Biological Control 54 (2010) 69–74

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Preliminary evaluation of the potential of the helminth parasite *Rhabdias elegans* as a biological control agent for invasive Puerto Rican coquís (*Eleutherodactylus coqui*) in Hawaii

Shenandoah R. Marr [a], Steve A. Johnson [b,c,*], Arnold H. Hara [a], Monica E. McGarrity [c]

[a] *Beaumont Agricultural Research Center, College of Tropical Agriculture and Human Resources, University of Hawaii at Mānoa, 875 Komohana Street, Hilo, HI 96720-4037, USA*
[b] *Department of Wildlife Ecology, University of Florida Institute of Food and Agricultural Sciences, 110 Newins-Ziegler Hall, P.O. Box 110430, Gainesville, FL 32611-0430, USA*
[c] *Gulf Coast Research and Education Center, University of Florida Institute of Food and Agricultural Sciences, 1200 North Park Road, Plant City, FL 33563-1540, USA*

ARTICLE INFO

Article history:
Received 11 August 2009
Accepted 25 April 2010
Available online 29 April 2010

Keywords:
Eleutherodactylus coqui
Rhabdias elegans
Anuran
Invasive
Biological control
Lung nematode
Growth
Survival
Locomotory performance

ABSTRACT

In Hawaii, the extremely loud mating calls of invasive Puerto Rican coquís (*Eleutherodactylus coqui* Thomas) depress real estate values in infested areas and reduce tourist appeal, with significant economic repercussions. Measures required to prevent inter-island transport of frogs also result in substantial costs for the floriculture industry. Classical biological control has been used successfully for over 100 years to combat invasive insects in Hawaii, and there is considerable interest in developing similar controls for coquís. Since Hawaii lacks native amphibian and reptile faunas (with the exception of marine species), the risk of unintended effects of a biological control agent on non-target species would be minimal. We identified *Rhabdias elegans* Gutierrez, a helminth parasite of native Puerto Rican populations of *E. coqui* that is not found in introduced Hawaiian populations, as a prime candidate for investigation as a potential biological control. We conducted laboratory experiments to evaluate overt effects of *R. elegans* on growth, survival, and locomotory performance of *E. coqui* from Hawaii. Experimental infection with *R. elegans* did not directly affect growth, survival, or endurance of *E. coqui* maintained under optimal laboratory conditions, but significantly reduced initial locomotory burst performance. We suggest that *R. elegans* holds limited potential as a biological control agent for eradication of *E. coqui*, but warrants additional investigation under more natural conditions of its potential for use as a management tool.

1. Introduction

Parasites often exert a negative influence on the behavior, growth, size, fecundity, and even survival of the host (reviewed by Minchella and Scott, 1991). Recently, such effects of parasites on amphibian hosts have received an increasing amount of attention (e.g., Tinsley et al., 2002; Johnson et al., 2006, 2008; Dare and Forbes, 2008; Koprivnikar et al., 2008). However, there is a paucity of experimental data on the majority of naturally occurring host–parasite relationships, particularly for those involving amphibian hosts. Many studies of amphibian parasites are descriptive and focus on parasite life cycles and taxonomy (e.g., Kuzmin et al., 2003, 2007; Dubey and Shine, 2008; Langford and Janovy, 2009) or host susceptibility (e.g., Dare and Forbes, 2009a,b) rather than on pathogenic effects on the host. Studies of pathogens thought to contribute to global amphibian decline (reviewed by Daszak et al., 2003) are among the only studies to evaluate effects of amphibian parasites, but largely ignore macroparasites. However, macroparasite effects on amphibian populations may be significant (Anderson, 1980), and analysis of these effects could provide valuable information for efforts to conserve imperiled species and manage invasive species.

Nematode macroparasites of the genus *Rhabdias* (Nematoda: Rhabdiasidae) commonly infect amphibians and reptiles throughout the world, living in association with a specific host (Anderson, 2000). Parasitic adults live as protandrous hermaphrodites (acting as functional males before becoming functional females) in the lungs of the host, where they deposit eggs that then pass up the respiratory tract to the oral cavity and are swallowed. The eggs hatch in the intestine, releasing first-stage larvae that accumulate in the colon and are voided in the feces. Free-living first-stage larvae undergo a series of molts in the external environment, eventually developing into infective third-stage larvae. These infective larvae penetrate the skin of a host and migrate into the body cavity where they grow into late third and fourth stages, and finally into parasitic subadults. Subadults must then invade the lungs in order to mature and produce eggs (Baker, 1979).

Investigations of the effects of nematodes of the genus *Rhabdias* suggest that this naturally-occurring parasite may have significant negative effects on some amphibian species (Goater, 1992, 1994;

* Corresponding author at: University of Florida/IFAS – GCREC, 1200 North Park Road, Plant City, FL 33563-1540, USA.
E-mail address: tadpole@ufl.edu (S.A. Johnson).

1049-9644/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2010.04.012

Goater and Ward, 1992; Goater et al., 1993; Goater and Vandenbos, 1997; Kelehear et al., 2009). Experimental infection of juvenile common toads (*Bufo bufo* L.) with varying doses of infective stage *Rhabdias bufonis* Schrank (Nematoda: Rhabdiasidae) larvae resulted in a decrease in toad growth, food intake, and survival, suggesting that *R. bufonis* infection could adversely affect host population size (Goater and Ward, 1992). Experimental infection with *R. bufonis* also reduced endurance of juvenile *B. bufo*; time and distance traveled by toads before exhaustion decreased significantly in both low and high parasite exposure groups (Goater et al., 1993). Similarly, experimental infection of cane toads (*Rhinella* [formerly *Bufo*] *marina* L.) with *Rhabdias* cf. *hylae* Barton (Nematoda: Rhabdiasidae) significantly reduced toad growth, survival, and locomotory performance (Kelehear et al., 2009). Heavy infections of *Rhabdias* spp. in captive amphibians have been found to cause pulmonary damage, predispose amphibians to secondary infections, and even cause death (Williams, 1960; Poynton and Whitaker, 2001). Analysis of the effects of parasite infection on non-native amphibians could provide valuable information for efforts to manage populations that are increasing rapidly and negatively impacting local ecosystems and/or economies, as is the case with Puerto Rican coquís in Hawaii.

The Puerto Rican coquí, *Eleutherodactylus coqui* Thomas (Anura: Leptodactylidae), was accidentally introduced to the Hawaiian Islands prior to 1988 via the ornamental plant trade, and populations rapidly expanded (Kraus and Campbell, 2002). This invasive frog is now ranked among the 100 most successful invaders in the world; one of only three amphibians classified as such (Lowe et al., 2000). Currently, there are more than 275 known *E. coqui* populations distributed throughout the Hawaiian Islands, primarily concentrated in lowland forests on the windward sides of the islands of Maui and Hawaii (Kraus and Campbell, 2002). Population density at some sites has been estimated to be as high as 89,000 frogs ha^{-1}, and average population density is up to three times the density seen in Puerto Rico (Woolbright et al., 2006). The extremely high density of *E. coqui* populations in Hawaii, coupled with the generalist diet of these frogs, suggests the potential for adverse effects on endemic invertebrates (Beard, 2007). These high density *E. coqui* populations also increase nutrient cycling rates, which could in turn impact native flora (Sin et al., 2008).

The main impacts of this invasive frog in Hawaii are economic, particularly when populations reach high densities, thus development of effective management strategies is of great importance. The mating calls of this species are extremely loud (80–90 dBA); as a result, tourist appeal has declined and real estate values have been reduced by up to 64% in infested areas (Beard and Pitt, 2005; Reaser et al., 2007). Hawaii's plant nursery and floriculture industries have also been affected by costly quarantine and treatment measures required in order to prevent inter-island transport of *E. coqui* on plants. Current control methods are limited to treatment of ornamental plants with citric acid or hot water (as lethal pesticides), modification of habitat to eliminate refuges, or the use of PVC pipe refuges as traps to capture and remove frogs (A. Hara, unpublished data). Other chemical pesticide treatments used on *E. coqui* in Hawaii have included caffeine, hydrated lime, and baking soda; however, these are not currently approved control treatments. Given the degree of potential economic impacts from this invasive frog, development of effective control methods is critical for long-term efforts to manage *E. coqui* in Hawaii, and to minimize spread to other areas.

Classical biological control has successfully been used in Hawaii for over 100 years against invasive exotic insect species (Funasaki et al., 1988), and is the subject of considerable interest for use against the invasive Puerto Rican coquí. However, biological control poses significant benefits and risks that must be thoroughly researched and carefully considered before an agent is introduced. Most importantly, the risks of impacts to non-target species and spread of the control agent to unintended areas must be minimal (Simberloff and Stiling, 1996; Hoddle, 2004; Messing and Wright, 2006). With the exception of marine species, all of Hawaii's herpetofaunal species are introduced ($\geqslant$11 species established), thus concerns regarding host specificity are minimal. Spread or dispersal of biological control agents is also a concern, but measures currently used to prevent the spread of *E. coqui* should also prevent transport and introduction of infected frogs and their associated parasites in sufficient numbers to maintain the parasite life cycle. Therefore, the potential benefits of using biological control agents to manage *E. coqui* in Hawaii warrant identification and evaluation of potential agents. When biological control is deemed a viable option for management of an invasive species, candidate biological control agents are selected from the natural predators, parasites, or pathogens of the invasive species, and should exert the maximum impact on the target species (Simberloff and Stiling, 1996; Hoddle, 2004; Messing and Wright, 2006). In the case of *E. coqui* in Hawaii, helminth parasites of the genus *Rhabdias* are a prime candidate for evaluation as a potential biological control.

In their native range in Puerto Rico, *E. coqui* have a diverse parasite fauna dominated by helminths (Dyer et al., 1995; Marr et al., 2008), with *Rhabdias elegans* Gutierrez detected at prevalences of >25% (Burrowes et al., 2004; Marr et al., 2008). However, parasite surveys of *E. coqui* in Hawaii have failed to detect *R. elegans* (Goldberg et al., 2007; Marr et al., 2008). Furthermore, comprehensive surveys of cane toads (*Rhinella marina*) and bullfrogs (*Lithobates* [formerly *Rana*] *catesbeianus* Shaw) in Hawaii have also failed to detect any *Rhabdias* spp. parasites (Barton and Pichelin, 1999; Barton and Riley, 2004; S. Marr, unpublished data). Introduced species often "lose" their natural parasites (i.e., those present in their native range), and may acquire few new ones; this reduction in parasitism may contribute to the invasion success of such species (Torchin et al., 2002, 2003; Torchin and Mitchell, 2004). In theory, introduction of naturally-occurring parasites from the host's native range could help to control invasive host populations.

We investigated the pathological effects of *R. elegans* on *E. coqui* as the first step in evaluating its potential as a biological control agent. The major objectives of our study were to evaluate the effects of *R. elegans* on (1) locomotory performance (both initial burst performance and endurance), (2) growth, and (3) survival of *E. coqui* in a laboratory setting.

2. Materials and methods

2.1. Collection and maintenance of frogs

We collected *E. coqui* from Lava Trees State Monument (19.5°N, −154.9°W) and a commercial nursery (19.6°N, −155.1°W) in June 2008, both located on Hawaii Island. We also collected adult *E. coqui* from the Caribbean National Forest (El Yunque) in Puerto Rico (18.3°N, −65.8°W). Frogs from Puerto Rico were used solely for collection of *R. elegans* eggs for culture – all experimentally infected and infection control animals were collected on Hawaii Island. We shipped frogs to the University of Florida/IFAS Gulf Coast Research and Education Center (GCREC) in Wimauma, FL, where we housed the frogs in temperature and light controlled "growth rooms." We set temperature to 24 °C (the maximum the system would allow), and set lighting to a 13-h light cycle (0600–1900 h) to approximate the natural photoperiod in Hawaii during the study (June/July). We housed each frog individually in a plastic deli container (23 × 19 × 8 cm) with a tightly sealed lid, and we provided a substrate of moist peat moss and half of a small plastic flowerpot as a refuge. We used a heated dissecting probe to create ~25 small holes around the container just below the lid (~2 cm) to

allow air exchange. For the duration of the experiments (60 days), we transferred frogs to clean containers with fresh peat moss and refuge every 10–14 days to prevent reinfection. In order to prevent incidental infection of control frogs, we housed control and treatment frogs on benches on opposite sides of the room, and we always handled control frogs first. We thoroughly cleaned and disinfected used containers and refuges with antibacterial soap and allowed them to air dry between uses. We used dry heat sterilization to rid used peat moss of nematodes (or eggs) prior to disposal. Twice weekly, we offered the smallest frogs (snout to vent length less than approximately 25 mm) six small crickets (6–8 mm long) and offered larger frogs (SVL > 25 mm) three medium-sized crickets (~12.5 mm long); as frogs grew larger than approximately 25 mm, they were offered fewer, larger prey. At the conclusion of our experiments, we euthanized frogs by immersion in a solution of MS-222, placed them into individually labeled plastic zipper bags, and shipped them on dry ice to the University of Hawaii at Hilo for necropsy.

2.2. Nematode culture and infection procedure

We euthanized *Rhabdias*-infected frogs collected in Puerto Rico ($n = 40$) by immersion in MS-222 and dissected each frog under a microscope to obtain gravid female *R. elegans* from the lungs. We placed each gravid female *R. elegans* individually into a Petri dish containing distilled water and lacerated the body with fine-tipped surgical scissors to release the eggs. We used a pipette to transfer the eggs to Petri culture dishes containing moist filter paper, placing the eggs directly onto frog feces located in the center of the dish. We used a pipette to recover infective third-stage larvae from the edges of the filter paper 2–4 days later. *Rhabdias* sp. nematodes collected from Puerto Rican frogs were sent to C. Bursey (Pennsylvania State University) for identification. Samples were identified as *R. elegans* based on the lengths of the postequatorial vulva and esophagus (C. Bursey, pers. comm.).

We randomly assigned frogs collected in Hawaii to treatment ($n = 54$) or control ($n = 54$) groups; the number of frogs less than 25 mm in length (SVL) in each group was approximately equal. We infected *E. coqui* with *R. elegans* by confining frogs for 24 h to individual Petri dishes lined with filter paper moistened with distilled water; we used a transfer pipette to add approximately 30–40 infective *R. elegans* larvae to the dishes of treatment frogs. Since natural exposure levels in the native range are unknown, we selected this treatment dosage based on intermediate levels used in similar studies of the effects of *Rhabdias* spp. on anuran hosts (Goater, 1992; Goater and Ward, 1992; Kelehear et al., 2009). We subjected control frogs to the same confinement procedure, but we did not add *R. elegans* larvae to the dishes of control frogs. After the infection period, we returned each frog to its individual container, and used distilled water to thoroughly rinse any remaining larvae from the filter paper into the Petri plate to facilitate counting under a microscope (larvae were difficult to see on white paper).

2.3. Evaluation of locomotory performance and growth

We conducted two sets of locomotory performance trials in an indoor arena. Pre-infection trials were conducted immediately prior to the 24 h infection period and post-infection trials were conducted 14 days (±1 day) after the infection period. We withheld food for 48 h before a trial, and allowed the frogs to acclimate to the temperature of the indoor testing area for 1 h before a trial. We covered a square arena (10 m × 10 m) on the floor of the indoor testing area with white paper to facilitate measurements. At the beginning of the trial, we placed each frog onto the paper near the center of the arena and encouraged it to jump until exhausted. We followed the frog and used a colored marker to record start point, landing points, and final end point for each frog. If a frog rested for more than 1 s between jumps, we tapped it lightly on the urostyle to encourage it to jump. If three subsequent taps failed to induce a jumping response, the frog was presumed exhausted and we recorded its stopping point as the final end point. We used a 60 cm × 90 cm cardboard barrier (held by an assistant at the edge of the arena) to prevent the frog from jumping off of the paper; if a frog jumped onto the barrier, the landing point was marked at the edge of the barrier. We used string to record the frog's initial position, the distance of each of the first four jumps, and the frog's final position (cumulative distance of all jumps), marking these distances directly onto the string with a permanent marker. We labeled each string and placed it into an individually labeled envelope for measuring at a later date. We also recorded the total number of jumps per frog, and measured SVL and weight of each frog immediately following pre- and post-infection locomotory performance trials (0 and 15 days, respectively). To evaluate growth rate, we weighed and measured all frogs again at 30 and 60 days post-infection.

2.4. Statistical analysis

We used repeated-measures analysis of covariance (ANCOVA) to evaluate the effects of *R. elegans* on locomotory performance of treatment and control groups of *E. coqui*. We evaluated three performance measures – mean distance of the first four "initial burst" jumps (cm), total distance traveled prior to exhaustion (cm), and total number of jumps prior to exhaustion. We found a significant positive correlation between body condition of frogs (weight per unit length, g/cm) and measures of locomotory performance – that is, performance increased with frog size. Therefore, we included body condition as a covariate in the ANCOVA. Values for all measures are presented as means with standard error.

We used repeated-measures analysis of variance (ANOVA) to evaluate the effects of *R. elegans* infection on growth of treatment and control groups of *E. coqui* under laboratory conditions, measured as SVL (cm), and weight (g). Data did not meet the sphericity assumption (Mauchly's Test), thus the Huyhn–Feldt epsilon correction for degrees of freedom was applied to the analysis of within-subjects effects.

3. Results

Based on the numbers of larvae recovered from Petri dishes following the infection period, we estimate that approximately 30 infective larvae penetrated each frog. At the conclusion of the experiment we examined a random sample of 43 treatment frogs. We found *R. elegans* in 68% of the treatment frogs we examined; 33% of frogs had adult *R. elegans* ($n = 4.0 \pm 7.9$ SD) present in the lungs only, 23% had larval *R. elegans* ($n = 2.0 \pm 3.1$ SD) in the intestine only, and 12% had *R. elegans* in both the lungs (adults) and the intestine (larvae). We found additional parasite species in 26% of the treatment frogs we examined; all (100% of the 43 frogs examined) had *Cosmocerca* sp. Diesing (Nematoda: Cosmocercdae) in the lower alimentary canal, and one frog (2%) also had *Physocephalus* sp. Diesing (Nematoda: Spirocercidae) nematodes in cysts on the stomach wall. We also examined a random sample of 21 control frogs and, as expected, did not find *R. elegans* in any of these frogs. However, we did find additional parasite species in 23% of the control frogs we examined; 19% had *Cosmocerca* sp. in the lower alimentary canal, and 4% (one frog) had *Physocephalus* spp. cysts on the stomach wall.

Frogs exhibited significant increases in both SVL ($F_{(1.87,\ 194.07)} = 420.00$, $p = 0.000$) and weight ($F_{(1.84,\ 191.19)} = 537.97$, $p = 0.000$)

over time, as anticipated. Growth did not differ significantly between treatment and control groups (SVL: $F_{(1,\ 104)} = 0.031$, $p = 0.861$; weight: $F_{(1,\ 104)} = 0.021$, $p = 0.885$), nor was there a significant interactive effect of treatment over time (SVL: $F_{(1.87,\ 194.07)} = 1.80$, $p = 0.171$; weight: $F_{(1.84,\ 191.19)} = 0.448$, $p = 0.623$) (Fig. 1). Survival was high, and was similar between treatment and control groups (98% and 100%, respectively). One treatment frog died before infection (excluded from survival estimates) and one treatment frog died before the post-infection (15-day) assessment of growth and locomotory performance. The remaining 52 treatment frogs and 54 control frogs lived throughout the 60-day study period.

The total distance jumped and number of jumps prior to exhaustion were similar for treatment and control groups (Fig. 2b and c), and increased with increasing frog size. There was no significant interactive effect of *Rhabdias* treatment over time on the total number of jumps ($F_{(1,\ 95)} = 0.476$, $p = 0.492$) or total distance jumped ($F_{(1,\ 103)} = 0.036$, $p = 0.850$). Burst performance of frogs in the control group also increased markedly over time as frog size increased. However, there was a significant interactive effect of *Rhabdias* treatment on burst performance of frogs over time ($F_{(1,\ 103)} = 6.38$, $p = 0.013$); post-infection burst performance of treatment frogs increased very little, and was much lower than that of control frogs (Fig. 2a).

In the weeks following completion of the performance trials (i.e., more than 14 days post-infection), we observed the gradual onset of a behavioral anomaly in two treatment frogs. Both of these frogs became increasingly uncoordinated during the remainder of the 60-day study period, seeming to lose neuromuscular control of their hind legs, but continued to feed and showed no other signs of stress, and therefore were not removed from the study.

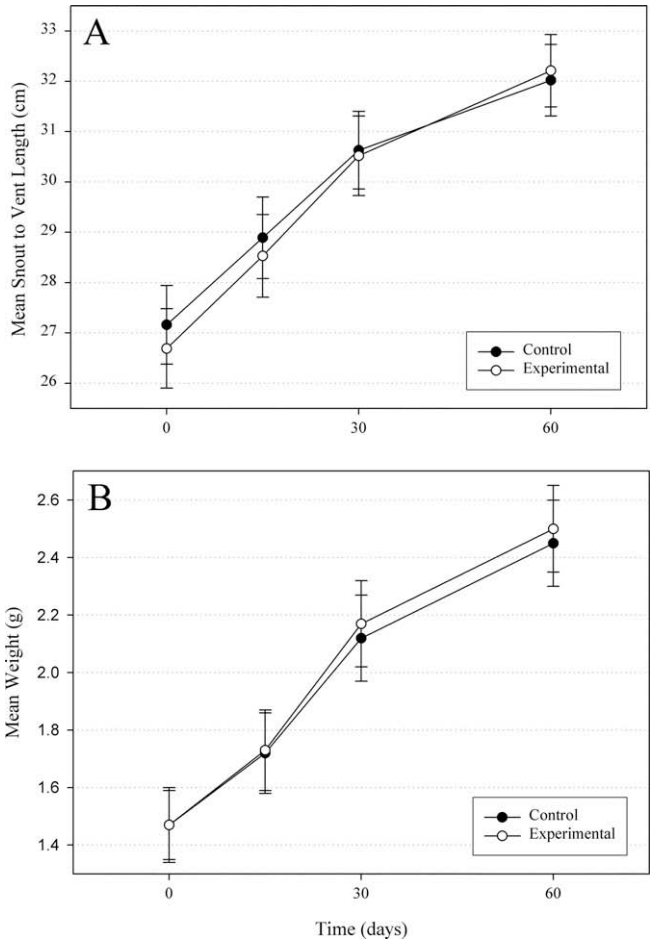

Fig. 1. Growth of control versus treatment coquís (*Eleutherodactylus coqui*) during the 60-day study period. Snout to vent length (A) and weight (B) are reported as mean ± one standard error of the mean.

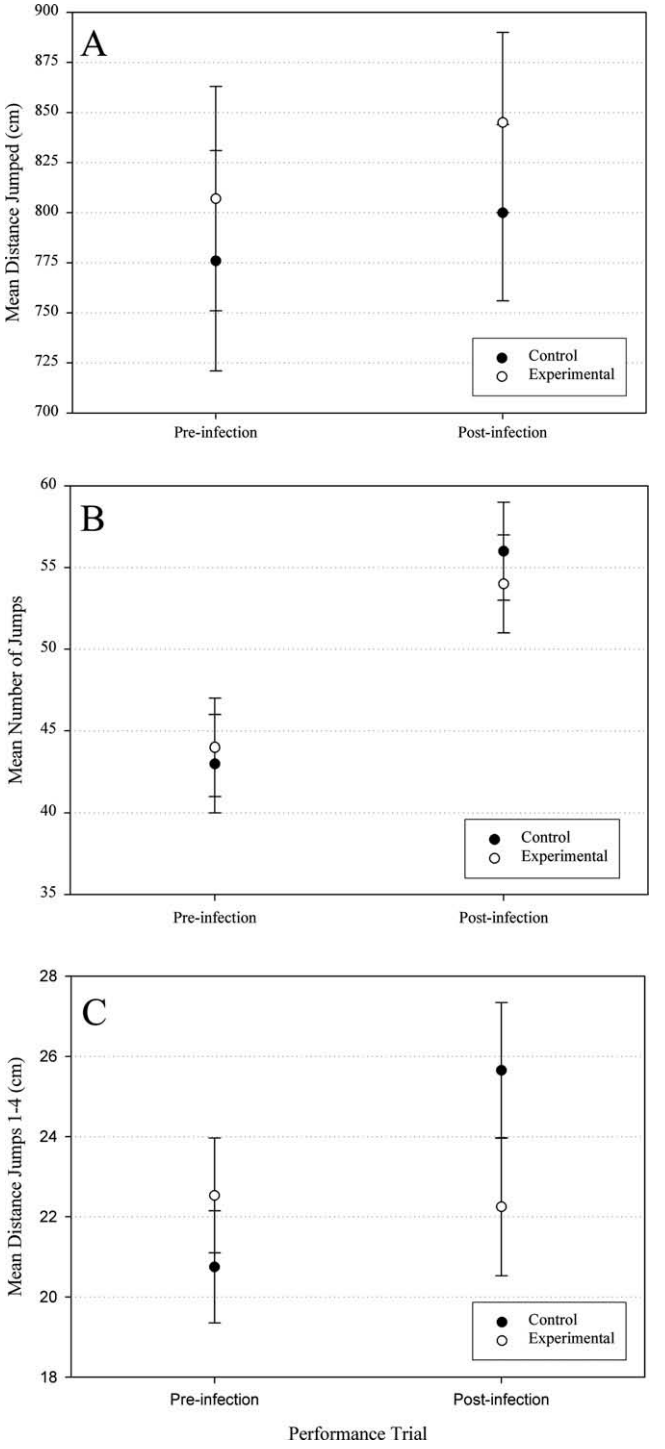

Fig. 2. Pre- and post-infection performance trial results for control versus treatment coquís (*Eleutherodactylus coqui*). Total distance jumped (A), total number of jumps (B), and initial burst (C; distance of jumps 1–4) are reported as ANCOVA estimated marginal mean ± one standard error of the mean. Post-infection trials were conducted 14 days (±1 day) after the infection period.

4. Discussion

Although *R. elegans* has been described in *Eleutherodactylus* frogs across the Caribbean, (Coy Otero and Ventosa, 1984;

Goldberg et al., 1998; Marr et al., 2008), the effects of *R. elegans* infection on these hosts have not previously been evaluated. We found that *R. elegans* had limited effects on physical performance of *E. coqui*, and there were no significant differences in survival or growth between experimentally infected and uninfected frogs. Of the measures of performance we evaluated (endurance and burst performance), only burst performance differed between groups over time. Frogs that were experimentally infected with *R. elegans* showed no increase in burst performance between pre- and post-infection periods despite a significant increase in body size, whereas the mean distance of the initial four jumps by uninfected frogs significantly increased by almost 5 cm. The lack of overt impacts on survivorship, growth, and physical performance sheds doubt on the efficacy of using *R. elegans* as a biological control agent to manage these invasive frogs in Hawaii. Nonetheless, for reasons we outline below, we do not feel it is appropriate to entirely discount the potential of this parasite to help manage coqui frogs.

Considering the extreme negative socioeconomic and potential ecological impacts of coqui frogs in Hawaii (Kraus and Campbell 2002; Beard and Pitt 2005; Beard 2007), it is vital that management strategies be developed for this invasive frog, and biological control should be considered. For a variety of reasons, Beard and O'Neill (2005) suggested that chytrid fungus (*Batrachochytrium dendrobatidis*) is not a good biological control for coqui frogs in Hawaii, although this pathogen may reduce fitness of coquís in their native range (Burrowes et al., 2010). Thus, *R. elegans* is perhaps one of most promising candidate biological control agents, and had not been evaluated prior to our research. This naturally-occurring parasite of *E. coqui* in Puerto Rico is not present in Hawaii, where these frogs have significantly fewer parasites (Marr et al., 2008) and are coincidentally found at densities up to three times those of native populations (Woolbright et al., 2006). Most importantly, the narrow host range of *Rhabdias* spp. parasites excludes native Hawaiian fauna, and transport of introduced nematodes from Hawaii holds minimal risk as *R. elegans* has been found only in frogs of the genus *Eleutherodactylus* and is already widespread in their native range (Coy Otero and Ventosa, 1984; Goldberg et al., 1998; Marr et al., 2008). There are also two novel parasites of *E. coqui* in Hawaii not found in Puerto Rico (Marr et al., 2008) that may warrant evaluation; however their effects are considered less pathogenic (*Cosmocercus* spp.; Hadfield and Whitaker, 2005) or are relatively unknown in amphibians (*Acanthocephala* spp.; McKenzie, 2007).

Even though there is considerable variation in the type and degree of host effects among the anuran–*Rhabdias* relationships that have been studied (Goater and Ward, 1992; Goater et al., 1993; Goater, 1994; Goater and Vandenbos, 1997; Kelehear et al., 2009), these parasites have been shown to have negative impacts on some species of frogs. Recently, Kelehear et al. (2009) showed that infection by *R. pseudosphaerocephala* reduced growth rates and survival, decreased locomotor performance, and curtailed prey intake of juvenile *Rhinella marina* (Cane Toads). As a result *R. pseudosphaerocephala* is actively being considered for use as a tool in the war against this invasive frog in Australia (Saunders et al., 2009). Additionally, experimental infection by *R. bufonis* has also been shown to have deleterious effects on growth and survival of juvenile *B. bufo* (Common Toad). Thus, frog life-history stage may have a significant influence on the effects of *Rhabdias* parasites and we suggest additional experiments with hatchling and juvenile *E. coqui*. In our study, tentative inclusion of size class ($\leqslant$25 mm SVL vs. >25 mm SVL) as a factor in analyses failed to find a significant interactive effect of frog size. However, the lack of a size-dependent effect of *R. elegans* in our study may have been due to the relatively large size of our smallest frogs; our smallest frog was 13 mm SVL, approximately twice the size of hatchlings (Townsend and Stewart, 1985). Experimental infection of recently hatched *E. coqui* might produce results comparable to those seen in young toads, but would require laboratory rearing of hatchlings from eggs in order to obtain a sufficient sample size (*E. coqui* is a direct-developing species and does not have a tadpole stage).

Under optimal laboratory conditions with ample food and minimal activity levels, infection with *R. elegans* did not overtly affect growth or survival of *E. coqui*. However, animals in the wild are exposed to a variety of stressors as they compete for food or refuges, avoid predation, and experience changes in environmental conditions. Furthermore, we sought to prevent artificially high levels of reinfection (in keeping with previous studies) and some frogs may have lost their parasites during our 60-day study period, whereas *E. coqui* in the wild exhibit a high degree of refuge fidelity (Stewart and Rand, 1991), and reinfection may be more likely in this species than in many other anurans. Thus, we also suggest additional experiments be conducted under more natural conditions, such as using mesocosms or enclosures in the frog's native range of Puerto Rico, where frogs must compete for food and experience natural reinfection rates.

Population density, climate, and other factors may compound the effects of a parasite on the host, and even the parasite infection itself may weaken the host's immune system over time and decrease resistance to the parasite, thus increasing the severity of infections and their effects (Zug and Zug, 1979; Carey, 1993; Christin et al., 2003). Therefore, the use of a biological control agent in combination with current control methods (i.e., habitat alteration) might add to the success of current methods; even if the effects of the biological control do not suggest that it could be used alone to significantly reduce population densities of these invasive frogs.

The efficacy of any biological control candidate for vertebrates must first be evaluated in the laboratory and then in the field under more natural conditions (Saunders et al., 2009). The objectives of our project were to investigate the impacts of *R. elegans* on *E. coqui* in a laboratory setting. Our lab experiments were meant as a first step in evaluating the potential for this parasite to be used as a biological control agent. However, a significant difference in burst performance alone between our two experimental groups of frogs does not suggest that *R. elegans* will have negative population-level impacts on *E. coqui* in Hawaii. As such, we conclude that infection with *R. elegans* holds little potential for use by itself as a biological control for management of *E. coqui*, but warrants additional investigation under more natural conditions as a management tool for use in combination with other methods.

Acknowledgments

We thank A. Dennis, C. Goater, C. Kelehear, and R. Niino-DuPonte for their assistance and advice. Financial support was provided by a grant from the County of Hawaii. Frog collection was permitted by the Hawaii Department of Agriculture (H-PL67), Departamento de Recursos Naturales y Ambientales de Puerto Rico (08-IC-020), and U.S. Department of Agriculture, Forest Service (CNF-2076). Frog exportation from Hawaii was permitted by the Hawaii Department of Land and Natural Resources (EX08-06), and importation into Florida was permitted by the Florida Fish and Wildlife Conservation Commission (ESC08-08). Animal care and use was approved by the Institutional Animal Care and Use Committees of the University of Hawaii (03-030-5) and the University of Florida/IFAS (013-08WEC).

References

Anderson, R.C., 2000. Nematode Parasites of the Vertebrates: Their Development and Transmission, second ed. CABI Publishing, Wallingford.

Anderson, R.M., 1980. Depression of host population abundance by direct life cycle macroparasites. Journal of Theoretical Biology 82, 283–311.

Baker, M.R., 1979. The free-living and parasitic development of *Rhabdias* spp. (Nematoda: Rhabdiasidae) in amphibians. Canadian Journal of Zoology 57, 161–178.

Barton, D.P., Pichelin, W., 1999. *Acanthocephalus bufonis* (Acanthocephala) from *Bufo marinus* (Bufonidae: Amphibia) in Hawaii. Parasite 6, 269–272.

Barton, D.P., Riley, J., 2004. *Raillietiella indica* (Pentastomida) from the lungs of the giant toad, *Bufo marinus* (Amphibia) in Hawaii, USA. Comparative Parasitology 71, 251–254.

Beard, K.H., 2007. Diet of the invasive frog, *Eleutherodactylus coqui*, in Hawaii. Copeia 2007, 281–291.

Beard, K.H., O'Neill, E.M., 2005. Infection of an invasive frog *Eleutherodactylus coqui* by the chytrid fungus *Batrachochytrium dendrobatidis* in Hawaii. Biological Conservation 126, 591–595.

Beard, K.H., Pitt, W.C., 2005. Potential consequences of the coqui frog invasion in Hawaii. Diversity and Distributions 11, 427–433.

Burrowes, P.A., Joglar, R.L., Green, D.E., 2004. Potential causes for amphibian declines in Puerto Rico. Herpetologica 60, 141–154.

Burrowes, P.A., Longo, A.V., Rodriguez, C.A., 2010. Potential fitness cost of *Batrachochytrium dendrobatidis* in *Eleutherodactylus coqui*, and comments on environment-related risk of infection. Herpetotropicos 4, 51–57.

Carey, C., 1993. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. Conservation Biology 7, 355–362.

Christin, M.-S., Gendron, A.D., Brousseau, P., Menard, L., Marcogliese, D.J., Cyr, D., Ruby, S., Fournier, M., 2003. Effects of agricultural pesticides on the immune system of *Rana pipiens* and on its resistance to parasitic infection. Environmental Toxicology and Chemistry 22, 1127–1133.

Coy Otero, A., Ventosa, L., 1984. Nematodos parasitos de anfibios Cubanos. Poeyana 269, 1–20.

Dare, O.K., Forbes, M.R., 2008. Rates of development in male and female wood frogs and patterns of parasitism by lung nematodes. Parasitology 135, 385–393.

Dare, O.K., Forbes, M.R., 2009a. Patterns of infection by lungworms, *Rhabdias ranae* and *Haematoloechus* spp. In northern leopard frogs: a relationship between sex and parasitism. Journal of Parasitology 95, 275–280.

Dare, O.K., Forbes, M.R., 2009b. Patterns of trematode and nematode lungworm infections in northern leopard frogs and wood frogs from Ontario, Canada. Journal of Helminthology 83, 339–343.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2003. Infectious disease and amphibian population declines. Diversity and Distributions 9, 141–150.

Dubey, S., Shine, R., 2008. Origin of the parasites of an invading species, the Australian cane toad (*Bufo marinus*): are the lungworms Australian or American? Molecular Ecology 17, 4418–4424.

Dyer, W.G., Williams, E.H., Bunkley-Williams, L., 1995. Nematode parasites of a Puerto Rican tree frog, *Eleutherodactylus coqui*. Transactions of the Illinois State Academy of Science 88, 39–41.

Funasaki, G.Y., Lai, P.-Y., Nakahara, L.M., Beardsley, J.W., Ota, A.K., 1988. A review of biological introductions in Hawaii: 1890 to 1985. Proceedings of the Hawaii Entomological Society 28, 105–160.

Goater, C.P., 1992. Experimental population dynamics of *Rhabdias bufonis* (Nematoda) in toads (*Bufo bufo*): density-dependence in the primary infection. Parasitology 104, 179–187.

Goater, C.P., 1994. Growth and survival of postmetamorphic toads: interactions among larval history, density, and parasitism. Ecology 75, 2264–2274.

Goater, C.P., Semlitsch, R.D., Bernasconi, M.V., 1993. Effects of body size and parasite infection on the locomotory performance of juvenile toads, *Bufo bufo*. Oikos 66, 129–136.

Goater, C.P., Vandenbos, R.E., 1997. Effects of larval history and lungworm infection on the growth and survival of juvenile wood frogs (*Rana sylvatica*). Herpetologica 53, 331–338.

Goater, C.P., Ward, P.I., 1992. Negative effects of *Rhabdias bufonis* (Nematoda) on the growth and survival of toads (*Bufo bufo*). Oecologia 89, 161–165.

Goldberg, S.R., Bursey, C.R., Kaiser, H., 1998. Gastrointestinal helminths of five species of *Eleutherodactylus* (Anura: Leptodactylidae) from the West Indies. Caribbean Journal of Science 34, 146–149.

Goldberg, S.R., Bursey, C.R., Kraus, F., 2007. *Eleutherodactylus coqui* endoparasites. Herpetological Review 38, 183–184.

Hadfield, C.A., Whitaker, B.R., 2005. Topics in medicine and surgery: amphibian emergency medicine and care. Seminars in Avian and Exotic Pet Medicine 14, 79–89.

Hoddle, M.S., 2004. Restoring balance: using exotic species to control invasive exotic species. Conservation Biology 18, 38–49.

Johnson, P.T.J., Hartson, R.B., Larson, D.J., Sutherland, D.R., 2008. Diversity and disease: community structure drives parasite transmission and host fitness. Ecology Letters 11, 1017–1026.

Johnson, P.T.J., Preu, E.R., Sutherland, D.R., Romansic, J.M., Han, B., Blaustein, A.R., 2006. Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. Ecology 87, 2227–2235.

Kelehear, C., Webb, J.K., Shine, R., 2009. *Rhabdias pseudosphaerocephala* infection in *Bufo marinus*: lung nematodes reduce viability of metamorph cane toads. Parasitology 136, 919–927.

Koprivnikar, J., Forbes, M.R., Baker, R.L., 2008. Larval amphibian growth and development under varying density: are parasitized individuals poor competitors? Oecologia 155, 641–649.

Kraus, F., Campbell, E.W., 2002. Human-mediated escalation of a formerly eradicable problem: the invasion of Caribbean frogs in the Hawaiian Islands. Biological Invasions 4, 327–332.

Kuzmin, Y., Tkach, V.V., Brooks, D.R., 2007. Two new species of *Rhabdias* (Nematoda: Rhabdiasidae) from the marine toad *Bufo marinus* (L.) (Lissamphibia: Anura: Bufonidae), in Central America. Journal of Parasitology 93, 159–165.

Kuzmin, Y., Tkach, V.V., Snyder, S.D., 2003. The nematode genus *Rhabdias* (Nematoda: Rhabdiasidae) from amphibians and reptiles of the Nearctic. Comparative Parasitology 70, 101–114.

Langford, G.J., Janovy Jr., J., 2009. Comparative life cycles and life histories of North American *Rhabdias* spp. (Nematoda: Rhabdiasidae) lungworms from snakes and anurans. Journal of Parasitology 95, 1145–1155.

Lowe, S., Browne, M., Boudjelas, S., de Poorter, M., 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. Published by the IUCN/SSC Invasive Species Specialist Group (ISSG) of the Species Survival Commission (SSC) of the World Conservation Union. Available online at http://www.issg.org/booklet.pdf.

Marr, S.R., Mautz, W.J., Hara, A.H., 2008. Parasite loss and introduced species: a comparison of the parasites of the Puerto Rican tree frog, *Eleutherodactylus coqui*, in its native and introduced ranges. Biological Invasions 10, 1289–1298.

McKenzie, V.J., 2007. Human land use and patterns of parasitism in tropical amphibian hosts. Biological Conservation 137, 102–116.

Messing, R.H., Wright, M.G., 2006. Biological control of invasive species: solution or pollution? Frontiers in Ecology and the Environment 4, 132–140.

Minchella, D.J., Scott, M.E., 1991. Parasitism: a cryptic determinant of animal community structure. Trends in Ecology and Evolution 6, 250–254.

Poynton, S.L., Whitaker, B.R., 2001. Protozoa and metazoa infecting amphibia. In: Wright, K.M., Whitaker, B.R. (Eds.), Amphibian Medicine and Captive Husbandry. Krieger Publishing Company, Malabar, pp. 193–222.

Reaser, J.K., Meyerson, L.A., Cronk, Q., de Poorter, M., Eldrege, L.G., Green, E., Kairo, M., Latasi, P., Mack, R.N., Mauremootoo, J., O'Dowd, D., Orapa, W., Sastroutomo, S., Saunders, A., Shine, C., Thrainsson, S., Vaiutu, L., 2007. Ecological and socioeconomic impacts of invasive alien species in island ecosystems. Environmental Conservation 34, 1–14.

Saunders, G., Cooke, B., McColl, K., Shine, R., Peacock, T., 2009. Modern approaches for the biological control of vertebrate pests: an Australian perspective. Biological Control 52, 288–295.

Sin, H., Beard, K.H., Pitt, W.C., 2008. An invasive frog, *Eleutherodactylus coqui*, increases new leaf production and leaf litter decomposition rates through nutrient cycling in Hawaii. Biological Invasions 10, 335–345.

Simberloff, D., Stiling, P., 1996. Risks of species introduced for biological control. Biological Conservation 78, 185–192.

Stewart, M.M., Rand, A.S., 1991. Vocalizations and the defense of retreat sites by male and female frogs, *Eleuterodactylus coqui*. Copeia 1991, 1015–1024.

Tinsley, R.C., Cable, J., Porter, R., 2002. Pathological effects of *Pseudodiplorchis americanus* (Monogenea: Polystomatidae) on the lung epithelium of its host, *Scaphiopus couchii*. Parasitology 125, 143–153.

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M., 2003. Introduced species and their missing parasites. Nature 421, 628–630.

Torchin, M.E., Lafferty, K.D., Kuris, A.M., 2002. Parasites and marine invasions. Parasitology 124, 137–151.

Torchin, M.E., Mitchell, C.E., 2004. Parasites, pathogens, and invasions by plants and animals. Frontiers in Ecology and the Environment 2, 183–190.

Townsend, D.S., Stewart, M.M., 1985. Direct development in *Eleutherodactylus coqui* (Anura: Leptodactylidae): a staging table. Copeia 2, 423–436.

Williams, R.W., 1960. Observations on the life history of *Rhabdias sphaerocephala* Goodey, 1924 from *Bufo marinus* L., in the Bermuda Islands. Journal of Helminthology 34, 93–98.

Woolbright, L.L., Hara, A.H., Jacobsen, C.M., Mautz, W.J., Benevides, F.L., 2006. Population densities of the Coqui, *Eleutherodactylus coqui*, (Anura:Leptodactylidae) in newly invaded Hawaii and in native Puerto Rico. Journal of Herpetology 40, 122–126.

Zug, G.R., Zug, P.B., 1979. The marine toad, *Bufo marinus*: a natural history resume of native populations. Smithson. Contributions to Zoology 284, 1–58.