# Monitoring Diphacinone Residues after an Eradication of Polynesian Rats from Lehua Island, Hawaii

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*This report replaces the previous version dated May 1, 2018, correcting the transposition of liver samples from a Pacific Golden Plover (NWRC Sample ID S180108-01) and a Red-footed Booby (S180108-2).

## Contents



## Introduction

Lehua is a 115-hectare island located 1.2 km off the northern shore of Niihau (a privately owned, 18,650 hectare island) in the Hawaiian Islands, Pacific Ocean. Lehua is a state-designated seabird sanctuary managed by the Hawaii Department of Land and Natural Resources (DLNR) and

federally owned by the U.S. Coast Guard (USCG). Lehua is one of Hawaii's most important seabird colonies because of its size and height above sea level, and offers an opportunity for restoring an island ecosystem within the main Hawaiian Islands.

In 2009, the State of Hawaii, in conjunction with U.S Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) Wildlife Services Hawaii State Office and the U.S. Fish and Wildlife Service Pacific Islands Fish and Wildlife Office (USFWS PIFWO), attempted to eradicate Polynesian rats (*Rattus exulans*) from Lehua via aerial application of a rodenticide bait (Diphacinone-50 Conservation 0.005% diphacinone pellets, HACCO, Randolph, WI). This attempt failed, and rat abundance recovered.

In August and September of 2017, The Hawaii Department of Land and Natural Resources (DLNR) Division of Forestry and Wildlife (DOFAW), in conjunction with USFWS as a federal co-lead, technical partner Island Conservation (IC), and cooperating members of the Lehua Island Restoration Steering Committee (LIRSC) undertook a second attempted eradication of Polynesian rats from Lehua Island, so that further restoration efforts can move forward in the future.

The purpose of this action was to eradicate non-native rats from Lehua and maintain its rodent-free status, which would facilitate the restoration of the natural island ecosystem. Rat eradication is expected to improve seabird nesting habitat and aid in the recovery of rare endemic seabirds such as Band-rumped Storm Petrels (*Oceanodroma castro*), Hawaiian Petrels (*Pterodroma sandwichensis*), Newell's Shearwaters (*Puffinus newelli*), and native coastal plants and insects. The proposed action was not anticipated to have any significant negative environmental effects; the State of Hawaii and the U.S. Fish and Wildlife Service each completed Environmental Assessments (EAs) and issued Findings of No Significant Impact (FONSIs) for the action.

This action included three aerial broadcast applications of a new rodenticide bait pellet formulation containing 0.005% diphacinone (50 parts per million) in a cereal-based bait matrix (DITRAC-50, Bell Laboratories, Madison, WI; EPA Reg. No. 12455-147). This product includes pyranine, a non-toxic biomarker that fluoresces under UV light, useful for assessing nontarget exposure to bait pellets. Pellets were broadcast into all potential rat territories on Lehua Island. Bait applications occurred in the summer dry season to maximize the probability of success by targeting rats when food resources were lowest and rat abundance was presumably declining. Conducting the operation during this period also minimized the risk of rain washing rodenticide pellets into the ocean.

Aerial bait applications occurred on 23 August, 30 August, and 12 September, 2017. These operations were observed and monitored by USDA National Wildlife Research Center (NWRC) personnel, with all significant events recorded and reported in Siers 2018. In total, approximately 8,536 kg of diphacinone bait was applied. By comparison, the 2009 operation consisted of only two bait applications, totaling 3,538 kg (Orazio et al. 2009). The 2017 operation also differed in that bait application was permitted directly up to the high tide mark, while in 2009 the Hawaii Department of Agriculture (HDOA) permit restrictions had imposed a mandatory buffer of 30 m from the shoreline, within which bait pellets could not be applied.

The rationale for such short-term contaminant inputs is that the environmental and human health risks of toxicant use are offset by the long-term ecological and societal benefits of invasive rodent removal (e.g., Jones et al. 2015, Le Corre et al. 2015, Russell and Broome 2016). The maintenance of this rationale requires that we continue to test assumptions about the actual primary and secondary adverse impacts of rodenticide use.

Before and after the 2009 bait applications, samples of seawater, soil, limpets, crabs and fish muscle tissue ('fillet') were collected and tested for diphacinone residues at a U.S. Geological Survey laboratory (Orazio et al. 2009). In these tests, there were no detections of diphacinone residues in any of the sampled materials. For the 2017 eradication attempt, we sought to replicate and augment this previous sampling scheme, to assess the potential persistence of diphacinone in various environmental compartments subsequent to this action.

Throughout this report and appendices we will refer to common English names for species. Latin, Hawaiian, or other vernacular names are listed in

Table 1.

## Sampling Methods

### Sampling sites

During the 2009 effort, samples were collected at the three sites depicted in Figure 2 of Orazio et al. 2009. These locations were the only places where the shore-based sampling crews could safely access the shoreline and where there would be potential public use of Lehua's near-shore resources. These sites also represent the most conservative circumstance for detection of residues, because the largest drainage gulches occur and enter the sea on the south slope of the island adjacent to the area of greatest human use. We conducted our land-based sampling at the same locations (Table 2, Figure 1). Additional boat-based sport fish sampling was conducted by the Hawaii Division of Aquatic Resources (DAR) within the caldera of Lehua's submerged cinder cone.

### Sampling occasions

Specimens were collected from each of the three selected sites on five sampling occasions: once prior to bait application (to establish a pre-treatment baseline); again one to four days following each of the three applications; and finally approximately two weeks after the last bait application (Table 3).

### Sample handling

Animal samples were stored whole. Necropsies or tissue collections did not occur on Lehua Island to minimize potential for sample contamination in the absence of a clean laboratory on site. All soil, water, and carcass samples were refrigerated in iced coolers and transported to freezers on Kauai at the earliest opportunity, from where they were shipped frozen to the NWRC Hawaii Field Station in Hilo for consolidation and forwarding to the NWRC Chemistry Lab Unit in Fort Collins, Colorado. Chain of custody forms were used to maintain record of possession en route to the laboratory. All animals were euthanized as approved by the NWRC Institutional Animal Care and Use Committee under protocol QA-2802.

### Seawater and soil sampling

Water samples were collected in chemically-cleaned 1-liter Nalgene bottles (Nalge Nunc International Corporation, Rochester, NY) with Teflon-lined lids. After removing the top, labeled collection bottles were submerged in water until the top was just below the water surface, allowing the bottle to fill almost completely, leaving headspace for freezing expansion, and the tops secured. Soil samples were collected in 125 ml bottles of the same construction. Bottles were filled using only the bottles to collect material (i.e., lids, cups, or trowels were not used to fill the bottles), scooping down 5-12 cm within the base of gulches that drain into the sea. The collector wore sterile gloves during sampling. One seawater and one soil sample were collected at each of the three sites during each of the five sampling periods (15 samples total for each).

### Limpet sampling

Limpets are single-shelled marine gastropod mollusks, and a valued human delicacy. They graze on algae growing on rocky substrates in intertidal zones. Composite samples of 5-8 whole limpets were collected at each of the sites during each sampling period. The intertidal habitat of limpets is inherently risky for human collection activities; successful collection during the targeted sampling interval is dependent upon tide and wave conditions and was not conducted when human safety was at risk. Limpets were collected by hand, by prying shells from the substrate and placing them into labeled plastic bags. Limpets are not known to be affected by diphacinone.

### Crab sampling

Natal lightfoot crabs are commonly eaten raw at parties. These crabs also occur in rocky intertidal zones, and are difficult to catch. Crabs were pinned with long poles or blinded with flashlights at night and collected by hand. Each sample was comprised of a composite of 2-3 crabs, and a sample was collected at each of the three sites during each of five sampling periods (15 total composite samples). Crabs are not known to be affected by diphacinone.

Table 1: Latin, Hawaiian, or other names for species referenced by common name throughout this report.

| Taxa | Common Name | Latin Name | Hawaiian/Other |
|---|---|---|---|
| Invertebrates | Limpet | *Cellana* sp. | Opihi |
| | Natal lightfoot crab | *Grapsus tenuicrustatus* | ʻA'ama |
| Fishes | Convict tang | *Acanthurus triostegus* | Manini |
| | Hawaiian chub | *Kyphosus* spp. | Nenue |
| | Stocky hawkfish | *Cirrhitus pinnulatus* | Po'opa'a |
| | Hawaiian hogfish | *Bodianus albotaeniatus* | ʻA'awa |
| | Hawaiian squirrelfish | *Sarogentron xantherythrum* | ʻAla'ihi |
| | Blue-lined squirrelfish | *Sargocentron tiere* | ʻU'u |
| | Sabre squirrelfish | *Sargocentron spiniferum* | ʻAla'ihi |
| | Soldierfish | *Myripristis* spp. | ʻU'u, menpachi |
| | Blacktail snapper | *Lutjanus fulvus* | None (nonnative) |
| | Bluestripe snapper | *Lutjanus kasmira* | None (nonnative) |
| | Goatfish | *Parupeneus* spp. | Moano |
| | Pinktail triggerfish | *Melichthys vidua* | Humuhumu hi'ukole |
| | Black triggerfish | *Melichthys niger* | Humuhumu ele ele |
| | Lei triggerfish | *Sufflamen bursa* | Humuhumu lei |
| | Small toothed jobfish | *Aphareus furca* | Wahanui |
| | Bluefin trevally | *Caranx melampygus* | ʻOmilu |
| | Blue-spotted grouper | *Cephalopholis argus* | Roi (Japanese) |
| | Flathead grey mullet | *Mugil cephalus* | Ama'ama |
| Birds* | Pacific Golden Plover | *Pluvialis fulva* | Kolea |
| | Ruddy Turnstone | *Arenaria interpres* | ʻAkekeke |
| | Red-footed Booby | *Sula sula* | ʻĀ |
| | Red-tailed Tropicbird | *Phaethon rubricauda* | Koa'e ʻula |
| | Wedge-tailed Shearwater | *Ardenna pacifica* | ʻUa'u kani |
| | Black Noddy | *Anous minutus* | Noio, ʻeki'eki |

*Bird names conform to the American Ornithological Union convention of using accepted, unambiguous common names as proper nouns and capitalizing them.

Table 2: Water, limpet, crab, and shore-based fish samples were collected within 25-50 m from the listed coordinates. Soil samples were collected 20-30 m upslope from these sites along gulch bases. Coordinates are in WGS 1984 decimal degrees.

| Site | Latitude | Longitude |
|---|---|---|
| 1 | 22.015005° | -160.096735° |
| 2 | 22.014903° | -160.095862° |
| 3 | 22.015097° | -160.095336° |

## Fish sampling

The potential for toxic residues in reef and game fish is among the greatest public concerns associated with rodenticide use on Lehua. For this reason, we placed greater emphasis on fish sampling compared to the 2009 effort. Our sampling protocol targeted three classes of fish:

1. **Resident (non-pelagic) reef fish.** This is the group of fish that were observed interacting with placebo bait pellets during prior site visits, constituting a 'worst case scenario' for fish likely to directly consume greater amounts of rodenticide pellets.

2. **Triggerfish.** A die-off of triggerfish on the coast of Niihau, coincident to the 2009 eradication attempt, is presumed to be unrelated to rodenticide use (Oishi 2009); however, public impression persists that the die-off was caused by diphacinone poisoning, despite a lack of supporting evidence.

3. **Prized near-shore game fish.** These are the fish most likely to be consumed by

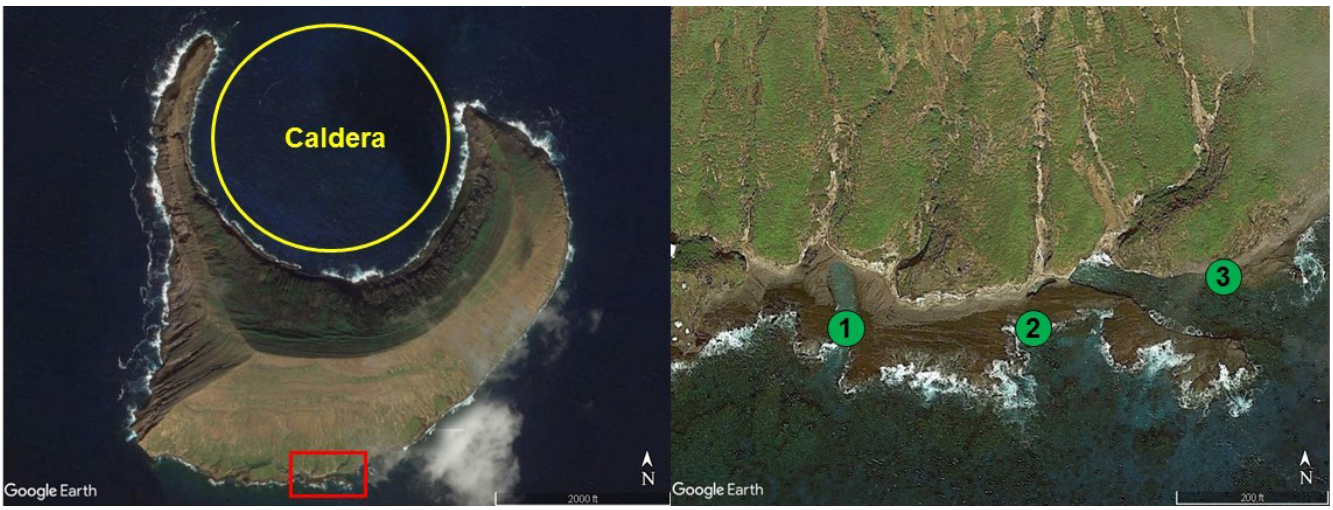


Figure 1: Location of fish collection sites: Hawaii Division of Aquatic Resources sport fish sampling occurred in the caldera (left); NWRC sampled fish from shore at three sites (red rectangle in left image, site locations 1-3 in right image).

Table 3: Sample collection dates relative to aerial bait applications ('App'). All dates 2017.

| Substrate | Pre-app | App 1 (23 Aug) | App 2 (30 Aug) | App 3 (12 Sep) | Final |
|---|---|---|---|---|---|
| Seawater | 1 Aug | 24 Aug | 1 Sep | 14 Sep | 29 Sep |
| Soils | 1 Aug | 24 Aug | 31 Aug | 14 Sep | 29 Sep |
| Limpets | 1 Aug | 25 Aug | 31 Aug | 13 Sep | 29 Sep |
| Crabs | 1 Aug | 25 Aug | 31 Aug | 13 Sep | 29 Sep |
| Shore fish | 1 Aug–2 Aug | 25 Aug | 31 Aug–1 Sep | 13 Sep | 29 Sep |
| Sport fish | 9 Aug | – | – | – | 26 Sep |

humans; they also tend to be higher trophic level predators that would be more likely to bioaccumulate toxins.

Although these were our objectives, it was recognized that reliable and species-specific collection of fish would be very difficult, and the actual samples collected would probably be highly variable.

We collected the majority of fish by hook and line from the shore of Lehua, with spearing as a supplementary method. Additional fish samples collected by DAR were caught from a boat. We placed whole fish into individually-labeled plastic bags, and did not conduct necropsies or tissue collections in the field in order to prevent diphacinone contamination.

Toxic residues in vertebrates are most highly concentrated in liver tissue; however, liver comprises a small component of fish mass, and "extrapolation from liver to muscle contaminant levels is fraught with uncertainty, and [such] data are essentially useless for [the] purpose [of assessing dietary contamination risk]" (Ahmed 1991). In most cases, livers of game fish are discarded. For the sake of balancing the most extreme potential concentrations of diphacinone with realistic potential for human exposure, we tested both liver and muscle tissue.

In addition to the fish sent off-island for diphacinone residue analysis, we also collected further fish samples from the Lehua near-shore environment on the day of bait applications for immediate necropsy to evaluate whether fish were feeding on bait pellets. After euthanizing these fish, we investigated stomach and gut contents for signs of bait ingestion. We looked for fluorescence under UV light to confirm presence of the pyranine biomarker. No known studies have documented the persistence of pyranine biomarker in fish; pyranine has been documented to persist for 3 to 10 days in mice (Pitt et al. 2013). We scored observations using the following scale: 0 = no indication of bait or pyranine fluorescence; 1 =

pyranine fluorescence only, no bait found; 2 = fluorescence and trace amount of bait (roughly equivalent to one pellet or less); 3 = moderate amount of bait (1-5 pellets); 4 = large amount of bait (>5 pellets).

Additional notes were made about condition of gut contents and any observations of suspected internal hemorrhaging (acknowledging that this determination would not be confirmed by qualified veterinary personnel). Attempts were made to obtain samples from a diversity of fish species. We did not submit these fish for diphacinone residue sampling, due to risk of contamination during necropsy.

### Non-target carcass surveys

Throughout the course of field activities associated with eradication efforts, Lehua team members collected any non-target organisms found dead (species other than rats) and submitted them for diphacinone residue analysis to assess whether the organism had been exposed to rodenticide intoxication (with birds being the primary taxa of concern). We conducted carcass surveys passively (collecting carcasses found while conducting other eradication monitoring activities) and actively (conducting carcass search transects throughout terrestrial habitat and along coastlines adjacent to the encampment area). Carcass searches included pre-treatment surveys to document natural mortality and to remove carcasses that could later be confused with mortalities due to rodenticide treatment.

### Bird necropsies

We sent bird carcasses that were relatively fresh when discovered to the Animal Industry Division of the Hawaii Department of Agriculture, where they were necropsied by state veterinarians to evaluate evidence as to whether the mortality was associated with rodenticide ingestion.

### Analytical chemistry

Diphacinone residues were assayed and quantified by liquid chromatography and mass spectrometry (LC-MS/MS) at the USDA APHIS WS NWRC Chemistry Lab Unit in Fort Collins, Colorado. Detection and quantitation limits for each sample type were established during analysis. Detailed descriptions of analytical processes are contained in the respective NWRC Analytical Services Reports for each sample type, as appendices to this report. The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not quantifiable as a reliable concentration value (otherwise known as the minimum level of detection, or 'MLOD' as referred to by Orazio et al.). The Quantitation Limit (QL) is the lowest concentration of analyte that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL. This was performed by comparing the analyte response observed in fortified control samples with the baseline noise observed at the same retention time in control samples. The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10.

## Results

Diphacinone concentrations are reported as ng/g (mass, wet weight) or ng/mL (volume), both of which can also be expressed as parts per billion (ppb). For reference, the concentration of diphacinone in the rodent bait pellets used on Lehua is 50 parts per million (ppm), or 50,000 ppb.

### Seawater, soil, and invertebrates

Results for seawater, soil, and invertebrate samples are summarized in Table 4. We found no detectable diphacinone residues in any of the seawater or soil samples collected before, during, and after rodenticide baiting operations; i.e., all samples were below the DL. We ran each seawater and soil sample twice, and each limpet and crab sample three times when enough tissue was available. No negative controls contained detectable levels of diphacinone.

We detected diphacinone in three crab samples, with the highest concentration being an average of 95.7 ng/g (average of three runs); all detections were within samples collected shortly after rodenticide applications. The final sample indicated no detectable diphacinone residues remaining after 29 September 2017. Fully-detailed results are

Table 4: Chemical analysis results for seawater, soil, and invertebrates. Sample counts: (number of samples) | (number > DL) | (number > QL). Grey cells highlight diphacinone detections.

| Material | Report | DL \| QL (ppb) | Pre-app | App 1 | App 2 | App 3 | Final |
|---|---|---|---|---|---|---|---|
| Seawater | 18-001/6 | 0.0044 \| 0.0147 | 3 \| 0 \| 0 | 3 \| 0 \| 0 | 3 \| 0 \| 0 | 3 \| 0 \| 0 | 3 \| 0 \| 0 |
| Soil | 18-001/7 | 0.59 \| 1.95 | 3 \| 0 \| 0 | 3 \| 0 \| 0 | 3 \| 0 \| 0 | 3 \| 0 \| 0 | 3 \| 0 \| 0 |
| Limpets | 18-001/2 | 8.4 \| 27.8 | 3 \| 0 \| 0 | 3 \| 0 \| 0 | 3 \| 0 \| 0 | 3 \| 0 \| 0 | 3 \| 0 \| 0 |
| Crabs | 18-001/3 | 8.0 \| 26.7 | 3 \| 0 \| 0 | 3 \| 2 \| 1 | 3 \| 1 \| 1 | 3 \| 0 \| 0 | 3 \| 0 \| 0 |

included in the respective Analytical Services Reports, attached as appendices.

## Fish

Diphacinone residue results for fish caught between 1 August and 13 September (pre-application though Application 3 periods) are reported in Analytical Services Report 18-001/4; DL and QL for these fish tissues were 9.1 and 30.4 ng/g (muscle) and 17 and 57.4 ng/g (liver). Results for fish tissues collected two weeks after the last bait applications ('Final', 26 and 29 September 2017) are detailed in Report 18-001/1; DL and QL were 5.6 and 18.7 ng/g (muscle) and 12 and 41.1 ng/g (liver). Tissue residue results are summarized in Table 5. We ran all fish tissue samples three times, with the exception of a few samples without enough tissue for three replicates. Values reported in this summary are averages of three replicates; repsective replicate values can be found in the original lab report appendices.

We detected no diphacinone in any pre-application fish samples. Throughout the course of aerial baiting operations, diphacinone residues were only detected in triggerfish, though these were the predominant fish we collected during this time. The highest level of diphacinone residues we recorded from fish was in a black triggerfish collected at Site 3 on 13 September 2017, with an average of 32.3 ng/g diphacinone in muscle tissue and 1360 ng/g in liver (NWRC ID # S171106-44). Of the 29 fish we collected two weeks after the last bait application, only two had detectable diphacinone residues, in liver only (blacktail snapper S171031-09 at 51.1 ng/g and bluestripe snapper S171031-12 at 295 ng/g).

We examined the stomach contents of 26 fish captured on the dates of bait applications, comprising 6 different species (Table 6). Of these, 10 were positive for the pyranine biomarker. One bluestripe snapper was the only fish captured that was estimated to have eaten more than 5 bait pellets. Eight of 10 triggerfish were positive for pyranine, 7 of which were observed to contain trace to moderate amounts of bait material in gastrointestinal contents. One of 5 stocky hawkfish consumed baits score = 3).

Bait material in fish stomachs and intestines (cereal-based pellets of a bluish color) generally appeared as a blue paste or a diffuse paste mixed with other food matter. Fish were very abundant and aggressive at biting hooks, particularly triggerfish. All captured fish appeared to be in good health and were very active, with no observable hemorrhaging or other notable pathology.

## Non-target fish mortalities

On 5 September 2017, in response to a social media post depicting dead fish and two dead birds, Lehua-based project personnel visited the large tide pool within the east arm of the caldera, where they found 45 small dead mullet-type fish and two dead immature boobies in the pool. All fish and birds were collected for analysis.

The dead fish appeared similar to flathead grey mullets, though identification could not be definitively made due to the advanced stage of tissue degradation. Twelve fish were bagged with a separate chain of custody form and provided to the Hawaii Department of Agriculture (HDOA) at their request. Of the remaining mullets, we sent the three samples in best condition to the NWRC Chemistry Lab Unit for diphacinone residue analysis. The remaining fish were physically examined as reported in Appendix H.

Per Report 18-001/4, no liver or other organ tissues could be salvaged from the three mullet samples sent to the NWRC Chemistry Lab Unit (Sample ID's S171106-55, -56, and -57). For chemical analysis, we attempted to harvest only

Table 5: Chemical analysis results for fish tissues. Sample counts: (number of samples) | (number > DL) | (number > QL). Grey cells highlight diphacinone detections.

| Taxa | Tissue | Pre-app | App 1 | App 2 | App 3 | Final |
|---|---|---|---|---|---|---|
| Small reef fish[1] | Muscle | – | – | – | – | 7\|0\|0 |
| | Liver | – | – | – | – | 7\|0\|0 |
| Squirrelfish | Muscle | 1\|0\|0 | – | – | – | 2\|0\|0 |
| | Liver | 1\|0\|0 | – | – | – | 2\|0\|0 |
| Soldierfish | Muscle | 3\|0\|0 | – | – | – | 1\|0\|0 |
| | Liver | 3\|0\|0 | – | – | – | 1\|0\|0 |
| Snapper | Muscle | 1\|0\|0 | 2\|0\|0 | – | – | 4\|0\|0 |
| | Liver | 1\|0\|0 | 2\|0\|0 | – | – | 4\|2\|2 |
| Goatfish | Muscle | 2\|0\|0 | – | – | – | 4\|0\|0 |
| | Liver | 2\|0\|0 | – | – | – | 4\|0\|0 |
| Triggerfish | Muscle | 4\|0\|0 | 4\|0\|0 | 6\|1\|0 | 5\|2\|1 | 8\|0\|0 |
| | Liver | 4\|0\|0 | 4\|4\|2 | 6\|5\|3 | 4\|3\|3 | 8\|0\|0 |
| Small toothed jobfish | Muscle | 2\|0\|0 | – | – | – | 2\|0\|0 |
| | Liver | 2\|0\|0 | – | – | – | 2\|0\|0 |
| Bluefin trevally | Muscle | 1\|0\|0 | – | – | – | 1\|0\|0 |
| | Liver | 1\|0\|0 | – | – | – | 1\|0\|0 |
| Blue-spotted grouper | Muscle | 1\|0\|0 | – | – | – | – |
| | Liver | 1\|0\|0 | – | – | – | – |

[1]Small reef fish: 1 convict tang; 3 Hawaiian chub; 2 stocky hawkfish; and 4 Hawaiian hogfish.

Table 6: Presence of biomarker and bait pellets in stomachs and intestines of fish caught on bait application days. Score: 0 = no indication of bait or biomarker fluorescence; 1 = biomarker only, no bait found; 2 = biomarker and trace amount of bait (roughly equivalent to one pellet or less); 3 = moderate amount of bait (1-5 pellets); 4 = large amount of bait (>5 pellets).

| | | Score | | | | |
|---|---|---|---|---|---|---|
| Species | N | 0 | 1 | 2 | 3 | 4 |
| Stocky hawkfish | 6 | 5 | – | – | 1 | – |
| Bigscale soldierfish | 8 | 8 | – | – | – | – |
| Black triggerfish | 1 | – | – | – | 1 | – |
| Pinktail triggerfish | 9 | 2 | 1 | 4 | 2 | – |
| Blacktail snapper | 1 | 1 | – | – | – | – |
| Bluestripe snapper | 1 | – | – | – | – | 1 |
| TOTAL | 26 | 16 | 1 | 4 | 4 | 1 |

muscle tissue that had not been exposed to air or water, to avoid contamination. Diphacinone residues were not detected in two of the three mullet muscle tissue samples. The third sample, S171106-56, had become so degraded as to turn black in color and had likely been exposed to the elements; this tissue had an average diphacinone concentration of 161 ng/g.

Despite passive and active carcass searches, the only other fish found dead during this period were two fish caught and released during shore fishing on 2 September, which were later found dead near the site of release: a stocky hawkfish and a lei triggerfish. The hawkfish (S171106-17) had no detectable residues, and the triggerfish (S171106-18) had residue concentrations in liver tissue only, below the QL.

## Non-target bird mortalities

Of bird carcasses we collected during aerial baiting operations, 14 were fresh enough to be submitted to HDOA for necropsy; after necropsy, the livers were sent to NWRC for diphacinone residue analysis. Another 24 whole bird carcasses, in poorer condition, were sent directly to NWRC for liver or tissue extraction, if possible. Residue analysis results are reported in Table 7.

Necropsies on the 14 freshest bird carcasses were performed by Travis Heskett, DVM, DACVP, of the State of Hawaii Department of Agriculture Animal Industry Division. His detailed reports are

Table 7: Bird carcasses collected during eradication operations. The target tissue for diphacinone concentration analysis was liver, but when liver tissue was not available decayed muscle tissue was collected. "Necro" indicates whether the carcass was necropsied by Hawaii Department of Agriculture veterinarians (see Table 8). "Conc" is the diphacinone concentration detected (ng/g); ND = <DL and * = <QL. Gray cells highlight diphacinone detections.

| NWRC ID | Collected | Tissue | Conc | Necro | Notes |
|---|---|---|---|---|---|
| Pacific Golden Plover (*Pluvialis fulva*) | | | | | |
| S180108-01 | 9/2/17 | Liver | 569[1] | Yes | Adult, fresh, external pyranine fluorescence |
| Ruddy Turnstone (*Arenaria interpres*) | | | | | |
| S180108-08 | 9/15/17 | Liver | 1880 | Yes | Adult, fresh, external pyranine fluorescence |
| S180108-09 | 9/15/17 | Liver | 3673 | Yes | Adult, fresh |
| Red-footed Booby (*Sula sula*) | | | | | |
| S180108-02 | 8/28/17 | Liver | ND[1] | Yes | Fresh |
| S180108-03 | 9/2/17 | Liver | ND | Yes | Fresh |
| S180108-04 | 9/5/17 | Liver | ND | Yes | Fresh, tide pool[2] |
| S180108-05 | 9/5/17 | Liver | ND | Yes | Fresh, tide pool[2] |
| S171031-47 | 9/5/17 | Decayed | ND | No | Insects |
| S171031-58 | 9/5/17 | – | – | No | Sun dried, no tissue available |
| S171031-39 | 9/6/17 | – | – | No | Sun dried, no tissue available |
| S171031-57 | 9/6/17 | Decayed | ND | No | Insects |
| S171031-52 | 9/8/17 | Decayed | ND | No | Insects |
| S171031-56 | 9/12/17 | Decayed | 11.2* | No | Insects |
| S180108-06 | 9/19/17 | Liver | ND | Yes | Fresh |
| S171031-51 | 9/21/17 | – | – | No | Insects; species uncertain (brown booby?) |
| Red-tailed Tropicbird (*Phaethon rubricauda*) | | | | | |
| S180108-07 | 8/30/17 | Liver | ND | Yes | Fresh |
| Wedge-tailed Shearwater (*Ardenna pacifica*) | | | | | |
| S180108-10 | 8/22/17† | Liver | – | Yes | Fresh, sample lost in prep (†pre-application) |
| S180108-11 | 8/28/17 | Liver | ND | Yes | Fresh |
| S171031-59 | 9/4/17 | – | – | No | Insects, no tissue available |
| S171031-45 | 9/5/17 | – | – | No | Insects, no tissue available |
| S180108-12 | 9/8/17 | Liver | ND | Yes | Fresh |
| S180108-13 | 9/8/17 | Liver | ND | Yes | Fresh |
| S180108-14 | 9/8/17 | Liver | ND | Yes | Fresh |
| S171031-40 | 9/8/17 | Decayed | ND | No | Insects |
| S171031-41 | 9/8/17 | – | – | No | Sun dried |
| S171031-36 | 9/10/17 | – | – | No | Insects, tissue too decayed |
| S171031-46 | 9/10/17 | – | – | No | Insects, no tissue available |
| S171031-49 | 9/10/17 | Decayed | ND | No | Appeared to be owl kill |
| S171031-43 | 9/12/17 | Decayed | ND | No | Appeared to be owl kill |
| S171031-42 | 9/14/17 | – | – | No | Insects, no tissue available |
| S171031-37 | 9/17/17 | Decayed | ND | No | Fresh, appeared to be owl kill |
| S171031-53 | 9/17/17 | Decayed | ND | No | Fresh, appeared to be owl kill |
| S171031-55 | 9/18/17 | – | – | No | Sun dried, no tissue available |
| S171031-44 | 9/19/17 | Decayed | ND | No | Insects |
| S171031-48 | 9/19/17 | – | – | No | Insects, no tissue available |
| S171031-54 | 9/19/17 | – | – | No | Sun dried, no tissue available |
| S171031-50 | 9/21/17 | Decayed | ND | No | Appeared to be owl kill |
| Black Noddy (*Anous minutus*) | | | | | |
| S171031-38 | 9/20/17 | – | – | No | Appeared to be owl kill, tissue too decayed |

[1] Accidental transposition of liver samples between S180108-01 and S180108-02 was evident from the size and color of the liver homegenate samples. This result is consistent with the internal pyranine fluorescence observed in the plover, no fluorescence observed in the booby, the residue concentrations in other shorebirds sampled here, and the unlikehood of pellet ingestion by seabirds due to their feeding habits. [2] These birds were found dead in the tide pool at the same time as the mullets described above.

included as Appendix I, and we have summarized the results in Table 8. Of note were one Pacific Golden Plover and two Ruddy Turnstones, all of the wading shorebird carcasses found, which exhibited internal biomarker fluorescence and gross pathology (hemorrhaging) consistent with direct bait consumption and anticoagulant intoxication. Additional notes on the physical condition and apparent age of the specimens were prepared by Dr. Michael Fry, U.S. Fish and Wildlife Service (Table 9)

Both Ruddy Turnstone carcasses exhibited high concentrations of diphacinone in liver tissues (1880-3670 ng/g), as did the only other shorebird, a Pacific Golden Plover (569 ng/g). All three shorebirds evidenced internal signs of pyranine biomarker fluorescence when examined under UV light. No diphacinone residues were detected in liver tissue of seabird carcasses found on Lehua Island. Diphacinone was detected in one decayed Red-footed Booby carcass with tissue levels so low as to be <DL in two of the three replicates, and <QL in the third.

## Discussion

Because of heightened public and regulatory scrutiny on this second eradication attempt, owing in part to the application of larger amounts of bait directly to the water's edge, we sampled more comprehensively during the 2017 eradication attempt than was previously conducted for the 2009 attempt. We also conducted sampling immediately following each of the three bait applications, to get a more complete picture of the pulse and decay of diphacinone residues in environmental compartments at each stage of the study. Further, our current analytical chemistry methods were able to achieve lower detection limits, for more sensitive testing. Sensitivity of analyses and summaries of detections for both operations (2009 and 2017) are listed and compared in Table 10.

Despite increased sensitivity over the 2009 analysis, no diphacinone concentrations were detected in any seawater, soil, or limpet samples from either operation. Our 2017 analysis detected diphacinone residues in 3 out of 12 post-baiting crab samples, while there were no detections in crab in 2009. Although our testing was more sensitive, our observed concentrations were higher than the DL for 2009, suggesting that such values would not have been "missed" in the 2009 sample. Although the differences in detection rates between 2009 and 2017 were not statistically distinguishable at $\alpha = 0.05$ (Fisher's exact test, P = 0.229), i.e., the difference could be due to sampling error, increased detections in crabs could be a result of baiting directly up to the shoreline.

No diphacinone was detected in any of 22 fish fillet samples from 2009, while we document 3 detections out of 46 samples; two of these detections averaged below the 2009 DL, and the proportion of detections between these samples was not statistically significant (P = 0.546). Additionally, all of our fish muscle tissue detections were from triggerfish, which were not sampled in 2009. By the time of our post-baiting sampling, there were no detectable residues in any of our 29 fish muscle tissue samples.

Detections of diphacinone in fish livers during baiting operations were relatively commonplace, particularly within triggerfish (though this family comprised the majority of fish caught during this period). However, by two weeks after the last bait operation, only 2 of 26 fish livers sampled contained detectable residues, the greatest of which averaged 296 ng/g (1/169th of the concentration in the rodenticide pellets). Liver tissues were not analyzed following the 2009 operation, so no comparisons can be made.

No bird carcass testing was included in the 2009 report by Orazio et al. Our results provide no evidence of diphacinone ingestion by seabirds. The single unreplicable detection of diphacinone in a highly-degraded booby carcass is consistent with environmental exposure. In contrast, the three shorebird carcasses found (two Ruddy Turnstones and one Pacific Golden Plover) exhibited clear signs of primary exposure (direct feeding on diphacinone pellets; see attached necropsy reports) and higher concentrations of diphacinone in liver tissue. Necropsy observations of hemorrhaging in these three birds is consistent with anticoagulant rodenticide ingestion, which may have contributed to death of the birds; similar shorebird mortalities have been observed in other island rodent eradication operations (e.g., Dowding et al. 2006).

Table 8: Summarized results from Hawaii Department of Agriculture necropsies of bird carcasses recovered from Lehua during eradication operations. "Fluorescence" indicates observation of fluorescence under UV light consistent with exposure to the pyranine biomarker included in the rodenticide bait. Original reports included in Appendix I.

| NWRC/HI ID | Fluor. | Specimen condition and significant findings |
|---|---|---|
| Pacific Golden Plover (*Pluvialis fulva*) | | |
| S180108-01 170824 | Internal | Moderately reduced post-mortem condition. Hemorrhages were observed within the subcutaneous tissues, coelomic cavity and ventriculus, which could have contributed to the death of this bird. Fluorescence was observed within the digestive tract and an anticoagulant rodenticide could have contributed to the hemorrhage. |
| Ruddy Turnstone (*Arenaria interpres*) | | |
| S180108-08 170831 | Internal and external | Moderately reduced post-mortem condition. Significant hemorrhages were observed within the skin, subcutaneous tissues, and muscles, which could have contributed to the death of this bird. Fluorescence was observed within the ventriculus, and an anticoagulant rodenticide could have contributed to the hemorrhage. |
| S180108-09 170832 | Internal | Moderately reduced post-mortem condition. Mild to moderate hemorrhages were identified in this bird. The severity of the hemorrhage present within the carcass would not have been anticipated to be fatal, but it is possible that additional blood loss could have occurred externally prior to the evaluation of the carcass. Fluorescence was observed within the digestive tract and an anticoagulant rodenticide could have contributed to the hemorrhage. |
| Red-footed Booby (*Sula sula*) | | |
| S180108-02 170825 | None | Moderately reduced post-mortem condition. There were no gross or microscopic lesions to explain the death of this bird. |
| S180108-03 170826 | None | Moderately reduced post-mortem condition. Dead ants. Gross or microscopic lesions to explain the death of this bird were not identified. |
| S180108-04 170827 | None | Advanced post-mortem decomposition. Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. |
| S180108-05 170828 | External | Moderately reduced post-mortem condition. A small focus of fluorescence is observed on the dorsum. Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. |
| S180108-06 170829 | External | Moderately reduced post-mortem condition. Moderate numbers of fly larvae present. Small amounts of fluorescence within feathers. Cause of the death of this bird could not be determined on gross or microscopic examination. Clinical significance of diagnosed lesions is uncertain. |
| Red-tailed Tropicbird (*Phaethon rubricauda*) | | |
| S180108-07 70830 | External | Moderately reduced post-mortem condition. Small amounts of fluorescence observed within feathers and on feet. There were no gross or microscopic lesions to explain the death of this bird. |
| Wedge-tailed Shearwater (*Ardenna pacifica*) | | |
| S180108-10 170833 | None | Advanced post-mortem decomposition. Multiple, irregular punctures and defects are present in the skin of the body wall and head, the coelomic cavity may have been entered, and the rib cage is collapsed. Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. |
| S180108-11 170834 | None | Advanced post-mortem decomposition. Coelomic cavity is opened and the integrity of the rib cage is disrupted. Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. |
| S180108-12 170835 | None | Advanced post-mortem decomposition. Coelomic cavity is opened. Fly larvae are present. Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. |
| S180108-13 170836 | External | Advanced post-mortem decomposition. Lumen of digestive tract empty. Marked autolysis, loss of detail due to freezing. One feather fluoresces. No diagnostic lesions. No cause of death identified. |
| S180108-14 170837 | External | Carcass flattened. Marked autolysis, loss of detail due to freezing. Coelomic cavity open and viscera granular and friable, with fly larvae. A few feathers fluoresce. No diagnostic lesions. No cause of death identified. |

Table 9: Additional notes on condition and apparent age for Red-footed Booby carcasses collected on Lehua. Notes by Dr. Michael Fry, U.S. Fish and Wildlife Service. Frozen specimens were thawed and examined by Dr. Fry and Travis Kaskett, DVM, and Raquel Wong, DVM, at the Hawaii Stae Veterinary Laboratory in Halawa. All carcasses had been previously frozen and thawed, necropsied by Dr. Heskett, bagged and refrozen.

| Notes |
|---|
| S180108-02[1]: The carcass was previously necropsied and tissue samples taken for toxicology and histology. Carcass is missing most viscera and internal organs, but skeleton, muscles, skin and feathers are intact. The bird is a nestling with growing primary and secondary feathers with prominent blood feather sheaths. Secondary feathers emerging from sheaths are about 2 cm in length. Primary feathers protrude about 5 cm from feather sheaths. This bird would not have been able to fly at time of death and would have been confined to the nest. |
| S180108-03: The carcass was previously necropsied and tissue samples taken for toxicology and histology. Carcass is missing most viscera and internal organs, but skeleton, muscles, skin and feathers are intact. The bird is a nestling with growing primary and secondary feathers with prominent blood feather sheaths. Secondary feathers emerging from sheaths are about 1.5 cm in length. Primary feathers protrude about 3 cm from feather sheaths. This bird would not have been able to fly at time of death and would have been confined to the nest. |
| S108108-04: This bird was collected dead from the tide pool on Lehua Island. The carcass was previously necropsied and tissue samples taken for toxicology and histology. Carcass is missing most viscera and internal organs, but skeleton, muscles, skin and feathers are intact. Tissues surrounding base of primary feathers have decomposed and feather shafts have pulled free from wing web. This bird is a hatching year 2018 fledgling, probably capable of flight. The primary, secondary and tail feathers are fully formed and "hard pinned" without feather sheaths and rachis is hollow with no blood or pulp. |
| S108108-05: This bird was collected dead from the tide pool on Lehua Island. The carcass was previously necropsied and tissue samples taken for toxicology and histology. Carcass is missing most viscera and internal organs, but skeleton, muscles, skin and feathers are intact. Most primary, secondary and tail feathers are almost fully formed, with feather sheaths still present, but feather sheaths do not appear to have pulp remaining. It is likely this bird would have been able to fly at time of death. |
| S108108-06: This bird was collected very fresh on Lehua Island. The carcass was previously necropsied and tissue samples taken for toxicology and histology. Carcass is missing most viscera and internal organs, but skeleton, muscles, skin and feathers are intact. Most primary, secondary and tail feathers are almost fully formed, with feather sheaths still present, but primaries 1-5 are blood feathers with pulp and emerging feathers. It is likely this bird would not have been able to fly at time of death. |

[1]This is the specimen for which the liver was transposed with S180108-01.

## Conclusions

To our knowledge, this is the most comprehensive and methodologically sensitive marine sampling effort associated with any aerial rodenticide application to date. There were no substantial nontarget mortality events clearly associated with this operation, with the exception of plausible implication in the deaths of three shorebirds. Although our results demonstrated a low pulse of diphacinone concentrations in crab, fish, and some bird tissues, our window for detecting residues was relatively short-lived, with very few detections persisting beyond two weeks after the last bait application. Animals that survive direct exposure quickly metabolize the majority of diphacinone ingested (Yu et al. 1982), and residual levels following operations such as the one monitored here are not likely to be biologically significant.

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Table 10: Comparison of analysis sensitivity and detections between the 2009 USGS analysis (Orazio et al. 2009) and the present 2017 NWRC analysis. "DL" = lower detection limit. "Detections" is the number of detections by (/) the number of samples tested*.

| | DL (ppb) | | Detections* | |
|---|---|---|---|---|
| Material | 2009 | 2017 | 2009 | 2017 |
| Seawater | 0.4000 | 0.0044 | 0/9 | 0/12 |
| Soil | 15 | 0.59 | 0/11 | 0/12 |
| Limpet | 34 | 8.4 | 0/11 | 0/12 |
| Crab | 13 | 8.0 | 0/9 | 3/12 |
| Fish muscle | 20 | 9.1\|5.6† | 0/22 | 3/46 |
| Fish liver | – | 17\|12† | – | 11/45 |
| Birds | – | 9.6 | – | 4/37 |

*Baseline (pre-baiting) samples were not included in the sample size summaries; there were no diphacinone detections in any pre-baiting baseline samples for either operation. †Fish samples from pre-baiting and Applications 1-3 (first value) were analyzed separately from the post-baiting sample (second value).

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## Appendices

**A.** NWRC Chemistry Lab Unit Report 18-001/6: Seawater

**B.** NWRC Chemistry Lab Unit Report 18-001/7: Soil

**C.** NWRC Chemistry Lab Unit Report 18-001/2: Limpets

**D.** NWRC Chemistry Lab Unit Report 18-001/3: Crabs

**E.** NWRC Chemistry Lab Unit Report 18-001/4: Fish (pre- and during baiting)

**F.** NWRC Chemistry Lab Unit Report 18-001/1: Fish (post-baiting)

**G.** NWRC Chemistry Lab Unit Report 18-001/1: Birds

**H.** Tide pool mullet mortality report

**I.** Bird carcass necropsy reports, Hawaii Department of Agriculture

| Wildlife Services NWRC National Wildlife Research Center Analytical Services Report | United States Department of Agriculture<br>Animal Plant Health Inspection Service<br>Wildlife Services<br>National Wildlife Research Center<br>Chemistry Lab Unit | Invoice #: 18-001/6<br>Date: March 6, 2018<br>Page: 1 of 4 |
|---|---|---|

To: Dr. Shane Siers
Hawaii Field Station Leader
NWRC

Subject: Diphacinone Residues in Seawater from Lehua Island (QA-2802)

Methods: New, non-GLP

Analysis Dates: 2/28 and 3/1/2018

Notebook Reference: AC-161, pp. 112, 134, 138, and 140-141

QC Notebook Reference: AC-162, p. 4

Analyst: Steve Volker

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**Sample Descriptions:**

Fifteen surface seawater samples from Lehua Island were received 10/31/2017 and 11/13/2017 for diphacinone residue analysis. Each sample consisted of 900 mL seawater contained in a 1-L polypropylene bottle. Control seawater from Hilo, HA was received 11/13/2017. All samples were stored frozen at -20°C until time of analysis.

**Sample Preparation:**

Water samples were thawed overnight and approximately 100 mL transferred to two 50-mL polypropylene Falcon tubes. The water was clarified by centrifuging at 1200 RCF for 2-3 minutes. Taking care to not disturb the tubes, thirty mL of supernatant was removed from each tube and added to a 125-mL separatory funnel. Surrogate analyte (20 µL, 16 µg/mL $D_4$-diphacinone in acetonitrile), 0.040 mL acetonitrile (ACN), 20 mL chloroform, sodium chloride (8.4-8.6 g), and 10 mL of 1M HCl were added and the mixture extracted by shaking three times for 10-15 s. The bottom chloroform layer was dispensed into a 25-mL glass tube and the solvent removed in a 60°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 300 µL ACN followed by 1200 µL pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC-MS/MS analysis.

| Analyst | Date | QC Specialist | Date | Reviewer | Date |
|---|---|---|---|---|---|
| | 3/6/2018 | | 3/6/18 | | 3/6/18 |

**Instrument method:**

Agilent 1290 Infinity II HPLC with G6470A QQQ

| | |
|---|---|
| Column | Xbridge C18, 2.5-µm, 2.1 x 50 mm, Waters P/N 186003085 |
| Mobile phase A | 90%(pH 9.5 20-mM ammonium acetate)/10%(Acetonitrile) |
| Mobile phase B | Acetonitrile |
| Flow rate | 0.700 mL/min |
| Column temp. | 60°C |
| Injection volume | 10 µL |
| Run time | 2.40 min |
| Source | AJS ESI, negative mode |
| Gas temp. | 300°C |
| Gas flow | 5 L/min |
| Nebulizer | 45 psi |
| Sheath gas | 250°C, 7 L/min |
| Capillary | -4500 V |
| Nozzle | -500 V |

| Time (min) | %A | %B |
|---|---|---|
| 0.00 | 90% | 10% |
| 0.30 | 90% | 10% |
| 1.40 | 0% | 100% |
| 1.90 | 0% | 100% |
| 1.91 | 90% | 10% |

| Analyte | Precursor Ion (m/z) | Product Ion (m/z) | Fragmentor (V) | Collision Energy (V) |
|---|---|---|---|---|
| Diphacinone | 339.1 | **167.1** | 100 | 23 |
| | | 145.0 | | 18 |
| $D_4$-Diphacinone | 343.1 | 167.1 | 120 | 23 |

**BOLD** = product ion used for quantitation

**Detection and Quantitation Limits:**

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value. The Quantitation Limit (QL) is the lowest concentration of analyte that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL. This was performed by comparing the analyte response observed in fortified control seawater (n=3) with the baseline noise observed at the same retention time in control seawater (n=3). The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10, respectively. The following table presents the average DL and QL concentrations determined for diphacinone in seawater (n=2).

Diphacinone Detection Limit (DL) and Quantitation Limit (QL)

| Control Matrix | DL (ng/mL) | QL (ng/mL) |
|---|---|---|
| Surface seawater | 0.0044 | 0.0147 |

**Results:**

All samples were tested in duplicate. Diphacinone residues are reported in units of ng/mL, equivalent to parts per billion (ppb). If no analyte response was recorded by the data acquisition software or if the observed concentration was less than the DL, an entry of "ND" is reported to indicate that the analyte was not detected.

**Diphacinone – Surface Seawater**

| NWRC ID | Sample ID, Collection Date | Analysis Date | Observed Diphacinone Concentration (ng/mL) |
|---|---|---|---|
| S171031-60 | Water 1-5, 9/29/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171031-61 | Water 2-5, 9/29/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171031-62 | Water 3-5, 9/29/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-23 | Water 1-1, 8/1/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-24 | Water 2-1, 8/1/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-25 | Water 3-1, 8/1/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-26 | Water 1-2, 8/24/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-27 | Water 2-2, 8/24/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-28 | Water 3-2, 8/24/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-29 | Water 1-3, 9/1/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-30 | Water 2-3, 9/1/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-31 | Water 3-3, 9/1/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-32 | Water 1-4, 9/14/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-33 | Water 2-4, 9/14/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| S171113-34 | Water 3-4, 9/14/2017 | 2/28/2018 | ND |
| | | 3/1/2018 | ND |
| | | DL (ng/mL) = | 0.0044 |
| | | QL (ng/mL) = | 0.0147 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

**QC Results:**

QC Recoveries – Seawater

| ID | Analysis Date | Theoretical Diphacinone Concentration (ng/mL) | Observed Diphacinone Concentration (ng/mL) | % Recovery |
|---|---|---|---|---|
| QC-213 | 2/28/2018 | 0.00 | ND | N/A |
| QC-217 | 3/1/2018 | 0.00 | ND | N/A |
| QC-214 | 2/28/2018 | 0.139 | 0.141 | 101% |
| QC-218 | 3/1/2018 | 0.139 | 0.141 | 101% |
| QC-215 | 2/28/2018 | 1.25 | 1.23 | 98.4% |
| QC-219 | 3/1/2018 | 1.25 | 1.26 | 101% |
| QC-216 | 2/28/2018 | 11.2 | 11.3 | 101% |
| QC-220 | 3/1/2018 | 11.2 | 10.8 | 96.4% |
| | | DL (ng/mL) = | 0.0044 | |
| | | QL (ng/mL) = | 0.0147 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

| Wildlife Services NWRC National Wildlife Research Center<br>Analytical Services Report | United States Department of Agriculture<br>Animal Plant Health Inspection Service<br>Wildlife Services<br>National Wildlife Research Center<br>Chemistry Lab Unit | Invoice #: 18-001/7<br>Date: March 23, 2018<br>Page: 1 of 5 |
|---|---|---|

To: Dr. Shane Siers
Hawaii Field Station Leader
NWRC

Subject: Diphacinone Residues in Soil from Lehua Island (QA-2802)

Methods: New, non-GLP

Analysis Dates: 3/21 and 3/22/2018

Notebook Reference: AC-161, pp. 112, 142-147

QC Notebook Reference: AC-162, p. 4

Analyst: Steve Volker

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**Sample Descriptions:**

Fifteen soil samples from Lehua Island were received 11/13/2017 for diphacinone residue analysis. Each sample was contained in a 125-mL polypropylene bottle. Control soil from Oahu, HI was also received 11/13/2017. All samples were stored at -20°C until time of preparation.

**Sample Preparation:**

Soil samples were warmed to room temperature over 3-4 hours, and then approximately 20g of each was transferred to 20-mL glass scintillation vials. Weights were recorded, samples dried for 18 hours at 105°C, and then reweighed to determine moisture loss. The dried samples were ground for 1 minute using a stainless steel Waring blender resulting in a homogenous powder. Ground samples were stored in vacuum sealed bags at room temperature until time of analysis. Several extraction methods were experimented with including column and dispersive solid phase extraction techniques. Initial extractions were performed with acidified acetone, but surrogate recoveries were low and variable in actual samples. Subsequent extraction with basic conditions produced higher surrogate recoveries and more consistent results. The extraction procedure was as follows:

Soil (1.9 – 2.1g) was weighed into a 25-mL glass tube and surrogate analyte (80 µL, 16 µg/mL $D_4$-diphacinone in acetonitrile) added. Deionized water (1.0 mL) was added and the sample vortex mixed for 4-5s. Excess NaCl (~3 g) was added followed by 10.0 mL acetone containing 0.5% trifluoroacetic acid. The sample was mechanically shaken in a horizontal position for 10 minutes. Ammonium hydroxide (0.20 mL, 50% v/v) was added and the sample vortex mixed twice for 4-5s. The sample was centrifuged at 1200 RCF for 1-2 minutes and 1.0 mL of supernatant transferred to a 1.5-mL microcentrifuge tube. The solvent was removed in a 60°C N-Evap with a gentle flow of nitrogen and the analytes reconstituted with 120 µL ACN followed by sonication for 2 min. Ammonium acetate buffer was added (480 µL, pH 9.5 20-mM), the sample vortex mixed for 3-4s, and lastly centrifuged at 12,000 RCF for 2-3s. An aliquot of the clarified supernatant was then transferred to an autosampler vial for LC-MS/MS analysis.

| Analyst | Date | QC Specialist | Date | Reviewer | Date |
|---|---|---|---|---|---|
| | 3/23/18 | | 3/23/18 | | 3/26/18 |

**Instrument method:**

Agilent 1290 Infinity II HPLC with G6470A QQQ

| | |
|---|---|
| Column | Xbridge C18, 2.5-µm, 2.1 x 50 mm, Waters P/N 186003085 |
| Mobile phase A | 90%(pH 9.5 20-mM ammonium acetate)/10%(Acetonitrile) |
| Mobile phase B | Acetonitrile |
| Flow rate | 0.700 mL/min |
| Column temp. | 60°C |
| Injection volume | 10 µL |
| Run time | 2.40 min |
| Source | AJS ESI, negative mode |
| Gas temp. | 300°C |
| Gas flow | 5 L/min |
| Nebulizer | 45 psi |
| Sheath gas | 250°C, 7 L/min |
| Capillary | -4500 V |
| Nozzle | -500 V |

| Time (min) | %A | %B |
|---|---|---|
| 0.00 | 90% | 10% |
| 0.30 | 90% | 10% |
| 1.40 | 0% | 100% |
| 1.90 | 0% | 100% |
| 1.91 | 90% | 10% |

| Analyte | Precursor Ion (m/z) | Product Ion (m/z) | Fragmentor (V) | Collision Energy (V) |
|---|---|---|---|---|
| Diphacinone | 339.1 | **167.1** | 100 | 23 |
| | | 145.0 | | 18 |
| $D_4$-Diphacinone | 343.1 | 167.1 | 120 | 23 |

**BOLD** = product ion used for quantitation

**Detection and Quantitation Limits:**

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value. The Quantitation Limit (QL) is the lowest concentration of analyte that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL. This was performed by comparing the analyte response observed in fortified control soil (n=3) with the baseline noise observed at the same retention time in control soil (n=3). The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10, respectively. The following table presents the average DL and QL concentrations determined for diphacinone in Soil from Oahu, HI.

Diphacinone Detection Limit (DL) and Quantitation Limit (QL)

| Control Matrix | DL (ng/g) | QL (ng/g) |
|---|---|---|
| Soil | 0.59 | 1.95 |

**Results:**

All samples were tested in triplicate. Diphacinone residues are reported in units of ng/g, equivalent to parts per billion (ppb). If no analyte response was recorded by the data acquisition software or if the observed concentration was less than the DL, an entry of "ND" is reported to indicate that no diphacinone was detected.

**Diphacinone – Soil**

| NWRC ID | Sample ID, Description, Collection Date | Water Content (% w/w) | Observed Diphacinone Concentration (ng/g) |
|---|---|---|---|
| S171113-08 | Soil 1-1, beach soil, 8/1/2017 | 5.9 | ND<br>ND<br>ND |
| S171113-09 | Soil 2-1, beach soil, 8/1/2017 | 3.6 | ND<br>ND<br>ND [a] |
| S171113-10 | Soil 3-1, beach soil, 8/1/2017 | 5.6 | ND<br>ND<br>ND |
| S171113-11 | Soil 1-2, beach soil, 8/24/2017 | 2.4 | ND<br>ND<br>ND |
| S171113-12 | Soil 2-2, beach soil, 8/24/2017 | 6.1 | ND<br>ND<br>ND |
| S171113-13 | Soil 3-2, beach soil, 8/24/2017 | 3.7 | ND<br>ND<br>ND |
| S171113-14 | Soil 1-3, beach soil, 8/31/2017 | 1.3 | ND<br>ND<br>ND |
| S171113-15 | Soil 2-3, beach soil, 8/31/2017 | 6.6 | ND<br>ND<br>ND |
| S171113-16 | Soil 3-3, beach soil, 8/31/2017 | 1.7 | ND<br>ND<br>ND |
| | | DL (ng/g) = | 0.59 |
| | | QL (ng/g) = | 1.95 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

[a] The first two replicate samples of S171113-09 had no detectable diphacinone, but the third sample had 0.81 ng/g. This was suspected as contamination so a fourth sample was prepared. The fourth preparation had no detectable diphacinone and confirmed the first two results. The third result was therefore omitted.

**Diphacinone – Soil**

| NWRC ID | Sample ID, Description, Collection Date | Water Content (% w/w) | Observed Diphacinone Concentration (ng/g) |
|---|---|---|---|
| S171113-17 | Soil 1-4, beach soil, 9/14/2017 | 1.9 | ND<br>ND<br>ND |
| S171113-18 | Soil 2-4, beach soil, 9/14/2017 | 5.6 | ND<br>ND<br>ND |
| S171113-19 | Soil 3-4, beach soil, 9/14/2017 | 3.8 | ND<br>ND<br>ND |
| S171113-20 | Soil 1-5, beach soil, 9/29/2017 | 1.2 | ND<br>ND<br>ND |
| S171113-21 | Soil 2-5, beach soil, 9/29/2017 | 6.2 | ND<br>ND<br>ND |
| S171113-22 | Soil 3-5, beach soil, 9/29/2017 | 1.0 | ND<br>ND<br>ND |
| | | DL (ng/g) = | 0.59 |
| | | QL (ng/g) = | 1.95 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

**QC Results:**

QC Recoveries – Control Soil from Oahu (S171113-07 [a])

| ID | Theoretical Diphacinone Concentration (ng/g) | Observed Diphacinone Concentration (ng/g) | % Recovery |
|---|---|---|---|
| QC-221 | 0 | ND | N/A |
| QC-222 | 0 | ND | N/A |
| QC-227 | 0 | ND | N/A |
| QC-223 | 10.4 | 9.71 | 93.4% |
| QC-228 | 10.3 | 10.1 | 98.1% |
| QC-229 | 10.1 | 9.09 | 90.0% |
| QC-224 | 277 | 258 | 93.1% |
| QC-225 | 271 | 258 | 95.2% |
| QC-230 | 277 | 255 | 92.1% |
| QC-226 | 1650 | 1560 | 94.5% |
| QC-231 | 1680 | 1560 | 92.9% |
| QC-232 | 1680 | 1550 | 92.3% |
| | DL (ng/g) = | 0.59 | |
| | QL (ng/g) = | 1.95 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

[a] Water content 8.8% (w/w)

| Wildlife Services NWRC National Wildlife Research Center<br>Analytical Services Report | United States Department of Agriculture<br>Animal Plant Health Inspection Service<br>Wildlife Services<br>National Wildlife Research Center<br>Chemistry Lab Unit | Invoice #: 18-001/2<br>Date: December 22, 2017<br>Page: 1 of 4 |
|---|---|---|

To: Dr. Shane Siers
Hawaii Field Station Leader
NWRC

Subject: Diphacinone Residues in Limpets from Lehua Island (QA-2802)

Methods: New, non-GLP

Analysis Dates: 12/20/2017

Notebook Reference: AC-161, pp. 112, 121-122

QC Notebook Reference: AC-162, p. 4

Analyst: Steve Volker

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**Sample Descriptions:**

Limpet (*cellana sp.*) from Lehua Island were received frozen on 10/31/2017 and 11/6/2017 for diphacinone residue analysis. Each of the fifteen samples is a composite of 8 individual limpets. All samples were stored at -20°C until time of analysis.

**Sample Preparation:**

Dissection and homogenization:

Whole limpet (n=8 for each sample) were thawed at room temperature for 30-45 minutes. Excess seawater was absorbed with paper towels before dissection. The flesh from each limpet was removed and the composite sample (n=8) homogenized with a SPEX 6875D liquid nitrogen freezer mill. Homogenates were transferred immediately to vacuum sealable bags and stored at -20°C.

Extraction and analysis of limpets:

The same extraction procedure and instrument method described in the Analytical Services report for fish muscle (Invoice 18-001/1 issued 12/12/2017) was used, except that 75 µL of DI water was added instead of 150 µL.

**Detection and Quantitation Limits:**

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value. The Quantitation Limit (QL) is the lowest concentration of analyte that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL. This was performed by comparing the analyte response observed in fortified control matrix (n=3) with the baseline noise observed at the same retention time in control matrix (n=3). Control limpet (*cellana sp.*, NWRC ID S171113-05) was used for Quality Control (QC) samples. The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10, respectively. The following table presents the average DL and QL concentrations determined for diphacinone in limpet.

| Analyst | Date | QC Specialist | Date | Reviewer | Date |
|---|---|---|---|---|---|
| | 12/22/17 | | 12/27/17 | | 12/27/17 |

Diphacinone Detection Limit (DL) and Quantitation Limit (QL)

| Control Matrix | DL (ng/g) | QL (ng/g) |
|---|---|---|
| Limpet (*cellana sp.*) | 8.4 | 27.8 |

**Results:**

Triplicate preparations of all samples were prepared. Diphacinone residues are reported in units of ng/g, equivalent to parts per billion (ppb). If no analyte response was recorded by the data acquisition software or if the observed concentration was less than the DL, an entry of "ND" is reported to indicate that the analyte was not detected. Results are reported to three significant figures.

**Diphacinone – Limpets**

| NWRC ID | Sample ID, Species, Count, Collection Date | Observed Diphacinone Concentration (ng/g) |
|---|---|---|
| S171031-01 | Opihi 1-5, *cellana sp.*, n=8 , 9/29/2017 | ND |
| | | ND |
| | | ND |
| S171031-02 | Opihi 2-5, *cellana sp.*, n=8 , 9/29/2017 | ND |
| | | ND |
| | | ND |
| S171031-03 | Opihi 3-5, *cellana sp.*, n=8 , 9/29/2017 | ND |
| | | ND |
| | | ND |
| S171106-19 | Opihi 1-1, *cellana sp.*, n=8 , 8/1/2017 | ND |
| | | ND |
| | | ND |
| S171106-20 | Opihi 2-1, *cellana sp.*, n=8 , 8/1/2017 | ND |
| | | ND |
| | | ND |
| S171106-21 | Opihi 3-1, *cellana sp.*, n=8 , 8/1/2017 | ND |
| | | ND |
| | | ND |
| S171106-22 | Opihi 1-2, *cellana sp.*, n=8 , 8/1/2017 | ND |
| | | ND |
| | | ND |
| | DL (ng/g) = | 8.4 |
| | QL (ng/g) = | 27.8 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

**Diphacinone – Limpets**

| NWRC ID | Sample ID, Species, Count, Collection Date | Observed Diphacinone Concentration (ng/g) |
|---|---|---|
| S171106-23 | Opihi 2-2, *cellana sp.*, n=8 , 8/25/2017 | ND |
| | | ND |
| | | ND |
| S171106-24 | Opihi 3-2, *cellana sp.*, n=8 , 8/25/2017 | ND |
| | | ND |
| | | ND |
| S171106-25 | Opihi 1-3, *cellana sp.*, n=8 , 8/31/2017 | ND |
| | | ND |
| | | ND |
| S171106-26 | Opihi 2-3, *cellana sp.*, n=8 , 8/31/2017 | ND |
| | | ND |
| | | N/A [a] |
| S171106-27 | Opihi 3-3, *cellana sp.*, n=8 , 8/31/2017 | N/A [a] |
| | | ND |
| | | ND |
| S171106-28 | Opihi 1-4, *cellana sp.*, n=8 , 9/13/2017 | ND |
| | | ND |
| | | ND |
| S171106-29 | Opihi 2-4, *cellana sp.*, n=8 , 9/13/2017 | ND |
| | | ND |
| | | ND |
| S171106-30 | Opihi 3-4, *cellana sp.*, n=8 , 9/13/2017 | ND |
| | | ND |
| | | ND |
| | DL (ng/g) = | 8.4 |
| | QL (ng/g) = | 27.8 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

[a] Sample spilled during preparation. No retest performed as replicates are both "ND".

**QC Results:**

QC Recoveries – Limpet (S171113-05)

| ID | Theoretical Diphacinone Concentration (ng/g) | Observed Diphacinone Concentration (ng/g) | % Recovery |
|---|---|---|---|
| QC69 | 0 | ND | N/A |
| QC70 | 0 | ND | N/A |
| QC71 | 0 | ND | N/A |
| QC72 | 62.1 | 51.9 | 83.6% |
| QC73 | 61.3 | 64.4 | 105% |
| QC74 | 65.6 | 62.2 | 94.8% |
| QC75 | 501 | 480 | 95.8% |
| QC76 | 495 | 408 | 82.4% |
| QC77 | 490 | 406 | 82.9% |
| QC78 | 3430 | 2670 | 77.8% |
| QC79 | 3730 | 3330 | 89.3% |
| QC80 | 3600 | 3380 | 93.9% |
| | DL (ng/g) = | 8.4 | |
| | QL (ng/g) = | 27.8 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

| Wildlife Services NWRC National Wildlife Research Center<br>Analytical Services Report | United States Department of Agriculture<br>Animal Plant Health Inspection Service<br>Wildlife Services<br>National Wildlife Research Center<br>Chemistry Lab Unit | Invoice #: 18-001/3<br>Date: January 10, 2018<br>Page: 1 of 5 |
|---|---|---|

To: Dr. Shane Siers
Hawaii Field Station Leader
NWRC

Subject: Diphacinone Residues in Crabs from Lehua Island (QA-2802)

Methods: New, non-GLP

Analysis Dates: 1/4/2018

Notebook Reference: AC-161, pp. 112, 123-124

QC Notebook Reference: AC-162, p. 4

Analyst: Steve Volker

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**Sample Descriptions:**

A'ama crabs (*Grapsus tenuicrustatus*) from Lehua Island were received frozen on 10/31/2017 and 11/6/2017 for diphacinone residue analysis. Each of the fifteen samples is a composite of 3 individual crabs. Control a'ama crabs from Hilo, HA were received 11/16/2017. All samples were stored at -20°C until time of analysis.

**Sample Preparation:**

Dissection and homogenization:

Whole crabs (n=3 for each sample) were thawed at room temperature for 20-30 minutes and excess water absorbed with paper towels before processing. Legs were removed and the meat extruded from each using a small steel roller. The carapace was removed and all fleshy contents removed. The leg meat and fleshy contents from each composite sample were combined and homogenized with a SPEX 6875D liquid nitrogen freezer mill. Homogenates were transferred immediately to vacuum sealable bags and stored at -20°C.

Extraction of crab tissue:

Homogenized sample (70-80 mg) was weighed into a 1.5-mL microcentrifuge tube, 75µL DI water added, and the sample vortex mixed at 2500 RPM for 20 minutes using an auto-vortexer to form a uniform slurry. Surrogate analyte (20 µL, 16 µg/mL $D_4$-diphacinone in acetonitrile) and 1.180 mL acetonitrile (ACN) were added and the sample vortex mixed again for 20 minutes. An excess of NaCl (~120 mg) was added and the sample vortex mixed 20 minutes to partition the water and ACN phases. The extract was clarified by centrifugation (12,000 RCF) and 0.900 mL of supernatant transferred to a dispersive solid-phase extraction (dSPE) tube containing $MgSO_4$ (150 mg), C18 sorbent (25 mg), and primary-secondary amine (PSA) sorbent (25 mg). The extract was exposed to the sorbents and $MgSO_4$ by vortex mixing for 4-5 s followed by centrifugation at 12,000 RCF for 2-3 s to clarify the supernatant. 0.400 mL of supernatant was then transferred to a 1.5-mL microcentrifuge tube and the solvent removed in a 60°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 100 µL ACN followed by 400 µL pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC-MS/MS analysis.

| Analyst | Date | QC Specialist | Date | Reviewer | Date |
|---|---|---|---|---|---|
| | 1/10/2018 | | 1/11/18 | | 1/11/18 |

**Instrument method:**

Agilent 1290 Infinity II HPLC with G6470A QQQ

| | |
|---|---|
| Column | Xbridge C18, 2.5-µm, 2.1 x 50 mm, Waters P/N 186003085 |
| Mobile phase A | 90%(pH 9.5 20-mM ammonium acetate)/10%(Acetonitrile) |
| Mobile phase B | Acetonitrile |
| Flow rate | 0.700 mL/min |
| Column temp. | 60°C |
| Injection volume | 10 µL |
| Run time | 2.40 min |
| Source | AJS ESI, negative mode |
| Gas temp. | 300°C |
| Gas flow | 5 L/min |
| Nebulizer | 45 psi |
| Sheath gas | 250°C, 7 L/min |
| Capillary | -4500 V |
| Nozzle | -500 V |

| Time (min) | %A | %B |
|---|---|---|
| 0.00 | 90% | 10% |
| 0.30 | 90% | 10% |
| 1.40 | 0% | 100% |
| 1.90 | 0% | 100% |
| 1.91 | 90% | 10% |

| Analyte | Precursor Ion (m/z) | Product Ion (m/z) | Fragmentor (V) | Collision Energy (V) |
|---|---|---|---|---|
| Diphacinone | 339.1 | **167.1** | 100 | 23 |
| | | 145.0 | | 18 |
| $D_4$-Diphacinone | 343.1 | 167.1 | 120 | 23 |

**BOLD** = product ion used for quantitation

**Detection and Quantitation Limits:**

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value. The Quantitation Limit (QL) is the lowest concentration of analyte that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL. This was performed by comparing the analyte response observed in fortified control matrix (n=3) with the baseline noise observed at the same retention time in control matrix (n=3). Control a'ama crab from Hilo, HA (NWRC ID S171116-01) was used for Quality Control (QC) samples. The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10, respectively. The following table presents the average DL and QL concentrations determined for diphacinone in crab tissue.

Diphacinone Detection Limit (DL) and Quantitation Limit (QL)

| Control Matrix | DL (ng/g) | QL (ng/g) |
|---|---|---|
| Crab tissue (*G. tenuicrustatus*) | 8.0 | 26.7 |

**Results:**

Triplicate preparations of all samples were prepared. Diphacinone residues are reported in units of ng/g, equivalent to parts per billion (ppb). If no analyte response was recorded by the data acquisition software or if the observed concentration was less than the DL, an entry of "ND" is reported to indicate that the analyte was not detected. Results are reported to three significant figures. . Results that are greater than the DL, but less than the QL are identified by an asterisk "*". Care should be taken when evaluating results below the QL as the variability will be significantly greater than the variability observed in quality control (QC) samples. Results above the QL are reported to three significant figures.

**Diphacinone – Crab Tissue**

| NWRC ID | Sample ID, Species, Count, Collection Date | Observed Diphacinone Concentration (ng/g) |
|---|---|---|
| S171031-04 | A'ama 1-5, G. tenuicrustatus, n=3, 9/29/2017 | ND |
| | | ND |
| | | ND |
| S171031-05 | A'ama 2-5, G. tenuicrustatus, n=3, 9/29/2017 | ND |
| | | ND |
| | | ND |
| S171031-06 | A'ama 3-5, G. tenuicrustatus, n=3, 9/29/2017 | ND |
| | | ND |
| | | ND |
| S171106-58 | A'ama 1-1, G. tenuicrustatus, n=3, 8/1/2017 | ND |
| | | ND |
| | | ND |
| S171106-59 | A'ama 2-1, G. tenuicrustatus, n=3, 8/1/2017 | ND |
| | | ND |
| | | ND |
| | DL (ng/g) = | 8.0 |
| | QL (ng/g) = | 26.7 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

**Diphacinone – Crab Tissue**

| NWRC ID | Sample ID, Species, Count, Collection Date | Observed Diphacinone Concentration (ng/g) |
|---|---|---|
| S171106-60 | A'ama 3-1, G. tenuicrustatus, n=3, 8/1/2017 | ND |
| | | ND |
| | | ND |
| S171106-61 | A'ama 1-2, G. tenuicrustatus, n=3, 8/25/2017 | 21.2 * |
| | | 20.6 * |
| | | 20.2 * |
| S171106-62 | A'ama 2-2, G. tenuicrustatus, n=3, 8/25/2017 | 45.9 |
| | | 46.3 |
| | | 50.9 |
| S171106-63 | A'ama 3-2, G. tenuicrustatus, n=3, 8/25/2017 | ND |
| | | ND |
| | | ND |
| S171106-64 | A'ama 1-3, G. tenuicrustatus, n=3, 8/31/2017 | ND |
| | | ND |
| | | ND |
| S171106-65 | A'ama 2-3, G. tenuicrustatus, n=3, 8/31/2017 | 93.3 |
| | | 98.7 |
| | | 95.0 |
| S171106-66 | A'ama 3-3, G. tenuicrustatus, n=3, 8/31/2017 | ND |
| | | ND |
| | | ND |
| S171106-67 | A'ama 1-4, G. tenuicrustatus, n=3, 9/13/2017 | ND |
| | | ND |
| | | ND |
| S171106-68 | A'ama 2-4, G. tenuicrustatus, n=3, 9/13/2017 | ND |
| | | ND |
| | | ND |
| S171106-69 | A'ama 3-4, G. tenuicrustatus, n=3, 9/13/2017 | ND |
| | | ND |
| | | ND |
| | DL (ng/g) = | 8.0 |
| | QL (ng/g) = | 26.7 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Observed diphacinone concentration less than Quantitation Limit (QL)

**QC Results:**

QC Recoveries – Crab Tissue (S171116-01)

| ID | Theoretical Diphacinone Concentration (ng/g) | Observed Diphacinone Concentration (ng/g) | % Recovery |
|---|---|---|---|
| QC153 | 0 | ND | N/A |
| QC154 | 0 | ND | N/A |
| QC155 | 0 | ND | N/A |
| QC156 | 62.1 | 65.1 | 105% |
| QC157 | 64.0 | 66.7 | 104% |
| QC158 | 60.7 | 70.1 | 115% |
| QC159 | 477 | 599 | 126% |
| QC160 | 469 | 543 | 116% |
| QC161 | 501 | 602 | 120% |
| QC162 | 3530 | 4090 | 116% |
| QC163 | 3570 | 3990 | 112% |
| QC164 | 3590 | 4110 | 114% |
| | DL (ng/g) = | 8.0 | |
| | QL (ng/g) = | 26.7 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

| Wildlife Services NWRC National Wildlife Research Center<br>Analytical Services Report | United States Department of Agriculture<br>Animal Plant Health Inspection Service<br>Wildlife Services<br>National Wildlife Research Center<br>Chemistry Lab Unit | Invoice #: 18-001/4<br>Date: February 2, 2018<br>Page: 1 of 8 |
|---|---|---|

To: Dr. Shane Siers
Hawaii Field Station Leader
NWRC

Subject: Diphacinone Residues in Fish from Lehua Island (QA-2802)

Methods: New, non-GLP

Analysis Dates: 1/11, 1/12, 1/18, 1/24, and 1/25/2018

Notebook Reference: AC-161, pp. 112-113, 125-133

QC Notebook Reference: AC-162, p. 4

Analyst: Steve Volker

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**Sample Descriptions:**

Forty whole fish from Lehua Island were received frozen on 11/06/2017 for diphacinone residue analysis of liver and muscle. Control Soldierfish (*Myripristis spp.*) from Hilo, HA were received 11/13/2017. All samples were stored at -20°C until time of analysis.

**Sample Preparation:**

Dissection and homogenization:

Whole fish were thawed overnight in a 4°C refrigerator. A portion of fillet muscle and entire liver were removed and then homogenized with a SPEX 6875D liquid nitrogen freezer mill. Homogenized samples were transferred immediately to vacuum sealable bags and stored at -20°C.

Extraction of fish muscle and liver:

Homogenized sample (70-80 mg) was weighed into a 1.5-mL microcentrifuge tube, DI water added (50µL for liver, 150µL for muscle), and the sample vortex mixed at 2500 RPM for 20 minutes using an auto-vortexer to form a uniform slurry. Surrogate analyte (20 µL, 16 µg/mL $D_4$-diphacinone in acetonitrile) and 1.180 mL acetonitrile (ACN) were added and the sample vortex mixed again for 20 minutes. An excess of NaCl (~120 mg) was added and the sample vortex mixed 20 minutes to partition the water and ACN phases. The extract was clarified by centrifugation (12,000 RCF) and 0.900 mL of supernatant transferred to a dispersive solid-phase extraction (dSPE) tube containing $MgSO_4$ (150 mg), C18 sorbent (25 mg), and primary-secondary amine (PSA) sorbent (25 mg). The extract was exposed to the sorbents and $MgSO_4$ by vortex mixing for 4-5 s followed by centrifugation at 12,000 RCF for 2-3 s to clarify the supernatant. 0.400 mL of supernatant was then transferred to a 1.5-mL microcentrifuge tube and the solvent removed in a 60°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 100 µL ACN followed by 400 µL pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC-MS/MS analysis.

| Analyst | Date | QC Specialist | Date | Reviewer | Date |
|---|---|---|---|---|---|
| | 2/2/2018 | | 2/2/18 | | 2/2/18 |

**Instrument method:**

Agilent 1290 Infinity II HPLC with G6470A QQQ

| | |
|---|---|
| Column | Xbridge C18, 2.5-µm, 2.1 x 50 mm, Waters P/N 186003085 |
| Mobile phase A | 90%(pH 9.5 20-mM ammonium acetate)/10%(Acetonitrile) |
| Mobile phase B | Acetonitrile |
| Flow rate | 0.700 mL/min |
| Column temp. | 60°C |
| Injection volume | 10 µL |
| Run time | 2.40 min |
| Source | AJS ESI, negative mode |
| Gas temp. | 300°C |
| Gas flow | 5 L/min |
| Nebulizer | 45 psi |
| Sheath gas | 250°C, 7 L/min |
| Capillary | -4500 V |
| Nozzle | -500 V |

| Time (min) | %A | %B |
|---|---|---|
| 0.00 | 90% | 10% |
| 0.30 | 90% | 10% |
| 1.40 | 0% | 100% |
| 1.90 | 0% | 100% |
| 1.91 | 90% | 10% |

| Analyte | Precursor Ion (m/z) | Product Ion (m/z) | Fragmentor (V) | Collision Energy (V) |
|---|---|---|---|---|
| Diphacinone | 339.1 | **167.1** | 100 | 23 |
| | | 145.0 | | 18 |
| $D_4$-Diphacinone | 343.1 | 167.1 | 120 | 23 |

**BOLD** = product ion used for quantitation

**Detection and Quantitation Limits:**

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value. The Quantitation Limit (QL) is the lowest concentration of analyte that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL. This was performed by comparing the analyte response observed in fortified control matrix (n=6) with the baseline noise observed at the same retention time in control matrix (n=6). Muscle and liver from Soldierfish (*Myripristis spp.*), NWRC ID S171113-03 and S171113-04 were used for controls. The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10, respectively. The following table presents the average DL and QL concentrations determined over two days for diphacinone in each matrix.

Diphacinone Detection Limit (DL) and Quantitation Limit (QL)

| Control Matrix | DL (ng/g) | QL (ng/g) |
|---|---|---|
| Muscle (Soldierfish) | 9.1 | 30.4 |
| Liver (Soldierfish) | 17 | 57.4 |

**Results:**

Triplicate preparations of all samples were prepared, except when sample size was insufficient. Diphacinone residues are reported in units of ng/g, equivalent to parts per billion (ppb). If no analyte response was recorded by the data acquisition software or if the observed concentration was less than DL, an entry of "ND" is reported to indicate that the analyte was not detected. Results that are greater than the DL, but less than the QL are identified by an asterisk "*". Care should be taken when evaluating results below the QL as the variability will be significantly greater than the variability observed in quality control (QC) samples. Results above the QL are reported to three significant figures.

**Diphacinone - Fish**

| NWRC ID | Sample ID, Common Name, Collection Date, Site | Analysis Dates | Observed Diphacinone Concentration (ng/g) Muscle | Liver |
|---|---|---|---|---|
| S171106-06 | DAR032, Sabre squirrelfish, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-07 | DAR011, Hawaiian hogfish, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-08 | DAR033, Soldierfish, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-09 | DAR015, Manybar goatfish, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11/18, N/A | ND | N/A [a] |
| S171106-10 | DAR037, Hawaiian chub, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-11 | DAR016, Bluefin trevally, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-12 | DAR042, Smalltooth jobfish, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-13 | DAR010, Blue-spotted grouper, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-14 | DAR020, Pinktail triggerfish, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-15 | DAR040, Smalltooth jobfish, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | DL (ng/g) = | 9.1 | 17 |
| | | QL (ng/g) = | 30.4 | 57.4 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

[a] Insufficient sample for three replicate samples.

**Diphacinone - Fish**

| NWRC ID | Sample ID, Common Name, Collection Date, Site | Analysis Dates | Observed Diphacinone Concentration (ng/g) Muscle | Liver |
|---|---|---|---|---|
| S171106-16 | DAR055, White saddle goatfish, 8/9/2017, Lehua Camp | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-17 | Fishby 01, Stocky hawkfish, 9/2/2017, 1 | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| | | 1/11, 1/18/18 | ND | ND |
| S171106-18 | Fishby 02, Lei triggerfish, 9/2/2017, 3 | 1/11, 1/18/18 | ND | 49.1* |
| | | 1/11, 1/18/18 | ND | 37.0* |
| | | 1/11, 1/18/18 | ND | 45.2* |
| S171106-31 | Fish 1-1, Pinktail triggerfish, 8/1/2017, 1 | 1/11, 1/18/18 | ND | ND |
| | | 1/12, 1/18/18 | ND | ND |
| | | 1/12, 1/18/18 | ND | ND |
| S171106-32 | Fish 1-2, Pinktail triggerfish, 8/25/2017, 1 | N/A, 1/18/18 | N/A [a] | 326 |
| | | 1/12, 1/18/18 | ND | 321 |
| | | 1/12, 1/18/18 | ND | 292 |
| S171106-33 | Fish 1-3, Pinktail triggerfish, 9/1/2017, 1 | 1/12, 1/18/18 | ND | 175 |
| | | 1/12, 1/18/18 | 9.8* | 162 |
| | | 1/12, 1/18/18 | 9.2* | 165 |
| S171106-34 | Fish 3-3, Pinktail triggerfish, 8/31/2017, 3 | 1/12, 1/18/18 | ND | 27.4* |
| | | 1/12, 1/18/18 | ND | 22.9* |
| | | 1/12, 1/18/18 | ND | 24.6* |
| S171106-35 | Fish 1-4, Pinktail triggerfish, 9/13/2017, 1 | 1/12, 1/18/18 | 12.0* | 250 |
| | | 1/12, 1/18/18 | 23.6* | 203 |
| | | 1/12, 1/18/18 | 15.6* | 225 |
| S171106-36 | Fish 2-3, Pinktail triggerfish, 8/31/2017, 2 | 1/12, 1/18/18 | ND | 179 |
| | | 1/12, 1/18/18 | ND | 182 |
| | | 1/12, 1/18/18 | ND | 185 |
| S171106-37 | Fish 2-4, Pinktail triggerfish, 9/13/2017, 2 [b] | 1/12/18, N/A | ND | N/A |
| | | 1/12/18, N/A | ND | N/A |
| | | 1/12/18, N/A | ND | N/A |
| | | DL (ng/g) = | 9.1 | 17 |
| | | QL (ng/g) = | 30.4 | 57.4 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Observed diphacinone concentration less than Quantitation Limit (QL)

[a] Sample spilled during preparation. No retest performed as replicates are both "ND".

[b] Fish 2-4 was a Pinktail triggerfish (*Melichthys vidula)* according to label included with the carcass, not Stocky hawkfish (*Cirrhitus pinulatus)* as stated on the client sample list. Additionally, the abdomen was crushed and no liver was identifiable.

**Diphacinone - Fish**

| NWRC ID | Sample ID, Common Name, Collection Date, Site | Analysis Dates | Observed Diphacinone Concentration (ng/g) Muscle | Liver |
|---|---|---|---|---|
| S171106-38 | Fish 2-1, Pinktail triggerfish, 8/1/2017, 2 | 1/12, 1/18/18 | ND | ND |
| | | 1/12, 1/18/18 | ND | ND |
| | | 1/12, 1/18/18 | ND | ND |
| S171106-39 | Fish 2-2, Black triggerfish, 8/25/2017, 2 | 1/12, 1/18/18 | ND | 62.8 |
| | | 1/12, 1/18/18 | ND | 51.7* |
| | | 1/12, 1/18/18 | ND | 47.0* |
| S171106-40 | Fish 3-4, Pinktail triggerfish, 9/13/2017, 3 [a] | 1/12, 1/18/18 | ND | 171 |
| | | 1/12, 1/18/18 | ND | 170 |
| | | 1/12, 1/18/18 | ND | 169 |
| S171106-41 | Fish 3-2, Black triggerfish, 8/25/2017, 3 | 1/12, 1/18/18 | ND | 47.7* |
| | | 1/12, 1/18/18 | ND | 44.6* |
| | | 1/12, 1/18/18 | ND | 51.1* |
| S171106-42 | Fish 3-1, Black triggerfish, 8/1/2017, 3 | 1/12, 1/18/18 | ND | ND |
| | | 1/12, 1/18/18 | ND | ND |
| | | 1/12, 1/18/18 | ND | ND |
| S171106-43 | FishA 2-4, Black triggerfish, 9/13/2017, 2 | 1/12, 1/25/18 | ND | ND |
| | | 1/12, 1/25/18 | ND | ND |
| | | 1/12, 1/25/18 | ND | ND |
| S171106-44 | FishA 3-4, Black triggerfish, 9/13/2017, 3 | 1/12, 1/25/18 | 36.3 | 1360 |
| | | 1/12, 1/25/18 | 36.9 | 1270 |
| | | 1/24, 1/25/18 | 23.6* | 1440 |
| S171106-45 | FishA 1-4, Lei triggerfish, 9/13/2017, 1 | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| S171106-46 | FishA 3-2, Bluestripe snapper, 8/25/2017, 3 | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| S171106-47 | FishA 2-2, Blacktail snapper, 8/25/2017, 2 | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| | | DL (ng/g) = | 9.1 | 17 |
| | | QL (ng/g) = | 30.4 | 57.4 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Observed diphacinone concentration less than Quantitation Limit (QL)

[a] Fish 3-4 was a Black triggerfish (*Melichthys niger*) according to label included with the carcass, not Pinktail triggerfish (*Melichthys vidula*) as stated on the client sample list.

**Diphacinone – Fish**

| NWRC ID | Sample ID, Common Name, Collection Date, Site | Analysis Dates | Observed Diphacinone Concentration (ng/g) Muscle | Liver |
|---|---|---|---|---|
| S171106-48 | FishA 3-3, Black triggerfish, 9/1/2017, 3 | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| S171106-49 | FishA 2-3, Black triggerfish, 9/1/2017, 2 | 1/24, 1/25/18 | ND | 53.7* |
| | | 1/24, 1/25/18 | ND | 67.1 |
| | | 1/24, 1/25/18 | ND | 65.9 |
| S171106-50 | FishA 1-3, Stocky hawkfish, 8/31/2017, 1 | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| S171106-51 | FishA 1-2, Black triggerfish, 8/25/2017, 1 | 1/24, 1/25/18 | ND | 124 |
| | | 1/24, 1/25/18 | ND | 139 |
| | | 1/24, 1/25/18 | ND | 124 |
| S171106-52 | FishA 1-1, Bigscale soldierfish, 8/2/2017, 1 | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| | | 1/24/18, N/A | ND | N/A [a] |
| S171106-53 | FishA 2-1, Bluestripe snapper, 8/2/2017, 2 | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| S171106-54 | FishA 3-1, Bigscale soldierfish, 8/2/2017, 3 | 1/24, 1/25/18 | ND | ND |
| | | 1/24, 1/25/18 | ND | ND |
| | | 1/24/18, N/A | ND | N/A [a] |
| S171106-55 | Fish 05, Flathead grey mullet , 9/5/2017, NE tide pool [b] | 1/24/18, N/A | ND | N/A |
| | | 1/24/18, N/A | ND | N/A |
| | | 1/24/18, N/A | ND | N/A |
| S171106-56 | Fish 07, Flathead grey mullet , 9/5/2017, NE tide pool [b] | 1/24/18, N/A | 144 | N/A |
| | | 1/24/18, N/A | 173 | N/A |
| | | 1/24/18, N/A | 166 | N/A |
| S171106-57 | Fish 23, Flathead grey mullet , 9/5/2017, NE tide pool [b] | 1/24/18, N/A | ND | N/A |
| | | 1/24/18, N/A | ND | N/A |
| | | 1/24/18, N/A | ND | N/A |
| | | DL (ng/g) = | 9.1 | 17 |
| | | QL (ng/g) = | 30.4 | 57.4 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Observed diphacinone concentration less than Quantitation Limit (QL)

[a] Insufficient sample for three replicate samples.

[b] No liver was identifiable in samples S171106-55, -56, and -57 as they were too decayed. An effort was made to harvest muscle from within the fish that had not been exposed to air and water, but S171106-56 was so deteriorated that muscle had turned brown to black in color and had likely been exposed to the elements. Additionally, the sample IDs and common names for these samples were obtained from the labels included with the carcasses.

**QC Results:**

**QC Recoveries – Fish Muscle (Soldierfish, S171113-03 and -04)**

| ID | Analysis Date | Theoretical Diphacinone Concentration (ng/g) | Observed Diphacinone Concentration (ng/g) | % Recovery |
|---|---|---|---|---|
| QC-81 | 1/11/2018 | 0 | ND | N/A |
| QC-82 | 1/11/2018 | 0 | ND | N/A |
| QC-89 | 1/12/2018 | 0 | ND | N/A |
| QC-90 | 1/12/2018 | 0 | ND | N/A |
| QC-97 | 1/24/2018 | 0 | ND | N/A |
| QC-98 | 1/24/2018 | 0 | ND | N/A |
| QC-83 | 1/11/2018 | 60.5 | 44.9 | 74.2% |
| QC-84 | 1/11/2018 | 61.7 | 64.4 | 104% |
| QC-91 | 1/12/2018 | 61.8 | 65.1 | 105% |
| QC-92 | 1/12/2018 | 63.0 | 63.7 | 101% |
| QC-99 | 1/24/2018 | 65.4 | 58.7 | 89.8% |
| QC-100 | 1/24/2018 | 66.2 | 60.6 | 91.5% |
| QC-85 | 1/11/2018 | 472 | 477 | 101% |
| QC-86 | 1/11/2018 | 474 | 487 | 103% |
| QC-93 | 1/12/2018 | 468 | 487 | 104% |
| QC-94 | 1/12/2018 | 475 | 462 | 97.3% |
| QC-101 | 1/24/2018 | 493 | 459 | 93.1% |
| QC-102 | 1/24/2018 | 512 | 461 | 90.0% |
| QC-87 | 1/11/2018 | 3510 | 2970 | 84.6% |
| QC-88 | 1/11/2018 | 3610 | 3560 | 98.6% |
| QC-95 | 1/12/2018 | 3580 | 3510 | 98.0% |
| QC-96 | 1/12/2018 | 3500 | 3450 | 98.6% |
| QC-103 | 1/24/2018 | 3890 | 3170 | 81.5% |
| QC-104 | 1/24/2018 | 3840 | 3200 | 83.3% |
| | | DL (ng/g) = | 9.1 | |
| | | QL (ng/g) = | 30.4 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

**QC Recoveries – Fish Liver (Soldierfish, S171113-03 and -04)**

| ID | Analysis Date | Theoretical Diphacinone Concentration (ng/g) | Observed Diphacinone Concentration (ng/g) | % Recovery |
|---|---|---|---|---|
| QC-117 | 1/18/2018 | 0 | ND | N/A |
| QC-118 | 1/18/2018 | 0 | ND | N/A |
| QC-125 | 1/18/2018 | 0 | ND | N/A |
| QC-126 | 1/18/2018 | 0 | ND | N/A |
| QC-133 | 1/25/2018 | 0 | ND | N/A |
| QC-134 | 1/25/2018 | 0 | ND | N/A |
| QC-119 | 1/18/2018 | 66.7 | 62.4 | 93.6% |
| QC-120 | 1/18/2018 | 68.1 | 65.5 | 96.2% |
| QC-127 | 1/18/2018 | 65.1 | 47.3 | 72.7% |
| QC-128 | 1/18/2018 | 67.8 | 46.5 | 68.6% |
| QC-135 | 1/25/2018 | 65.4 | 47.6 | 72.8% |
| QC-136 | 1/25/2018 | 66.2 | 45.7 | 69.0% |
| QC-121 | 1/18/2018 | 526 | 386 | 73.4% |
| QC-122 | 1/18/2018 | 515 | 424 | 82.3% |
| QC-129 | 1/18/2018 | 527 | 410 | 77.8% |
| QC-130 | 1/18/2018 | 483 | 389 | 80.5% |
| QC-137 | 1/25/2018 | 493 | 383 | 77.7% |
| QC-138 | 1/25/2018 | 512 | 394 | 77.0% |
| QC-123 | 1/18/2018 | 3910 | 2670 | 68.3% |
| QC-124 | 1/18/2018 | 4000 | 2770 | 69.3% |
| QC-131 | 1/18/2018 | 3840 | 3100 | 80.7% |
| QC-132 | 1/18/2018 | 3940 | 3110 | 78.9% |
| QC-139 | 1/25/2018 | 3890 | 3060 | 78.7% |
| QC-140 | 1/25/2018 | 3840 | 3060 | 79.7% |
| | | DL (ng/g) = | 17 | |
| | | QL (ng/g) = | 57.4 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

| Wildlife Services NWRC National Wildlife Research Center Analytical Services Report | United States Department of Agriculture<br>Animal Plant Health Inspection Service<br>Wildlife Services<br>National Wildlife Research Center<br>Chemistry Lab Unit | Invoice #: 18-001/1<br>Date: December 12, 2017<br>Page: 1 of 7 |
|---|---|---|

To: Dr. Shane Siers
Hawaii Field Station Leader
NWRC

Subject: Diphacinone Residues in Fish from Lehua Island, Post-baiting (QA-2802)

Methods: New, non-GLP

Analysis Dates: 11/27, 11/28, 12/5, and 12/6/2017

Notebook Reference: AC-161, pp. 110-118

QC Notebook Reference: AC-162, p. 4

Analyst: Steve Volker

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**Sample Descriptions:**

Twenty-nine whole fish from Lehua Island were received frozen on 10/31/2017 for diphacinone residue analysis of liver and muscle. All samples were stored at -20°C until time of analysis.

**Sample Preparation:**

Dissection and homogenization:

Whole fish were thawed overnight in a 4°C refrigerator. A portion of fillet muscle and entire liver were removed and then homogenized with a SPEX 6875D liquid nitrogen freezer mill. Homogenized samples were transferred immediately to vacuum sealable bags and stored at -20°C.

Extraction of fish muscle and liver:

Homogenized sample (70-80 mg) was weighed into a 1.5-mL microcentrifuge tube, DI water added (50µL for liver, 150µL for muscle), and the sample vortex mixed at 2500 RPM for 20 minutes using an auto-vortexer to form a uniform slurry. Surrogate analyte (20 µL, 16 µg/mL $D_4$-diphacinone in acetonitrile) and 1.180 mL acetonitrile (ACN) were added and the sample vortex mixed again for 20 minutes. An excess of NaCl (~120 mg) was added and the sample vortex mixed 20 minutes to partition the water and ACN phases. The extract was clarified by centrifugation (12,000 RCF) and 0.900 mL of supernatant transferred to a dispersive solid-phase extraction (dSPE) tube containing $MgSO_4$ (150 mg), C18 sorbent (25 mg), and primary-secondary amine (PSA) sorbent (25 mg). The extract was exposed to the sorbents and $MgSO_4$ by vortex mixing for 4-5 s followed by centrifugation at 12,000 RCF for 2-3 s to clarify the supernatant. 0.400 mL of supernatant was then transferred to a 1.5-mL microcentrifuge tube and the solvent removed in a 60°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 100 µL ACN followed by 400 µL pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC-MS/MS analysis.

| Analyst | Date | QC Specialist | Date | Reviewer | Date |
|---|---|---|---|---|---|
| | 12/12/2017 | | 12/12/17 | | 12/12/17 |

**Instrument method:**

Agilent 1290 Infinity II HPLC with G6470A QQQ

| | |
|---|---|
| Column | Xbridge C18, 2.5-µm, 2.1 x 50 mm, Waters P/N 186003085 |
| Mobile phase A | 90%(pH 9.5 20-mM ammonium acetate)/10%(Acetonitrile) |
| Mobile phase B | Acetonitrile |
| Flow rate | 0.700 mL/min |
| Column temp. | 60°C |
| Injection volume | 10 µL |
| Run time | 2.40 min |
| Source | AJS ESI, negative mode |
| Gas temp. | 300°C |
| Gas flow | 5 L/min |
| Nebulizer | 45 psi |
| Sheath gas | 250°C, 7 L/min |
| Capillary | -4500 V |
| Nozzle | -500 V |

| Time (min) | %A | %B |
|---|---|---|
| 0.00 | 90% | 10% |
| 0.30 | 90% | 10% |
| 1.40 | 0% | 100% |
| 1.90 | 0% | 100% |
| 1.91 | 90% | 10% |

| Analyte | Precursor Ion (m/z) | Product Ion (m/z) | Fragmentor (V) | Collision Energy (V) |
|---|---|---|---|---|
| Diphacinone | 339.1 | **167.1** | 100 | 23 |
| | | 145.0 | | 18 |
| $D_4$-Diphacinone | 343.1 | 167.1 | 120 | 23 |

**BOLD** = product ion used for quantitation

**Detection and Quantitation Limits:**

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value. The Quantitation Limit (QL) is the lowest concentration of analyte that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL. This was performed by comparing the analyte response observed in fortified control matrix (n=6) with the baseline noise observed at the same retention time in control matrix (n=6). Muscle and liver from Bluestripe snapper (*Lutjanus kasmira*), NWRC ID S171113-01 and S171113-02 were used for controls. The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10, respectively. The following table presents the average DL and QL concentrations determined over two days for diphacinone in each matrix.

Diphacinone Detection Limit (DL) and Quantitation Limit (QL)

| Control Matrix | DL (ng/g) | QL (ng/g) |
|---|---|---|
| Muscle (Bluestripe snapper) | 5.6 | 18.7 |
| Liver (Bluestripe snapper) | 12 | 41.1 |

**Results:**

Triplicate preparations of all samples were prepared, except when sample size was insufficient. Diphacinone residues are reported in units of ng/g, equivalent to parts per billion (ppb). If no analyte response was recorded by the data acquisition software or if the observed concentration was less than the DL, an entry of "ND" is reported to indicate that the analyte was not detected. Results are reported to three significant figures.

**Diphacinone - Fish**

| NWRC ID | Sample ID, Common Name, Collection Date, Site | Analysis Dates | Observed Diphacinone Concentration (ng/g) Muscle | Liver |
|---|---|---|---|---|
| S171031-07 | Sportfish 2-1, Hawaiian squirrelfish, 9/29/2017, 1 | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | N/A [a] |
| S171031-08 | Sportfish 2-2, Bluestripe squirrelfish, 9/29/2017, 2 | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| S171031-09 | Sportfish 1-1, Blacktail snapper, 9/29/2017, 1 | 11/27, 12/5/17 | ND | 48.2 |
| | | 11/27, 12/5/17 | ND | 54.2 |
| | | 11/27, 12/5/17 | ND | 50.9 |
| S171031-10 | Sportfish 3-1, Blacktail snapper, 9/29/2017, 3 | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| S171031-11 | Sportfish 3-2, Bigscale soldierfish, 9/29/2017, 3 | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| S171031-12 | Sportfish 3-3, Bluestripe snapper, 9/29/2017, 3 | 11/27, 12/5/17 | ND | 326 |
| | | 11/27, 12/5/17 | ND | 254 |
| | | 11/27, 12/5/17 | ND | 309 |
| S171031-13 | Fish 1-5, Pinktail triggerfish, 9/29/2017, 1 | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| S171031-14 | Fish 2-5, Pinktail triggerfish, 9/29/2017, 2 | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| S171031-15 | Fish 3-5, Black triggerfish, 9/29/2017, 3 | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | ND |
| S171031-16 | FishA 1-5, Stocky hawkfish, 9/29/2017, 1 | 11/27, 12/5/17 | ND | ND |
| | | 11/27, 12/5/17 | ND | N/A [a] |
| | | 11/27, 12/5/17 | ND | N/A [a] |
| | | DL (ng/g) = | 5.6 | 12 |
| | | QL (ng/g) = | 18.7 | 41.1 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

[a] Insufficient sample for three replicate samples.

**Diphacinone - Fish**

| NWRC ID | Sample ID, Common Name, Collection Date, Site | Analysis Dates | Observed Diphacinone Concentration (ng/g) Muscle | Liver |
|---|---|---|---|---|
| S171031-17 | FishA 2-5, Bluestripe snapper, 9/29/2017, 2 | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | ND |
| S171031-18 | FishA 3-5, Black triggerfish, 9/29/2017, 3 | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | ND |
| S171031-19 | DAR116, Pinktail triggerfish, 9/26/2017, Caldera | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | ND |
| S171031-20 | DAR120, Hawaiian hogfish, 9/26/2017, Caldera | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | N/A [b] |
| S171031-21 | DAR119, Chub, nenue, 9/26/2017, Caldera | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | ND |
| | | 11/28, 12/5/17 | ND | ND |
| S171031-22 | DAR082, Hawaiian hogfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-23 | DAR078, Pinktail triggerfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-24 | DAR060, Manybar goatfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-25 | DAR101, Chub, nenue, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-26 | DAR109, Manybar goatfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-27 | DAR108, Manybar goatfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | N/A [a] |
| | | 11/28, 12/6/17 | ND | N/A [a] |
| | DL (ng/g) = | | 5.6 | 12 |
| | QL (ng/g) = | | 18.7 | 41.1 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

[a] Insufficient sample for three replicate preparations.

[b] Sample spilled during preparation. No retest performed as replicates are both "ND".

**Diphacinone - Fish**

| NWRC ID | Sample ID, Common Name, Collection Date, Site | Analysis Dates | Observed Diphacinone Concentration (ng/g) Muscle | Liver |
|---|---|---|---|---|
| S171031-28 | DAR103, Convict tang, Manini, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-29 | DAR098, Small toothed jobfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-30 | DAR099, Small toothed jobfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-31 | DAR079, Pinktail triggerfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-32 | DAR059, Manybar goatfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-33 | DAR077, Hawaiian hogfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-34 | DAR081, Black triggerfish, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| S171031-35 | DAR084, Bluefin trevally, 9/26/2017, Caldera | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | 11/28, 12/6/17 | ND | ND |
| | | DL (ng/g) = | 5.6 | 12 |
| | | QL (ng/g) = | 18.7 | 41.1 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

**QC Results:**

**QC Recoveries – Fish Muscle (Bluestripe snapper, S171113-01 and -02)**

| ID | Analysis Date | Theoretical Diphacinone Concentration (ng/g) | Observed Diphacinone Concentration (ng/g) | % Recovery |
|---|---|---|---|---|
| QC-1 | 11/27/2017 | 0 | ND | N/A |
| QC-2 | 11/27/2017 | 0 | ND | N/A |
| QC-9 | 11/28/2017 | 0 | ND | N/A |
| QC-10 | 11/28/2017 | 0 | ND | N/A |
| QC-17 | 11/28/2017 | 0 | ND | N/A |
| QC-18 | 11/28/2017 | 0 | ND | N/A |
| QC-3 | 11/27/2017 | 60.4 | 60.7 | 100% |
| QC-4 | 11/27/2017 | 61.1 | 58.1 | 95.1% |
| QC-11 | 11/28/2017 | 61.5 | 56.6 | 92.0% |
| QC-12 | 11/28/2017 | 61.5 | 60.1 | 97.7% |
| QC-19 | 11/28/2017 | 64.5 | 60.4 | 93.6% |
| QC-20 | 11/28/2017 | 64.2 | 70.6 | 110% |
| QC-5 | 11/27/2017 | 480 | 350 | 72.9% |
| QC-6 | 11/27/2017 | 496 | 460 | 92.7% |
| QC-13 | 11/28/2017 | 463 | 457 | 98.7% |
| QC-14 | 11/28/2017 | 500 | 480 | 96.0% |
| QC-21 | 11/28/2017 | 477 | 459 | 96.2% |
| QC-22 | 11/28/2017 | 465 | 453 | 97.4% |
| QC-7 | 11/27/2017 | 3460 | 3280 | 94.8% |
| QC-8 | 11/27/2017 | 3620 | 2900 | 80.1% |
| QC-15 | 11/28/2017 | 3650 | 3450 | 94.5% |
| QC-16 | 11/28/2017 | 3520 | 3370 | 95.7% |
| QC-23 | 11/28/2017 | 3590 | 3110 | 86.6% |
| QC-24 | 11/28/2017 | 3570 | 3350 | 93.8% |
| | | DL (ng/g) = | 5.6 | |
| | | QL (ng/g) = | 18.7 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

**QC Recoveries – Fish Liver (Bluestripe snapper, S171113-01 and -02)**

| ID | Analysis Date | Theoretical Diphacinone Concentration (ng/g) | Observed Diphacinone Concentration (ng/g) | % Recovery |
|---|---|---|---|---|
| QC33 | 12/5/2017 | 0 | ND | N/A |
| QC34 | 12/5/2017 | 0 | ND | N/A |
| QC35 | 12/5/2017 | 0 | ND | N/A |
| QC45 | 12/6/2017 | 0 | ND | N/A |
| QC46 | 12/6/2017 | 0 | ND | N/A |
| QC47 | 12/6/2017 | 0 | ND | N/A |
| QC36 | 12/5/2017 | 61.9 | 62.9 | 102% |
| QC37 | 12/5/2017 | 63.5 | 51.0 | 80.3% |
| QC38 | 12/5/2017 | 64.2 | 53.2 | 82.9% |
| QC48 | 12/6/2017 | 60.2 | 48.1 | 79.9% |
| QC49 | 12/6/2017 | 63.8 | 47.3 | 74.1% |
| QC50 | 12/6/2017 | 62.6 | 52.2 | 83.4% |
| QC39 | 12/5/2017 | 480 | 399 | 83.1% |
| QC40 | 12/5/2017 | 471 | 397 | 84.3% |
| QC41 | 12/5/2017 | 486 | 409 | 84.2% |
| QC51 | 12/6/2017 | 494 | 447 | 90.5% |
| QC52 | 12/6/2017 | 503 | 479 | 95.2% |
| QC53 | 12/6/2017 | 477 | 419 | 87.8% |
| QC42 | 12/5/2017 | 4330 | 3780 | 87.3% |
| QC43 | 12/5/2017 | 4220 | 3850 | 91.2% |
| QC44 | 12/5/2017 | 4400 | 3810 | 86.6% |
| QC54 | 12/6/2017 | 4170 | 3360 | 80.6% |
| QC55 | 12/6/2017 | 4540 | 3800 | 83.7% |
| QC56 | 12/6/2017 | 4280 | 3890 | 90.9% |
| | | DL (ng/g) = | 12 | |
| | | QL (ng/g) = | 41.1 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

| Wildlife Services NWRC National Wildlife Research Center Analytical Services Report | United States Department of Agriculture<br>Animal Plant Health Inspection Service<br>Wildlife Services<br>National Wildlife Research Center<br>Chemistry Lab Unit | Invoice #: 18-001/5<br>Date: February 13, 2018<br>Page: 1 of 6 |
|---|---|---|

To: Dr. Shane Siers
Hawaii Field Station Leader
NWRC

Subject: Diphacinone Residues in Birds from Lehua Island (QA-2802)

Methods: New, non-GLP

Analysis Dates: 2/8 and 2/12/2018

Notebook Reference: AC-161, pp. 112, 134-137

QC Notebook Reference: AC-162, p. 4

Analyst: Steve Volker

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**Sample Descriptions:**

Fourteen bird livers and 24 decayed bird carcasses from Lehua Island were received frozen on 10/31 and 12/28/2017 for diphacinone residue analysis. Control Barn Owl (*Tyto alba*) from Hawaii (S150226-14) was used for all QC samples. All samples were stored at -20°C until time of analysis.

**Sample Preparation:**

Dissection and homogenization:

Thirteen of the 24 bird carcasses were too decayed to obtain tissue. Tissue was collected from the remaining 11 birds. An attempt was made to identify liver tissue in each, but the tissue collected may consist of other organs, insects, and detritus. All samples were homogenized with a SPEX 6875D liquid nitrogen freezer mill. Homogenized samples were transferred immediately to vacuum sealable bags and stored at -20°C.

Extraction of bird liver and tissue:

Homogenized sample (70-80 mg) was weighed into a 1.5-mL microcentrifuge tube, 75 µL DI water added, and the sample vortex mixed at 2500 RPM for 20 minutes using an auto-vortexer to form a uniform slurry. Surrogate analyte (20 µL, 16 µg/mL $D_4$-diphacinone in acetonitrile) and 1.180 mL acetonitrile (ACN) were added and the sample vortex mixed again for 20 minutes. An excess of NaCl (~120 mg) was added and the sample vortex mixed 20 minutes to partition the water and ACN phases. The extract was clarified by centrifugation (12,000 RCF) and 0.900 mL of supernatant transferred to a dispersive solid-phase extraction (dSPE) tube containing $MgSO_4$ (150 mg), C18 sorbent (25 mg), and primary-secondary amine (PSA) sorbent (25 mg). The extract was exposed to the sorbents and $MgSO_4$ by vortex mixing for 4-5 s followed by centrifugation at 12,000 RCF for 2-3 s to clarify the supernatant. 0.400 mL of supernatant was then transferred to a 1.5-mL microcentrifuge tube and the solvent removed in a 60°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 100 µL ACN followed by 400 µL pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC-MS/MS analysis.

| Analyst | Date | QC Specialist | Date | Reviewer | Date |
|---|---|---|---|---|---|
| | 2/13/2018 | | 2/21/18 | | 2/14/18 |

**Instrument method:**

Agilent 1290 Infinity II HPLC with G6470A QQQ

| | |
|---|---|
| Column | Xbridge C18, 2.5-µm, 2.1 x 50 mm, Waters P/N 186003085 |
| Mobile phase A | 90%(pH 9.5 20-mM ammonium acetate)/10%(Acetonitrile) |
| Mobile phase B | Acetonitrile |
| Flow rate | 0.700 mL/min |
| Column temp. | 60°C |
| Injection volume | 10 µL |
| Run time | 2.40 min |
| Source | AJS ESI, negative mode |
| Gas temp. | 300°C |
| Gas flow | 5 L/min |
| Nebulizer | 45 psi |
| Sheath gas | 250°C, 7 L/min |
| Capillary | -4500 V |
| Nozzle | -500 V |

| Time (min) | %A | %B |
|---|---|---|
| 0.00 | 90% | 10% |
| 0.30 | 90% | 10% |
| 1.40 | 0% | 100% |
| 1.90 | 0% | 100% |
| 1.91 | 90% | 10% |

| Analyte | Precursor Ion (m/z) | Product Ion (m/z) | Fragmentor (V) | Collision Energy (V) |
|---|---|---|---|---|
| Diphacinone | 339.1 | **167.1** | 100 | 23 |
| | | 145.0 | | 18 |
| $D_4$-Diphacinone | 343.1 | 167.1 | 120 | 23 |

**BOLD** = product ion used for quantitation

**Detection and Quantitation Limits:**

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value. The Quantitation Limit (QL) is the lowest concentration of analyte that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL. This was performed by comparing the analyte response observed in fortified control matrix (n=6) with the baseline noise observed at the same retention time in control matrix (n=6). Liver from Barn Owl (*Tyto alba.*), NWRC ID S150226-14 was used for controls. The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10, respectively. The following table presents the average DL and QL concentrations determined over two days for diphacinone.

Diphacinone Detection Limit (DL) and Quantitation Limit (QL)

| Control Matrix | DL (ng/g) | QL (ng/g) |
|---|---|---|
| Liver (Barn Owl) | 9.6 | 32.0 |

**Results:**

Triplicate preparations of all samples were prepared of all available samples. Diphacinone residues are reported in units of ng/g, equivalent to parts per billion (ppb). If no analyte response was recorded by the data acquisition software or if the observed concentration was less than DL, an entry of "ND" is reported to indicate that the analyte was not detected. Results that are greater than the DL, but less than the QL are identified by an asterisk "*". Care should be taken when evaluating results below the QL as the variability will be significantly greater than the variability observed in quality control (QC) samples. Results above the QL are reported to three significant figures.

**Diphacinone – Birds**

| NWRC ID | Sample ID, Common Name, Collection Date, Tissue Type | Analysis Date | Observed Diphacinone Concentration (ng/g) | |
|---|---|---|---|---|
| S180108-01 | PGPL-01, Pacific golden plover, 9/2/17, Liver | 2/8/18 | ND | * |
| | | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| S180108-02 | RFBO-01, Red-footed booby, 8/28/17, Liver | 2/8/18 | 590 | |
| | | 2/8/18 | 539 | |
| | | 2/8/18 | 578 | |
| S180108-03 | RFBO-02, Red-footed booby, 9/2/17, Liver | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| S180108-04 | RFBO-04, Red-footed booby, 9/5/17, Liver | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| S180108-05 | RFBO-05, Red-footed booby, 9/5/17, Liver | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| S180108-06 | IC-06, Red-footed booby, 9/19/17, Liver | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| S180108-07 | RTTB-01, Red-tailed tropicbird, 8/30/17, Liver | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| | | 2/8/18 | ND | |
| S180108-08 | RUTU-01, Ruddy turnstone, 9/15/17, Liver | 2/8/18 | 1890 | |
| | | 2/8/18 | 1920 | |
| | | 2/8/18 | 1830 | |
| S180108-09 | RUTU-02, Ruddy turnstone, 9/15/17, Liver | 2/8/18 | 3770 | |
| | | 2/8/18 | 3650 | |
| | | 2/8/18 | 3600 | |
| S180108-10 | WTSH-01, Wedge-tailed shearwater, 8/22/17, Liver | - | N/A [a] | |
| | | DL (ng/g) = | 9.6 | |
| | | QL (ng/g) = | 32.0 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

[a] Canister broke during homogenization and sample lost.

*It was discovered that these samples had been transposed based on the size and consistency of liver homogenates, the necropsy results for the plover sowing signs of rodenticide ingestion. See QA-2802 Final Report text.

**Diphacinone – Birds**

| NWRC ID | Sample ID, Common Name, Collection Date, Tissue Type | Analysis Date | Observed Diphacinone Concentration (ng/g) |
|---|---|---|---|
| S180108-11 | WTSH-02, Wedge-tailed shearwater, 8/28/17, Liver | 2/8/18 | ND |
| | | 2/8/18 | ND |
| | | 2/8/18 | ND |
| S180108-12 | WTSH-06, Wedge-tailed shearwater, 9/8/17, Liver | 2/8/18 | ND |
| | | 2/8/18 | ND |
| | | 2/8/18 | ND |
| S180108-13 | WTSH-07, Wedge-tailed shearwater, 9/8/17, Liver | 2/8/18 | ND |
| | | 2/8/18 | ND |
| | | 2/8/18 | ND |
| S180108-14 | WTSH-08, Wedge-tailed shearwater, 9/8/17, Liver | 2/8/18 | ND |
| | | 2/8/18 | ND |
| | | 2/8/18 | ND |
| S171031-36 | WTSH-10, Wedge-tailed shearwater, 9/10/17, No Tissue Available | - | N/A [a] |
| S171031-37 | IC-02, Wedge-tailed shearwater, 9/17/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| S171031-38 | IC-08, Black Noddy, 9/20/17, No Tissue Available | - | N/A [a] |
| S171031-39 | RFBO-07, Red-footed booby, 9/6/17, No Tissue Available | - | N/A [a] |
| S171031-40 | WTSH-09, Wedge-tailed shearwater, 9/8/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| S171031-41 | WTSH-05, Wedge-tailed shearwater, 9/8/17, No Tissue Available | - | N/A [a] |
| S171031-42 | WTSH-14, Wedge-tailed shearwater, 9/14/17, No Tissue Available | - | N/A [a] |
| S171031-43 | WTSH-13, Wedge-tailed shearwater, 9/12/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| S171031-44 | IC-07, Wedge-tailed shearwater, 9/19/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | DL (ng/g) = | 9.6 |
| | | QL (ng/g) = | 32.0 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

[a] No tissue collected. Sample too decayed.

**Diphacinone – Birds**

| NWRC ID | Sample ID, Common Name, Collection Date, Tissue Type | Analysis Date | Observed Diphacinone Concentration (ng/g) |
|---|---|---|---|
| S171031-45 | WTSH-04, Wedge-tailed shearwater, 9/5/17, No Tissue Available | - | N/A [a] |
| S171031-46 | WTSH-11, Wedge-tailed shearwater, 9/10/17, No Tissue Available | - | N/A [a] |
| S171031-47 | RFBO-03, Red-footed booby, 9/5/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| S171031-48 | IC-05, Wedge-tailed shearwater, 9/19/17, No Tissue Available | - | N/A [a] |
| S171031-49 | WTSH-12, Wedge-tailed shearwater, 9/10/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| S171031-50 | IC-09, Wedge-tailed shearwater, 9/21/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| S171031-51 | IC-10, Red-footed booby, 9/21/17, No Tissue Available | - | N/A [a] |
| S171031-52 | RFBO-09, Red-footed booby, 9/8/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| S171031-53 | IC-01, Wedge-tailed shearwater, 9/17/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| S171031-54 | IC-04, Wedge-tailed shearwater, 9/19/17, No Tissue Available | - | N/A [a] |
| S171031-55 | IC-03, Wedge-tailed shearwater, 9/18/17, No Tissue Available | - | N/A [a] |
| S171031-56 | RFBO-10, Red-footed booby, 9/12/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | 11.2* |
| S171031-57 | RFBO-08, Red-footed booby, 9/6/17, Decayed Tissue | 2/12/18 | ND |
| | | 2/12/18 | ND |
| | | 2/12/18 | ND |
| S171031-58 | RFBO-06, Red-footed booby, 9/5/17, No Tissue Available | - | N/A [a] |
| S171031-59 | WTSH-03, Wedge-tailed shearwater, 9/4/17, No Tissue Available | - | N/A [a] |
| | | DL (ng/g) = | 9.6 |
| | | QL (ng/g) = | 32.0 |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

[a] No tissue collected. Sample too decayed.

* Observed diphacinone concentration less than Quantitation Limit (QL)

**QC Results:**

**QC Recoveries – Bird Liver (Barn Owl, S150226-14)**

| ID | Analysis Date | Theoretical Diphacinone Concentration (ng/g) | Observed Diphacinone Concentration (ng/g) | % Recovery |
|---|---|---|---|---|
| QC-177 | 2/8/2018 | 0 | ND | N/A |
| QC-178 | 2/8/2018 | 0 | ND | N/A |
| QC-179 | 2/8/2018 | 0 | ND | N/A |
| QC-189 | 2/12/2018 | 0 | ND | N/A |
| QC-190 | 2/12/2018 | 0 | ND | N/A |
| QC-191 | 2/12/2018 | 0 | ND | N/A |
| QC-180 | 2/8/2018 | 60.7 | 57.8 | 95.2% |
| QC-181 | 2/8/2018 | 62.7 | 60.2 | 96.0% |
| QC-182 | 2/8/2018 | 61.4 | 56.5 | 92.0% |
| QC-192 | 2/12/2018 | 61.7 | 60.7 | 98.4% |
| QC-193 | 2/12/2018 | 60.5 | 52.3 | 86.4% |
| QC-194 | 2/12/2018 | 61.5 | 63.5 | 103% |
| QC-183 | 2/8/2018 | 471 | 463 | 98.3% |
| QC-184 | 2/8/2018 | 472 | 441 | 93.4% |
| QC-185 | 2/8/2018 | 486 | 442 | 90.9% |
| QC-195 | 2/12/2018 | 465 | 441 | 94.8% |
| QC-196 | 2/12/2018 | 486 | 478 | 98.4% |
| QC-197 | 2/12/2018 | 475 | 433 | 91.2% |
| QC-186 | 2/8/2018 | 3530 | 3170 | 89.8% |
| QC-187 | 2/8/2018 | 3560 | 3310 | 93.0% |
| QC-188 | 2/8/2018 | 3510 | 3210 | 91.5% |
| QC-198 | 2/12/2018 | 3590 | 3120 | 86.9% |
| QC-199 | 2/12/2018 | 3560 | 3420 | 96.1% |
| QC-200 | 2/12/2018 | 3480 | 3290 | 94.5% |
| | | DL (ng/g) = | 9.6 | |
| | | QL (ng/g) = | 32.0 | |

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

# Tide Pool Mullet Mortality Report

Shane R. Siers[1†]

September 16, 2017

[1] USDA APHIS Wildlife Services National Wildlife Research Center, Hawaii Field Station, Hilo, HI

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## Introduction

Following posting of a video on social media showing dead fish and bait pellets in a tide pool along the eastern arm of the island crescent of Lehua, within the caldera (Figure 1), Chris Niebuhr (NWRC) investigated the area. Access to this area by land is difficult and dangerous and it was not regularly patrolled during the post-application monitoring.

Dr. Niebuhr collected the dead fish he could find, approximately 45 mullet-type fish, reporting them to be in poor condition varying from dried on land to waterlogged in the pool. Twelve fish were provided to the Hawaii Department of Agriculture at their request; the remaining fish were shipped to the USDA NWRC Hawaii Field Station in Hilo for forwarding to the NWRC Chemistry Lab Unit in Fort Collins, Colorado, for diphacinone residue analysis.

## Methods

At the request of Gregg Howald, Island Conservation, the NWRC sample was submitted to additional physical examination and sample collection, to assess for indications that the fish may have originated elsewhere and been staged on Lehua. The following sampling protocol was established:

- External examination for signs of trauma (e.g. fish hook injuries) or hemorrhaging (anticoagulant intoxication)
- Removal and disposal of skin to eliminate potential contamination of internal tissues by external diphacinone residues
- Examination of GI tract contents with UV illumination to determine the presence of the pyranine biomarker incorporated in the diphacinone bait matrix
- Removal of the GI tract and preservation for future stomach contents analysis, to determine whether contents were consistent with feeding near Lehua or elsewhere
- Removal of liver for diphacinone residue analysis
- Retention of remaining fish tissue mass for diphacinone residue analysis

Necropsies and tissue collections were conducted by myself and Robert T. Sugihara (NWRC) at the NWRC Hawaii Field Station in Hilo. The Hawaii Department of Agriculture lab was requested to follow the same procedures.

### Results

Upon thawing, the fish samples were found to be in severely degraded condition (Figure 2). Fish ranged in size from approximately 15 to 40 cm from snout to caudal peduncle (inferred for specimens lacking heads). No fish body cavities


Figure 1: Location of tide pool where fish were collected.

contained identifiable internal organs with the exception of the heart. The majority of the samples were degraded to a mushy, pasty consistency with heads completely disintegrated or separated from the body mass.

Skin removal proved impossible without severe disintegrating of the body mass. GI tracts and livers were completely degraded and could not be collected. Given the limitations posed by sample condition, fish were only examined for external and internal signs of UV fluorescence from the pyranine biomarker incorporated into bait pellets. Fish tissues were re-frozen for chemical residue analysis. The majority of fish samples indicated a sheen of pyranine fluorescence over the entire exterior surface (Figure 3).

On examination of the internal organ cavity and muscle tissues, all but eight samples exhibited diffuse fluorescence throughout the interior surfaces, ranging from dull to very vibrant in intensity (Figure 4). The general pattern of fluorescence was fine yellow speckles in the anterior and thoracic region and a diffuse orange glow throughout muscle tissue. Specimens with less interior fluorescence exhibited yellow fluorescence only in the anterior thorax region. As specimens increased in degradation, the perfusion and intensity of fluorescence increased; the softest, most water-logged specimens generally fluoresced the most.

Discussion Precise species identification of these fish remains lacking. I am not authoritative enough to make any statements as to whether the location of the fish carcass collection is consistent with the habitat requirements of the particular species. Hopes for determining feeding ecology from examination of GI tract contents are negated by the complete degradation of internal organs in these specimens. Pyranine is incorporated into the bait matrix as a constituent ingredient along with diphacinone and the other inert ingredients. To my knowledge, there is no inherent association of diphacinone and pyranine molecules, and no expectation that pyranine behavior upon dissolution of the pellet is correlated to the fate of diphacinone molecules. In other words, intensity of pyranine fluorescence cannot be expected to correlate with intensity of diphacinone molecules. Diphacinone has low solubility in water (0.30 g/L, expected to be lower in saltwater), while pyranine and its fluorescent properties appear highly soluble (see Figure 5).

No specimen exhibited the pattern of localized fluorescence within the GI tract expected to result from ingestion of diphacinone bait pellets containing pyranine. This could be an artefact of the highly degraded nature of the samples. However, what is clear is that the majority of the samples underwent prolonged immersion in pyranine solution resulting from dissolved pellets in the tide pool. It is my opinion that the high level of UV fluorescence in these degraded fish tissues resulted from this immersion.

Yellow pyranine fluorescence tended to start toward the anterior (head) portion of the thorax in minimally fluorescent samples, with greater perfusion of yellow throughout the thorax in specimens with greater levels of fluorescence. This could be consistent with permeation of pyranine

Figure 2: Range of sample conditions.

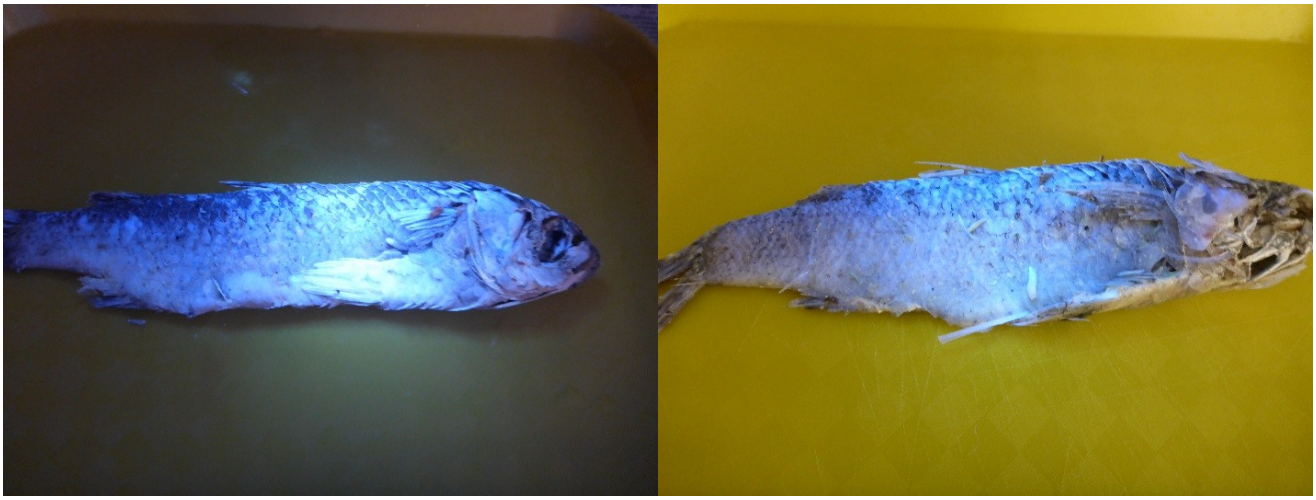

Figure 3: External pyranine fluorescence.

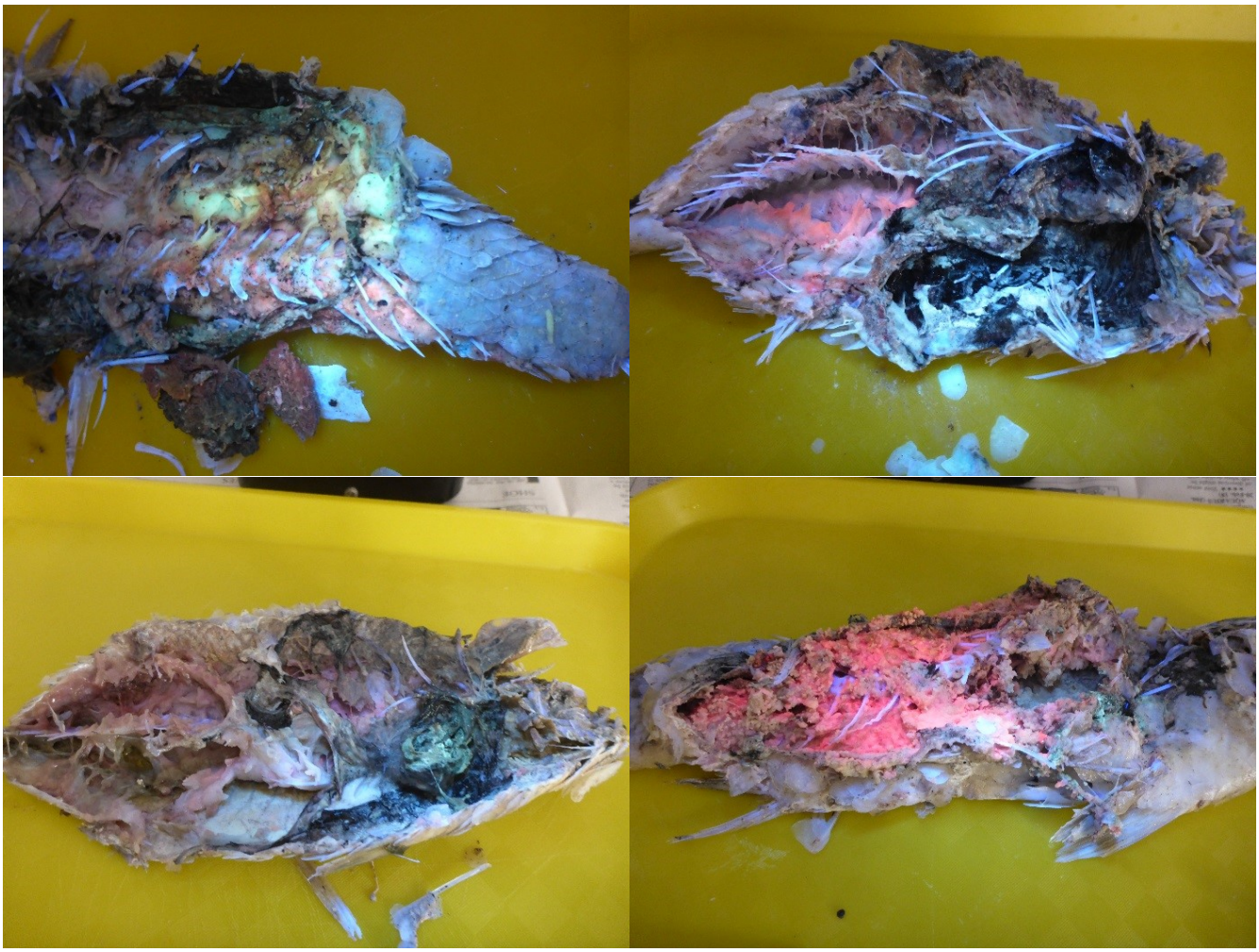

Figure 4: Fluorescence of internal tissues. The general pattern was yellow speckles toward the anterior thoracic cavity and diffuse orange throughout the muscle tissue, particularly within the tail.

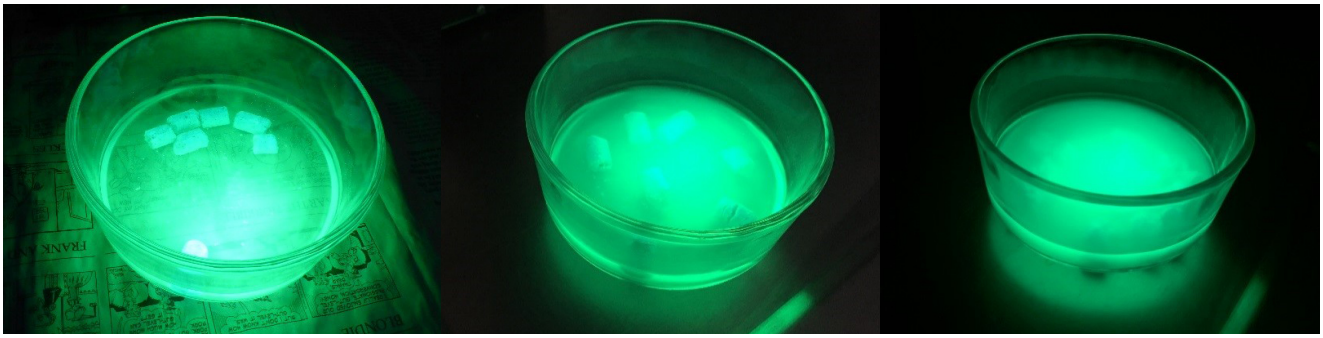

Figure 5: Pyranine fluorescence in solution. Six diphacinone pellets in tap water under UV lighting at 10 minutes (left), 24 hours (center), and 24 hours with water-softened pellets mashed to release interior pyranine solute.

solution into the body cavity through the gill region and more delicate/early-degrading soft tissues around the head (most samples had no heads and exposed anterior thoracic cavities).

The orange fluorescence in muscle tissue was unexpected and remains unexplained. It is unclear what physical process would lead to the yellow-fluorescing pyranine permeating muscle tissue and fluorescing a different color. It should be considered that the orange fluorescence may be unrelated to pyranine exposure and potentially be a result of bacterial fluorescence.

The deduction that pyranine fluorescence resulted from immersion in water retention areas containing bait pellets sheds little light on the probability that fish mortality was due to diphacinone exposure. Chemical analysis of these degraded tissues is likely to do little to answer this question; the ability to detect residues degrades rapidly along with tissue condition, and the context of the tissues soaking in a bait pellet solution, as suggested by perfuse pyranine fluorescence, would lead us to expect positive detections of diphacinone residues without being able to determine whether diphacinone was ingested or absorbed by a living fish leading to anticoagulant intoxication. Such chemical residue results will prove inconclusive as to the cause of death.

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Accession #: 170824
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

## FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | PGPL-01 | Pacific golden plover | 1 | 386672 2434901 | 09/02/17 | | Fresh | adult |

On bag: QA 2802 9-2-17 EP DKF PGPL-01 Bird Bycatch

In bag: Bird Species: Pacific golden plover Collection date: 9-2-2017 Sample ID#: PGPL-01 Collection site: 0386672 2434901 Quantity: 1 Collector: Erin P.
Notes: Found dead along shore Freshly dead Green bird poop seen in the area

## LABORATORY FINDINGS/DIAGNOSIS

1. Hemorrhage, multifocal, acute, moderate, coelomic cavity, ventriculus, and subcutaneous tissues of the legs and vent.
2. Reduced nutritional condition, mild, total body as a whole.
3. Autolysis, moderate.

## ACCESSION SUMMARY

Hemorrhages were observed within the subcutaneous tissues, coelomic cavity and ventriculus, which could have contributed to the death of this bird. Fluorescence was observed within the digestive tract and an anticoagulant rodenticide could have contributed to the hemorrhage.

## GROSS FINDINGS

GENERAL EXAMINATION: The Pacific Golden Plover was received frozen on 12/20/17. A necropsy is performed on 12/21/17. The carcass is thawed and is in moderately reduced

post-mortem condition. Fluorescence is not observed on examination of the carcass. The eyes are moderately recessed in their orbits and the keel is moderately increased in prominence. The subcutaneous tissues of the legs are dark red. The subcutaneous tissues of the ventral coelomic cavity and vent contain moderate amounts of dark red gelatinous material. The coelomic adipose tissue contains moderate amounts of dark red-gray gelatinous material. Small amounts of ingesta are present in the lumen of the ventriculus. The ventricular muscle is multifocally dark red. The ventriculus and proventriculus fluoresce under UV light.
SPECIAL EXAMINATIONS:

Liver is collected for toxicology.

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**HISTOPATHOLOGY**

There is a moderate reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

Cross section of the head including brain: A cross section of an apparent ectoparasite is present in the choanal slit.

Vent: Moderate numbers of erythrocyte nuclei along with moderate amounts of deeply eosinophilic, homogenous material are present in the deeper tissues.

Coelomic adipose tissue: Moderate numbers of erythrocyte nuclei along with moderate amounts of deeply eosinophilic, homogenous material are present in the coelomic adipose tissue. Adipocytes are multifocally, mildly reduced in size.

Proventriculus: There are no significant findings.

Ventriculus: Groups of myofibers in the muscularis are multifocally separated by moderate numbers of erythrocytes.

The following tissues are examined and there are no significant findings: lung, heart, liver, kidney, ovary and oviduct, proventriculus and intestine.

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Electronically signed by: Travis Heskett, DVM, DACVP

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Accession #: 170825
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 03/29/18
Submission summary: 1 red footed booby carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided on the chain of custody form, and included on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2 | RFBO-01 | Red footed booby | 1 | 386558 2434934 | 08/28/17 | | Fresh | immature |

In bag: Species: Red footed booby Collection date: August 28, 2017 Sample ID#: RFBO-01 Collection site: 0386558 2434934 Quantity: 1 Collector: Mele Khalsa
Notes: Found dead, recent mort Processed by DKF

## LABORATORY FINDINGS/DIAGNOSIS

1. Glial nodule, focal, brain.
2. Autolysis, moderate.

## ACCESSION SUMMARY

There were no gross or microscopic lesions to explain the death of this bird.

## GROSS FINDINGS

GENERAL EXAMINATION: The Red Footed Booby was received frozen on 12/20/17. A necropsy is performed on 12/21/17. The carcass is thawed and is in moderately reduced post-mortem condition. Fluorescence is not observed on examination of the carcass. A small amount of tan, gelatinous to rubbery material suggestive of squid is present in the esophagus, and moderate numbers of squid beaks are present in the ventriculus. Fluorescence is not observed on examination of the gastrointestinal tract.
SPECIAL EXAMINATIONS:
Liver is collected for toxicology.

## HISTOPATHOLOGY

There is a moderate reduction in cellular detail and distortion of tissues architecture secondary to freezing and thawing.

Brain: Focally, a small glial nodule is present within the parenchyma.

Spleen: Rarely, small amounts of fibrin are present in the red pulp.

The following tissues are examined and there are no significant findings: head, trachea, lung, thymus, heart, lung, liver, kidney, adrenal gland, skeletal muscle, skin, esophagus, proventriculus, ventriculus, and intestine.

---

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Accession #: 170826
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 03/29/18
Submission summary: 1 carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3 | RFBO-02 | Red footed booby | 1 | 386818 2435004 | 09/02/17 | | Fresh | chick |

On bag: QA 2802 RFBO-02 9-2-17 DKF

In bag: Species: RFBO Collection date: 9-2-2017 Sample ID#: RFBO-02
Collection site: 040 0386818 2435004 Quantity: 1 Collector: Mele Khalsa
Notes: Recently deceased, found dead above camp to the east (in nest in a bush).

## LABORATORY FINDINGS/DIAGNOSIS

1. Proventriculitis, heterophilic, multifocal, acute, minimal, proventriculus.
2. Autolysis, moderate.

## ACCESSION SUMMARY

Gross or microscopic lesions to explain the death of this bird were not identified.

## GROSS FINDINGS

GENERAL EXAMINATION: The Red Footed Booby was received frozen on 12/20/17. A necropsy is performed on 12/21/17. The carcass is thawed and is in moderately reduced post-mortem condition with moderate autolysis. Dead ants are present on the carcass. Fluorescence is not observed on examination of the carcass with a UV light. The keel is moderately increased in prominence. Small amounts of adipose tissue are present in the subcutaneous tissues and in the coelomic cavity. Moderate numbers of squid beaks are

present in the lumen of the ventriculus. Fluorescence is not observed on examination of the gastrointestinal contents with a UV light.
SPECIAL EXAMINATIONS:

Liver is collected for toxicology.

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**HISTOPATHOLOGY**

There is a moderate reduction in cellular detail and distortion of tissues architecture secondary to freezing and thawing.

Proventriculus: In one section, small numbers of heterophils are multifocally present within glands. Adipocytes within the coelomic adipose tissue are of adequate size.

The following tissues are examined and there are no significant findings: brain, head, trachea, thymus, lung, air sac, heart, liver, kidney, adrenal gland, oviduct, skin, bursa of Fabrecius, uropygial gland, esophagus, ventriculus, and intestine.

---

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Accession #: 170827
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 03/29/18
Submission summary: 1 carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4 | RFBO-04 | Red footed booby | 1 | 387395 2435928 | 09/05/17 | CNN | Fresh | immature |

On bag: QA 2802 9-2-17 EP DKF PGPL-01 Bird Bycatch

In bag: Species: Red footed booby Collection date: 9-5-2017 Sample ID#: RFBO-04
In 2nd bag: Species: Bird-booby Collection date: 5 Sep 2017 Collection site: Lehua, HI
0387395 2425928 Quantity: 1 Collector: CNN Notes: North end Lehua, HI enters tidal flats

## LABORATORY FINDINGS/DIAGNOSIS

1. No diagnostic lesions; marked autolysis.

## ACCESSION SUMMARY

Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. There was advanced post-mortem decomposition of this carcass.

## GROSS FINDINGS

GENERAL EXAMINATION: The Red Footed Booby was received frozen on 12/20/17. A necropsy is performed on 12/21/17. The carcass is thawed and is in markedly reduced post-mortem condition. There is a loss of skin over the head, cervical vertebrae, wings and coelomic cavity. There is a loss of integrity of the body wall and fly larvae are present superficially and within the coelomic cavity. There is marked distortion of the coelomic

viscera. Fluorescence is not observed on examination of the exterior or interior of the carcass under UV light.
SPECIAL EXAMINATIONS:
Liver is collected for toxicology.

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**HISTOPATHOLOGY**

There is a marked reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

The following tissues are identifiable and there are no significant findings: heart, muscle, adipose tissue, and skin.

---

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Accession #: 170828
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

## FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5 | RFBO-05 | Red footed booby | 1 | 387395 2435928 | 09/05/17 | CNN | Fresh | immature |

In bag: Species: Red footed boobies Collection date: 9-9-17 Sample ID#: RFBO-05 Collection site: tidepool Notes: Found dead in tidepool with RFBO-04

In bag: Birds Species: Boobies? Collection date: 9-5-2017 Collection site: 0387395 2435928 Quantity: 2 boobies, 1 wing Collector: CNN Notes: Immature red-foot boobies & wing collected in eastern tidal flat W/TH crater Lehua

## LABORATORY FINDINGS/DIAGNOSIS

1. No diagnostic gross or microscopic lesions; moderate autolysis.

## ACCESSION SUMMARY

Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. In the absence of inflammation, the fungal hyphae within one of the sections of lung, and circular aggregates of bacteria within multiple sections of liver, are interpreted as post-mortem microbial proliferations, which did not contribute to the death of this bird.

## GROSS FINDINGS

GENERAL EXAMINATION: The Red Footed Booby was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in moderately reduced post-mortem condition. The exterior of the beak and feathers are separating from the carcass and the eyes are missing. There appear to be reduced amounts of subcutaneous and coelomic

adipose tissue. The lumen of the gastrointestinal tract is empty. A small focus of fluorescence is observed on the dorsum associated with feather loss. Fluorescence is not observed on examination of the interior of the carcass under UV light.
SPECIAL EXAMINATIONS:

Liver is collected for toxicology.

---

**HISTOPATHOLOGY**

There is a moderate reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

Lung: In one section, large numbers of fungal hyphae are present in the parenchyma without any associated inflammatory response (presumed post-mortem proliferation).

Liver: Multifocally, there are circular aggregates of bacteria in the liver without any associated inflammation.

Kidney: Small amounts of mineralized material are multifocally present within tubular lumina.

Cloaca: Small numbers of apparent nematode ova are present within the lumen of the cloaca.

The following tissues are examined and there are no significant findings: brain, trachea, heart, spleen, ovary, skeletal muscle, skin, adipose tissue (adipocytes appear to be of adequate size), proventriculus, and intestine.

---

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Accession #: 170829
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | IC-06 | Red footed booby | 1 | 386633 2435338 | 09/19/17 | | Fresh |

In bag: Species: RFBO Collection date: 9/19/17 Sample ID#: IC6 Collection site: 386633 2435338 Quantity: 1 Collector: MK & CC. Notes: Super fresh.

## LABORATORY FINDINGS/DIAGNOSIS

1. Myocarditis, interstitial, multifocal, subacute, mild, heart.
2. Mineralization, interstitial, multifocal, subacute, mild, kidney.
3. Urate deposits (presumptive), multifocal, subacute, mild, liver.
4. Autolysis, moderate.

## ACCESSION SUMMARY

The cause of the death of this bird could not be determined on gross or microscopic examination. The clinical significance of the diagnosed lesions is uncertain. A cause for the mild inflammation within the heart was not identified. Although small numbers of urate deposits were presumptively identified within the liver, they were not identified elsewhere, and gout appears unlikely to have contributed to the death of this bird.

## GROSS FINDINGS

GENERAL EXAMINATION: The Red Footed Booby was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in moderately reduced post-mortem condition. Moderate numbers of fly larvae are present on the surface of the carcass and in the oral cavity. Small amounts of fluorescence are observed within the feathers which

does not completely resolve with washing. Small amounts of adipose tissue are present in the coelomic cavity. Moderate numbers of squid beaks and small amounts of yellow mucoid material are present within the lumen of the proventriculus. The surfaces of the kidneys are pale. Fluorescence is not observed on examination of the interior of the carcass under UV light.

SPECIAL EXAMINATIONS:

Liver is collected for toxicology.

---

**HISTOPATHOLOGY**

There is a moderate reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

Lung: In one section of lung, the parenchyma is effaced by erythrocytes and homogenous, eosinophilic material.

Heart: Mildly increased numbers of lymphocytes and macrophages are infrequently present in the interstitium.

Liver: Small numbers of aggregates of radiating acicular clefts are multifocally present in the liver (presumptive urate deposits). There is diffuse karyolysis within the liver (autolysis).

Kidney: Infrequently, there are small foci of mineralization within the interstitium.

The following tissues are examined and there are no significant findings: brain, trachea, spleen, adrenal gland, skeletal muscle, skin, ventriculus, and intestine (mesenteric adipocytes appear to be of adequate size).

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Accession #: 170830
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7 | RTTB-01 | Red tailed tropicbird | 1 | 386429 2434738 | 08/30/17 | MK | Fresh | adult |

On bag: 04Q 0386429 2434938 RTTB Chick fledgling.

In bag: Species: Red Tailed Tropic Bird Collection date: August 30 2017 Sample ID#: RTTB-01 Collection site: UTM 04Q 0386429 2434738. Quantity: 1 chick Collector: Mele Khalsa Notes: Found dead along shoreline.

## LABORATORY FINDINGS/DIAGNOSIS

1. No diagnostic lesions; moderate autolysis.

## ACCESSION SUMMARY

There were no gross or microscopic lesions to explain the death of this bird.

## GROSS FINDINGS

GENERAL EXAMINATION: The Red Tailed Tropic Bird was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in moderately reduced post-mortem condition. Small amounts of fluorescence are observed within the feathers and on the feet, which does not completely resolve with washing. The lumen of the gastrointestinal tract is empty. The bird is female. The spleen is small and purple. The gallbladder is distended with green fluid. Pale streaks are present on the surface of the kidneys. Fluorescence is not observed on examination of the interior of the carcass under UV light.

SPECIAL EXAMINATIONS:

Liver is collected for toxicology.

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**HISTOPATHOLOGY**

There is a moderate reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

The following tissues are examined and there are no significant findings: brain, trachea, lung, heart, liver, gallbladder, spleen, kidney, skeletal muscle, skin, esophagus, proventriculus, ventriculus, and intestine.

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Electronically signed by: Travis Heskett, DVM, DACVP

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Accession #: 170831
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 03/29/18
Submission summary: 1 carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8 | RUTU-01 | Ruddy turnstone | 1 | 386420 2434953 | 09/15/17 | CNN | Fresh | adult |

In bag: Species: RUTU-01 Collection date: 15 Sep 2017 Collection site: 0386420 2434953 Quantity:1 Collector: CNN Notes: Ruddy turnstone, on shore, fresh + pyranine UV

## LABORATORY FINDINGS/DIAGNOSIS

1. Hemorrhage, multifocal, acute, moderate to marked, skin, subcutaneous tissues and skeletal muscles of legs, vent, and dorsum.
2. Fibroplasia, focal, chronic, mild, skeletal muscle.
3. Autolysis, moderate.

## ACCESSION SUMMARY

Significant hemorrhages were observed within the skin, subcutaneous tissues, and muscles, which could have contributed to the death of this bird. Fluorescence was observed within the ventriculus, and an anticoagulant rodenticide could have contributed to the hemorrhage.

## GROSS FINDINGS

GENERAL EXAMINATION: The Ruddy Turnstone was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in moderately reduced post-mortem condition. Focal fluorescence is observed on external examination of the carcass in the caudal, ventral coelom. This fluorescence is still observed after washing. Marked

hemorrhages are multifocally present within the skeletal muscles and subcutaneous tissues of the legs, and extend over the dorsum. The lumen of the esophagus and proventriculus is empty. The ventriculus contains moderate numbers of squid beaks. The ventriculus fluoresces under UV light. Additional fluorescence is observed within the intestines.
SPECIAL EXAMINATIONS:

Liver is collected for toxicology.

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**HISTOPATHOLOGY**

There is a moderate reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

Skin, subcutaneous tissues, and skeletal muscle: Multifocal, acute, moderate to marked hemorrhages are present in the skin, subcutaneous tissues and muscle. In one section of muscle, there are increased numbers of fibroblasts separating myofibers.

The following tissues are examined and there are no significant findings: cross section of the head including brain, trachea, lung, heart, liver, kidney, proventriculus, ventriculus, and intestine.

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Electronically signed by: Travis Heskett, DVM, DACVP

**DAVID Y. IGE**
Governor

**SHAN S. TSUTSUI**
Lt. Governor

**SCOTT E. ENRIGHT**
Chairperson, Board of Agriculture

**PHYLLIS SHIMABUKURO-GEISER**
Deputy to the Chairperson

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Accession #: 170832
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

## FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 9 | RUTU-02 | Ruddy turnstone | 1 | 386828 2434907 | 09/15/17 | CNN | Fresh | adult |

In bag: Species: RUTU-02 Collection date: 15 Sep 2017 Collection site: 0386828 2434907 Quantity: 1 Collector: CNN Notes: Ruddy Turnstone, fresh, along shoreline, ext UN neg.

## LABORATORY FINDINGS/DIAGNOSIS

1. Hemorrhages, multifocal, acute, mild to moderate, subcutaneous tissues and muscles of the caudal dorsum, vent, left wing, left leg, and coelomic cavity.
2. Autolysis, moderate.

## ACCESSION SUMMARY

Mild to moderate hemorrhages were identified in this bird. The severity of the hemorrhage present within the carcass would not have been anticipated to be fatal, but it is possible that additional blood loss could have occurred externally prior to the evaluation of the carcass. Fluorescence was observed within the digestive tract and an anticoagulant rodenticide could have contributed to the hemorrhage.

## GROSS FINDINGS

GENERAL EXAMINATION: The Ruddy Turnstone was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in moderately reduced post-mortem condition. Fluorescence is not observed externally on evaluation of the carcass under UV light. Moderate hemorrhages are multifocally present in the subcutaneous tissues of the caudal dorsum, and multifocal, mild hemorrhages are present in the muscles of the left wing

and left leg. Small amounts of red fluid are present in the coelomic cavity. The lungs are pale and the spleen is small. The lumen of the esophagus and proventriculus are empty. The lumen of the ventriculus contains small numbers of pebbles. The serosa of the intestines is multifocally reddened and the lumen of the intestine contain small amounts of red and tan mucoid material. The ventriculus and intestines fluoresce under UV light.
SPECIAL EXAMINATIONS:

Liver is collected for toxicology.

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**HISTOPATHOLOGY**

There is a moderate reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

Adrenal gland: Multifocally, there are moderate hemorrhages in the adipose tissue adjacent to the adrenal gland.

Vent: Multifocally, there are moderate hemorrhages in the subcutaneous tissues.

Skeletal muscle: Multifocally, there are mild hemorrhages between groups of myofibers and in the adjacent adipose tissue.

The following tissues are examined and there are no significant findings: cross section of the head including the brain, heart, trachea, lung, liver, kidney, proventriculus, ventriculus, and intestine.

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Electronically signed by: Travis Heskett, DVM, DACVP

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Accession #: 170833
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 10 | WTSH-01 | Wedge-tailed shearwater | 1 | 386416 2434968 | 08/22/17 | | Fresh |

On bag: MESH001 8.21.17 Juv Wedgetail Shearwater. Pre-drop. Found dead. QA 2802. Collector Mele K. I.C.

In bag: Species: Wedgetail shearwater Collection date: 8-21-17 Predrop Sample ID# WESH001 Collection site: Iphone 22,01565 -160,10047 Quantity: 1 Collector: Mele Khalsa GMK/I.C. Notes: Found dead, juvenile, fresh no rigor, very hot day.

## LABORATORY FINDINGS/DIAGNOSIS

1. No diagnostic lesions; marked autolysis.

## ACCESSION SUMMARY

Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. There was advanced post-mortem decomposition of this carcass.

## GROSS FINDINGS

GENERAL EXAMINATION: The Wedgetail Shearwater was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in markedly reduced post-mortem condition. Multiple, irregular punctures and defects are present in the skin of the body wall and head, the coelomic cavity may have been entered, and the rib cage is collapsed. The internal viscera are brown, fractured, and friable. The lumen of the ventriculus

contains a small number of seeds. Fluorescence is not observed externally or internally on examination under UV light.
SPECIAL EXAMINATIONS:
Liver is collected for toxicology.

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**HISTOPATHOLOGY**

There is a marked reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

The following tissues are examined and there are no significant findings: cross section of the head including brain, heart, skeletal muscle, subcutaneous tissues, skin, proventriculus, ventriculus, and intestine.

---

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Accession #: 170834
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 11 | WTSH-02 | Wedge-tailed shearwater | 1 | 386570 2434945 | 08/28/17 | | Fresh | chick |

In bag: Species: Wedgetail shearwater Collection date: August 28 2017 Sample ID#: WESH-02 Collection site: 0386570 2434945 Quantity: 1 Collector: Mele Khalsa Notes: Chick found dead. Bleeding when moved with a stick. Processed by DKF.

## LABORATORY FINDINGS/DIAGNOSIS

1. No diagnostic lesions; marked autolysis.

## ACCESSION SUMMARY

Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. There was advanced post-mortem decomposition of this carcass.

## GROSS FINDINGS

GENERAL EXAMINATION: The Wedgetail Shearwater was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in markedly reduced post-mortem condition. The carcass is laterally compressed and is markedly autolyzed. The skin is friable and easily separates. The coelomic cavity is opened and the integrity of the rib cage is disrupted. The lumen of the ventriculus contains a small number of pebbles. The liver is light red and granular. Fluorescence is not observed externally or internally on examination under UV light.
SPECIAL EXAMINATIONS:
Liver is collected for toxicology.

**HISTOPATHOLOGY**

There is a marked reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

The following tissues are examined and there are no significant findings: cross section of the head including the brain, heart, liver, skeletal muscle, skin, and ventriculus.

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Accession #: 170835
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 | WTSH-06 | Wedge-tailed shearwater | 1 | 386973 2435068 | 09/08/17 | CNN | Fresh |

In bag: Collection date 9-8-17 Sample ID# WTSH-06

In bag: Species: Bird WTSH chick Collection date: 8 Sept 2017 Collection site: 0386973 2435068 Quantity:1 Collector: CNN Notes: fresh, in sun, insects.

## LABORATORY FINDINGS/DIAGNOSIS

1. No diagnostic lesions; marked autolysis.

## ACCESSION SUMMARY

Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. There was advanced post-mortem decomposition of this carcass.

## GROSS FINDINGS

GENERAL EXAMINATION: The Wedgetail Shearwater was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in markedly reduced post-mortem condition. The carcass is markedly autolyzed. The coelomic cavity is opened. Fly larvae are present inside the left leg and in the coelomic cavity. The lumen of the ventriculus contains a small number of squid beaks and pebbles. Fluorescence is not observed externally or internally on examination under UV light.
SPECIAL EXAMINATIONS:
Liver is collected for toxicology.

**HISTOPATHOLOGY**

There is a marked reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

Kidney: Small amounts of mineralized material are multifocally present within tubular lumina.

The following tissues are examined and there are no significant findings: cross section of the head including the brain, heart, trachea, lung, skeletal muscle and skin, esophagus, proventriculus, and intestine.

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Accession #: 170836
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

# FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 13 | WTSH-07 | Wedge-tailed shearwater | 1 | 387120 2435016 | 09/08/17 | CNN | Fresh |

In bag: Collection date: 9-8-17 Sample ID# WTSH-07

In bag: Species: Bird-WTSH chick Collection date: 8 Sep 2017 Collection site: 0387120 2435016 Quantity: 1 Collector: CNN Notes: fresh, in sun.

## LABORATORY FINDINGS/DIAGNOSIS

1. No diagnostic lesions, marked autolysis.

## ACCESSION SUMMARY

Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. There was advanced post-mortem decomposition of this carcass.

## GROSS FINDINGS

GENERAL EXAMINATION: The Wedgetail Shearwater was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in markedly reduced post-mortem condition. A single feather fluoresces under UV light. There is marked autolysis and the coelomic viscera is multifocally green. There is a mild increase in the prominence of the keel. The lumen of the digestive tract is empty. Fluorescence is not observed internally under UV light.

SPECIAL EXAMINATIONS:

Liver is collected for toxicology.

**HISTOPATHOLOGY**

There is a marked reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

The following tissues are examined and there are no significant findings: cross section of the head, brain, heart, trachea, lung, liver, kidney, skeletal muscle, skin, esophagus, proventriculus, and intestine. Coelomic adipocytes appear to be adequately sized.

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Accession #: 170837
Submitter: USDA/APHIS
Date received: 12/20/17
Date of report: 3/29/18
Submission summary: 1 carcass

## FINAL REPORT

## SUBMITTED HISTORY

Birds found dead and collected during a diphacinone rodenticide application on Lehua Island, HI.

The following information is provided with the chain of custody form and on and/or in the bag:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 14 | WTSH-08 | Wedge-tailed shearwater | 1 | 387296 2435180 | 09/08/17 | CNN | Fresh | |

In bag: Collection date: 9-8-17 Sample ID#: WTSH-08

In bag: Species: Bird WTSH chick. Collection date: 8 Sep 2017 Collection site 0387296 2435180 Quantity: 1 Collector: CNN Notes: fresh, in sun, insects.

## LABORATORY FINDINGS/DIAGNOSIS

1. No diagnostic lesions, marked autolysis.

## ACCESSION SUMMARY

Gross or microscopic findings suggestive of the cause of the death of this bird were not identified. There was advanced post-mortem decomposition of this carcass.

## GROSS FINDINGS

GENERAL EXAMINATION: The Wedgetail Shearwater was received frozen on 12/20/17. A necropsy is performed on 12/22/17. The carcass is thawed and is in markedly reduced post-mortem condition. A few feathers fluoresce under UV light. The carcass is dorsoventrally flattened. The coelomic cavity is opened and viscera are granular and friable. Fly larvae are present within the coelomic cavity. Fluorescence is not observed internally under UV light.
SPECIAL EXAMINATIONS:
Liver is collected for toxicology.

**HISTOPATHOLOGY**

There is a marked reduction in cellular detail, and distortion of architectural structure, secondary to freezing, thawing, and autolysis.

The following tissues are examined and there are no significant findings: cross section of the head including brain, heart, kidney, muscle, skin, proventriculus, ventriculus, and intestine.

Electronically signed by: Travis Heskett, DVM, DACVP