Final Report, November 2007

Title of Project: Ecological consequences of the coqui frog invasion into Hawaii
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The following report describes the research that I conducted in part with the grant I received from the Hawaii Invasive Species Council, Department of Land and Natural Resources. I have broken this report into sections that describe each of the research projects that were conducted. Under each section, I present for each study, the objectives, general approach, results, and location where the results are available. With this report, I have also included pdfs of published manuscripts that are already in press as a result of this research. As you will see the Hawaii Invasive Species Council, Department of Land and Natural Resources was acknowledged for funding support, as appropriate.

Small-scale experiment to determine ecological impacts

<u>Experiment #1: Enclosure experiment to determine effects on invertebrates and ecosystem processes</u>

Objectives: I conducted research on *Eleutherodactylus coqui* in Puerto Rico and found that it reduces phytophagous arthropods and herbivory rates, and increases nutrients available to decomposers, leaf-litter decomposition rates, and plant production rates. The objective of this study was to repeat the experiment that I conducted in Puerto Rico, in Hawaii, so that we could determine the effects of *E. coqui* on invertebrates, nutrient cycling, and ecosystem processes in Hawaii.

Approach: To address this objective, we conducted an enclosure experiment in two locations (Leilani and Bryson's Cinder) on the Island of Hawaii. We used the same methods that were used in Puerto Rico so that results were comparable. At each location, 20 1 x 1 x 1-m enclosures were constructed using a PVC frame covered by plastic mesh. Enclosures were placed on the forest floor in pairs. One enclosure in each pair contained frogs at natural densities and the other contained no frogs. Enclosures were monitored for 6 months and the following measurements were taken: arthropod densities and composition, herbivory rates, growth rates of both native and invasive plants, throughfall water chemistry, and leaf-litter decomposition rates.

Results: Results showed that aerial, herbivorous, and leaf litter invertebrates were reduced with *E. coqui* at the Leilani site, but not at the Bryson site. Herbivory was reduced for all plants species (*Psychotria mariniana*, *Melastoma candidum*, and *Psidium cattleianum*) at both sites with *E. coqui*. Across sites, *E. coqui* increased NH_4^+ and P concentrations in throughfall, increased Mg, N, P, and K in decomposing leaf litter, increased new leaf production of *P. cattleianum*, and increased leaf litter decomposition rates of *M. polymorpha.* In summary, *E. coqui* effects on invertebrates differed by site, but *E. coqui* effects on ecosystem processes were similar across sites. Path analyses suggest that *E. coqui* increased the number of new leaves and leaf litter decomposition rates by making nutrients more available to plants and microbes rather than through changes in the invertebrate community. Results suggest that *E.*

coqui have the potential to reduce endemic invertebrates and increase nutrient cycle rates, which may confer a competitive advantage to invasive plants.

The results of this study are published in: *Sin*, *H*.*, K.H. Beard, and W.C. Pitt. 2007. An invasive frog, *Eleutherodactylus coqui*, increases new leaf production and leaf litter decomposition rates through nutrient cycling in Hawaii. *Biological Invasions* (Accepted) 11 June 2007 doi: 10.1007/s10530-007-9133-x. Available on-line.

<u>Experiment #2: Common garden experiment to determine relative importance of leaf litter and coqui frogs on the invertebrate community</u>

Objectives: We determined the relative importance of litter resources, from an invasive nitrogen-fixer, *Falcataria moluccana*, and a native tree, *M. polymorpha*, and predation effects of an invasive terrestrial frog, *E. coqui*, on leaf litter invertebrate abundance and composition in a Hawaiian lowland forest. We also determined whether different leaf litter types limit the number of prey items available to *E. coqui* and whether this in turn influences *E. coqui* survivorship and reproduction.

Approach: We conducted an enclosure experiment at the Leilani site, Big Island, Hawaii. Forty 1 x 1 x 1-m enclosures constructed using a PVC frame and covered by plastic mesh were placed on the forest floor. There were 10 enclosures with each of the four treatments: *M. polymorpha* with frogs, *M. polymorpha* without frogs, *F. moluccana* with frogs, and *F. moluccana* without frogs. Enclosures were monitored for 5 months and the following measurements were taken: leaf litter arthropod densities and composition, leaf-litter decomposition rates, and growth and survivorship of adult frogs.

Results: Principle component analysis revealed that *F. moluccana* litter creates an invertebrate community that greatly differs from the invertebrate community found in *M. polymorpha* litter. More specifically, we found that *F. moluccana* increased non-native fragmenter (Amphipoda and Isopoda) abundance four-fold, non-native predaceous ant (Hymenoptera: Formicidae) abundance two-fold, and microbivore (mostly Acari and Collembola) abundance two-fold. In contrast, *E. coqui* had little effect on the litter invertebrate community, except that it reduced microbivores by 40% and non-native ants by 30% in *F. moluccana*. Furthermore, *E. coqui* stomach contents were similar in abundance and composition in *F. moluccana* and *M. polymorpha* litter treatments, despite the dramatic differences in the invertebrate community. Electivity analyses showed that *E. coqui* prefer non-native Amphipoda and avoid non-native Isopoda across litter types. Finally, our results suggest that invertebrate community differences between litter types did not cascade to influence *E. coqui* growth or survivorship. In conclusion, the results suggest that an invasive tree species with leaf litter that is high in nitrogen content and decomposes rapidly has a greater influence on litter invertebrate community abundance and composition than the invasive predator, *E. coqui*.

We plan to publish results in: Tuttle, N.C., K.H. Beard, and W.C. Pitt. (in preparation) Litter resources, not an invasive insectivore, determine invertebrate communities in a Hawaiian lowland forest. *Diversity and Distributions.* To be submitted: 05 December 2007.

Large-scale experiments to determine ecological impacts:

<u>Experiment #3: Coqui diet study</u>

Objectives: Because *E. coqui* is a generalist insectivore, one of the mostly likely impacts that it will have in Hawaii is a reduction of invertebrate populations. This is of concern because there are a large number of endemic and endangered invertebrates in Hawaii. The objectives of this study were: (1) to quantify the amount and types of invertebrate that *E. coqui* consume in different forest types, (2) to identify *E. coqui* prey preferences, and (3) to relate *E. coqui* densities to prey availability and habitat structure.

Approach: E. coqui diets were compared to invertebrate abundances in seven sites on the Islands of Hawaii and two sites on Maui in the summer of 2004. At each site, 22-119 frogs from 20 x 20-m plots, and invertebrates in light traps, beating trays, and leaf litter samples were collected. Prey items in frog stomachs and invertebrates collected in environmental samples were identified to scientific order or family.

Results: Multivariate analyses of these data suggest that *E. coqui* is mostly foraging in 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 *E. coqui* stomachs 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 Collembola (springtails), Acarina (mites), Coleoptera (beetles), and Diptera (flies), which each made up greater than 2% of the *E. coqui* diet. Dominant prey items in frog stomachs differed among study sites suggesting that frogs are opportunistic feeders and prey-switch to abundant prey items. The results suggest that *E. coqui* management should focus on native-dominated forests and areas with new invasions because it is in these locations where *E. coqui* are most likely to consume endemic invertebrates and have the greatest impact.

The results of this study are published in: Beard, K.H. 2007. Diet of the invasive frog, *Eleutherodactylus coqui*, in Hawaii. *Copeia* 2007(2):281-291.

Experiment #4: Predator diet study

Objective: The objective of this study was to determine if *E. coqui* are an important food resource for known bird predators.

Approach: Small Indian mongooses (*Herpestes auropunctatus*), black rats (*Rattus rattus*) and Polynesian rats (*R. exulans*) were trapped at Lava Tree State Park, a site known to have high *E. coqui* densities. We used a trap web design to concurrently estimate mammal population densities and conduct stomach analyses. The trap web has a total of 256 traps, half of which are designed to capture rats and half of which are designed to capture mongoose. We also hand captured *Bufo marinus* (cane toads). All stomach contents of trapped individuals were identified.

Results: Of the 44 rats trapped, we found that no *E. coqui* were consumed. Of the 22 mongoose trapped, three contained a total of 16 *E. coqui*. These *E. coqui* represented 6.6 % of mongoose prey items by weight. We suggest that a *H. javanicus* consumption rate of 3 frogs ha^{-1} day^{-1} is unlikely to significantly reduce *E. coqui* populations. We collected 27 *Bufo marinus* and found that they did not consume *E. coqui*, but may be competing with *E. coqui* for prey. We suggest that *E. coqui* is not bolstering populations of rats or cane toads at this site, but may be influencing mongoose populations.

The results of this study are published in: Beard, K.H. and W.C. Pitt. 2006. Potential predators of an invasive frog (*Eleutherodactylus coqui*) in Hawaiian forests. *Journal of Tropical Ecology* 22(4):1-3.

Experiment #5: Continued long-term monitoring of established populations

Objective: Starting in 2004, we have been estimating *E. coqui* populations at eight locations on the Island of Hawaii. These measures allow us to develop a better understanding of the growth and spread of *E. coqui* populations. To determine possible mechanisms of control, we also related these densities to elevation, snout-vent length (SVL), habitat structure in the understory, and invertebrate abundance collected at each location.

Approach: We used mark-recapture methods to estimate *E. coqui* survival and abundance, and determined growth rates of adult male and female frogs. We analyzed this data using complex population models and the program MARK. We related densities to elevation, SVL, habitat structure in the understory, and invertebrate abundance using ANOVA models in SAS.

Preliminary Results: While density estimates for adult *E. coqui* vary across sites, estimates of long-term averages from Puerto Rico range from 1-33 adults/100 m^2. We found that for all sites surveyed, except for OL and KP, mean adult estimates were greater than the highest long-term estimates from Puerto Rico. In fact, we found that adult densities were about three times greater at three of our study sites (around 100 adults/100 m^2) than the highest long-term estimates from Puerto Rico. Our highest estimates for sampling periods were 133 and 138 adults/100 m^2 in Lava Tree and Manuka Natural Area Reserve, respectively, and were higher than the highest estimate for a sampling period in Puerto Rico, 114 adults/100 m^2.

We found that, averaged over the three years, total *E. coqui* density across sites ranged from was 2,200-50,000 frogs/ha. At four of the eight study sites, total frog densities were at least 1.7 times greater (35,000 frogs/ha) than mean long-term estimates for the eastern mountains of Puerto Rico (20,570/frogs ha; elevation 350 m). Our highest total density estimate was for Manuka Natural Area Reserve in 2004, where we estimated there were 91,000 frogs/ha.

Mean growth rate was 0.0078 mm/day (± 0.007 SD, n = 87) for males and 0.0097 mm/day (± 0.0088 SD, n = 11). Growth rates were smaller for adult females in Hawaii than adult females of similar size in Puerto Rico. Results suggest there is no relationship between elevation and *E. coqui* density or SVL, or between *E. coqui* density and invertebrate abundance. However, there was a positive relationship between *E. coqui* density and understory structure. The strong relationship between *E. coqui* density and understory structure suggests that control should be targeted to areas with great understory structure and that removing understory structure should reduce *E. coqui* densities.

We plan to publish results in: Beard, K.H., R. Al-Chokhachy, *N.C. Tuttle**, and *E.M. O'Neill** (in preparation) Population density and growth rates of *Eleutherodactylus coqui* in Hawaii. *Journal of Herpetology* To be submitted 05 December 2007

Experiment #6: Effect of aerially applied citric acid on *E. coqui* populations

Objective: Citric acid is approved for controlling frogs, but has been limited to accessible terrain. The objective of this study was to determine the effectiveness of helicopter applications of 16% citric acid and repeated 11% citric acid treatments.

Approach: We did this by monitoring frogs before and after treatment using toe clips. There were two control plots (C1 and C2) that were never treated with citric acid and two treated plots (T1 and T2). We also evaluated the effects of toe clipping and weather changes on population parameter estimates.

Results: We found that a 16% citric acid treatment reduced adult frog density 3-fold in a plot, T1, completely covered with citric acid, but did not reduce adult density in a plot, T2, where

6% of the plot was unintentionally not treated. Preadults were reduced 3- to 5-fold in treated plots. The reduction in adults in T1 lasted at least five months. Repeated treatments of 11% citric acid were studied in T2 and reduced adults 440-fold while preadults were reduced 9-fold. Frogs with fewer toes clipped had greater recapture probability and weather changes had no effect on parameter estimates. In summary, we found that 16% and 11% citric acid treatments can reduce frog density, treatment effects can last five months for adults, and repeated treatments appear more effective than single applications.

Results have been submitted to: Tuttle, N.C., K.H. Beard, and R. Al-chonsky. (in review) Aerially applied citric acid reduces an invasive frog. *Wildlife Research.* Submitted: 05 September 2007

Genetic analyses on* E. coqui*:

<u>Experiment #7: Potential biocontrol agent for *E. coqui*</u>

Objective: The objective of this study was to determine if chytrid fungus was already present on *E. coqui* in Hawaii because managers had suggested using chytrid fungus as a biocontrol agent.

Approach: We used *E. coqui* collected from the Big Island and Maui and used genetic analyses to identify the marker known to reveal chytrid presence.

Results: We found that a small percent of *E. coqui* in Hawaii already carry chytrid fungus. We suggest that it not be introduced to Hawaii to control *E. coqui* as it does not appear to negatively affect them and increased prevalence could increase the risk of chytrid escaping to other Pacific Islands.

The results of this study are published in: Beard, K.H. and *E.M. O'Neill**. 2005. Infection of an invasive frog *Eleutherodactylus coqui* by the chytrid fungus *Batrachochytrium dendrobatidis* in Hawaii. *Biological Conservation* 126(4): 591-595.

<u>Experiment #8: Understanding the *E. coqui* invasion</u>

Objective: I have also been working with a number of colleagues to determine the source of the *E. coqui* introduction, whether there have been bottlenecks, and where multiple introductions might have taken place.

Approach: We have not completed this research yet, but we have made a lot of progress towards achieving these goals. Using mtDNA from Hawaii and Puerto Rico, we have identified the origin of frogs in Hawaii. We have also identified microsatellite markers for *E. coqui*, something that had not been previously done. We are in the process of using these markers to identify bottlenecks, diversity, and sources of introduction across Hawaii.

Preliminary Results: This project is still in the analysis phase. *Results from this research can be found in:*

Velo-Antón, *G*., P.A. Burrowes, R. Joglar, I. Martínez-Solano, K.H. Beard, and G. Parra-Olea. 2007. Phylogenetic study of *Eleutherodactylus coqui* (Anura: Leptodactylidae) reveals deep genetic fragmentation in Puerto Rico and origins in Hawaii. *Molecular Phylogenetics and Evolution* 45(2007):716-728.

Peters, M., K.H. Beard, C. Hagen, *E.M. O'Neill**, K.E. Mock, W.C. Pitt, and T.C. Glenn. Isolation of microsatellite loci from the coqui frog, *Eleutherodactylus coqui*. *Molecular Ecology Notes* (Accepted) 3 June 2007. doi:10.1111/j.1471-8286.2007.01899.x Available on-line.

DELIVERABLES:

GRADUATE STUDENTS AND DEGREES:

Five graduate students have been involved in and greatly contributed to this research:

- Hans Sin, MS Wildlife Biology, fall 2004-fall 2006 (Thesis title: *Eleutherodactylus coqui* influences lowland forest invertebrate communities and ecological processes in Hawaii, Graduation Date: December 2006)
- Nathania C. Tuttle, MS Ecology, spring 2005-fall 2007 (Thesis title: Assessing mechanisms of invasion and control of *Eleutherodactylus coqui* in Hawaii, Graduation Date: December 2007)
- Eric M. O'Neill, PhD Biology, spring 2006-present (Thesis title: Genetic and phenotypic variation in the frog, *Eleutherodactylus coqui*, in its native and introduced ranges, Expected Graduation Date: December 2008)
- Simon Bisrat, PhD Ecology, fall 2004-present (Thesis title: Climatic envelope of coqui frogs from Puerto Rico predicts their distribution in Hawaii, Expected Graduation Date: May 2009)
- Emily Price, PhD Ecology, fall 2007-present (Thesis title: Social and political factors influencing the coqui frog invasion in Hawaii, Expected Graduate Date: December 2012)

The following have been submitted to peer-reviewed journals or to books:

PUBLICATIONS:

- * plus italics indicate students for whom I am the primary advisor, italics alone indicates graduate/undergraduate students for whom I am not the primary advisor.

PEER-REVIEWED IN PRESS PUBLICATIONS:

1. *Sin*, *H.**, **K.H. Beard**, and W.C. Pitt. An invasive frog, *Eleutherodactylus coqui*, increases new leaf production and leaf litter decomposition rates through nutrient cycling in Hawaii. *Biological Invasions* (Accepted) 11 June 2007 doi:10.1007/s10530-007-9133-x
2. Peters, M., **K.H. Beard**, C. Hagen, *E.M. O'Neill**, K.E. Mock, W.C. Pitt, and T.C. Glenn. Isolation of microsatellite loci from the coqui frog, *Eleutherodactylus coqui*. *Molecular Ecology Notes* (Accepted) 3 June 2007 doi:10.1111/j.1471-8286.2007.01899.x

PEER-REVIEWED PUBLICATIONS:

* plus italics indicate students for whom I am the primary advisor, italics alone indicates

1. *Velo-Antón*, *G*., P.A. Burrowes, R. Joglar, I. Martínez-Solano, **K.H. Beard**, and G. Parra-Olea. 2007. Phylogenetic study of *Eleutherodactylus coqui* (Anura: Leptodactylidae) reveals deep genetic fragmentation in Puerto Rico and origins in Hawaii. *Molecular Phylogenetics and Evolution* 45(2007):716-728.
2. **Beard, K.H.** 2007. Diet of the invasive frog, *Eleutherodactylus coqui*, in Hawaii. *Copeia* 2007(2):281-291.
3. **Beard, K.H.** and W.C. Pitt. 2006. Potential predators of an invasive frog (*Eleutherodactylus coqui*) in Hawaiian forests. *Journal of Tropical Ecology* 22(4):1-3.
4. **Beard, K.H.** and *E.M. O'Neill**. 2005. Infection of an invasive frog *Eleutherodactylus coqui* by the chytrid fungus *Batrachochytrium dendrobatidis* in Hawaii. *Biological Conservation* 126(4): 591-595.
5. **Beard, K.H.** and W.C. Pitt. 2005. Potential consequences of the coqui frog invasion in Hawaii. *Diversity and Distributions* 11(5):427-433 (**cover article**)

PEER-REVIEWED IN REVIEW/PREPARATION:

1. *Tuttle, N.C.**, **K.H. Beard**, and R. Al-Chokhachy. (in review) Aerially applied citric acid reduces an invasive frog. *Wildlife Research.* Submitted: 05 September 2007

2. *Tuttle, N.C.**, **K.H. Beard**, and W.C. Pitt. (in preparation) Litter resources, not an invasive insectivore, determine invertebrate communities in a Hawaiian lowland forest. *Diversity and Distributions.* Submitted: 05 December 2007
3. **Beard, K.H.**, R. Al-Chokhachy, *N.C. Tuttle**, and *E.M. O'Neill** (in preparation) Population density and growth rates of *Eleutherodactylus coqui* in Hawaii. *Journal of Herpetology* To be submitted 05 December 2007

BOOK CHAPTERS:

1. **Beard, K.H.** 2006. Case Study Box: Puerto Rico and Hawaii: Wet tropical forests and the dilemma of coqui frog conservation and eradication. Pp: 135-137. In: Forests and Society: Sustainability and life cycles of forests in human landscapes Eds. K.A. Vogt, J. Honea, D.J. Vogt, M. Andreu, R. Edmonds, J. Berry, R. Sigurdardóttir, T. Patel-Weynand.

PRESENTATIONS:

PRESENTATIONS AT NATIONAL AND INTERNATIONAL MEETINGS W/PUBLISHED ABSTRACTS:

1. *Tuttle*, *N.C.** and **K.H. Beard**. Bottom-up control determines invertebrate community composition and abundance in Hawaii, Ecological Society of America, San Jose, California, August 2007.
2. **Beard, K.H.** and A. Kulmatiski. Disturbance contingent plant-soil feedback: the passenger takes the wheel, Ecological Society of America, San Jose, California, August 2007.
3. **Beard, K.H.**, *H. Sin**, and W. C. Pitt. An invasive frog influences ecosystem processes similarly in its native and introduced ranges. Ecological Society of America, Memphis, Tennessee, August 2006.
4. *Sin*, *H.**, **K.H. Beard**, and W. C. Pitt, Effects of an invasive frog on invertebrate community and ecosystem processes in Hawaii, Vertebrate Pest Conference, Berkeley, California, March 2006.
5. **Beard, K.H.** and *Tuttle*, *N.C.** Direct effects of coqui frogs (*Eleutherodactylus coqui*) on invertebrate communities in Hawaii, Hawaii Conservation Conference, Honolulu, Hawaii, July 2005.
6. **Beard, K.H.** and W. C. Pitt, Potential consequences of the coqui frog invasion in Hawaii, ***Invited Symposium***: Introduced Amphibians and Reptiles, Joint Meeting of Ichthyologists and Herpetologists, Tampa, Florida, July 2005.

PRESENTATIONS AT NATIONAL AND INTERNATIONAL MEETINGS WITHOUT PUBLISHED ABSTRACTS:

7. *Sin*, *H.**, **K.H. Beard**, and W.C. Pitt. Top-down effects of coquis in lowland forests in Hawaii. Hawaii Ecosystem Meeting, University of Hawaii, Hilo, Hawaii, June 2007.
8. **Beard, K.H.**, *H. Sin**, and W. C. Pitt. Effects of coqui frogs on ecosystem processes in Hawaii. Hawaii Ecosystem Meeting, Volcanoes National Park, Hawaii, July 2006.
9. *Tuttle*, *N.C.** and **K.H. Beard.** Direct effects of coqui frogs on arthropods in Hawaii. Annual Berryman Symposium, Logan, Utah State University, August 2005.
10. *Sin*, *H.**, **K.H. Beard**, and W.C. Pitt. Effects of coqui frogs on nutrient cycling in Hawaii. Annual Berryman Symposium, Logan, Utah State University, August 2005.

INVITED PRESENTATIONS AT UNIVERSITIES:

11. **Beard, K.H.** Can an invasive frog influence ecosystem processes? Department of Ecology, Montana State University, Bozeman, Montana. December 6, 2007.
12. **Beard, K.H.** Mechanisms and consequences of coqui frog invasions in Hawaii. Department of Wildland Resources, Utah State University, Logan, Utah. March 1, 2007.
13. **Beard, K.H.** Mechanism of coqui frog invasions in Hawaii. Department of Biology, Utah State University, Logan, Utah. April 25, 2006.
14. **Beard, K.H.** Ecological consequences of the coqui invasion. Department of Biology, University of Hawaii, Hilo. November 9, 2004.

OTHER INVITED PRESENTATIONS:

15. **Beard, K.H.** Effects of coqui frogs in Hawaii. Kaloka Coqui Community Coalition. Kaloko Mauka, Kona, Hawaii. October 22, 2005.
16. **Beard, K.H.** Mechanisms of invasion: Coquis in Hawaii. Department of Agriculture, Plant Quarantine Branch, Honolulu, Hawaii. October 14, 2005.
17. **Beard, K.H.** Ecological consequences of the coqui frog invasion: is there support for the hype? U.S. Forest Service, Hilo, Hawaii. November 11, 2004.
18. **Beard, K.H.** Coqui effects on prey and ecosystem processes in Puerto Rico. U.S. Fish and Wildlife Service, Honolulu, Hawaii. June 6, 2003.
19. **Beard, K.H.** Coqui effects on their prey and ecosystem processes in their native community. Nurserymen Association, Hilo, Hawaii. June 4, 2003.
20. **Beard, K.H.** Coqui effects on prey and ecosystem processes in their native community. Coqui Working Group, Hilo, Hawaii. June 3, 2003.

NEWSLETTERS AND NEWSPAPER ARTICLES:

Beard, K.H. Spring 2007. Coqui frogs as predators and prey in Hawai'i. Newsletters of the Maui Invasive Species Council. Invited article for the "New Science" column.