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Diet of the Invasive Frog, *Eleutherodactylus coqui*, in Hawaii

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Because of their high densities and generalist feeding behaviors, the introduced frog, *Eleutherodactylus coqui*, has been hypothesized to consume and potentially reduce endemic invertebrates in Hawaii. To address this hypothesis, I compared *E. coqui* diets to invertebrate abundances in 11 sites on the Islands of Hawaii and Maui in the summer of 2004. At each site, I collected between 22 and 119 frogs from 20 × 20-m plots, and invertebrates from light traps, beating trays, and leaf litter samples. Prey items in frog stomachs were identified to order, and invertebrates collected in environmental samples were identified to the lowest taxonomic category possible. Multivariate analyses of diet content and invertebrates collected at each site suggest that most prey was from the leaf litter. Non-native ants (Hymenoptera: Formicidae) and amphipods (Amphipoda: Talitridae) comprised 30% and 22%, respectively, of the total prey items consumed. These non-native invertebrates were more abundant in stomachs of *E. coqui* than in the environment indicating a preference for these species. There was little evidence that *E. coqui* were reducing important invertebrate pests. No mosquitoes (Diptera: Culicidae) were found in stomachs, and termites (Isoptera) comprised <1% of the total prey items. Arthropod orders containing endemic species that appear most vulnerable to *E. coqui* predation include Acarina (mites), Coleoptera (beetles), Collembola (springtails), and Diptera (flies), which each made up >2% of the diet of *E. coqui*. Dominant prey items in frog stomachs differed among study sites suggesting that frogs are opportunistic feeders and forage on abundant prey items. *Eleutherodactylus coqui* management should focus on areas with endemic invertebrates of concern because it is these locations where *E. coqui* may have the greatest impact.

A frog endemic to Puerto Rico, *Eleutherodactylus coqui*, has invaded Florida and several islands in the Caribbean and was accidentally introduced to Hawaii presumably via nursery plants in the late 1980s (Kraus et al., 1999). *Eleutherodactylus coqui* is now established on all four main Hawaiian Islands, but most populations (>250) are located on the islands of Hawaii and Maui, in lowland forests (0–500 m) on the windward sides (Kraus and Campbell, 2002). Direct development, year-round breeding, and the lack of a need for aquatic habitat to breed are thought to have contributed to their rapid spread (Beard and O'Neill, 2005). Attempts to control *E. coqui* have been generally unsuccessful (Beard and Pitt, 2005), primarily because of delayed responses to introductions (Kraus and Campbell, 2002).

Research from Puerto Rico suggests that *E. coqui* can attain extremely high densities (20,570 individuals/ha on average) and consume an estimated 114,000 prey items/ha/night, primarily invertebrates (Woolbright, 1991; Stewart and Woolbright, 1996). There is evidence that their densities in Hawaii can be 2–3 times greater than they are in Puerto Rico (Beard and Pitt, 2005; Woolbright et al., 2006). Thus, the most obvious ecological consequence of the invasion is the consumption, and potential reduction, of invertebrate prey (Kraus et al., 1999). A reduction of invertebrate populations in Hawaii could be devastating because invertebrates comprise the large majority of the endemic fauna (Eldredge and Miller, 1995). Alternatively, it has been suggested that predation by *E. coqui* could result in a reduction of undesirable, non-native invertebrates (Fullington, 2001; Singer, 2001).

Eleutherodactylus coqui has already been the subject of a wide variety of studies in its native Puerto Rico dealing with its feeding ecology and behavior (Townsend, 1985; Woolbright, 1985; Woolbright and Stewart, 1987; Townsend, 1989; Woolbright, 1989). *Eleutherodactylus coqui* has been characterized as an extreme sit-and-wait nocturnal predator (Woolbright and Stewart, 1987). Studies focused on the diet of *E. coqui* in Puerto Rico suggest that they primarily consume foliage invertebrates and some litter invertebrates (Stewart and Woolbright, 1996). Experiments conducted in Puerto Rico show that they can also control flying arthropods (Beard et al., 2003a). However, information on food preferences and foraging behavior of *E. coqui* in Puerto Rico may not apply to introduced populations of *E. coqui* in Hawaii.

In this study, I determine dominant prey taxa and prey preferences of *E. coqui* in Hawaii. Based on the results, I identify invertebrate orders

TABLE 1. DESCRIPTION OF STUDY SITES. Study site elevation, year *Eleutherodactylus coqui* was introduced to each study site, and the number of frogs collected at each site for stomach content analyses. *Year introduced based on USDA/Wildlife Services and Hawaii Invasive Species Council hotlines.

Site, Island	Coordinates	Elevation (m)	Year*	Males	Females	Subadults
Akaka Falls State Park (AK), Hawaii	19°51.29′N, 155°09.18′W	405	2001	12	9	1
Hawaiian Paradise Park (PP), Hawaii	19°35.89′N, 154°59.16′W	50	2000	48	12	45
Humane Society (HS), Hawaii	19°36.28′N, 155°01.15′W	135	1998	33	38	16
Kaumana Caves Park (KC), Hawaii	19°41.19′N, 155°07.86′W	323	1999	19	6	0
Kihei Nursery (MKN), Maui	20°43.80′N, 156°26.99′W	16	2000	29	19	4
Kurtistown (KT), Hawaii	19°35.52′N, 154°04.68′W	308	1995	38	8	21
Lava Tree State Park (LT), Hawaii	19°28.99′N, 154°54.20′W	181	1996	30	14	47
Maliko Gulch (MMG), Maui	20°52.33′N, 156°19.00′W	440	1997	51	18	50
Manuka Natural Area Reserve (MP), Hawaii	19°06.58′N, 155°49.53′W	556	2000	14	5	26
Puainako Street/Safeway (PK), Hawaii	19°41.81′N, 155°03.52′W	45	2001	29	16	8
Waipio Overlook (WO), Hawaii	20°07.03′N, 155°35.08′W	303	2000	16	5	9
			Total	319	150	227

containing endemic species that are most likely to be affected by the invasion. I also determine microhabitats used by *E. coqui* for foraging and nocturnal perches. Finally, I compare the results on diet and microhabitat use by *E. coqui* found in this study to those found in previous studies conducted in Puerto Rico.

MATERIALS AND METHODS

Study sites.—Research was conducted in nine sites on the Island of Hawaii, USA, 10–21 May 2004, and two sites on Maui, Hawaii, USA, 20–25 August 2004. These sites were selected because they had established populations of *E. coqui* and because they captured a diversity of forest types, elevation, and geologic history (Table 1). Dominant overstory trees differed across sites and included *Cecropia obtusifolia* (sites abbreviated after species name have this species as a dominant: HS and KT), *Chrysalidocarpus lutescens* (MKN), *Falcataria mollucana* (LT), *Melaleuca quinquenervia* (WO), *Metrosideros polymorpha* (AK, KC, and MP), and *Psidium cattleianum* (PK, PP, and MMG). Dominant understory plants also differed across sites: *Archontophoenix alexandrae* (HS), *Clidemia hirta* (KT and LT), *Monstera deliciosa* (AK), *Pennisetum purpureum* (MMG), *Phoenix roebelenii* (MKN), *P. cattleianum* (MP, PK, and WO), *Syzygium jambos* (PP), or *Dicranopteris linearis* (KC).

Frog sampling.—Each study site was visited once. A 20 × 20-m plot was established at each study site. Each 20 × 20-m plot was divided into four 5-m wide transects. Frog collections began at 2000 h, after *E. coqui* had sufficient time to move to their nocturnal perches (Woolbright, 1985). Each transect was searched by two researchers for 30 min, not including handling time. When a frog was collected, height from the forest floor (up to 3 m) and microhabitat use when first observed were recorded. The 11 microhabitat categories were branch, fallen branch, fallen leaf (not on forest floor), fallen trunk, leaf, leaf litter (on the forest floor), rock or root, soil, stem (herbaceous), trunk, and other (flower pot, plastic crate). After collection, frogs were immediately euthanized with CO_2 and then frozen.

Within 24 h, frogs were dissected, and opened stomachs were placed in 70% ethanol until further examination. Each individual was measured (snout–vent length [SVL], to the nearest 0.1 mm with dial calipers) and sexed by direct examination of gonads. Individuals were considered adults when SVL >25 mm (Woolbright, 2005). For each stomach, prey items were counted and identified to order with a dissecting microscope. Where possible, prey items were identified to family.

To determine prey volume, each prey item was measured to 0.1 mm^2 and then volume was estimated using the formula for a prolate spheroid (Vitt, 1991; Vitt et al., 1996). Prey importance values (I) were calculated as: (%F +%N +%V)/3, where F = frequency (number) of stomachs that contain a particular prey item, N = total number of that prey item, and V = total volume of that prey item (Biavati et al., 2004). Vegetation in stomachs could not be properly counted (N), and, therefore, was not considered in the calculation of I. Egg masses were considered one prey item. Prey diversity was measured as the number of different food items identified in Table 2.

Invertebrate sampling.—Invertebrates were sampled from the environment adjacent to plots

TABLE 2. DIET SUMMARY FOR *Eleutherodactylus coqui* IN HAWAII. Number of frogs with prey item (F), number of items (#), volume of items (mm^3), and importance (I) for each food category in the diet of *Eleutherodactylus coqui* (n = 696) collected from 11 sites in Hawaii (nine on the Island of Hawaii and two on Maui). *Identifications are based on Borror et al. (1989).

Class* Order Family	F (%)	# (%)	Volume (%)	I
Amphibia				
Anura	4 (0.57)	4 (0.07)	133.07 (0.43)	0.36
E. coqui eggs	10 (1.44)	10 (0.18)	1711.96 (5.55)	2.39
Arachnida				
Acarina	178 (25.57)	393 (7.06)	236.25 (0.77)	11.13
Araneae	62 (8.91)	71 (1.28)	177.67 (0.58)	3.59
Chilopoda	53 (7.61)	68 (1.22)	313.88 (1.02)	3.28
Diplopoda	26 (3.74)	28 (0.50)	200.28 (0.65)	1.63
Gastropoda	74 (10.63)	99 (1.78)	485.97 (1.58)	4.66
Insecta				
Blattodea	4 (0.57)	4 (0.07)	237.38 (0.77)	0.47
Coleoptera—other	103 (14.80)	124 (2.23)	1071.47 (3.47)	6.83
Coccinellidae	9 (1.29)	9 (0.16)	126.68 (0.41)	0.62
Curculionidae	14 (2.01)	22 (0.40)	96.49 (0.31)	0.91
Scotylidae	55 (7.90)	113 (2.03)	347.81 (1.13)	3.69
Collembola	174 (25.00)	570 (10.24)	361.41 (1.17)	12.14
Dermaptera	37 (5.32)	46 (0.83)	637.77 (2.07)	2.74
Diptera	52 (7.47)	98 (1.76)	414.84 (1.35)	3.57
Tipulidae	34 (4.89)	47 (0.84)	278.46 (0.90)	2.21
Hemiptera	66 (9.48)	88 (1.58)	540.90 (1.75)	4.27
Homoptera	28 (4.02)	40 (0.72)	80.08 (0.26)	1.67
Hymenoptera—other	4 (0.57)	4 (0.072)	88.33 (0.28)	0.31
Formicidae	398 (57.18)	1679 (30.17)	2466.65 (8.86)	32.07
Isoptera	6 (0.86)	8 (0.14)	134.14 (0.43)	0.48
Lepidoptera—adult	2 (0.29)	5 (0.09)	52.03 (0.17)	0.18
Lepidoptera—larvae	22 (3.16)	29 (0.52)	1508.10 (4.89)	2.86
Phthiraptera	1 (0.14)	1 (0.02)	0.17 (0.00)	0.05
Pscocptera	44 (6.32)	75 (1.35)	59.86 (0.19)	2.62
Thysanoptera	5 (0.72)	5 (0.09)	1.02 (0.00	0.27
Unknown eggs	1 (0.14)	1 (0.02)	9.76 (0.03)	0.06
Unknown larvae	20 (2.87)	28 (0.50)	25.98 (0.08)	1.15
Malacostraca				
Amphipoda	309 (44.40)	1185 (21.29)	13238.94 (42.93)	36.21
Isopoda	173 (24.86)	443 (7.96)	2486.52 (8.06)	13.63
Pseudoscorpionida	13 (1.87)	14 (0.25)	29.53 (0.10)	0.74
Unidentifiable remains	162 (23.28)	256 (4.60)	832.95 (2.70)	10.19
Vegetation	245 (35.20)	—	2451.79 (7.95)	—
Total	—	5567 (100)	30838.14 (100)	—

just prior to or during frog collections. Aerial (flying) insects were collected from 1930 h to 2230 h using a portable UV light trap (BioQuip, Products, Inc., Rancho Dominguez, CA) placed on the north side of each plot. Foliage invertebrates were collected from four randomly selected plants of the dominant understory species at each site (listed above in the Study site section) using a 0.8 m^2 beating tray. Litter was collected from four randomly selected 0.6 m^2 areas of the forest floor, and invertebrates were extracted using Berlese–Tullgren funnels. All collected invertebrates were stored in 70% ethanol. Invertebrates were counted and sorted to order and recognizable taxonomic unit (RTU). When possible, RTUs were identified to the lowest taxonomic category possible, often species.

Statistical analyses.—Factorial ANOVAs were used in a completely randomized design to evaluate the fixed effects of site (11 levels) and class (three levels: subadult, adult male, adult female) on total prey items and total prey diversity as response variables. Because of a significant association between frog SVL and total prey volume ($R^2 = 0.03$, $F_{1,693} = 23.84$, $P < 0.0001$), a factorial ANCOVA, with SVL as the covariate, was used to evaluate the fixed effects of site and class upon prey volume. To analyze microhabitat use differences by class and site, numbers of individuals on each structure were compared using Pearson's chi-square exact test.

When necessary to meet assumptions of normality and homogeneity of variance, data were log-transformed. All means comparison tests were conducted using the Tukey-Kramer procedure. Because there were often class differences and the number of subadults collected varied by site (Table 1), means comparison tests across sites were conducted using adults only. Because no subadults were collected at KC, means comparison tests across classes did not include this site.

A principle components analysis (PCA) was conducted to assess microhabitats where *E. coqui* forage (i.e., flying, foliage, or litter). Additional PCAs were conducted to determine if stomach contents of *E. coqui* were more similar to each other and/or invertebrate communities at each site. Principle components (PCs) with eigenvalues greater than 0.1 or less than −0.1 are presented. Ordinations were tested with a random permutation test. The PCA outputs were used as response variables in factorial ANOVAs to evaluate the fixed effects of site and method (levels: stomach, flying, foliage, and litter samples) as appropriate.

Prey selection at each site and environmental sample was determined using Strauss' Linear Selection Index (L): $L_i = (p_i - e_i)*100$, where $i = 1$ to n prey taxa, p_i is the numerical proportion consumed, and e_i is the numerical proportion in the prey resource sample (Strauss, 1979). Negative values indicate avoidance, positive values indicate selection, and values near zero indicate predation at a rate proportional to the abundance of the taxa. Mean across-site L-values greater than 3 or less than −3 are presented.

Except for PCAs, all statistical analyses were conducted using SAS v.9 for Windows (SAS Institute, Cary, North Carolina). Principle components analyses were conducted using pca and ordtest functions in the labdsv library in R 2.0.1 (R Development Core Team, R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2004). Means ± 1 SD are presented to describe populations. Means ± 1 SE are presented where means tests were conducted.

Results

Population descriptions.—I collected a total of 696 *E. coqui* (319 males, 150 females, and 227 subadults; Table 1). The sex ratio was biased towards males. Mean SVL for subadults was 15.6 ± 4.5 mm (min: 3 mm), for adult males was 29.7 ± 2.3 mm (max: 36.7 mm), and for adult females was 32.4 ± 3.9 mm (max: 46.6 mm). Across sites, females were 10% larger than males ($P < 0.05$). Adults were collected at a greater height from the forest floor than subadults (0.9 ± 0.03 m vs. 0.5 ± 0.03 m; $F_{2,664} = 10.41$, $P < 0.0001$). Adult male and female collection heights were not different (0.9 ± 0.03 vs. 0.8 ± 0.05 m; $P > 0.05$).

Microhabitat use.—Subadults and adults used different microhabitat structures ($\chi^2 = 156.8$, df = 10, $P < 0.0001$). Across sites, subadults were mostly found on leaves (80.4 ± 10.5%), while also being found on herbaceous stems (5.3 ± 4.1%) and trunks (5.5 ± 4.1%; Fig. 1). Remaining structural categories each accounted for <4% of all subadults collected. Adults were more evenly distributed between trunks and leaves (43.6 ± 8.0% and 36.9 ± 8.4%, respectively) and also collected on branches (4.1 ± 1.7%; Fig. 1). Remaining structural categories each accounted for <4% of all adults collected.

Diet descriptions.—Of adult males collected, 21 (6.5%) had empty stomachs. Of adult females, five (3.3%) had empty stomachs. Only one (0.4%) subadult stomach was empty. Plant material was found in 245 (35.2%) of the stomachs examined. A total of 5310 invertebrates in stomachs were identifiable, representing 34 prey categories (Table 2). On average, frogs contained 7.6 ± 7.6 prey items (max: 53 ants and one Coleoptera, female at MKN) per stomach. The diversity of prey items was on average 2.8 ± 1.8 (max: 10). Total prey volume per stomach was, on average, 44.3 ± 77.0 mm^3 (max: 722.0 mm^3, 17 Amphipoda and two ants, male at PP).

Total number of prey items was greater for subadults than for adults (10.7 ± 0.5 vs. 6.2 ± 0.3; $F_{2,664} = 22.9$, $P < 0.0001$). There was no difference in the total number of prey items consumed by adult males and females (6.5 ± 0.4 vs. 5.5 ± 0.5). Subadult diets had greater diversity than adults (4.0 ± 0.1 vs. 2.3 ± 0.7; $F_{2,664} = 20.9$, $P < 0.0001$). There was no difference in prey diversity between adult males and females (2.2 ±

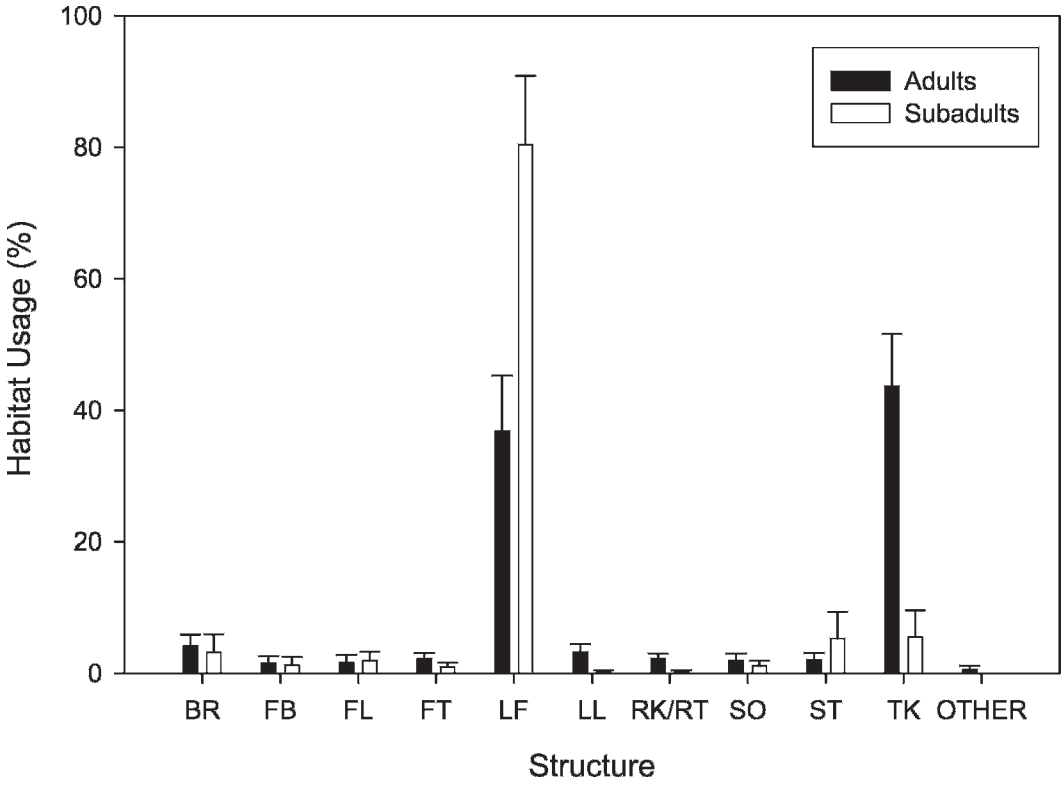

Fig. 1. Percent of adult and subadult frogs (+ SE) collected on microhabitat structures across 11 study sites in Hawaii. BR = branch, FB = fallen branch, FL = fallen leaf, FT = fallen trunk, LF = leaf, LL = leaf litter, RK/RT = rock/root, SO = soil, ST = stem, TK = trunk, and OTHER.

0.1 vs. 2.4 ± 0.1). Independent of SVL, adult female and subadult prey volumes per stomach were greater than adult male prey volumes (71.6 ± 9.0 mm^3, 30.2 ± 2.7 mm^3, and 41.5 ± 4.2 mm^3, respectively; $F_{2,681} = 9.2$, $P = 0.0001$).

Subadults consumed more Acarina, Amphipoda, Araneae, Collembola, Hymenoptera, and Pseudoscorpionida than adults ($P < 0.05$). Subadults consumed more Isopoda than adult males, but not adult females ($P < 0.05$). Adult females consumed more Diplopoda than adult males, but not more than subadults ($P < 0.05$). Adult females also consumed more Blattodea and Lepidoptera larvae than males or subadults ($P < 0.05$).

Differences by site.—Heights where frogs were collected varied by site ($F_{10,447} = 4.7$, $P < 0.0001$), with means ranging from 0.5 m at MMG to 1.3 m at AK. Subadults used different microhabitats in different sites ($\chi^2 = 254.0$, df = 81, $P = 0.01$). At KT, MKN, LT, and PP 100, 100, 97.9, and 97.8% of subadult frogs were collected on leaves; no other structural category was used by >35% of subadults in any site. Adults also used different microhabitats in different sites ($\chi^2 = 420.4$, df = 100, $P < 0.0001$). At LT, MKN, and MMG, 72, 81, and 62% of adults were collected on leaves, whereas at MP, WO, PK, and PP, 74, 71, 67, and 70% of adults were collected on trunks.

There was a different number of total prey items consumed per frog by site ($F_{10,447} = 5.27$, $P < 0.0001$), with means ranging from 2.2 at WO to 12.8 at MKN (means for adults only). Prey diversity per frog also differed across sites ($F_{10,447} = 5.3$, $P < 0.0001$), with means ranging from 1.4 at WO to 3.7 at AK. Independent of SVL, prey volume per stomach differed by site ($F_{10,445} = 4.9$, $P < 0.0001$), with means ranging from 21.4 mm^3 at KT to 118.0 mm^3 at AK.

Site differences were common when each prey item was analyzed separately (Table 3). For example, at MKN, frogs consumed a significantly greater number of ants (92% of the total prey items) than at any other site. At PK, frogs consumed the greatest number of Collembola (38%) and Diplopoda (3%) compared to any other site. However, at these two sites (MKN and PK), no Amphipoda were consumed or present in the environmental samples. Frogs at MP

TABLE 3. *Eleutherodactylus coqui* DIETS ACROSS STUDY SITES. Percent of prey items identified in *Eleutherodactylus coqui* stomach contents by site for 11 study sites in Hawaii. *Mean values followed by the same lower case letters are not significantly different when comparing across site (Tukey-Kramer comparisons of means, $P < 0.05$).

Class Order	Study sites*										
	AK	HS	KC	KT	LT	MKN	MMG	MP	OL	PK	PP
Amphibia											
Anura	0.00^{a}	0.00^{a}	0.00^{a}	0.26^{a}	0.16^{a}	0.00^{a}	0.14^{a}	0.00^{a}	0.00^{a}	0.22^{a}	0.00^{a}
E. coqui eggs	0.00^{a}	0.00^{a}	0.00^{a}	0.52^{a}	0.16^{a}	0.15^{a}	0.14^{a}	0.00^{a}	1.35^{a}	0.00^{a}	0.24^{a}
Arachnida											
Acarina	1.53^{a}	5.96^{a}	0.00^{a}	5.68^{a}	5.27^{a}	0.15^{a}	7.44^{a}	25.39^{a}	8.11^{a}	4.21^{a}	10.28^{a}
Araneae	5.61^{a}	0.61^{b}	0.00^{b}	2.84^{b}	1.44^{b}	0.15^{b}	1.69^{b}	2.17^{b}	1.35^{b}	0.22^{b}	1.04^{b}
Chilopoda	0.00^{b}	0.76^{b}	0.00^{b}	2.58^{ab}	0.64^{b}	0.15^{b}	5.20^{a}	0.00^{ab}	0.00^{b}	0.67^{b}	0.64^{b}
Diplopoda	0.00^{b}	0.00^{b}	0.00^{b}	0.26^{b}	0.96^{b}	0.00^{b}	0.56^{b}	0.62^{b}	0.68^{b}	2.88^{a}	0.08^{b}
Gastropoda	1.02^{abc}	0.00^{c}	0.00^{bc}	0.26^{bc}	0.96^{bc}	0.44^{bc}	2.25^{ab}	2.79^{abc}	0.00^{bc}	4.66^{a}	3.27^{ab}
Insecta											
Blattodea	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.14^{b}	0.93^{a}	0.00^{b}	0.00^{b}	0.00^{b}
Coleoptera	2.04^{ab}	14.53^{a}	4.76^{ab}	5.94^{ab}	4.63^{b}	1.45^{b}	2.95^{b}	2.48^{ab}	4.05^{ab}	4.88^{ab}	3.43^{b}
Collembola	6.12^{b}	11.16^{bc}	2.38^{bc}	5.43^{bc}	6.23^{bc}	0.00^{c}	5.90^{bc}	0.31^{bc}	6.08^{bc}	38.14^{a}	15.78^{bc}
Dermaptera	2.55^{a}	0.15^{a}	0.79^{a}	1.29^{a}	0.00^{a}	0.00^{a}	2.67^{a}	0.00^{a}	1.35^{a}	1.11^{a}	0.64^{a}
Diptera	9.69^{a}	1.83^{b}	0.79^{b}	2.07^{b}	3.67^{b}	1.89^{b}	2.81^{b}	1.86^{b}	2.03^{b}	3.55^{b}	1.91^{b}
Hemiptera	0.00^{ab}	3.21^{a}	0.79^{ab}	2.33^{ab}	1.44^{ab}	0.00^{b}	1.69^{ab}	1.24^{ab}	0.68^{ab}	2.22^{ab}	1.67^{ab}
Homoptera	0.00^{b}	0.15^{b}	0.79^{b}	2.33^{a}	0.64^{b}	0.44^{b}	2.95^{ab}	0.00^{b}	0.00^{b}	0.22^{b}	0.00^{b}
Hymenoptera—other	0.00^{a}	0.00^{a}	0.00^{a}	0.52^{a}	0.00^{a}	0.00^{a}	0.14^{a}	0.00^{a}	0.00^{a}	0.22^{a}	0.00^{a}
Formicidae	18.88^{bc}	35.02^{bc}	66.67^{b}	40.83^{bc}	23.96^{bc}	92.44^{a}	13.48^{bc}	8.05^{c}	18.92^{bc}	20.62^{bc}	11.31^{bc}
Isoptera	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.28^{a}	1.55^{a}	0.00^{a}	0.22^{a}	0.00^{a}
Lepidoptera—adult	1.53^{a}	0.31^{a}	0.00^{a}	1.29^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Lepidoptera—larvae	1.71^{b}	0.00^{b}	0.00^{b}	0.00^{b}	2.08^{a}	0.15^{b}	0.56^{b}	0.62^{ab}	1.35^{ab}	0.44^{b}	0.00^{b}
Phthiraptera	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.16^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Pscocptera	0.00^{ab}	1.22^{ab}	0.00^{ab}	1.03^{ab}	0.96^{b}	0.00^{b}	1.40^{ab}	0.93^{ab}	0.00^{ab}	5.54^{a}	1.51^{ab}
Thysanoptera	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.16^{a}	0.00^{a}	0.28^{a}	0.62^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Unknown eggs	0.00^{ab}	0.00^{b}	0.00^{ab}	0.00^{ab}	0.00^{ab}	0.00^{ab}	0.00^{b}	0.00^{ab}	0.00^{ab}	0.22^{a}	0.00^{ab}
Unknown larvae	0.00^{a}	0.76^{a}	0.79^{a}	0.00^{a}	0.96^{a}	0.73^{a}	0.00^{a}	0.31^{a}	0.00^{a}	0.67^{a}	0.56^{a}
Malacostraca											
Amphipoda	35.20^{ab}	19.11^{cd}	11.11^{bcd}	14.47^{cd}	36.74^{abc}	0.00^{d}	29.07^{bc}	22.91^{cd}	5.41^{d}	0.00^{d}	32.03^{a}
Isopoda	4.59^{bc}	0.61^{c}	0.00^{bc}	2.58^{bc}	2.40^{c}	1.16^{bc}	15.17^{b}	17.96^{a}	27.03^{ab}	1.55^{bc}	14.66^{bc}
Pseudoscorpionida	0.00^{a}	0.15^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.44^{a}	0.28^{a}	1.86^{a}	1.35^{a}	0.00^{a}	0.00^{a}

consumed a greater number of Isopoda (18%) and Blattodea (1%), but fewer Hymenoptera (8%) and Collembola (<1%) compared to other sites. At AK, frogs consumed more Diptera (10%) and Araneae (6%) than at any other site.

Prey preferences.—A total of 13,662 invertebrates was collected and identified in the environmental samples (Table 4). The first PCA was conducted to determine which environmental samples were similar to stomach samples. The first PC (PC-1) separated foliage samples from flying samples (Fig. 2), loading positively on Collembola (0.90) and negatively on Coleoptera (−0.22), Diptera (−0.34), and Lepidoptera (−0.13). The second PC (PC-2) separated litter samples from foliage or flying samples, loading positively on Amphipoda (0.16), Hymenoptera (0.46), and Isopoda (0.26), and negatively on Collembola (−0.19), Coleoptera (−0.23), Diptera (−0.41), and Lepidoptera (−0.15). The first two PCs captured 62% of the total variation in invertebrate composition of these samples. Differences among the four samples were detected (ordtest: $P = 0.0001$). The analysis suggests that invertebrates collected in litter samples were most similar to stomach samples; however, as identified in the PCA using only foliage samples (Fig. 3A), stomachs were more similar to foliage samples at PK.

To better analyze preferences for invertebrates in litter, another PCA was conducted using stomach and leaf litter samples only (Fig. 3B). Principle component one separated Hymenop-

TABLE 4. INVERTEBRATES COLLECTED IN ENVIRONMENT SAMPLES. Mean number of individuals for each category (± SE) collected from 11 sites in Hawaii using light traps, beating trays, and extracted from leaf litter (n = 1471, 4489, and 7702 individuals collected, respectively). *Beating tray and leaf litter subsamples were averaged within site before averaging across sites.

Class Order	Collection method Light trap	beating tray*	Leaf litter*
Arachnida			
Acarina	0 (0)	7.07 (2.08)	36.41 (8.10)
Araneae	0.27 (0.19)	0.73 (0.32)	1.77 (0.93)
Chilopoda	0 (0)	0 (0)	0.34 (0.15)
Diplopoda	0 (0)	0.09 (0.09)	1.05 (0.65)
Gastropoda	0 (0)	2.11 (1.11)	5.32 (2.34)
Insecta			
Blattodea	0 (0)	0.07 (0.04)	0 (0)
Coleoptera	25.55 (8.30)	0.25 (0.13)	4.55 (1.65)
Collembola	0.82 (0.54)	66.57 (24.24)	10.59 (2.50)
Dermaptera	0.18 (0.18)	0 (0)	0.70 (0.30)
Diptera	45.09 (16.17)	0.20 (0.09)	0.23 (0.10)
Embiidina	0 (0)	0 (0)	0.05 (0.05)
Hemiptera	11.36 (4.70)	0.59 (0.17)	0.55 (0.24)
Homoptera	6.36 (3.91)	0.75 (0.21)	0.91 (0.34)
Hymenoptera	2.18 (1.32)	19.93 (10.39)	70.80 (27.87)
Isoptera	6.18 (4.35)	0 (0)	0 (0)
Lepidoptera	25.45 (9.08)	0.02 (0.02)	0.18 (0.14)
Nueroptera	0.09 (0.09)	0.02 (0.02)	0 (0)
Orthoptera	0.18 (0.18)	0.14 (0.09)	0.11 (0.11)
Pscocptera	7.64 (7.34)	1.05 (0.47)	0.11 (0.05)
Thysanoptera	0 (0)	0.20 (0.09)	0.36 (0.23)
Trichoptera	2.09 (1.09)	0.02 (0.02)	0 (0)
Unknown larvae	0 (0)	1.73 (0.79)	5.52 (2.58)
Malacostraca			
Amphipoda	0.18 (0.18)	0.02 (0.02)	14.70 (4.04)
Isopoda	0 (0)	0.30 (0.08)	19.91 (3.92)
Oligochaeta	0 (0)	0.02 (0.02)	0.25 (0.13)
Pseudoscorpionida	0 (0)	0 (0)	0.05 (0.03)
Symphyla	0 (0)	0 (0)	0.09 (0.07)
Total	133.73 (31.95)	102.00 (33.65)	175.02 (29.26)

tera from other common litter invertebrates, loading positively on Acarina (0.32), Amphipoda (0.14), and Isopoda (0.16), and negatively on Hymenoptera (−0.92). Principle component two mostly separated Acarina and Amphipoda, loading positively on Acarina (0.62), Hymenoptera (0.11), and larvae (0.13), and negatively on Amphipoda (−0.76). These two PCs captured 79% of the total variation in invertebrate composition of these samples. No differences among the stomach and litter samples or among sites were detected (ordtest: P = 0.10, 0.12, respectively). To determine if stomach contents were more similar to each other than to litter invertebrates, and if stomach contents and litter invertebrates were more similar to each other at each site than contents and invertebrates collected at other sites, I conducted ANOVAs using PCA outputs. These analyses revealed that for PC-1, stomach contents and litter samples were not different, and that there were no site differences for stomach and litter samples ($F_{1,10}$ = 0.81, P = 0.39, $F_{10,10}$ = 2.53, P = 0.080). For PC-2, however, stomach and litter samples were different, and stomach and litter samples were different by site ($F_{1,10}$ = 19.80, P = 0.0012, $F_{10,10}$ = 3.12, P = 0.044).

Relative proportions of invertebrates consumed differed from invertebrates collected for most taxa. In the leaf litter, Amphipoda (L = 8.4), Collembola (3.6), and Hymenoptera (4.7) were over-represented in stomach samples,

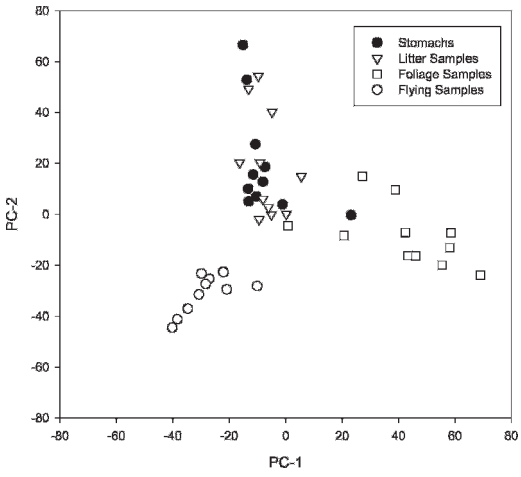

Fig. 2. PCA of invertebrate categories found in stomachs of *E. coqui* and flying, foliage, and litter invertebrate communities sampled in each of 11 study sites in Hawaii. Invertebrates were categorized as in Table 4.

whereas Acarina (−15.7) and Isopoda (−3.9) were under-represented in stomach samples. On foliage, Coleoptera (4.5) and Hymenoptera (22.1) were over-represented in stomach samples, whereas Acarina (−6.1), Collembola (−48.5), and Gastropoda (−4.1), were under-represented. All taxa in light trap samples were under-represented in stomachs (Coleoptera [−20.1], Diptera [−30.2], and Lepidoptera [−13.8]).

Discussion

Amphipoda (amphipods), Formicidae (ants), and Isopoda (isopods) were the three most important prey categories for *E. coqui*. These three categories represented nearly 60% of the diet of *E. coqui* across the 11 sites. In the environmental samples, there were eight species of non-native ants, one non-native amphipod (Talitridae: *Talitroides topitotum*), and one non-native isopod (Porcellionidae: *Porcellio laevis*). Thus, nearly 60% of their diet consisted of non-native species. It is not surprising that non-natives constitute the majority of their diet because most of the study sites have disturbed native vegetation and are dominated by non-native plants. It is important to note that *E. coqui* had been established in each of the study sites for at least three years prior to sampling. Sites with established populations of *E. coqui* were chosen because areas where populations can persist are desirable for diet analyses. It is possible that endemic prey were a more important component of the diet of *E. coqui* when it first invaded these

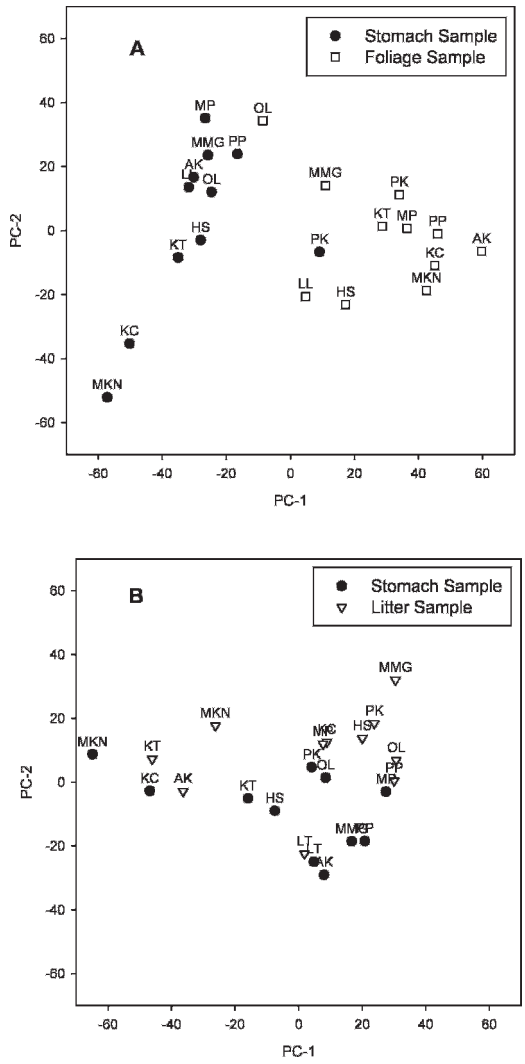

Fig. 3. PCA of invertebrate categories found in stomachs of *E. coqui* and (A) foliage invertebrate communities, and (B) litter invertebrate communities in each of 11 study sites in Hawaii.

sites, and that these populations were reduced or depleted prior to sampling.

It has been suggested that *E. coqui* may reduce non-native Culicidae (mosquitoes) and Isoptera (termites) in Hawaii (Fullington, 2001; Singer, 2001). However, there is little evidence that *E. coqui* are reducing these arthropods. No Culicidae, adults or larvae, were found in 696 stomachs. Similarly, *E. coqui* has not been observed to consume many mosquitoes in Puerto Rico (Stewart and Woolbright, 1996). Of the Diptera found in stomachs, 32% were in the family Tipulidae (crane flies). Crane flies are similar morphologically to mosquitoes, and their discovery in stomachs suggests that mosquitoes

would have been observed if they were present. Similar to data from Puerto Rico (Stewart and Woolbright, 1996), termites were found to constitute a small percentage of the diet of *E. coqui* (<1% of the total prey items).

Determining the types and amount of endemic invertebrates that *E. coqui* consume is necessary because their invasion is likely to impact invertebrates. This is straightforward for some groups that are only represented by non-natives (e.g., ants, termites) in Hawaii, but requires identifying invertebrates to lower taxonomic categories for groups that are represented by both endemics and non-natives. Endemic Acarina (mites), Coleoptera (beetles), Collembola (springtails), Diptera (flies), and Gastropoda (snails) appear to be the most vulnerable to predation; each composed more than 1.5% of their diet and had importance values >4.5. Of the 46 beetle species collected, 24 (52%) were identified as possibly endemic (Michael Ivie, pers. comm.). Of the 15 species of springtails collected in environmental samples, nine (60%) were identified as possibly endemic (David Preston, pers. comm.). Of the 106 flies species collected, 64 (60%) were identified as possibly endemic (David Preston, pers. comm.). Of the 21 snail species collected, 12 (57%) were identified as possibly endemic (Robert Cowie, pers. comm.). I was unable to find an expert who could identify the >70 mite species as endemic or non-native. Additionally, springtails in litter and beetles on foliage may be particularly vulnerable because they were over-represented in stomach samples compared to environment samples, indicating a preference for these orders.

As a caveat, any conclusions regarding prey preferences, both positive and negative, assume that invertebrate samples accurately reflect what prey is available to foraging frogs. In fact, it is virtually impossible to design a sampling system that accurately reflects what invertebrates are available to frogs. In this study, light traps used to sample flying insects are the most obviously biased method; beating trays and litter extraction probably provided better estimates. It is important to note that if the methods used for sampling invertebrates were biased, this would influence the preferences determined in this study.

With this in mind, some non-native invertebrates (ants and amphipods) were more abundant in stomachs of *E. coqui* than in the environment. For example, ants and amphipods in litter and ants on foliage were over-represented in stomachs compared to environmental samples. However, the results also suggest that *E. coqui* is not dependent on the presence of these prey to invade a site. Ants were present in stomachs at every site, but their percentage of total prey ranged from 8 to 92% across sites. Amphipods were not present in stomachs or the environment in two of the 11 invaded sites. In general, the results reveal some prey preferences that appear to create consistencies in diets across sites; however, the results also reveal that *E. coqui* are opportunistic and can change dominant prey depending on availability.

Multivariate analyses strongly suggest that *E. coqui* in Hawaii forage mostly in the leaf litter. In this study, frog collections started at 2000 h to maximize sample sizes. Woolbright and Stewart (1987) showed that stomach passage time is approximately 12 h. Thus stomachs collected between 2000 h and 2200 h include prey consumed during the day and for 1–3 hrs after dark. Stomach data from Puerto Rico collected at 0600 h show that leaf litter invertebrates are underrepresented in stomachs, and that foliage invertebrates, such as Blattodea, Homoptera, and Orthoptera, are more important than what was found in this study (Stewart and Woolbright, 1996). It has been suggested that *E. coqui* in Puerto Rico only capture prey from litter while moving from diurnal retreat sites, which are often close to or in leaf litter, to nocturnal perch sites, and that they actively forage while on vegetation at night (Stewart and Woolbright, 1996). If the stomach contents presented here mostly reflect diurnal foraging, when *E. coqui* are in their retreats on the ground, then the difference in collection times could explain why the Puerto Rico studies found mainly foliage invertebrates and this study found mainly litter invertebrates.

However, if the difference in collection times caused the difference in primary foraging microhabitats observed between Puerto Rico and Hawaii, then the results from this study highlight the importance of diurnal feeding for *E. coqui* in Hawaii. In Puerto Rico, adults and subadults consume around three and six prey items per night, respectively (Townsend, 1985; Woolbright, 1985); whereas, in this study, adults and subadults had six and 11 prey items in their stomachs, respectively. Furthermore, in Puerto Rico, 16% of female and male adults collected at 0600 h had empty stomachs (Woolbright and Stewart, 1987), while in this study the percentage is comparatively less (3% and 7% for females and males, respectively).

Another potential explanation for the difference in foraging microhabitat between Puerto Rico and Hawaii might be the use of different microhabitat structures at night, when they are expected to be actively foraging (Stewart and Woolbright, 1996). However, this did not appear

to be the case because microhabitat use by adults and subadults was consistent across ranges. In both Puerto Rico and Hawaii, adults are typically found on trunks, branches, or leaves (Townsend, 1989; Beard et al., 2003b), and subadults are typically found on leaves (Townsend, 1985; Beard et al., 2003b). Furthermore, heights from the forest floor at which adults and subadults are found are similar between Puerto Rico and Hawaii (Pough et al., 1983; Townsend, 1985; Beard et al., 2003b). More research is needed to determine the mechanism driving the difference in foraging microhabitat in Puerto Rico and Hawaii; some of the questions posed here could be addressed if this study was repeated with frogs collected at 0600 h.

Many results from stomach content analyses were different between Puerto Rico and Hawaii; however, there were some important similarities. In both ranges, subadults consume more, smaller prey items (mites, springtails, and spiders) and have greater prey diversity than adults (Townsend, 1985). More adults, especially males, have empty stomachs than subadults (Woolbright and Stewart, 1987; Stewart and Woolbright, 1996). This is thought to occur because males that are actively calling spend less time foraging (Woolbright and Stewart, 1987). The importance of ants in the diet of *E. coqui* was also similar. In this study, 30% of prey items and 8% of prey volume were ants. In Puerto Rico, ants make up 38% of prey items and 6% of prey volume (Stewart and Woolbright, 1996). One major difference is that the most important prey item in Hawaii, amphipods, is not present in Puerto Rico.

Because sampling was not replicated within sites across time, it is possible that daily weather differences affected the behavior and foraging of frogs and some of the site differences found in this study. However, sampling across sites did occur close together in time, and weather across sites during collections differed only slightly: by 1–2 C, by 10% relative humidity, and between 1–2 days since the last rain event (K. Beard, unpubl. data). Furthermore, at seven of the study sites, *E. coqui* have been further studied, and microhabitat use has not been found to vary within sites across time, but rather appears to reflect habitat availability (K. Beard, unpubl. data). Nevertheless, diet studies should be repeated at these sites across time to determine the robustness of diet differences by site found in this study.

Further study is needed to determine the consequences of the *E. coqui* invasion on invertebrates in Hawaii. Stomach analyses have a known bias toward invertebrates with robust body parts (Iverson et al., 2004). Thus, it may be important to confirm results from stomach analyses with perhaps isotopic analyses of tissue from prey and *E. coqui* to determine which prey have sustained use. Canopy foraging should be explored in future studies because *E. coqui* in the canopy have been found to consume different prey from those that forage in the understory (Stewart, 1985; Stewart and Woolbright, 1996). It is important to determine how invertebrate communities have changed in areas where *E. coqui* have invaded, perhaps by conducting retrospective analyses where invertebrate communities were studied prior to invasion. Finally, this study did not reveal the indirect effects of *E. coqui* predation on other parts of the invertebrate community or on ecosystem processes (Beard and Pitt, 2005). Many invertebrates that *E. coqui* consume play important roles in ecosystem processes, such as pollination, herbivory, and decomposition of plant material (Beard et al., 2002, 2003a). Future research should use experimental techniques to determine these indirect effects.

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