Assessment of snail exposure to the anticoagulant rodenticide brodifacoum in the Galapagos Islands

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Abstract Eradication of invasive rodents has become a powerful tool to protect native island biota. Use of brodifacoum, an anticoagulant rodenticide, has contributed to hundreds of successful invasive rodent eradication efforts on islands. Application of bait containing brodifacoum for this purpose requires appropriate consideration of adverse effects on non-target wildlife. Thus, a priori identification of non-target risks and, where needed, approaches to mitigate these to acceptable levels, is now an essential component of eradication planning and implementation. As part of the plan for eradicating invasive rats and mice from Floreana Island in the Galapagos, we experimentally tested the effect of brodifacoum on the Galapagos endemic land snail species *Naesiotus unifasciatus*. Importantly, the trials were designed to evaluate effects of particular components of the bait pellets, namely the active brodifacoum, the pyranine biomarker, and a blue dye. We found no evidence for increased snail mortality following exposure to any of these bait components. We review results of past toxicity studies on terrestrial molluscs and find that, as for our own study, there is likely to be little impact of anticoagulant rodenticide on terrestrial mollusc survival as the result of application of brodifacoum bait. However, given the limited taxonomic representation in the toxicity tests performed on terrestrial molluscs so far, we recommend the continued used of captive toxicity trials to assess potential effect of any rodenticide applications on native malacological fauna on a case-by-case basis where large-scale eradication programmes are planned and undertaken.

Keywords: anticoagulant, brodifacoum, Bulimulidae, islands, restoration, rodent eradication

INTRODUCTION

Invasive mammal eradications are powerful conservation tools to protect biodiversity and prevent extinctions on islands (Lorvelec & Pascal, 2005; Bellingham, et al., 2010; Nogales, et al., 2013). Three rat species (*Rattus rattus*, *R. norvegicus*, *R. exulans*) and house mice (*Mus musculus*) are the most common rodents introduced to islands worldwide (Atkinson, 1985). These species are responsible for population declines and extinctions of insular flora and fauna, and they are known to interrupt ecosystem processes with negative cascading effects (Fukami, et al., 2006; Steadman, 2006; Towns, et al., 2006; Jones, et al., 2008; Kurle, et al., 2008; Varnham, 2010; Dunlevy, et al., 2011; St Clair, 2011). To recover endangered species and restore ecosystem processes, invasive rodents on islands are increasingly targeted for eradication, with at least 637 successful rodent eradications to date (based on DIISE island data ranked as good or satisfactory; DIISE, 2015). Ninety-seven percent of successful rodent eradications have involved the use of rodenticide, with brodifacoum having been used in 76% of them.

The common mode of toxicity of anticoagulant rodenticides in mammals and birds is to inhibit Vitamin K metabolism in liver, which in turns prevents the formation of chemical factors essential to blood coagulation (e.g., Rattner, et al., 2014). In mammals and birds a lethal exposure will cause these clotting factors to deplete to a level so that blood can no longer coagulate, resulting in death through internal haemorrhage (MacNicoll, 1993). 'First-generation' anticoagulants, such as warfarin, are most effective against rodents in multiple feeds but their intensive use as rodenticides resulted in the development of heritable resistance in some rodent populations (Rattner, et al., 2014). This prompted the development of the more potent 'second generation' anticoagulants, such as brodifacoum, which are effective against target rodents in a single feed (Rattner, et al., 2014). Sublethal or chronic effects of anticoagulants are not well described in wildlife (Rattner, et al., 2014), but sublethal exposure may result in the retention of residual anticoagulant concentrations in liver tissue. In this regard the 'second generation' anticoagulant rodenticides are more persistent in animal tissues, especially liver, than 'first-generation' anticoagulants (Fisher, et al., 2003). The second generation anticoagulant brodifacoum has been the most commonly used rodenticide for eradicating invasive rodents from islands, with a high success rate (Howald, et al., 2007; Parkes, et al., 2011; DIISE, 2015). Brodifacoum, incorporated at 20–50 ppm (0.002–0.005%) into cereal or wax baits, is applied to every rodent territory via bait stations, or broadcasted by hand or from a modified agricultural spreader bucket suspended from a helicopter. Large-scale broadcast of bait has facilitated increasingly large and complex island restoration projects involving the eradication of invasive rodents (e.g., Towns & Broome, 2003), but it also raises concerns about environmental contamination and adverse effects on non-target wildlife (Pain, et al., 2000; Eason, et al., 2002). Thus, *a priori* identification of non-target risks and the potential mitigation of these to acceptable levels is now an essential step to inform feasibility of large-scale eradication projects.

Invasive rats are known to prey upon terrestrial invertebrates (e.g., St Clair, 2011). On Galapagos, endemic land snails are particularly vulnerable (Clark, 1981), and recent field collections of land snail shells suggest that rats are particularly voracious snail predators on Floreana (Parent, unpublished data). Although the eradication of invasive rodents would likely benefit terrestrial molluscs, the potential impact of bait on non-target species should be evaluated. Indeed, a range of terrestrial invertebrate species, including snails and slugs, have been found to feed on cereal-based baits used for rodent control (e.g., Spurr & Drew, 1999; Johnston, et al., 2005). Reports that bait containing brodifacoum caused mortality in captive introduced and endemic snails (*Achatina fulica* and *Pachnodus silhouettanus*) from the Seychelles Islands that fed on the baits, and suspected field mortality of *Pachystyla bicolor* snails following operational baiting

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(anecdotally reported in Gerlach & Florens (2000a; 2000b) and Gerlach (2005)) raised concerns for other native and endemic snail species on islands where rodent eradication using anticoagulants was proposed. Limited information suggests that invertebrates are generally less susceptible to brodifacoum toxicity than mammals and birds (Booth, et al., 2001; Eason & Spurr, 1995), but current knowledge of snail physiology is insufficient to predict with confidence its effect on snails. To assess the feasibility of using brodifacoum to eradicate rats and mice from Floreana Island in the Galapagos (Island Conservation, 2013), a need to investigate risk to endemic land snails was therefore identified.

Land snails are known for their remarkable diversity in island systems (Cameron, et al., 2013). On Galapagos Islands, the land snail fauna comprises 103 endemic species distributed in 13 genera. Approximately 80 species and subspecies belong to the genus *Naesiotus* (Family Bulimulidae) and form the most species-rich adaptive radiation of these islands (Parent, et al., 2008). Recent field and genetic work suggests that most (if not all) Galapagos bulimulid species are single-island endemics (Parent & Crespi, 2006; Parent, unpublished data). Twenty species (and eight subspecies) of endemic land snails are known from Floreana Island. Eight of these species are critically endangered, and three are endangered (IUCN, 2015), whereas others remain to be evaluated. Thus, given the conservation status of Floreana endemic snails, we identified the need to evaluate whether exposure of these endemic snails to the brodifacoum bait type proposed for rodent eradication was likely to cause mortality.

Four previous experimental studies, together assessing twelve species of terrestrial molluscs, have failed to find significant effect of brodifacoum exposure on individual short-term mortality. The only exception to this trend is the study reported by Gerlach & Florens (2000a; 2000b) mentioned above. Therefore, a precautionary approach demands that the effects of exposure to brodifacoum bait should be tested on island endemic snails prior to large-scale rodent eradication measures on islands. Importantly, the rodenticide baits are composed of more than 99% inert ingredients, most of which is compacted cereal grains but may include other inert ingredients such as dye or biomarkers. Past studies failed to explicitly test the effect of inert ingredients on land snails. Thus, the main objectives of the study are to: (1) test various bait formulations to identify which component(s) of the baits are responsible for any mortality that might be observed in land snails, and (2) review and synthesize the literature on experimental toxicity tests of bait-based rodenticides on terrestrial molluscs.

MATERIALS AND METHODS

Site description

Floreana Island is part of the Galapagos archipelago, which straddles the equator approximately 1,000 km off the western coast of Ecuador. The islands are oceanic and have never been connected to any continent. Floreana is volcanic in origin, and at 17,253 ha is the sixth largest island in the archipelago. The maximum elevation of the island is 640 m, and its generally conical shape results in two distinct habitat types: dry lowlands and lush central highlands, which meet and overlap to some extent into what is referred to as a transition zone (McMullen, 1999). Over 98% of the island land area is Galapagos National Park, with the remaining 2% divided between a small town in the lowlands and agricultural and pastoral areas in the highlands (DPNG, 2014). The island is home to an estimated 140 residents as of 2014.

Snail population

Snails were collected at Cerro Pajas on Floreana (Latitude: 01.2968°S, Longitude: 90.4559° W) in November 2012. The Cerro Pajas site was selected based on the relatively high density of snails found there (Parent, unpublished data). We collected snails opportunistically from leaf litter on the ground and from low (< 0.5 m) vegetation. We chose to collect snails near the ground because those snails would be more likely to encounter bait pellets on or near the ground. There are at least three Galapagos endemic species of land snails occurring at that particular site (*Naesiotus nux*, *N. unifasciatus*, and *Succinea brevior*; endangered, critically endangered, and unknown status, respectively), and these species are expected to be either detritivores or to consume algae and lichens scraped from the substrate. For the present study, we used adult individuals of *N. unifasciatus* since the population density of this species was the highest and we felt confident that our sampling would not impact the survival of the population at that particular location (100 adult individuals were collected, less than 5% of the individuals encountered over a period of approximately one hour). We did not collect specimens from any other snail species because their population density was such that we would have had to collect more than 5% of the adult individuals encountered for our experiments. It is important to note that the information gathered from our toxicity experiments will by far outweigh the potential detrimental effects of our population sampling of *N. unifasciatus*. Bulimulid snails are hermaphrodites and therefore sexing them was not applicable.

Experimental design

We housed snails in small cylindrical plastic containers (11 cm high, 17 cm and 14 cm in diameter at the top and base of the container, respectively) replacing lids with tightly covering mesh secured with rubber bands to prevent snail escapes. Two sheets of task wipe (Kimwipe ®) paper were placed on the bottom of each container and kept moist for the duration of the experiment. A small amount of litter from which the snails were collected was sifted and visually inspected to remove any other small invertebrates. Approximately 10 grams of sifted litter was added to each container as a source of natural food and shelter. Each container held five snails at the beginning of the experiment.

We used three types of pelleted bait as experimental treatments: (1) non-toxic baits containing a blue dye (well less than 1% of pellet content) that is a standard proprietary component of the bait formulation, (2) non-toxic baits containing pyranine (a fluorescent marker dye allowing easy detection of metabolized bait in snails' bodies, faeces and slime trails when exposed to ultraviolet (UV) light, also representing well less than 1% of pellet content); and (3) bait containing blue dye and 50 ppm brodifacoum. For treatment groups, one moistened bait pellet was placed in each container at the start of the trial. Control group containers had no pellets, but were otherwise the same as treatment containers. We prepared five containers per treatment and for the control group (total n = 100 snails). All containers were kept on Floreana, at sea level in the shade at ambient temperature (25-28°C). In parallel, we kept five pellets in a container under the same conditions but without snails to evaluate the effect of the containers on the pellets themselves. All containers were opened twice daily to increase circulation of fresh air and to remoisten the tissue paper and bait pellet by spraying water, as necessary.

The experiment was conducted in two parts; the first over 10 days during which all containers with snails were monitored daily for mortality. A 10-day period was

selected as slightly longer than the four to eight days that bait pellets are likely to be available to snail populations in the Floreana highlands (Island Conservation, unpubl. data) and substantially longer than the maximum of 72 hours over which snail mortality occurred in the study reported by Gerlach & Florens (2000a and 2000b).

Snail activity was noted twice a day by recording whether the snails were immobile and firmly attached to substrate (i.e., estivating) or moving in the containers. Snails found estivating were moved onto the vegetation and sprayed with water; this reliably caused the snails to become active once again. Snails found dead were frozen immediately in individual vials to preserve tissue and be dissected later if any statistically significant mortality effects were to be detected in our study. In addition, containers were visually inspected daily by illumination with a UV light to detect any fluorescent traces of pyranine in the slime trails and faeces of the snails in containers of treatment 2 which would have indicated ingestion of this bait by the snails. Control containers were inspected in the same manner. Finally, we also monitored bait consumption by noting any changes to the surfaces of the bait pellets. At the end of the 10-day period, living snails from the two treatment groups using non-toxic bait types were returned to the location where they were collected.

In the second part of the experiment, the remaining snails in the control group and the treatment group with brodifacoum baits were monitored for an additional 11 days (for a total of 21 days, well in excess of the period over which signs of poisoning and mortality would have occurred in mammals) and any snails alive were euthanized by freezing at the end of this second part of the experiment to use in subsequent residue content analysis if any significant mortality was observed.

Statistical analyses

We used a logistic regression approach with a Bernoulli (binomial) distribution to evaluate the effect of each bait component on the survival of the snails. We used post-hoc tests to determine whether any of the bait components had a significant effect on snail survival. We implemented all statistical analyses in R version 3.1.0 (R Core Team, 2014).

RESULTS

Snails remained active (i.e., not estivating) throughout the experiment. Individuals were confirmed to consume baits, either by direct observation (snails on bait pellet) or evidence of blue dye or pyranine fluorescence in the snails' bodies, faeces or slime trails. We did not track each individual snail's consumption of bait, but the observed evidence suggested that most snails consumed or were in direct physical contact with bait when available.

The survival of snails over the course of the bait treatments did not differ significantly from the survival of snails in the control groups without baits (Fig. 1). Because our treatment groups did not represent all possible combinations of bait type (with/without brodifacoum, presence/absence of blue dye, and presence/absence of pyranine), we could not directly compare the individual effect of each of these bait components on the snails. However, we found that when all treatments were analysed simultaneously, none of the components had a significant effect on snail survival (Fig. 1). In contrast, in a post-hoc test for individual effects of each component, we found that the survival of snails exposed to bait containing pyranine was greater than the survival of snails exposed to bait without pyranine (Fig. 2; Welch two-tailed t-test for unequal sample size, $t = 3.056$, d.f. = 14, $P < 0.01$). The survival of the snails over 21 days in the containers with bait containing brodifacoum did not significantly differ from the control (Welch two-sample two-tailed *t*-test, $t = 1.497$, d.f. = 5.611, $P > 0.05$).

DISCUSSION

The goal of our study is to quantify the short-term impacts of anticoagulant rodenticide bait on Galapagos endemic land snails. Importantly, any potential short-term impact the bait might have has to be considered against the long-term benefits that rodenticide bait application can bring to terrestrial malacofauna. These potential benefits include, for example, release from invasive rodent predation and general habitat improvement.

Our results suggest that none of the baits tested were toxic to the snails over the 10-day exposure period (i.e.

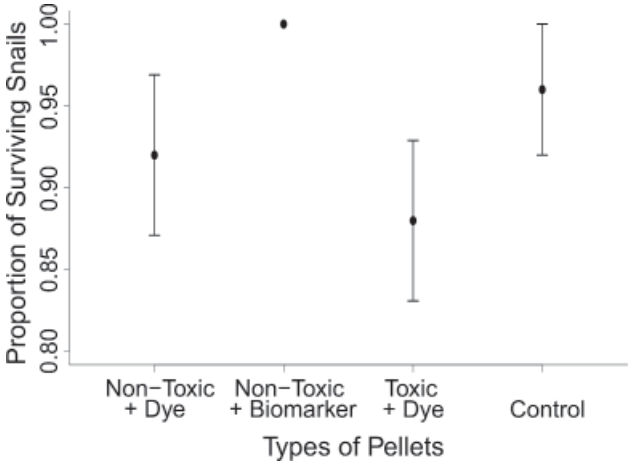

Fig. 1 Survival of snails exposed to different types of pellets: non-toxic pellets with blue dye, non-toxic pellets with biomarker, pellets with 50 ppm brodifacoum and blue dye, and control populations without pellets. Survival for all treatments was not significantly different than the survival of the snails in the control populations ($P > 0.05$). Error bars represent standard errors.

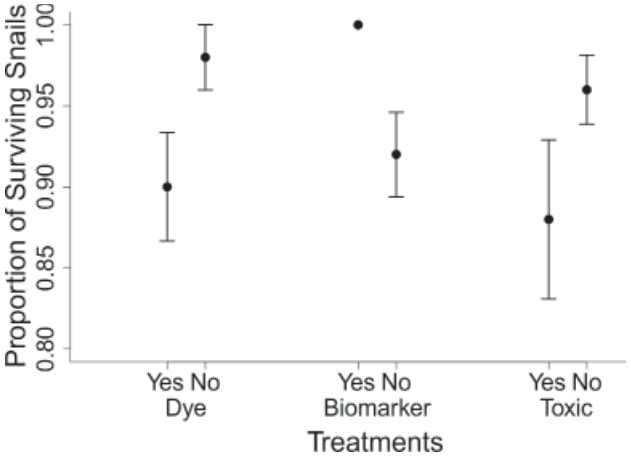

Fig. 2 Survival of snails as a function of presence/absence of pellet components: blue dye, biomarker, and brodifacoum. A logistic regression approach with a Bernoulli (binomial) distribution including all samples at once reveals no significant effect for any of the components ($P > 0.05$). However, a post-hoc t-test indicates that snails exposed to pellets with biomarkers have significantly higher survival than snails exposed to pellets without biomarker ($P < 0.01$). Error bars represent standard errors.

Table 1 Terrestrial mollusc species experimentally tested for the effects of anticoagulant rodenticides.

Species	Family	Locality	Use of control(s)?	Number of individuals tested	Anticoagulant rodenticide	Brodifacoum exposure time (max no of days)	Effect on organism survival	Reference
Oxychilus oglasicola	Zonitidae	Montecristo Island, Italy	Information not available	12	Brodifacoum 0.005%, bromadiolone	Information not available	Not significant	Sposimo et al., 2011
Ciliellopsis oglasae	Hygromiidae	Montecristo Island, Italy	Information not available	4	Brodifacoum 0.005%, bromadiolone	Information not available	Not significant	Sposimo et al., 2011
Deroceras laeve	Agriolimacidae	Hawaii, USA	yes	15	HACCO Ramik ® Green (0.005% diphacinone)	7	Not significant	Johnston et al., 2005
Oxychilus spp.	Zonitidae	Hawaii, USA	yes	15	HACCO Ramik ® Green (0.005% diphacinone)	7	Not significant	Johnston et al., 2005
Limax maximus	Limacidae	Hawaii, USA	yes	15	HACCO Ramik ® Green (0.005% diphacinone)	7	Not significant	Johnston et al., 2005
Orobophana solidula	Helicinidae	Henderson Is, South Pacific	yes	28	Pestoff 20R (0.002% brodifacoum)	10	Not significant	Brooke et al., 2011
Pacifella sp., Tornatellides sp., Lamellidae sp., Tubuaia hendersoni	Achatinellidae	Henderson Island, South Pacific	yes	43	Pestoff 20R (0.002% brodifacoum)	10	Not significant	Brooke et al., 2011
Pupisoma orcula	Pupilidae	Henderson Is, South Pacific	yes	1	Pestoff 20R (0.002% brodifacoum)	10	Not significant	Brooke et al., 2011
Helix aspersa	Helicidae	New Zealand	yes	24	Talon 20P (0.002% brodifacoum)	14	Not significant	Booth et al., 2003
Pachnodus silhouettanus, Achatina fulica	Cerastidae Achatinidae	Fregate Island, Seychelles	Information not available	Information not available	0.01 - 0.2mg brodifacoum	4	Mortality was observed but not statistically tested	Gerlach and Florens, 2000a & 2000b
Naesiotus unifasciatus	Bulimulidae	Floreana Island, Ecuador	yes	25	Bell Laboratories Brodifacoum 50D Conservation Blue FP2015 (0.005% brodifacoum)	21	Not significant	This study

survival of snails exposed to any bait treatment was not significantly different than 1.0). For the purposes of a conservative risk assessment, our experiment simulated a 'worst case' exposure to snails through use of 50 ppm brodifacoum in bait, compared to the lower concentrations proposed for the rodent eradication on Floreana (25 ppm) and used in previous similar experiments with snails (e.g., Booth, et al., 2003; Brooke, et al., 2011; Table 1). In confining snails under conditions favourable for foraging and in close proximity to bait, we also simulated a worst-case exposure potential, in comparison to the expected availability of bait to snails following an operational aerial application. Application rates for rodent eradication on Floreana Island remain to be determined, nonetheless relatively few snails are expected to encounter and consume bait before it is removed by other animals or breaks down naturally. Additionally, the operation will occur during the driest time of the year, corresponding to the time when snails are more likely to be estivating (Parent, unpublished data).

An apparent absence of toxic effects of brodifacoum bait on snails was further supported by survival being significantly higher in a post-hoc test in snails exposed to non-toxic baits that contained pyranine compared to snails exposed to baits without the biomarker during the first 10 days of the experiment. It is possible that one or some of inert components of the bait types used in our experiment provided a nutritional supplement benefiting the snails. Any such benefits from a boost in diet would become more evident over time. However, this pattern of increased survival did not carry over in snails that were kept for the full-length (21 days) of the experiment.

Our results add to a growing body of research suggesting that exposure to rodenticide bait formulations containing brodifacoum does not cause significant mortality in snails (Table 1). Reports by Gerlach & Florens (2000a; 2000b) and Gerlach (2005) appear to be exceptions, but are also brief and lacking in detail that would allow statistical evaluation. While the absolute toxicity of brodifacoum and its mechanism in snails remain to be established, in the context of potential exposure to rodenticide baits it is important to also consider the possible effects of other, nominally inert, ingredients of specific bait formulations (e.g., binders, preservatives, emulsifiers, pH regulating, flavouring or colouring agents). In designing our study we sought to account for some of these factors and recommend that future studies of the effects of rodenticides on invertebrates contain a mechanism to ensure any observed mortality is in fact due to the active anticoagulant agent and not other bait components or experimental conditions.

We caution that our tests were performed on a single species of land snail, and are therefore not extensive enough to confirm that exposure to brodifacoum bait would not have adverse effects in other terrestrial malacofauna on Galapagos or elsewhere. However, given that most Galapagos endemic snail species are of the same genus as the species tested here, we feel confident that at least this important group will not be affected by exposure to brodifacoum bait if it was applied for eradication of invasive rodents on Floreana and other Galapagos Islands. Most importantly, these snails are known to be consumed by introduced rats (Clark, 1981; Parent, unpublished data), and therefore eradication of invasive rodents is more likely to result in positive effects on Galapagos endemic snail populations.

Secondary exposure pathways must be considered when assessing non-target risk and when developing measures to prevent non-target mortality (Eason, et al., 1999). We did not test for residual brodifacoum concentrations in the bodies of exposed snails in our study, but Booth et al. (2003) measured brodifacoum residues in the bodies of some snails that had consumed bait. We expect that any snails that consume bait on Floreana Island could constitute a secondary exposure pathway for their predators such as some of the larger land birds. The only Galapagos birds that have been verified to be preying on endemic snails are the Galapagos mocking birds which have been extirpated from Floreana Island. There are no other known potential secondary exposure pathways for non-target species on Floreana Island involving the endemic snails as intermediate.

Evidence to date and our results indicate that rodent bait containing brodifacoum does not present a high risk of non-target mortality to terrestrial snails. However, our study is limited to the detection of mortality (i.e. we did not monitor for other potentially negative effects) and was over a short period of time. Given the general trend across terrestrial molluscs of the effect of brodifacoum on snail mortality, we recommend a re-evaluation of this effect for the species included in the study reported by Gerlach & Florens (2000a; 2000b) that would incorporate a more complete set of treatments and controls. More specifically, we recommend more toxicity tests on the invasive giant African snails (*Achatina fulica*) given its broad distribution (tests could be conducted in a range of localities on continents and islands) and the negligible impact these tests would have on this highly invasive species (Lowe, et al., 2004). We conclude that it is prudent to continue to assess toxicity risk on a species by species basis, where rodent eradication using brodifacoum or other rodenticides is planned. Trials to determine whether captive snails would eat baits and whether exposure to baits results in measurable mortality are a relatively straightforward and low-cost means to test theoretical assessments of non-target risk.

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