No detection of brodifacoum residues in the marine and terrestrial food web three years after rat eradication at Palmyra Atoll, Central Pacific

A. Wegmann[1]*, G. Howald*[1]*, S. Kropidlowski*[2]*, N. Holmes*[1] *and A.B. Shiels*[3]

[1]*Island Conservation, 2100 Delaware Ave, Suite 1, Santa Cruz, CA 95060, USA. <alex.wegmann@tnc.org>.* [2]*U.S. Fish and Wildlife Service, Pacific Remote Islands Marine National Monument, Palmyra Atoll National Wildlife Refuge, USA.* [3]*USDA, APHIS, WS, National Wildlife Research Center, Fort Collins, CO 80521, USA.*

Abstract Invasive alien species represent one of the greatest threats to native plants and animals on islands. Rats (*Rattus* spp.) have invaded most of the world's oceanic islands, causing lasting or irreversible damage to ecosystems and biodiversity. To counter this threat, techniques to eradicate invasive rats from islands have been developed and applied across the globe. Eradication of alien rats from large or complex island ecosystems has only been successful with the use of bait containing a rodenticide. While effective at eradicating rats from islands, rodenticide can persist in the ecosystem longer than the time required to eradicate the target rat population and can potentially harm non-target species. However, the persistence of rodenticides in ecosystems following rat eradication campaigns is poorly understood, though predictions can be made based on the chemical properties of the rodenticide and the environment it is applied in. Brodifacoum, a relatively persistent second-generation anticoagulant, was used to successfully eradicate rats from Palmyra Atoll. With this study, we evaluated the persistence of brodifacoum residues in terrestrial and marine species at Palmyra Atoll (Northern Line Islands) three years after rat eradication. We collected 44 pooled samples containing 121 individuals of the following: mullet (*Moolgarda engeli*), cockroaches (*Periplaneta* sp.), geckos (*Lepidodactylus lugubris*), hermit crabs (*Coenobita perlatus*), and fiddler crabs (*Uca tetragonon*). Despite detection of brodifacoum residue in all five of the species sampled in this study 60 days after the application of bait to Palmyra Atoll in 2011, brodifacoum residue was not found in any of the pooled samples collected three years after bait application. Our study demonstrates how brodifacoum residues are unlikely to persist in the marine and terrestrial food web, in a wet tropical environment, three years after rat eradication.

Keywords: aerial rodenticide broadcast, best practice, brodifacoum anticoagulant rodenticide, land crabs, *Rattus rattus*, risk assessment, tropical island

INTRODUCTION

Invasive alien species represent a key threat to native plants and animals on islands (Tershy, et al., 2015). In particular, invasive rodents are known to have widespread negative impacts following introduction to islands (Towns, et al., 2006), and rodents have been introduced to most of the world's island groups (Atkinson, 1985). In prior decades, techniques to eradicate invasive rodents from islands have been developed and applied across the globe, most using anticoagulant rodenticides (Howald, et al., 2007). Demonstrable conservation benefits are common following successful eradication (Jones, et al., 2016; Brooke, et al., 2017).

To date, rat (*Rattus* spp.) eradications on tropical islands experience a lower success rate than those in temperate regions (Russell & Holmes, 2015). Lack of seasonality and warm temperatures in tropical latitudes can provide year-round breeding opportunities and a consistent abundance of alternative food sources that rodents may choose instead of the offered bait. Tropical regions also host land crab populations which readily compete with rats for bait (Wegmann, et al., 2011; Holmes, et al., 2015). In 2011, Palmyra was the site of a successful eradication of *R. rattus* (US Fish and Wildlife Service, 2011). The planning and implementation of the rat eradication required novel techniques, including direct baiting of the tree canopy, and two aerial broadcast applications, each at rates of 75 and 85 kg/ha, of bait containing brodifacoum (0.0025%) (Wegmann, et al., 2012). Ecotoxicology monitoring undertaken during and after the project detected residual brodifacoum in soil, water and biota (Pitt, et al., 2015). Sampling ceased 60 days after the bait application before undetectable levels of brodifacoum were reached (Pitt, et al., 2015). Resources to continue the monitoring were not secured until three years after the bait application for rat eradication, providing the opportunity to investigate longer-term persistence of brodifacoum within the Palmyra food web.

METHODS

Study site and animals

Palmyra Atoll (5°53' N, 162°05' W) is located at the northern end of the Line Islands in the Central Pacific Ocean. Palmyra is a wet atoll containing approximately 235 ha of emergent land primarily covered in thick rainforest. The atoll is an incorporated, unorganised territory of the United States that is managed in partnership by The Nature Conservancy (TNC) and the US Fish and Wildlife Service (USFWS). TNC's preserve includes Cooper/Menge (94.3 ha) and Barren (4.6 ha) islands. Most of the remaining emergent land is owned and managed by USFWS as Palmyra Atoll National Wildlife Refuge, which includes all marine habitats to 12 nm offshore.

Palmyra's islets support a regional flora that is typical of Central Pacific wet forests (Wester, 1985). Heavily influenced by the Intertropical Convergence Zone, Palmyra receives an average of 450 cm of rain each year. Palmyra is a refuge for 11 species of seabirds and is home to a robust community of land crabs comprised of nine species. Black rats (*Rattus rattus*) were inadvertently brought to Palmyra during WWII. In 2011, Palmyra's rat population was eradicated through two strategic applications of compressed-grain bait containing the second-generation anticoagulant rodenticide, brodifacoum, at 0.0025% (25 ppm) (Wegmann, et al., 2012). Pitt et al. (2015) collected and analysed fifty-one animal samples representing 15 species of birds, fish, reptiles, and invertebrates for brodifacoum residue out to 60 days after the initial bait application.

Environmental monitoring methodology

We followed the sampling methods outlined in Pitt et al. (2015) to assess brodifacoum residue concentrations three years after bait application in cockroaches

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(*Periplaneta* sp.), fiddler crabs (*Uca tetragonon*), hermit crabs (*Coenobita perlatus*), and geckos (*Lepidodactylus lugubris*). Limited time and resource restrictions did not allow sampling of black-spot sergeant fish (*Abudefduf sordidus*) or ants, as undertaken in 2011; however, we harvested mullet (*Moolgarda engeli*)), which were opportunistically collected as carcasses in 2011 following the eradication and their tissues were found to contain brodifacoum (Pitt, et al., 2015). All biological samples were collected at Palmyra Atoll between 4 and 19 June 2014. Biological samples were frozen immediately after collection.

Sampling site selection (Fig. 1) was determined by ease of access to the target species. All emergent land at Palmyra has relatively similar characteristics and vegetation and was treated with the same baiting prescription during the 2011 eradication campaign. We therefore assumed that site location would not be an influential factor in brodifacoum residue concentrations three years after bait was applied. Biological samples were collected at least 500 m from The Nature Conservancy's research station where rodenticide bait is maintained in bait stations for biosecurity when planes and ships arrive.

All biological samples were collected with gloved-hands and segregated in sterile sample bags. Captured hermit crabs were placed in a freezer (-4 C) for 24 hours and then removed from their gastropod shells and stored in sterile sample bags. Mullet were collected by dip-nets and fence-nets from several shoreline locations around Palmyra's central lagoon. Geckos and cockroaches were captured at night from the leaves of *Scaevola taccada* shrubs, and fiddler crabs were collected from lagoon flats at low tide. American Veterinarian Medical Association guidelines for euthanasia were followed with all collections. All samples were pooled (Table 1) to increase probability of detecting brodifacoum within the funding limits of this project and to ensure minimum amounts of sample material were provided for analysis (e.g. cockroach samples required two individuals to achieve the 2 g minimum for brodifacoum residue analysis). Samples were shipped frozen to US Department of Agriculture's National Wildlife Research Center (NWRC) in Fort Collins, Colorado, for brodifacoum residue analysis. Samples were prepared and analysed according to methods established by USDA NWRC for detection of brodifacoum in animal tissue, and these methods, as well as the laboratory conducting the analyses, were the same as those used in Pitt et al. (2015). Same-species pooled carcasses' samples were homogenised for analysis.

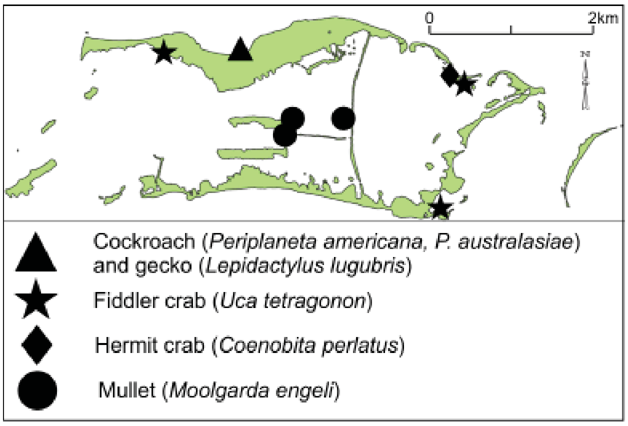

Fig. 1 Locations of sample collections that were used to investigate persistence of residual brodifacoum three years after the implementation of the 2011 eradication of rats from Palmyra Atoll.

Brodifacoum residue analysis methodology

The whole bodies of geckos and fish were homogenised. Cockroaches (whole bodies), as well as fiddler crabs and hermit crabs were homogenised in a liquid nitrogen freezer mill and 0.25 g of homogenate was placed into 25 ml glass tubes for further extraction and analysis following methods of Pitt, et al. (2015). Aliquots (0.5 g) of each homogenised gecko and fish sample were placed in MARS vessels for microwave extraction (Pitt, et al., 2015). Samples were clarified by centrifugation prior to HPLC analysis.

Brodifacoum analyses were performed with Agilent 1100 and 1200 HPLC systems (Pitt, et al., 2015). Brodifacoum concentrations were determined from the peak area ratio of brodifacoum to surrogate in each extracted sample and were compared to the average peak area ratio from replicate injections of a working standard. Samples with analytical concentrations above the linear range were re-diluted into the linear region.

RESULTS

We collected 44 pooled samples containing 121 total individuals (Table 1). Brodifacoum residues were not detected (detection levels reported in Table 1) in any of the pooled samples of mullet, geckos, cockroaches, hermit crabs, or fiddler crabs.

DISCUSSION

Ecotoxicology monitoring is uncommon for rodent eradication projects using rodenticides, but future projects are dependent on the collective knowledge gained from toxicological monitoring efforts. The Palmyra rat eradication used substantially higher rodenticide application rates compared to other rodenticide-based rodent eradication projects on islands and provided a unique opportunity to follow residue persistence in the environment over time. Brodifacoum residues were detected in soil, water and biota up to 60 days after the first aerial broadcast application (Pitt, et al., 2015) but were no longer detectable in the range of biota studied three years later, indicating rodenticides break down in this ecosystem over time. Resource availability did not allow complete repetition of the 2011 sampling, thus we chose to sample animals with known residue concentrations, as this had the most biologically useful outcome for management.

The use of second generation anticoagulant rodenticides can pose significant risks to non-target species (Howald, et al., 2007), particularly birds and mammals. However, knowledge gaps exist, particularly for taxa less sensitive to rodenticides, such as reptiles and invertebrates (Hoare & Hare, 2006). The distribution and longevity of rodenticide residue within a food web will be a function of rodenticide properties and how it is applied, environmental

Table 1 Biological samples analysed in 2014 for brodifacoum residue analysis following the 2011 eradication of rats from Palmyra Atoll. "Pooled" represents the number of individuals contained in each sample; "MLOD" is the mean level of brodifacoum detection.

Organism	Samples analysed	Pooled	MLOD (µg/g)
Mullet	9	2–3	0.013
Gecko	5	5	0.011
Cockroach	15	1–2	0.011
Hermit crab	5	3	0.0057
Fiddler crab	10	3	0.0057

compartments it ultimately resides within (e.g. soil, animals), open pathways to transfer residue (e.g. scavenger consumption of poisoned carcasses), and exposure to environmental conditions (e.g. temperature, precipitation, ultraviolet radiation, and fungi) that impact its persistence. Ultimately, the breakdown of rodenticides is believed to be accelerated in soil rich in organic matter with healthy populations of microbiological organisms. Different island ecosystems can be expected to have different timescales of residue longevity, and we expect our results will transfer most closely to other wet tropical atolls and low islands, rather than dry and/or temperate island environments.

Rodenticides are known to temporarily infiltrate the food web when undertaking rat eradications as happened with the Palmyra rat eradication. Brodifacoum residues were found in ocean water, soil, and marine and terrestrial biota within 60 days of the initial baiting, indicating diverse food web integration (Pitt, et al., 2015). Other studies document brodifacoum residues in various compartments of the food web after brodifacoum bait was applied to eradicate rats from islands (e.g. Dowding, et al., 1999; Masuda, et al., 2014; Masuda, et al., 2015; Pitt, et al., 2015; Siers, et al., 2015; Rueda, et al., 2016; Shiels, et al., 2017). Although few studies include long-term (>1 year) sampling for residues after brodifacoum application, there are three recent studies that report residues in animals two years (Rueda, et al., 2016), three years (Siers, et al., 2015), and four years (Shiels, et al., 2017) post-application. Brodifacoum persisted in lava lizards (*Microlophus duncanensis*) in the Galápagos Islands for 2.1 years (Rueda, et al., 2016), where liver residue levels were <0.200 µg/g (mean level of detection [MLOD] = 0.010 µg/g). On Wake Island in the Pacific Ocean, three years after rat eradication (Siers, et al., 2015), two out of 69 fish samples had detectable levels of brodifacoum in their livers, with concentrations 0.0038 µg/g and 0.0086 µg/g (MLOD = 0.0035 µg/g); the two fish were caught within an intermittently land-locked pond. Finally, on Desecheo Island, Puerto Rico, detectable levels of brodifacoum were found in seven animal samples (three endemic lizards, two black rats, one forest bird, and one cockroach sample [18 individuals]) four years after bait application (Shiels, et al., 2017). The range of brodifacoum residues in these seven samples was 0.027-0.134 µg/g (MLOD = 0.0054-0.012 µg/g, depending on species. Desecheo, Wake, and the Galápagos islands receive less rainfall than Palmyra (e.g. Desecheo = 1,020 mm/yr, Wake = 906 mm/yr; Pinzon, Galápagos = <1,100 mm/yr; Palmyra = 3,500 mm/yr), and this may contribute to the lack of detectable levels of brodifacoum in the Palmyra food web three years after bait application. We hypothesise that warmer and wetter environments, and soils with more diverse microbiological communities support microbiological processes breaking down residues faster. This remains an important research avenue, including decomposition experiments in a laboratory setting.

Undertaking eradications of invasive species from islands should only proceed where expected benefits outweigh expected costs (Broome, et al., 2014), including consideration of the environmental impacts of the method used (Empson & Miskelly, 1999). Potential non-target impacts were anticipated as part of the environmental impact assessment for the Palmyra rat eradication, but the decision to proceed was based on negative impacts ceasing shortly after the bait application and positive benefits accruing over a longer time-span (US Fish and Wildlife Service, 2011). Immediately following bait application, brodifacoum residues were detected within multiple levels of the food web, and were attributed to mortality of birds, fish, and crabs (Pitt, et al.., 2015). Our results show undetectable levels of residue three years later, suggesting this short-term impact is no longer present. Longer-term changes to native species populations following the removal of rat impacts are emerging, including increased seedling recruitment of several native tree species and the non-native coconut palm (*Cocos nucifera*), the elimination of a non-native mammal-biting mosquito population (*Aedes albopictus*), as well as the discovery of two new-to-Palmyra land crab species (*Geograpsus grayi* and *Ocypode cordimanus*). These short and long-term changes are consistent with management expectations, and the rat eradication has proven to be a baseline restoration activity to advance natural resource management goals.

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