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Research Article

Brodifacoum residues in fish three years after an island-wide rat eradication attempt in the tropical Pacific

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Abstract

Invasive rats are known to threaten natural resources and human health and safety. Island-wide rat eradication attempts have been increasing in number and scale during the past several decades, as has the frequency of eradication success. The most common method to remove all rats from an island is to broadcast anticoagulant rodenticide bait into every rat's home range on the island. Broadcast of toxicants can put humans and other nontarget species in marine and terrestrial environments at risk of exposure. The persistence of anticoagulant residues is somewhat unknown, particularly in marine environments. Three years after ~ 18,000 kg of 25 mg/kg brodifacoum bait was broadcast across Wake Atoll to eliminate rats, we collected whole-body fish samples from six near-shore sites and one intermittently land-locked pond to test for brodifacoum residues. Of the 69 samples tested using high-performance liquid chromatography with fluorescence detection, 20 were suspected of brodifacoum contamination and therefore subject to more selective liquid chromatography-mass spectrometry analysis. Of those 20 fish, brodifacoum was detected in two individuals of blacktail snapper (*Lutjanus fulvus*), although at levels too low to be accurately quantified. Both fish containing detectable brodifacoum residues were caught within an intermittently land-locked pond in an area of the island that received heavy brodifacoum baiting, and not truly in the "marine environment". Brodifacoum was not detected in any of the samples collected within the lagoon of the atoll or within near-shore waters outside the lagoon. These results demonstrate that under some circumstances very low levels of brodifacoum can occur in a low proportion of fish tissues for as long as three years after the application of the rodenticide to an environment. Such information is valuable in assessing the relative environmental risks associated with rodenticide use in rodent eradications for protection of threatened species and restoration of island ecosystems. The overall result is one of declining incidence and magnitude of residue concentrations over time and within limited environmental circumstances.

Key words: bioaccumulation, contamination retention, marine food web, nontarget effects, risk management, rodenticide

Introduction

Biological invasions of island ecosystems by commensal rodents, particularly rats (*Rattus* spp.), have had profound negative consequences for species

and ecosystems (Townsend et al. 2006; Jones et al. 2008; Shiels et al. 2014). Eradication of invasive rats from islands can be a powerful conservation intervention for protection of threatened species and recovery of ecosystem function (Whitworth et al. 2013; Russell and Holmes 2015; Graham et al. 2018); ecosystem response to rat eradication can be rapid and dramatic (Le Corre et al. 2015; Wolf et al. 2018). Using principles and techniques pioneered and improved over several decades (Howald et al. 2007; Russell and Broome 2016) and following established best management practices (Broome et al. 2014; Keitt et al. 2015), island rodent eradications are increasing in frequency, size, and complexity (Howald et al. 2007; Russell and Broome 2016; Martin and Richardson 2019).

Rat eradications are achieved primarily by the aerial broadcast of cereal-based pellets containing rodenticide (Howald et al. 2007; Witmer et al. 2007; Broome et al. 2014). A study summarizing rodent eradication attempts between 1971 and 2011 demonstrated that brodifacoum was the chosen toxicant in 396 of 546 cases (72.5%); this is partly due to its status as one of the few rodenticides registered for aerial use against rodents (Parkes et al. 2011). While the use of brodifacoum baits has proven successful in most rat eradication attempts, nontarget species in marine and terrestrial environments can be exposed and are therefore at risk of toxic effects. There is a growing body of literature on anticoagulant residue monitoring in water, soil, and aquatic environments (Ogilvie et al. 1997; Orazio et al. 2009; Fisher et al. 2010; Siers et al. 2018a) and the nontarget consequences and fate of toxicant residue from rodent eradication operations (e.g., Dowding et al. 1999; Sztukowski and Kesler 2013; Masuda et al. 2014; Pitt et al. 2015; Rueda et al. 2016; Coeurdassier et al. 2018). The duration of risk from anticoagulant residues is less well-known (however, see Wegmann et al. 2019).

Brodifacoum is a second generation anticoagulant that is extremely effective at inhibiting the reconstitution of active vitamin K, yielding a 100-fold decrease in vitamin K-dependent coagulation factors when compared with warfarin (a pharmaceutical anticoagulant) at the same molar dose (Lipton and Klass 1984).

For use in rat eradications, brodifacoum is typically incorporated into a cereal matrix-based rodenticide formulation at 25 or 50 mg/kg and is available in small (< 3 cm in longest length) pellets with dyes ranging from bright red, green, or blue (Fang et al. 2012). Brodifacoum may enter the marine food web through direct consumption of baits having drifted or washed into the marine environment, or by indirect means (e.g., invertebrates eating the bait then in turn being eaten by fish). A review by Fisher (2010) indicated that aerial applications of brodifacoum for eradication of introduced rodents on New Zealand islands have led to no detectable brodifacoum residues in fresh water, and conclude that factors such as brodifacoum's low water-solubility (especially at acidic and neutral pH), the adsorption of brodifacoum to organic particles, and dilution with water

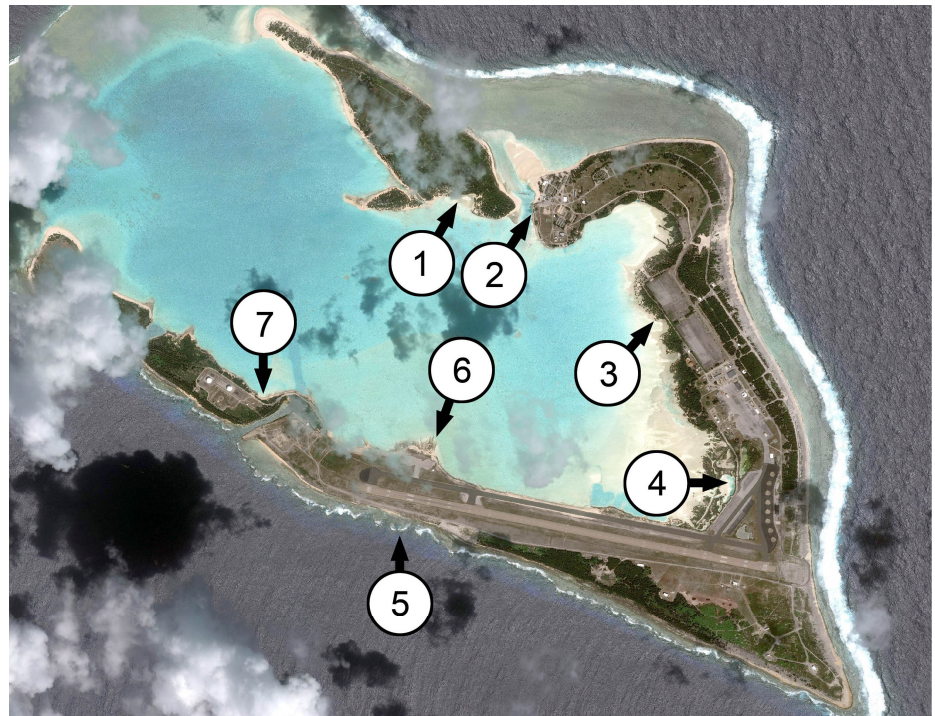


Figure 1. Wake Island with the seven sampling locations marked by arrows. Site names include: 1 – Peale Lagoon Side, 2 – Ioke Beach House, 3 – Waterplant Outfall, 4 – Battery Dump Pond, 5 – Southern Runway Windssock (not sampled in this study), 6 – Old AF Beach House, 7 – Nitro Rock. Image courtesy of U.S. Air Force Civil Engineer Center.

volume and flow rate likely contributed to this result. To our knowledge, the longest documented residence period of brodifacoum in the marine environment (2.2 years) was from an accidental spill of 20 tons of brodifacoum bait directly into the near-shore environment (Primus et al. 2005). No brodifacoum residues were detected by Wegmann et al. (2019) three years after an unusually intensive rat eradication baiting on Palmyra Atoll (Pitt et al. 2015).

Another example of an eradication attempt comes from Wake Atoll (hereafter referred to as Wake Island or Wake), which is between Hawaii and Guam in the northern Pacific Ocean (19°18'N; 166°38'E; maximum elevation of 6 m above sea level), and is made up of Wake Island proper (525 ha), Peale Island (95 ha), and Wilkes Island (76 ha; Figure 1). Wake is an unincorporated U.S. territory that is managed by the Department of Defense, U.S. Air Force, and the 50–100 people that generally reside on Wake are military personnel and contractors. Wake has approximately 19 km of coastline and is an important breeding area for many species of seabirds. The coastline is also fished by the local residents for sport and food. Two species of rats were inadvertently introduced onto Wake Island, the Polynesian rat (*Rattus exulans* Peale, 1848) and Asian house rat (*Rattus tanezumi* Temminck, 1844), and these rats are known to consume eggs and chicks of seabirds. To protect natural resources, infrastructure, human health and safety, and biosecurity risks posed by a high abundance of invasive rats, an eradication effort was conducted on Wake Island in the

summer of 2012 (Island Conservation 2013; Griffiths et al. 2014). Brodifacoum 25W: Conservation anticoagulant rodenticide bait (25 mg/kg brodifacoum, manufactured by Bell Laboratories, Madison, Wisconsin), was applied by helicopter and hand-broadcast at approximately 27 and 18 kg/ha for each of two applications in summer 2012. *Rattus tanezumi* was successfully extirpated, but *R. exulans* survived the eradication attempt and is now widespread on the island. Possible causes for this partial failure include insufficient bait availability or palatability, availability of alternative food sources for rats, operational complexity including above- and below-ground structures, insufficient sanitation by the resident workforce, and prohibitions on sowing bait in tidally-inundated habitats to prevent contamination of the nearshore marine environment (Brown et al. 2013; Siers et al. 2018b; Hanson et al. 2019).

Some bait entering the ocean during these island-wide rat eradication operations is inevitable (directly or indirectly, e.g., by tidal activity; Pitt et al. 2015). Three months after the 2012 rat eradication attempt, fish and land crabs were sampled for brodifacoum residues (Musashino Keisoku 2012) to evaluate risk of human exposure through consumption by the resident workforce. Musashino Keisoku (2012) documented no detections in 2 eels (species not listed), 11 bonefish (*Albula glossodonta*), 16 milkfish (*Chanos chanos*), 1 goatfish (species not listed) or 6 “land crabs” (probably *Coenobita* spp.). Some fish (1 of 8 bluefin trevally, *Caranx* sp., and 4 of 4 blacktail snapper, *Lutjanus fulvus*, all from within the lagoon) had low but detectable levels of brodifacoum residues. How long these residues persist in the environment, affecting the marine food web, and to what extent they persist in fish that are caught by Wake Island residents for sport and consumption, is uncertain. The objectives of the current study were to re-sample the same near-shore environments on Wake Island in 2015, approximately 3 years post-rodenticide application, to determine the levels of brodifacoum residue in a suite of fish species commonly caught by island residents. Based on the relatively low incidence of brodifacoum detection approximately 3 months after rodenticide application in 2012 (e.g., 5 out of 48 samples had “detectable levels”, which were > 0.001 mg/kg), we expected even lower incidence of detectable brodifacoum in the 2015 samples.

Our sampling effort was sponsored by Pacific Air Force Regional Support Center. Potential human health implications from brodifacoum use and environmental residues, and any risk mitigation strategies (e.g., fishing moratoria), were evaluated by Air Force occupational health and safety personnel. Fish consumption advisories for the atoll were in existence prior to the inception of eradication operation (PACAF 2002) and remain in effect; these advisories were not associated with brodifacoum use. The intention of this article is not to evaluate human health risks but rather to report the observed frequency and concentration of residues so that they might be interpreted in terms of potential impacts to environmental health and natural resources.

Table 1. General characteristics of sampled fish species on Wake Atoll.

Species	Trophic Characteristics	Size	Longevity
Milkfish	Juveniles and adults eat cyanobacteria, soft algae, small benthic invertebrates, and pelagic fish eggs and larvae	To 180 cm, commonly 100 cm	To 15 years
Goatfish	Feed on crustaceans, mollusks, worms, heart urchins and foraminiferans	To 60 cm, commonly 30 cm	To 5 years ^{a*}
Blacktail snapper	Adults feed at night on fishes, shrimps, crabs, holothurians and cephalopods	To 40 cm, commonly 25 cm	To 34 years ^b
Bluefin trevally	Feeds mainly on other fishes, also crustaceans. Often contain ciguatoxins when reaching lengths of more than 50 cm	To 120 cm, commonly 60 cm	To 11 years ^{a*}
Bonefish	Feeds on invertebrates, benthic species, mollusks and small crustaceans	To 90 cm	To 20 years, usually 5 to 10 ^{c*}
Soldierfish	Feed mainly on plankton such as crab larvae	To 60 cm, commonly 18 cm	To 14 years ^{d*}
Flounder	Feed on fishes, crabs and shrimps	To 51 cm	No information

Source: FishBase (Froese and Pauly 1994) unless otherwise noted. ^aAnAge 2015; ^bShimose and Nanami 2014; ^cCrabtree et al. 1996; ^dDee and Radtke 1989. * Estimated from information on closely related species.

Materials and methods

Fish sampling methods

Fish samples for whole-body analysis were collected in April 2015 from Wake Island at the same locations that were sampled in 2012 (Musashino Keisoku 2012) (Figure 1). These locations were originally chosen because they are representative of where fishing typically occurs, with the exception of an intermittently landlocked saltwater pond around, which bait was applied at higher concentrations (> 45 kg/ha; Island Conservation 2013). The same species were sampled as in 2012, with the exception of soldierfish and flounder sampled instead of eel and crab. The species targeted for this study were: milkfish (*Chanos chanos*); goatfish (*Mulloidichthys flavolineatus*, *Parupeneus barberinus*, or *Upeneus arge*); blacktail snapper (*Lutjanus fulvus*); bluefin trevally (*Caranx* sp., probably *C. melampygus*); bonefish (*Albula glossodonta*); soldierfish (*Myripristis murdjan*); and flounder (*Bothus* sp., probably *B. mancus*). Select characteristics of these species are summarized in Table 1. In the 2012 sampling, only land crabs were collected from sampling location 5; our study focused on aquatic environments so no land crabs were collected and there would be no comparative value for fish sampling at this site. Instead, a small number of fish (9) were collected opportunistically from a small number of other coastal locations outside the lagoon: Heal Point, Marina Channel, North Shore Peale, and North Shore Wake.

Our target sample size for 2015 sampling (as was in 2012) was 10 samples per species (total of 70 samples). In most cases, a sample represented an individual; however, in those instances when an individual caught weighed < 100 g, multiple individuals were pooled to make a sample (typically up to three individuals). Individuals < 10 g were released. We planned to sample across the locations as evenly as possible for most fish

species; however, some species only reside in particular locations so it was expected that all 10 samples for a given species might be collected from a subset of the locations.

The primary sampling method was fishing pole (hook and line), or secondarily by cast nets or spearing. Fish removed from nets or from hooks were stunned with a blow to the head and then euthanized by pithing in accordance with American Veterinary Medical Association guidelines for euthanasia of fish. Nontarget captures were released. Fish were photographed, weighed, and measured (total length) immediately following capture. Individual fish were placed in a plastic bag, then pooled, if necessary (as outlined above), and placed into a larger plastic bag labeled by location and time period. Samples were shipped frozen to the USDA-APHIS-WS-NWRC field station in Hilo, Hawai'i, and then on to the NWRC Chemistry Lab Unit in Fort Collins, Colorado, for brodifacoum analysis.

Brodifacoum residue analytical methods

Because the intention of the initial sampling by Musashino Keisoku (2012) was to inform evaluation of health risk from consuming fish caught by anglers following the eradication attempt, analyte concentration estimation was based on whole-body homogenates including both muscle and liver tissue. Contaminant concentrations are typically much higher in liver than muscle tissues (Ahmed 1991). Anglers typically eat only the muscle tissues; however, some smaller fish are eaten whole and other body parts may be included in soups, so testing of whole-body homogenates was seen as a conservative approach for evaluating potential human exposure. Testing liver and muscle tissues independently requires multiple tests per specimen and increased sampling in order to obtain enough liver tissue for analysis, and therefore was considered beyond the scope of the sponsor's interest for our study. Whole-body analysis has been used in similar food web residue monitoring studies as it also represents the typical consumption pattern of predators (Pitt et al. 2015; Wegmann et al. 2019).

HPLC/FLD

Fish samples were preliminarily analyzed by high-performance liquid chromatography with fluorescence detection (HPLC/FLD) using the method described in Pitt et al. (2015), except that a chlorine substituted analog of brodifacoum (Richman Chemical, Lower Gwynedd, PA, USA) was used as the surrogate analyte instead of difenacoum. Homogenized whole fish (~ 1.0 g) was microwave extracted into acetonitrile (ACN) and cleaned-up using mixed-mode weak anion exchange/reversed phase solid-phase extraction (SPE). Method accuracy using control homogenized fish from fish markets in Hilo, Hawaii (bluestripe snapper, *Lutjanus kasmira*; soldierfish,

Myripristis sp.) fortified at 0.1 mg/kg brodifacoum ranged from 69.8%–89.0% ($n = 12$). Brodifacoum detection and quantitation limits estimated from 3X and 10X the signal-to-noise ratio in control fish were 0.0035 and 0.012 mg/kg, respectively.

LC-MS/MS

Samples from the HPLC/FLD analysis with responses at the retention time for brodifacoum were subsequently analyzed with liquid chromatography-tandem mass spectrometry (LC-MS/MS) to confirm and quantify brodifacoum concentrations. The method was based on the procedure described in Franklin et al. (2018) with the following changes: Homogenized whole fish (0.20–0.25 g) was weighed into a 15-mL polypropylene tube and the surrogate analyte flocoumafen (Honeywell Fluka, Morris Plains, NJ; 20 μ L, 33 μ g/mL in acetonitrile) added. DI water (40 μ L) was added and the sample vortex mixed 15–20 s. ACN (3 mL) was added and the sample extracted 30 min by mechanical shaking. Excess NaCl (~ 250 mg) was added to produce a water:ACN phase separation and the sample mechanically shaken an additional 30 min. The supernatant was clarified by centrifugation (2150 RCF) and 1.5 mL transferred to a dispersive solid-phase extraction (dSPE) tube (Agilent Technologies, Santa Clara, CA, USA) containing magnesium sulfate, C18 sorbent, and primary-secondary amine (PSA) sorbent. A portion of the supernatant (0.4 mL) was reduced to dryness and reconstituted in mobile phase. Each sample (10 μ L) was injected into an Agilent LC-MS/MS consisting of a 1200 Series HPLC coupled to a 6410B triple-quadrupole mass spectrometer with atmospheric pressure chemical ionization (APCI) source. The gradient program was held at 10% B for 0.5 min, and then increased linearly to 69% B over 4.5 min. The source drying gas (N_2) was 350 °C with a flow of 4 L/min and a nebulizer pressure of 20 psi. The vaporizer was 400 °C with a corona current of –10 μ A. The capillary voltage was –4500V. Multiple reaction monitoring (MRM) was used to quantify brodifacoum by detecting the ion transition 522.9 \rightarrow 80.9 m/z (541.0 \rightarrow 382.1 for flocoumafen). The fragmentor and collision energies for brodifacoum were 165V and 50V, respectively (157V and 23V for flocoumafen). The identity of brodifacoum was also confirmed by detecting the qualifier ion transition 522.9 \rightarrow 135.0 m/z (collision energy 44V). The brodifacoum/flocoumafen peak area response ratio versus brodifacoum concentration (6-level standard curve ranging from 1.4–1050 ng/mL) was fit to a second order logarithmic function ($R^2 = 0.999$) and used to quantify samples. Method accuracy using control homogenized fish (bluestripe snapper) fortified with 0.026, 0.24, and 3.2 mg/kg brodifacoum was 103%, 92.9%, and 89.2%, respectively. Brodifacoum detection and quantitation limits estimated from 3X and 10X signal-to-noise ratio in control fish were 0.0034 and 0.0112 mg/kg, respectively.

Table 2. Distribution of sampled fish among species and sampling locations.

Common name	Latin name	Counts of Samples by Location							Total	
		1	2	3	4	5	6	7		Other
Milkfish	<i>Chanos chanos</i>			5	6					11
Goatfish	<i>Mulloidichthys flavolineatus</i> , <i>Parupeneus barberinus</i> or <i>Upeneus arge</i>	4					1	2	1 ^b ,1 ^c	9
Blacktail Snapper	<i>Lutjanus fulvus</i>	2	2		3		4	1		12
Bluefin trevally	<i>Caranx</i> sp., probably <i>C. melampygus</i>	1						2	2 ^a ,1 ^b ,2 ^d	8
Bonefish	<i>Albula glossodonta</i>	2			4		4			10
Soldierfish	<i>Myripristis murdjan</i>			7					2 ^{b*}	9
Flounder	<i>Bothus</i> sp., probably <i>B. manthus</i>	1	2	2			1	4		10
Total		10	4	14	13	0	10	9	9	69

Sampling locations are as follows: 1 – Peale Lagoon Side, 2 – Ioke Beach House, 3 – Waterplant Outfall, 4 – Battery Dump Pond, 5 – Southern Runway Windsock (not sampled), 6 – Old AF Beach House, 7 – Nitro Rock. These seven locations are depicted on the map in Figure 1. The “Other” category signifies supplemental collection sites and are labeled with superscripts: a – Heal Point; b – Marina Channel; c – North Shore Peale; d – North Shore Wake. Results reported with an asterisk (*) denotes that one soldierfish at this site was *Myripristis chryseres*.

Results

Fish sampling

Between 21 March 2015 and 2 April 2015, 69 fish samples were collected from Wake Island. Samples were of individual fish, with the exception of 5 milkfish samples pooled from small individuals. The samples are summarized in Table 2.

Brodifacoum analysis

For both HPLC-FLD and LC-MS/MS, “detection limit” (DL) is the concentration above which brodifacoum could be detected and the “quantitation limit” (QL) is the concentration above which measurements are relatively reliable. Concentration estimates lower than the DL are not distinguishable from background noise and are reported as “not detected” (ND). Concentrations higher than the DL but lower than the QL are reported as detections without listing the concentration measurements because they are too low to be reliably interpreted as true concentrations. Values above the QL are reported in units of mg/kg (= PPM) and are relatively reliable for comparisons. Full laboratory methods and results are included in the Analytical Chemistry Report provided as Appendix 1, with raw analytical chemistry data in Supplementary material Table S1.

The 69 whole fish samples were initially analyzed by HPLC with fluorescence detection (FLD), resulting in 20 suspected detections at levels that were above the DL (0.0035 mg/kg) but less than the QL (0.012 mg/kg). These results are detailed in Table S2.

The samples with suspected brodifacoum residues were subsequently tested by an LC-MS/MS method that is highly selective for brodifacoum. Of these 20 samples, 18 indicated no brodifacoum contamination or were below the detection limit of the method (DL = 0.0034 mg/kg). Two

samples (S150413-35 and -36) tested positive for brodifacoum but, as with the HPLC/FLD method, both were at concentrations below the quantitation limit of the method (QL = 0.0112 mg/kg). Both fish with detectable contamination were blacktail snappers, *Lutjanus fulvus*, from the Battery Dump Pond (Site 4), which is an intermittently land-locked pond.

The 2012 sample indicated 5 brodifacoum detections from 48 fish, including 4 of 4 blacktail snappers from Site 7; they collected no snappers from the Battery Dump Pond (Site 4), which was the location of our only brodifacoum detections. The current (2015) sample indicated 2 brodifacoum detections (though below the quantitation limit) in a sample of 69 fish (2 of 12 blacktail snappers). Qualitatively, the proportion of detections in the 2015 sample ($2/69 = 0.0290$) appears less than the proportion in the 2012 sample ($5/48 = 0.104$); however, these proportions cannot be statistically distinguished (Fisher's exact test, one-tailed, $p = 0.100$). Among blacktail snappers sampled, the 2015 sample contained a lower ratio ($2/12 = 0.17$) than the 2012 sample ($4/4 = 1.0$), a difference that is statistically significant (one-tailed, $p = 0.005$).

Discussion

Three years after an island-wide application of brodifacoum bait (Island Conservation 2013), 2 fish samples (out of 69 sampled around the island) indicated levels of brodifacoum above the detection limit but below the quantitation limit (between 0.0034 and 0.0112 mg/kg). Both fish with detectable brodifacoum residues were blacktail snappers. This is particularly noteworthy because 4 of the 5 fish identified as containing brodifacoum residues in the 2012 sample were also blacktail snappers, and every snapper in that sample tested positive. This may indicate that blacktail snappers either had more exposure to brodifacoum (through direct consumption of bait, consumption of other organisms exposed to brodifacoum, or both), retained brodifacoum in body tissues longer, are longer lived, or exhibited greater site fidelity than other fish. Snappers are predators of fishes, shrimps, crabs, holothurians, and cephalopods, placing them in a trophic level with potential for bioaccumulation of brodifacoum. Although age estimates of *L. fulvus* and *L. kasmira* were not available, other *Lutjanus* species appear relatively long-lived based on Heupel et al. (2010) determining modal ages of 7–10 years, and maximum ages of 12–23 years, after harvesting 3,334 individuals of three *Lutjanus* species from tropical waters. Snappers typically grow to about 25 cm, sometimes 40 cm, and may live as long as 34 years (Table 1). All blacktail snappers tested in this study ranged from 24 to 32.5 cm (positive samples 28 and 30 cm); although we know nothing about migration of individuals into and out of the landlocked pond area, it seems that individuals of this size could be old enough to have been within the pond during the bait applications three years prior to sampling. The

only other species sampled from the landlocked pond were milkfish and bonefish. Of the 6 milkfish samples, 5 were pooled from small individuals and were not likely to have been alive in the pond at the time of brodifacoum applications. The 1 remaining milkfish and 4 bonefish were large enough to possibly be present at the time of bait applications. Beyond this reasoning, we don't have enough evidence to speculate further on relative influence of possible factors leading to the appearance of a pattern of high snapper contamination.

Given the chemical properties of brodifacoum, the potential for bioconcentration in aquatic organisms is high (USNLM 2016); however, 3 years after whole-island brodifacoum bait application ceased, 67 of 69 of the marine fishes that we sampled (97%) did not show evidence of this toxicant. Similar to our findings on Wake, there were no detectable levels of brodifacoum in the 44 pooled samples of 121 mullet, gecko, cockroach, and crab individuals from the marine and terrestrial food web on another tropical Pacific island (Palmyra Atoll) 3 years post-application of brodifacoum (Wegmann et al. 2019). Therefore, residual brodifacoum appears to move and/or degrade from the majority of the marine and terrestrial food webs on these tropical islands within 3 years, but some residues may be detected as long as 3 years after bait application. Significant exposures to brodifacoum via Wake fish 3 years after rat eradication would be highly unlikely for humans or marine predators of fish. Sufficient data do not exist to characterize the lethal or sublethal health effects of chronic exposure to trace levels of brodifacoum in humans or suitable animal models.

No brodifacoum residues were detectable in fish caught within the lagoon of the atoll or within near-shore waters outside the lagoon. Both of the 2015 brodifacoum detections were from fish captured within the inland water feature known as the Battery Dump Pond. Although connected to the marine environment in the lagoon via a culvert that is frequently obstructed by sediment buildup, this cannot truly be considered representative of the marine environment. Fish captured in this pond also had higher levels of heavy metal contamination (chromium, cobalt, nickel, copper, zinc, antimony, barium, and lead) than fish captured within the lagoon (Siers et al. 2016). During the rat eradication operation, habitat adjacent to this pond received some of the highest application rates (> 45 kg of bait/ha) (Island Conservation 2013). Bait drift from heavy treatment of nearby habitat into the relatively stagnant or infrequently-flushed pond likely led to higher contamination rates of fish as opposed to those in the open and frequently-flushed lagoon environment. Migration of fish in and out of the pond was potentially impeded by frequent and prolonged clogging of culverts by sediment, restricting emigration and containing fish within an environment where brodifacoum residues were not being flushed, prolonging exposure. Also, prolonged persistence of brodifacoum residues in Battery Dump Pond fish could be partially attributable to continued

exposure through erosion of brodifacoum-laden soils from nearby treated terrestrial habitats into the often-stagnant pond. Brodifacoum is persistent in soils, with a soil half-life of 157 days (USEPA 1998); however, there is no published evidence of soil-bound brodifacoum entering the aquatic food web. These factors, coupled with blacktail snapper characteristics of having a longer lifespan and feeding at a higher trophic level than some other Battery Dump Pond fish (Table 1), likely contributed to this result of prolonged contamination. None of the five 2012 brodifacoum detections by Musashino Keisoku originated from the Battery Dump Pond (1 trevally from Site 2 and 4 blacktail snappers from Site 7), and none of the 11 milkfish samples from the Battery Dump Pond tested positive for brodifacoum; the 2012 sample included no snappers from the Battery Dump Pond.

Brodifacoum has only been used on Wake during the 2012 rat eradication campaign, during a very small-scale USDA-run cage trial that was contained in a building, and for biosecurity purposes in a small number of bait stations at the marina (near the east end of Wake Island, over 3 km from the location of our two brodifacoum detections; J. Helm, US Air Force, *personal communication*); therefore, it appears almost certain that the brodifacoum residues detected were a result of the rat eradication attempt in 2012. The 2012 rat eradication campaign involved large numbers of bait stations and the aerial and hand broadcast of brodifacoum pellets across the terrestrial expanse of the islands, during which over 18,000 kg of brodifacoum bait was applied (Island Conservation 2013). While great care was taken in applying brodifacoum bait along coastlines, small amounts of pellets were observed to have directly entered the marine environment. Although it is possible that routine use of brodifacoum in bait stations could lead to the movement of brodifacoum traces into the marine environment, potentially through consumption and dispersal by invertebrates such as crabs, such transmission of anticoagulant residues has not been documented; it is far more likely that any marine residues of brodifacoum are a result of the 2012 landscape-scale application of brodifacoum pellets.

Monitoring of brodifacoum residues in the Palmyra Atoll food web before, and less than 2 months after, a 2011 rat eradication campaign has demonstrated contamination of fish in the near-shore environment (Pitt et al. 2015), probably via bait drift directly into coastal waters, making it directly available to a wide variety of marine organisms. Blackspot sergeant (*Abudefduf sordidus*) were sampled before the first brodifacoum application (8 samples from 26 fish) and after the second application (10 samples from 30 fish); brodifacoum was not detected in any of the pre-treatment samples, but 10–14 days after initial brodifacoum application there was 9 of 10 samples that contained detectable brodifacoum residues (mean = 0.143 mg/kg, SE = 0.027 mg/kg). Twenty-four samples of dead mullet (*Moolgarda engeli* or *Liza vaigiensis*, 47 fish) and 1 dead pufferfish (species ID unknown) were opportunistically collected during and up to 3 weeks

after eradication operations. All dead fish samples tested positive for brodifacoum residues, with concentrations ranging from 0.058 to 1.160 mg/kg (mean = 0.337 mg/kg), with the highest concentrations in the earlier recovered samples and declining over time. The lack of dead organisms before the initial bait broadcast and the concentrations of brodifacoum in carcasses recovered after baiting began suggests that brodifacoum played a role in those mortalities. Mullet are frequently fed upon by predatory fish, demonstrating potential for accumulation of brodifacoum residues at higher trophic levels.

Masuda et al. (2015) also report on brodifacoum residues in coastal marine species following a 2011 Norway rat (*R. norvegicus*) eradication on Ulva Island, New Zealand, finding initially low but detectable levels of brodifacoum in two blue cod (*Paraperis colias*) liver samples (0.026 and 0.092 mg/kg; no detections in muscle tissue), four composite mussel samples (*Mytilus edulis*, 0.001–0.022 mg/kg), and four composite limpet samples (*Cellana ornata*; 0.001–0.016 mg/kg). By 77 days post-operation, no fish tissues were found to contain brodifacoum; by day 176, no limpets contained detectable residues while one composite mussel sample tested positive (0.018 mg/kg). By day 274, no residues were detected in any marine species samples (Masuda et al. 2015).

As a follow-up to the short-term brodifacoum residues study on Palmyra Atoll (Pitt et al. 2015), Wegmann et al. (2019) evaluated the persistence of residues of brodifacoum in terrestrial and marine species at Palmyra Atoll 3 years after rat eradication. After collections and analysis of 121 individuals of mullet, cockroaches (*Periplaneta* sp.), fiddler crabs (*Uca tetragonon*), hermit crabs (*Coenobita perlatus*), and geckos (*Lepidodactylus lugubris*), comprising 44 samples pooled by species, there were no detectable levels of brodifacoum found (DL was 0.0057–0.013 mg/kg). There are several possible explanations for differences in brodifacoum detections between 3 years post-rat eradications in the Palmyra Atoll study (Wegmann et al. 2019) and in our Wake Island study. First is that there was only one fish species (mullet) analysed in the Palmyra Atoll study, whereas we analysed 7 species on Wake and none were mullet. In our study, as well as Musashino Keisoku (2012), it was only snappers (genus *Lutjanus*) that had persistent detectable anticoagulant residues; thus, differences in fish species appear important. Second, microbial breakdown of brodifacoum may be accelerated on Palmyra Atoll relative to Wake in part because of the warmer and 4 times wetter conditions at Palmyra Atoll than at Wake (Wegmann et al. 2019) Third, although the same laboratory completed the brodifacoum residue analysis on Palmyra Atoll and Wake Island, the brodifacoum DL was slightly higher for fish collected from Palmyra (0.013 mg/kg) than Wake (0.0035 mg/kg), which opens the possibility that very low levels of brodifacoum residue in mullet 3 years after the Palmyra Atoll rat eradication

may have gone undetected; our 2 detections in this study were below the DL for the Palmyra study. However, it is also quite likely that a lack of detectable residues years after rodenticide use is indeed the general pattern, and that our 2 detections were the exception, being found in one species of fish in a landlocked and potentially highly-contaminated pond. Given that 97% of the sampled fish on Wake did not have detectable levels of brodifacoum 3 years after rat eradication, and that no persistent residues were detected on Palmyra Atoll under similar conditions, it is unlikely that brodifacoum generally persists in the marine food web, and for the few exceptions it would be at very low levels (Primus et al. 2005; Wegmann et al. 2019).

A follow-up Polynesian rat eradication, incorporating lessons learned and information gained since the 2012 effort (Griffiths et al. 2014; Niebuhr et al. 2018; Siers et al. 2018b; Hanson et al. 2019) continues to be one of the objectives in the U.S. Air Force's Integrated Natural Resources Management Plan for Wake Atoll (PACAF 2015). Pitt et al. (2015) concluded from the Palmyra results that any future eradication efforts, such as may occur on Wake, should include monitoring for toxicant residues in fish, insects, crabs, and other organisms for at least 180 days following rodenticide broadcast. Our results suggest that it may be advisable to extend this recommendation in low-turnover aquatic environments that may be contaminated by bait drift to a full year or until no potentially harmful residue concentrations are detected from a comprehensive sampling regime. Given variations in detection and quantitation limits of various methods, and specifics of acceptable risk (e.g., human consumption versus ecological food webs), what is considered detectable and harmful will be subjective and based on the specific context and objectives of a particular operation and level of risk aversion of the agencies involved. Our sampling did not include all wildlife that may have been exposed to brodifacoum or potentially impacted by residues. Brodifacoum may have vastly different effects on individual wildlife species, from negligible or unknown impacts on some species to lethal impacts on others (Eason and Spurr 1995; Dowding et al. 1999; Hoare and Hare 2006; Fang et al. 2012). It remains possible that low level re-exposure or persistence of residues in tissues may impact long-term survival and reproduction by individuals of some species, or have impact on other species through trophic interactions. Sublethal effects of wildlife exposure to brodifacoum remain poorly understood and should be considered an area of further research and risk evaluation.

During aerial rat eradication operations, exposure of the nearshore marine environment to anticoagulant rodenticides may occur through bait drift or accidental discharge from aerial pellet spreaders, bouncing or rolling of pellets from steep shoreline features, runoff from rainfall, movement of pellets by rats and other organisms (particularly crabs),

carcasses of intoxicated rats, or movement of residues throughout the food web. With relatively little known about consequence of anticoagulant contamination of the marine environment, excess of caution can increase risk of eradication failure by instigating prohibition of bait application within buffers around the coastline or other exclusion zones resulting in undertreatment of coastal habitats (Brown et al. 2013; Siers et al 2018b). As documented to date, the negative effects of nontarget exposure and residue persistence during island rodent eradications have been relatively limited and short-term, and must be weighed against the dramatic and enduring ecosystem responses documented after successful eradications (Le Corre et al. 2015; Graham et al. 2018; Wolf et al. 2018) when assessing the relative risks and rewards of anticoagulant use for island rodent eradications. Despite very low levels of brodifacoum in a small number of samples from a landlocked pond detected a full three years after the Wake eradication attempt, our study did not document detectable persistence of brodifacoum in the marine food web within the lagoon as measured by sampling of game fishes. Both Wake and Palmyra represent relatively high-risk scenarios for contamination of the nearshore marine environment, requiring moderate to high target bait application rates, relatively low-turnover marine environments (lagoons), and complex coastlines that include intermittently-inundated rat habitats. We suggest that these results should be interpreted as adding detail to an overall picture of minimal persistent risk of marine contamination associated with island rodent eradications using anticoagulants. Nonetheless, all reasonable efforts should be made to minimize unnecessary environmental and nontarget exposures (e.g., through precise application methods) and all risk assessments must consider the specific context of proposed action.

Conclusions

Brodifacoum residues that were detected in 2 of 69 fish samples from nearshore Wake Island in 2015 were below or near the limits of detectability, and were restricted to an inland pond that is frequently cut off from water exchange with the marine environment in the adjacent lagoon. Between the 2012 sampling and the results reported here, 6 of 7 suspected brodifacoum detections (85.7%) occurred in a single species, the blacktail snapper (*Lutjanus fulvus*), a species that comprised only 13.6% of the fish sampled. Our findings should inform the decisions of management of nearshore marine ecosystems and occupational health personnel when making determinations of acceptable risk and contemplating issuance of fish consumption advisories when brodifacoum rat bait is used. These results will also bear consideration when assessing potential risks in the degradation rates and movement of environmental traces of brodifacoum when considering future rodent eradication actions.

Abbreviations

HPLC/FLD: High-performance liquid chromatography with fluorescence detection

ACN: Acetonitrile

LC-MS/MS: Liquid chromatography-tandem mass spectrometry

DL: Detection limit

QL: Quantitation Limit

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Supplementary material

The following supplementary material is available for this article:

Table S1. Analytical Chemistry Data. This file contains a summary of the analytical chemistry results, calibration data, and LC-MS/MS output.

Table S2. Brodifacoum Residue Results.

Appendix 1. Analytical Chemistry Report. This file contains the formal lab report summarizing the analytical chemistry results.

This material is available as part of online article from:

http://www.reabic.net/journals/mbi/2020/Supplements/MBI_2020_Siers_etal_Table_S1.xlsx

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