
Arachnida				
Acari				
Oribatida*	33 (13.87)	41 (2.19)	1.60 (0.02)	5.36
Acari other*	21 (8.82)	28 (1.49)	5.69 (0.06)	3.46
Araneae*	39 (16.39)	50 (2.67)	131.38 (1.36)	6.80
Pseudo-scorpiones*	6 (2.52)	6 (0.32)	8.13 (0.08)	0.97
Clitellata				
Oligochaeta	0 (0.00)	0 (0.00)	0 (0.00)	0.00
Chilopoda*	14 (5.88)	18 (0.96)	51.07 (0.53)	2.46
Diplopoda*	7 (2.94)	7 (0.37)	126.11 (1.30)	1.54
Gastropoda*	9 (3.78)	13 (0.69)	148.67 (1.53)	2.00
Insecta				
Blattodea				
Isoptera	2 (0.84)	3 (0.16)	15.45 (0.16)	0.39
Other	18 (7.56)	25 (1.33)	1,820.78 (18.78)	9.23
Coleoptera*				
Adult	70 (29.41)	130 (6.93)	1,286.58 (13.27)	16.54
Larvae	3 (1.26)	3 (0.16)	33.58 (0.35)	0.59
Collembola*	55 (23.41)	123 (6.56)	33.64 (0.35)	10.01
Dermaptera	9 (3.78)	9 (0.48)	330.14 (3.41)	2.56
Diptera*	32 (13.45)	45 (2.40)	71.63 (0.74)	5.53
Hemiptera*	51 (21.43)	78 (4.16)	981.58 (10.12)	11.90
Homoptera*	18 (7.56)	23 (1.23)	66.77 (0.69)	3.16
Hymenoptera*				
Formicidae	125 (52.52)	952 (50.77)	968.05 (9.98)	37.76
Other*	10 (4.20)	13 (0.69)	53.89 (0.56)	1.82
Larvae unknown*	3 (1.26)	4 (0.21)	1.61 (0.02)	0.50
Lepidoptera*				
Adult	11 (4.62)	14 (0.75)	591.90 (6.10)	3.82
Larvae	24 (10.08)	37 (1.97)	263.42 (2.72)	4.92
Neuroptera*	2 (0.84)	2 (0.11)	9.96 (0.10)	0.35
Orthoptera*	2 (0.84)	2 (0.11)	31.68 (0.33)	0.42
Psocoptera*	34 (14.29)	61 (3.25)	19.27 (0.20)	5.91
Thysanoptera*	1 (0.42)	2 (0.11)	0.32 (0.00)	0.18
Malacostraca				
Amphipoda	35 (14.71)	58 (3.09)	550.82 (5.68)	7.83
Isopoda	27 (11.34)	52 (2.77)	416.95 (4.30)	6.14
Reptilia				
Squamata	2 (0.84)	2 (0.11)	560.75 (5.78)	2.24
Unidentified*	33 (13.87)	37 (1.97)	130.75 (1.35)	5.73
Total	-	1,875	9,695.56	-
Detritus	11	12	29.59	-
Vegetation	233	554	581.41	-
Rock	35	14	14.17	-

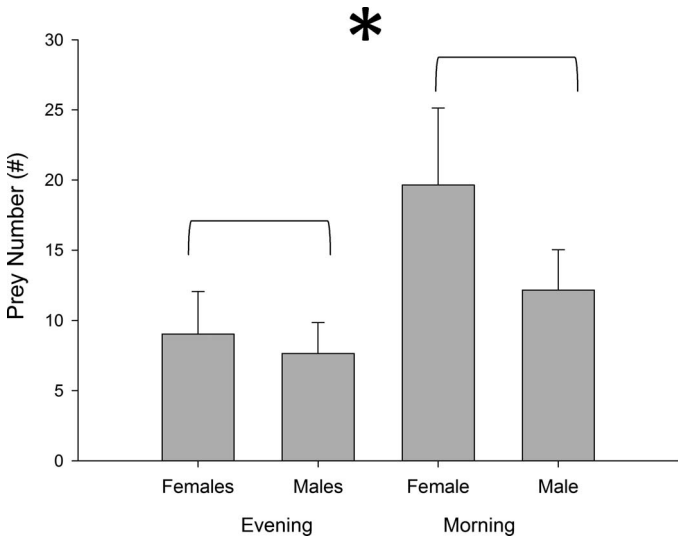

FIG. 2. Mean (±SE) prey number for females and males across collection periods. Mean prey number was greater in morning-collected frogs than in the evening-collected frogs ($P = 0.030$; $n = 5$ sites).

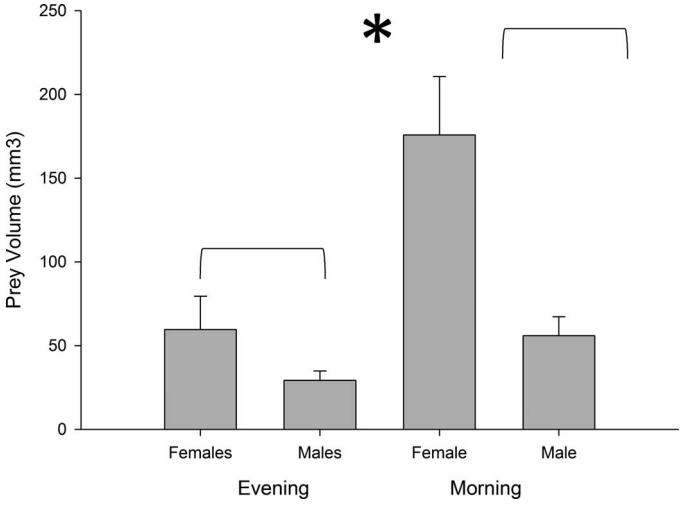

FIG. 3. Mean (±SE) prey volume for females and males across collection periods. Mean prey volume was greater in morning-collected frogs than in the evening-collected frogs ($P = 0.031$; $n = 5$ sites).

dissimilarity statistic (to determine if diet composition by collection period [morning vs. evening], site, or sex were different from one another). We conducted a principle component analysis (PCA) to test whether the composition of prey items in the stomachs resembled invertebrate communities collected in the environment (i.e., the potential microhabitat where Coquis forage). We present all loadings for the first two axes. We tested ordinations with a random permutation test.

We conducted ANOVAs in SAS v.9.4 for Windows (SAS Institute, Inc., Cary, North Carolina USA), ordination analyses in R 3.1.1 (R Core Team, 2014), and used $\alpha = 0.05$ for all tests. We present summary statistics as mean ± SE throughout.

RESULTS

We collected a total of 435 frogs across the five sites (SVL = 31.0 ± 0.17 mm); 289 (66.4%) males (SVL = 29.4 ± 0.12 mm) and 146 (33.6%) females (SVL = 34.0 ± 0.33 mm). We collected 95 frogs at Lava Tree State Park, 64 frogs at Kaumana Caves State Park, 123 frogs at Humane Society, 65 frogs at Akaka Falls State Park, and 88 frogs at Manuka State Park. While Coquis are thought to have a 1:1 sex ratio, often more males are found because they are vocal and often more exposed than females (Woolbright, 1985).

Diet Generalities.—Of the 435 collected individuals, 20 had empty stomachs; 13 males (3 in the morning and 10 in the evening; 4.8% of males) and 7 females (2 in the morning and 5 in the evening; 4.5% of females). We identified a total of 4,520 invertebrate items from the 415 stomachs that had prey items. On average, frogs had 10.4 ± 0.52 prey items per stomach. Mean prey volume per stomach was 61.28 ± 4.57 mm^3. While not considered prey items, plant material was present in 228 (52%) stomachs and rocks in 10 (2.3%) stomachs.

Prey Categories.—The most important prey categories were Formicidae, Coleoptera, Amphipoda, Hemiptera, and Collembola, respectively (Tables 1,2; Appendix 1). Formicidae made up 45.7% of the total number of prey items ingested followed by Amphipoda (9.3%), Collembola (8.9%), Coleoptera (5.8%), and Hemiptera (4.1%) (Appendix 1). Amphipoda represented 16.3% of the prey volume followed by Blattodea (13.9%), Coleoptera (13.8%), Hemiptera (9.4%), and Formicidae (8.6%) (Appendix 1).

Diet by Time of Day and Sex.—Coquis collected in the morning had more prey items in their stomach (13.43 ± 0.87) than did frogs collected during the evening (7.88 ± 0.57; $F_{1,4} = 10.93$, $P = 0.030$; Fig. 2). Females consumed more prey items (12.8 ± 1.08) than did males (9.17 ± 0.54; $F_{1,8} = 9.57$, $P = 0.015$), but there was no significant interaction between collection period and sex ($F_{1,8} = 3.19$, $P = 0.11$; Fig. 2). The main items consumed were Formicidae (46.63% total prey items) and Amphipoda (13.96%) for females and Formicidae (44.98% of total prey items) and Collembola (9.92%) for males.

Coquis collected in the morning had a greater volume of prey (86.09 ± 8.08 mm^3) than did frogs collected during the evening (40.74 ± 4.62 mm^3; $F_{1,4} = 10.71$, $P = 0.031$; Fig. 2). The volume of prey per stomach was higher in females (108.16 ± 11.36 mm^3) compared to males (37.60 ± 2.21 mm^3; $F_{1,8} = 22.11$, $P = 0.0015$), but there was no significant interaction between collection period and sex ($F_{1,8} = 2.81$, $P = 0.1322$; Fig. 2). Males often have fewer prey items and prey volume in their stomachs than do females because of the cost of calling (Woolbright and Stewart, 1987).

Diet Composition by Time of Day, Sex, and Site.—In the NMDS, stomach contents did not differ among collection periods (ANOSIM statistic = 0.085, $P = 0.10$) or sex (ANOSIM statistic = −0.015, $P = 0.48$); however, they did differ among sites (ANOSIM statistic = 0.77, $P < 0.001$; Fig. 3). The first dimension of the NMDS separated stomach contents from Lava Tree State Park and Akaka Falls State Park whereas the second dimension separated stomach contents from the Humane Society and Manuka State Park from the other sites (Fig. 3).

In the PCA, stomach contents differed from black light and beating trap samples (ordtest: $P < 0.001$) but were not different than the leaf litter samples (ordtest: $P = 0.955$; Fig. 4). The PC-1 separated the black light from the beating trap samples by loading positively on Collembola (0.91) and negatively on Diptera (−0.35), Lepidoptera (−0.15), and Amphipoda (−0.11; Fig. 4); PC-2 separated leaf litter samples and stomachs from the other samples by loading positively on Formicidae (0.81) and negatively on Diptera (−0.49), Lepidoptera (−0.20), Collembola (−0.17), and Coleoptera (−0.10; Fig. 4). Stomach contents also did not differ between the two collection periods in this analysis (ordtest: $P = 0.557$) but did differ across sites (ordtest: $P = 0.011$).

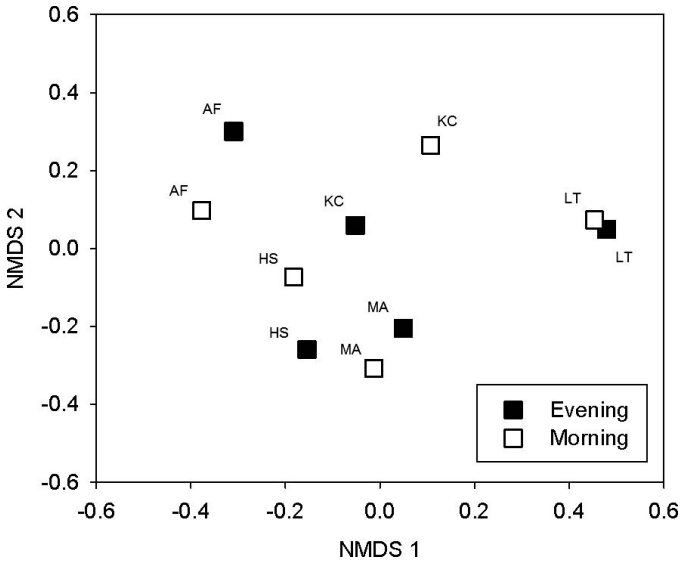

FIG. 4. Nonmetric multidimensional scaling (NMDS) of *Eleutherodactylus coqui* stomach contents from frogs collected in the morning and evening across sites in Hawaii ($n = 5$ sites).

Formicidae was the most numerous prey item consumed at Lava Tree State Park (81.3%). At the Humane Society and Manuka State Park, Formicidae (21.8%; 27.8%, respectively) and Amphipoda (26.4%, 24.4%, respectively) were almost equally important. At Kaumana Caves, Collembola (33.1%) and Formicidae (25.8%) dominated the diet. Frogs at Akaka Falls State Park had the most distinctive diet in that Formicidae made up a small percentage (<3%) and Hemiptera (21.0%), Coleoptera (14.7%), and Collembola (13.9%) were the dominant prey.

DISCUSSION

We found more prey and a greater volume of prey in Coqui stomachs of morning-collected frogs than in evening-collected frogs. More specifically, frogs collected in the morning had 1.7 times more prey items and 2.1 times greater prey volume than those collected at night. Where densities are as high as 91,000 frogs/ha, Beard et al. (2008) estimate that Coqui adults and juveniles consume 690,000 invertebrates/night. This approximation used an average of 7.6 prey items per night and considered adults as well as juveniles, which often have more prey items in their stomachs than adults (Townsend, 1985; Beard, 2007). Using the mean of 13.4 prey items/stomach in morning-collected frogs found in this study, which did not include any juvenile frogs that would have likely increased this estimate, Coquis were found to consume more like 1,200,000 prey items/ha/night. Therefore, previous studies have greatly underestimated the amount of invertebrates that Coquis consume in Hawaii when using estimates from evening-collected frogs.

We also found that stomach contents of Coquis collected at night and in the morning did not have different prey composition. This result was supported by the NMDS analysis, the PCA, and visual comparison of Tables 1 and 2. This result is surprising considering that Coquis use retreat sites during the day often near the forest floor and then at night they often move to understory perch sites (Woolbright and Stewart, 1987). This study suggests that despite the seemingly different microhabitats that frogs use over the course of a day (Beard, 2007), their diet composition does not change. Diet may not differ between evening- and morning-collected frogs because 1) Coquis select particular prey regardless of the microhabitat used, or 2) Coquis do not forage in different microhabitats between the two collection periods. In support of the first explanation, Beard (2007) suggested that Coquis prefer Formicidae (ants), their dominant prey, particularly on foliage, and ants do occur both on foliage and in the leaf litter. Similar arguments could be made for Collembola and Acari.

There is, however, more evidence for the second explanation that Coquis mostly forage in the leaf litter during the day and at night. First, even though across the five sites the main prey items in frogs collected in the morning and evening were surprisingly similar (Tables 1,2), the greatest difference in prey importance between these frogs was for Amphipoda (a change of 15.85), which was a much more important prey item in morning-collected frogs. We found no other difference >3 for prey importance between morning- and evening-collected frogs. Because Amphipoda at these sites is represented by one nonnative, ground-dwelling species (*Talitroides topitotum*) (Beard, 2007), this increase strongly suggests that Coquis are foraging on the forest floor throughout the night. Second, multivariate analyses also suggest that Coquis are foraging mostly in the leaf litter throughout the night (Fig. 5). Stomach contents were not different between morning- and evening-collected frogs, and they did not differ from leaf-litter invertebrate samples. Stomach contents from morning- and evening-collected frogs were different, however, from flying and foliage invertebrate samples. If frogs collected in the morning had more foliage invertebrates in their stomachs than did evening-collected frogs (Stewart and Woolbright, 1996), this shift should have been consistent across sites in the PCA.

There are several potential explanations why Coquis in their introduced range may have shifted away from foraging on the foliage, as they do in their native Puerto Rico (Stewart and Woolbright, 1996), toward foraging in the leaf litter (Beard, 2007). First, prey availability appears greater and more diverse on the forest floor than in the foliage in Hawaii, and because Coquis are sit-and-wait predators, they are likely to prefer habitat with more prey (Beard, 2007). Second, some preferred prey items from the foliage, such as Orthoptera, Homoptera, and Blattodea (Stewart and Woolbright, 1996), appear more common in the nocturnal forests of Puerto Rico than they do in many of the sites Coquis have invaded in Hawaii (Beard, 2007). Finally, Stewart and Woolbright (1996) suggested that Coquis in Puerto Rico avoid the leaf litter at night because there are more predators in the leaf litter than on the foliage. There are potentially fewer predators in the leaf litter in Hawaii than in Puerto Rico (Beard and Pitt, 2005).

While previous studies show that Coquis mostly forage in the leaf litter, they also show that Coquis shift prey composition across sites (Beard, 2007; Choi and Beard, 2012). Similarly, we found that stomach contents differed across sites. For some sites, such as Lava Tree State Park and Manuka State Park, stomach content samples were very similar to leaf litter samples whereas for other sites, such as Akaka State Park, stomach content samples did not appear to resemble any invertebrate sample. Therefore, the importance of leaf litter prey in the diet vs. prey from other microhabitats can shift across sites in Hawaii, and the shift likely reflects prey availability (Beard, 2007).

This study supports previous research showing the majority of Coqui diets in Hawaii consist of nonnative species (Beard, 2007). In addition, because we did not find a compositional shift

TABLE 2. Frequency of prey items (%), total number of prey items (%), volume of prey items (mm^3; %), and importance (I) of each item in the diet of *Eleutherodactylus coqui* collected from five sites in the island of Hawaii based on the analysis of 197 stomachs collected during the morning period of the study. Asterisks indicate a terrestrial taxon with native representative(s) on the island of Hawaii according to Nishida (2002) and at these sites according to Beard (2007).

Prey category	Frequency (%)	Number (%)	Volume (%)	I
Amphibian				
Eleutherodactylus frogs	1 (0.51)	7 (0.26)	135.22 (0.80)	0.52
Eleutherodactylus eggs	7 (3.55)	46 (1.74)	1,043.08 (6.15)	3.81
Arachnida				
Acari Oribatida*	23 (11.68)	30 (1.13)	2.76 (0.02)	4.28
Acari other*	11 (5.58)	12 (0.45)	2.18 (0.01)	2.02
Araneae*	39 (19.80)	62 (2.34)	207.33 (1.22)	7.79
Pseudoscorpiones*	4 (2.03)	6 (0.23)	5.02 (0.03)	0.76
Clitellata				
Oligochaeta	1 (0.51)	1 (0.04)	0 (0.00)	0.19
Chilopoda*	15 (7.61)	17 (0.64)	554.01 (3.27)	3.84
Diplopoda*	16 (8.12)	17 (0.64)	150.79 (0.89)	3.22
Gastropoda*	15 (7.61)	17 (0.64)	795.79 (4.69)	4.32
Insecta				
Blattodea				
Isoptera	0 (0.00)	0 (0.00	0 (0.00)	0.00
Other	18 (9.14)	19 (0.72)	1,888.27 (11.13)	7.00
Coleoptera*				
Adult	64 (32.49)	130 (4.91)	2,401.81 (14.16)	17.19
Larvae	2 (1.02)	2 (0.08)	27.35 (0.16)	0.42
Collembola*	44 (22.34)	281 (10.62)	80.28 (0.47)	11.14
Dermaptera	14 (7.11)	19 (0.72)	290.40 (1.71)	3.18
Diptera*	26 (13.20)	76 (1.74)	206.60 (1.22)	5.76
Hemiptera*	46 (23.35)	108 (4.08)	1,519.65 (8.96)	12.13
Homoptera*	26 (13.20)	37 (1.40)	184.10 (1.09)	5.23
Hymenoptera*				
Formicidae	112 (56.85)	1112 (42.04)	1,326.03 (7.82)	35.57
Other*	9 (4.57)	12 (0.45)	103.04 (0.61)	1.88
Larvae unknown*	11 (5.58)	11 (0.42)	30.26 (0.18)	2.06
Lepidoptera*				
Adult	13.00 (6.60)	13 (0.49)	205.05 (1.21)	2.77
Larvae	11 (5.58)	55 (2.08)	1,412.52 (8.33)	6.85
Neuroptera*	2 (1.02)	2 (0.08)	0.10 (0.00)	0.36
Orthoptera*	3 (1.52)	3 (0.11)	41.89 (0.25)	0.63
Psocoptera*	33 (16.75)	76 (2.87)	36.98 (0.22)	6.61
Thysanoptera*	1 (0.51)	1 (0.04)	0.33 (0.00)	0.18
Malacostraca				
Amphipoda	69 (35.03)	362 (13.69)	3,786.76 (22.33)	23.68
Isopoda	32 (16.24)	79 (2.99)	353.31 (2.08)	7.10
Reptilia				
Squamata	0 (0.00)	0 (0.00)	0.00 (0.00)	0.00
Unidentified*	28 (14.21)	32 (1.21)	165.12 (0.97)	5.47
Total	-	2,645	16,960.5	-
Detritus	1	1	2.83	-
Vegetation	103	588	747.63	-
Rock	22	29	26.59	-

in diet between morning- and evening-collected frogs, the high proportion of nonnative species in their diet appears consistent throughout the day. Several taxonomic units are solely represented by nonnative species in our samples and, more generally, in Hawaii; these include amphibians, reptiles, Amphipoda, Blattodea, Dermaptera, Formicidae, Isopoda, and Oligochaeta (Table 2). Based on our knowledge of these sites and the invertebrates that live there (Beard, 2007), at a minimum 61.6% of their prey items are nonnative species (Appendix 1). This percentage is likely to be much higher because many groups such as Acari, Collembola, and Coleoptera have nonnative representatives, but we did not identify these prey items to species, and therefore cannot determine what portion of prey items in these categories were nonnative.

While the majority of their diet is nonnative species, there are categories of invertebrates that contain native species at invaded sites, such as Acari, Araneae, Collembola, Coleoptera, and Hemiptera, which remain vulnerable to the invasion. Coquis have the potential to reduce native invertebrates through direct predation and make populations more susceptible to extinction. This may be of particular concern for species of special interest, such as the Happy Face Spider (*Theridion grallator*) (Howarth et al., 1988). Coquis also could have positive indirect effects on the invertebrate community if they reduce nonnative species. Ants, for example, are known predators of native invertebrates in Hawaii, and their reduction could have a positive effect on native invertebrates (Krushelnycky and Gillespie, 2008); so far, however, research has not revealed any benefit of Coquis to native invertebrates (Choi and Beard, 2012). Because Coquis mostly reduce leaf-litter invertebrates (Acari, Coleoptera, Collembola, and Amphipoda), and in particular fragmenters (Choi and Beard, 2012), Coquis also could reduce leaf litter decomposition rates and hence the availability of nutrients in these ecosystems. Previous research, however, suggests that

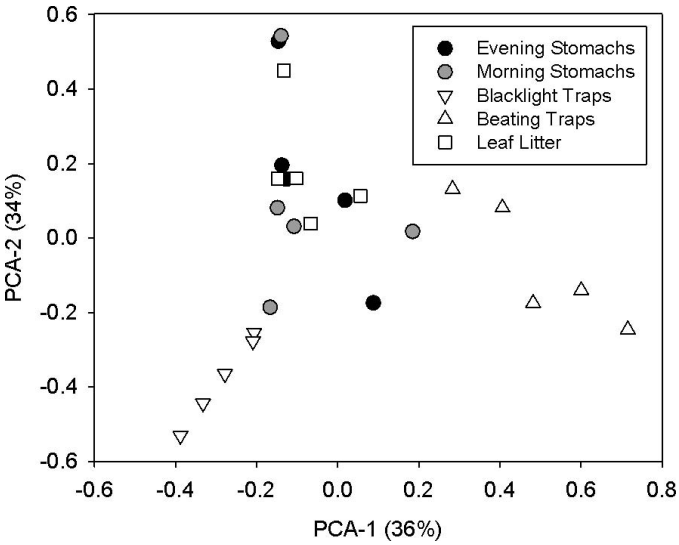

FIG. 5. Principle components analysis (PCA) of invertebrates found in *Eleutherodactylus coqui* stomach contents and invertebrates collected in the environmental samples in five sites on the island of Hawaii. Circles = evening/morning stomach content; upside down triangles = flying invertebrates collecting using a black light; triangles = foliage invertebrates collected using a beating trap; and squares = leaf-litter invertebrates extracted from the leaf litter ($n = 5$ sites).

Coquis increase decomposition rates where they invade by increasing nutrient recycling rates (i.e., the turnover of invertebrates into more-available nutrient forms) despite changes in the invertebrate community (Sin et al., 2008). Increased rates of nutrient cycling could be detrimental to native plant species, such as Ohia (*Metrosideros polymorpha*), that are evolved to nutrient-poor conditions (Sin et al., 2008).

In summary, because diet composition does not differ between morning- and evening-collected frogs, and because both time periods reflect consumption of nonnative, leaf-litter invertebrates, previously stated hypotheses and predictions about the impacts of Coquis remain relevant (Beard and Pitt, 2005; Choi and Beard, 2012). While supporting this previous research, this study provides a much better estimate of their prey number and prey volume. Furthermore, the lack of temporal change in diet composition suggests that Coquis in Hawaii mostly forage in the leaf litter and not on foliage as Coquis do in Puerto Rico (Stewart and Woolbright, 1996). Because introduced species diets and foraging behaviors can differ substantially from those of the native range (Tillberg et al., 2007), this study highlights the need to characterize the diet of introduced amphibians so as to understand potential impacts where they invade.

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APPENDIX 1. Frequency of prey items (%), total number of prey items (%), volume of prey items (mm^3; %), and importance (I) of each item in the diet of *Eleutherodactylus coqui* collected from five sites in the island of Hawaii based on the analysis of 435 stomachs. Asterisks indicate a terrestrial taxon with native representative(s) on the island of Hawaii according to Nishida (2002) and at these sites according to Beard (2007).[a]

Prey category	Frequency (%)	Number (%)	Volume (%)	I
Amphibian				
Eleutherodactylus frogs	2 (0.46)	8 (0.18)	327.82 (1.23)	0.62
Egg mass	14 (3.22)	82 (1.81)	1,833.89 (6.88)	3.97
Arachnida				
Acari Oribatida*	56 (12.87)	71 (1.57)	4.37 (0.02)	4.82
Acari other*	32 (7.36)	40 (0.88)	7.87 (0.03)	2.76
Araneae*	78 (17.93)	112 (2.48)	338.71 (1.27)	7.23
Pseudoscorpiones	10 (2.30)	12 (0.27)	13.15 (0.05)	0.87
Clitellata				
Oligochaeta	1 (0.23)	1 (0.02)	4.48 (0.02)	0.09
Chilopoda*	29 (6.67)	35 (0.77)	605.07 (2.27)	3.24
Diplopoda*	23 (5.29)	24 (0.53)	276.91 (1.04)	2.29
Gastropoda*	24 (5.52)	30 (0.66)	944.46 (3.54)	3.24
Insecta				
Blattodea				
Isoptera	2.00 (0.46)	3 (0.07)	15.45 (0.06)	0.19
Other	36 (8.28)	44 (0.97)	3,709.05 (13.91)	7.72
Coleoptera*				
Adult	134 (30.80)	260 (5.75)	3,688.39 (13.84)	16.80
Larvae	5 (1.15)	5 (0.11)	60.93 (0.23)	0.50
Collembola*	99 (22.76)	404 (8.94)	113.92 (0.43)	10.71
Dermaptera*	23 (5.29)	28 (0.62)	620.54 (2.33)	2.74
Diptera*	58 (13.33)	121 (2.68)	278.23 (1.04)	5.68
Hemiptera*	97 (22.30)	186 (4.12)	2,501.23 (9.38)	11.93
Homoptera	44 (10.11)	60 (1.33)	250.88 (0.94)	4.13
Hymenoptera				
Formicidae	237 (54.48)	2,064 (45.66)	2,294.08 (8.61)	36.25
Other*	19 (4.37)	25 (0.55)	156.93 (0.59)	1.84
Larvae unknown	14 (3.22)	15 (0.33)	31.87 (0.12)	1.22
Lepidoptera				
Adult	24 (5.52)	27 (0.60)	796.94 (2.99)	3.03
Larvae	44 (10.11)	92 (2.04)	1,675.93 (6.29)	6.15
Neuroptera	4 (0.92)	4 (0.09)	10.07 (0.04)	0.35
Orthoptera	5 (1.15)	5 (0.11)	73.57 (0.57)	0.51
Psocoptera	67 (15.40)	137 (3.03)	56.25 (0.21)	6.21
Thysanoptera	2 (0.46)	3 (0.07)	0.66 (0.00)	0.18
Malacostraca				
Amphipoda*	104 (23.91)	420 (9.29)	4,337.59 (16.27)	16.49
Isopoda*	59 (13.56)	131 (2.90)	770.26 (2.89)	6.45
Reptilia				
Squamata	2 (0.46)	2 (0.04)	560.75 (2.10)	0.87
Unidentified	61 (14.02)	69 (1.53)	295.87 (1.11)	5.55
Total	-	4,520	26,656.07	-
Detritus	11	13	32.42	
Vegetation	233	1,142	1,327.69	
Rock	35	46	41.04	

[a] Dermaptera at the sites were all nonnative *Euborellia annulipes* and *Chelisoches morio*. Isopoda were nonnative *Porcellio laevis*. Amphipoda were nonnative *Talitroides topitotum*.