A next-generation sequencing study of arthropods in the diet of Laysan Teal *Anas laysanensis*

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Abstract

The critically endangered Laysan Teal *Anas laysanensis* (known as koloa pōhaka in the Hawaiian language), in the Northwestern Hawaiian Islands has wild populations on Kamole (Laysan Island), Kuaihelani (Midway Atoll NWR) and Hōlanikū (Kure Atoll). The Laysan Teal face a new risk on Sand Island in Kuaihelani: non-target poisoning via a pending House Mouse *Mus musculus* eradication programme. After mice were observed attacking and depredating Laysan Albatross *Phoebastria immutabilis* (mōlī) in 2015, plans to eradicate the mice were developed to protect this seabird species. This approach, however, risks poisoning the Laysan Teal. To reduce exposure, teal will be translocated during mouse eradication. Even so, there remains a potential risk of secondary poisoning for teal by ingesting arthropods that feed on mouse bait. We therefore used next-generation sequencing (NGS) to identify which arthropods teal consume. From August 2019 to February 2020, we collected 71 fresh teal faecal samples on Sand Island, and successfully extracted DNA from 21 samples. Via NGS, we found that teal most frequently consumed cockroaches (order: Blattodea), freshwater ostracods (Cyprididae), midges (Chironomidae) and isopods (Porcellionidae). To a lesser degree, teal also ate spiders (Araneae), moths (Lepidoptera), beetles (Coleoptera), springtails (Entomobryomorpha), thrips

(Thysanoptera) and crabs (Decapoda). Notably, the teal on Sand Island showed differences in diet from those on Kamole, which mainly eat flies (Diptera) and brine shrimp (Anostraca, *Artemia* sp.). Our study serves as a model for risk mitigation during invasive rodent eradication.

Key words: arthropods, diet, Laysan Teal, Midway Atoll, next-generation sequencing (NGS).

The Laysan Teal *Anas laysanensis* (known as koloa pōhaka in the Hawaiian language, and also as the Laysan Duck) is a Hawaiian endemic and among the world's most endangered waterfowl (Reynolds *et al.* 2008). Currently, this species is only found in the Northwestern Hawaiian Islands, with wild populations on Kamole (Laysan Island), Kuaihelani (Midway Atoll NWR), and Hōlanikū (Kure Atoll). Overall population numbers for Laysan Teal have increased since its listing as an endangered species in 1967 (Federal Register in the Library of Congress 32 FR 4001), but several threats remain – such as epizootic outbreaks (Work *et al.* 2010) – which can cause rapid population declines. Laysan Teal now face a new risk that requires mitigation: non-target poisoning from a pending House Mouse *Mus musculus* eradication on Sand Island in Kuaihelani (Hamer Environmental 2019).

In 2015, introduced House Mice were observed attacking and depredating adult, nesting Laysan Albatross *Phoebastria immutabilis* (mōlī) on Sand Island. During the following winter, the number of injured and mortally wounded Laysan Albatross from these mouse attacks increased substantially (Duhr *et al.* 2019), prompting efforts to eradicate mice (Hamer Environmental 2019). Given that Kuaihelani is home to 73% of the global Laysan Albatross population (and 36% of the world's Black-footed Albatross *Phoebastria nigripes* [ka'upu] population), mouse removal is critical to secure seabird breeding grounds (Duhr *et al.* 2019).

To eradicate mice on Sand Island, a pelleted rodenticide bait (Brodifacoum-25D Conservation) will be spread across the island by helicopter in the summer of 2023, presumably when mouse abundance is in decline and food resources are less available (Hamer Environmental 2019). However, Laysan Teal and other federally listed and at-risk non-target taxa may be exposed to brodifacoum (see Eason *et al.* 2002). To reduce the risk of primary poisoning via bait consumption, the teal will be captured and temporarily translocated to nearby Eastern Island in Kuaihelani (where mice are absent). There, caretakers will clip the teal's wings and maintain them in enclosures (with supplemental food and water) to reduce the possibility of the birds returning to Sand Island until the risk of poisoning there is low (Breeden & Goodale 2021; Hamer Environmental 2019). When pellets are no longer observed on the ground on Sand Island, the risk of primary poisoning is assumed to be low; however, teal may still be at risk of secondary poisoning, notably by ingesting arthropods that feed on brodifacoum bait (Antaky 2022).

Invertebrates can feed on anticoagulant rodenticides with little or no negative effects to them, while accruing toxin in their tissues (Brooke *et al.* 2013; Shiels *et al.* 2019). The length of time that brodifacoum may remain in invertebrates is unclear, which poses a serious risk to insectivorous species. Residue levels can take at least four weeks to return to background levels in invertebrates, and trace levels are detectable up to 10 weeks following bait deployment (Hoare & Hare 2006). Because arthropods compose much of the Laysan Teal diet, teal therefore may potentially accumulate a lethal dose of brodifacoum through secondary exposure, or experience sub-lethal effects (Hamer Environmental 2019).

To reduce the risk of non-target poisoning and secure the safe release of Laysan Teal following mouse eradication, several strategies will be used. The US Fish and Wildlife Service (USFWS) may elect to release teal when: 1) no pellets remain visible in the environment; 2) brodifacoum residue levels in arthropods reach levels considered to be low enough to avoid sub-lethal effects; 3) migratory shorebirds returning from their breeding grounds stay healthy upon arrival, and; 4) sentinel Atlantic Canaries *Serinus canaria* and Common Mynas *Acridotheres tristis* released post-eradication show no ill effects (Hamer Environmental 2019). Focal arthropods will be sampled regularly for several weeks after bait application to monitor brodifacoum levels, until residue levels have dropped to a safer level (*i.e.* field-derived ED_5, where 5% of the exposed population may be affected by coagulopathy but not necessarily death; Hamer Environmental 2019). However, there have been no diet studies on the Kuaihelani population of Laysan Teal and it is unknown which arthropods they consume. Such information will help managers to monitor specific arthropods for brodifacoum residue, to reduce the likelihood of secondary poisoning before the teal are released.

All previous diet information of Laysan Teal is from Kamole (Laysan Island), where they rely heavily on flies (Diptera) and brine shrimp (Anostraca, *Artemia* sp., found in Kamole's hypersaline lake), and to a lesser degree on seeds and plant material (Reynolds *et al.* 2006). Less common food items are ants (Formicidae), moth (Lepidoptera) larvae, and adult beetles (Coleoptera) (Reynolds *et al.* 2006). Earlier dietary studies on Kamole reported that teal mainly eat Ephydrid Flies *Neoscatella sexnotata* and moth *Agrotis* sp. larvae and pupae (Butler & Usinger 1963; Caspers 1981; Moulton & Weller 1984; Warner 1963). Teal on Kamole also show differences in diet among habitats: teal consume ants, lepidopteran larvae, and seeds in terrestrial, vegetated habitats, but mainly they rely on adult dipterans and brine shrimp in and around the hypersaline lake (Reynolds *et al.* 2006).

Kuaihelani's landscape differs from that of Kamole (*e.g.* in having widespread modification from wartime eras and no hypersaline lake), and Kuaihelani also has a high proportion of introduced arthropod and plant taxa absent from Kamole (Rauzon 2000). Thus, Laysan Teal may eat different prey on Kuaihelani than they do on Kamole. To address this knowledge gap, we aimed to determine which arthropods are consumed by Laysan Teal using next-generation sequencing (NGS) to analyse DNA of prey remains in faecal samples (McInnes *et al.* 2017; Pompanon *et al.* 2012; Taberlet *et al.* 2018). Compared to traditional diet analysis methods, such as microhistological or observational techniques (see Reynolds *et al.* 2006), NGS can provide high-resolution, accurate identification of food sources. Based on previous dietary studies of Laysan Teal, we expected that the teal would consume similar prey as on Kamole (as available), including cockroaches (Blattodea) and moths (Lepidoptera). Given that Laysan Teal forage extensively at freshwater sources on Kuaihelani, we also suspected that they would commonly feed on aquatic invertebrates.

Methods

Study site

Kuaihelani (Midway Atoll National Wildlife Refuge) is a remote Pacific atoll located near the end of the Northwestern Hawaiian Islands (Kūpuna Islands; 28°12'N, 177°22'W) and lies within the Papahānaumokuākea Marine National Monument (EO No. 13022, Presidential Proclamation No. 8031; Fig. 1A). The atoll consists of three islands: Sand Island (460 ha; mean elevation 3.2 m; Fig. 1B), Spit Island (5.8 ha; mean elevation 1.5 m), and Eastern Island (138 ha; mean elevation 2.6 m) (Reynolds *et al.* 2015). Between 2004 and 2005, 42 Laysan Teal were translocated to Kuaihelani and the population grew rapidly to *c.* 200 individuals by 2007 (Reynolds *et al.* 2008). The Kuaihelani population is susceptible to severe declines, as observed following the 2011 Tōhoku Japan earthquake-generated tsunami and also Avian Botulism type C. *Clostridium botulinum* outbreaks (Reynolds *et al.* 2017a; Work *et al.* 2010). In 2015, the teal population on Kuaihelani was estimated at between 314–435 individuals (Reynolds *et al.* 2017b), with recent surveys estimating 870–1,008 birds (J.H. Plissner, pers. comm. 2022).

Sample collection

Given that mouse eradication efforts on Sand Island were planned for 1 July–15 August 2023, our samples were collected over a six-month period, from August–January 2023, to cover the anticipated maximum length of time that brodifacoum residue could remain in arthropod prey following bait application. The long-term phase decay of brodifacoum does, however, depend on several factors, including environmental attributes (weather, habitat distribution, potential sinks), application rate, number of secondary consumers, and microbial processes. Some studies have reported brodifacoum residues in non-target organisms anywhere from 11 weeks after bait application (in cockroaches *Periplaneta* sp. and Mourning Geckos *Lepidodactylus lugubris*; Wegmann *et al.* 2019) to seven months (in Northern Pintail *Anas acuta*; Pitt *et al.* 2015). Indeed, on Wake Atoll, brodifacoum residue was documented in Blacktail Snapper *Lutjanus fulvus* up to three years after bait application (Siers *et al.* 2020). On Sand Island, brodifacoum is expected to decay rapidly and should be flushed from the system relatively quickly (especially from water and soil). Brodifacoum has extremely low solubility in water and binds tightly to soil, where the chemical compound is

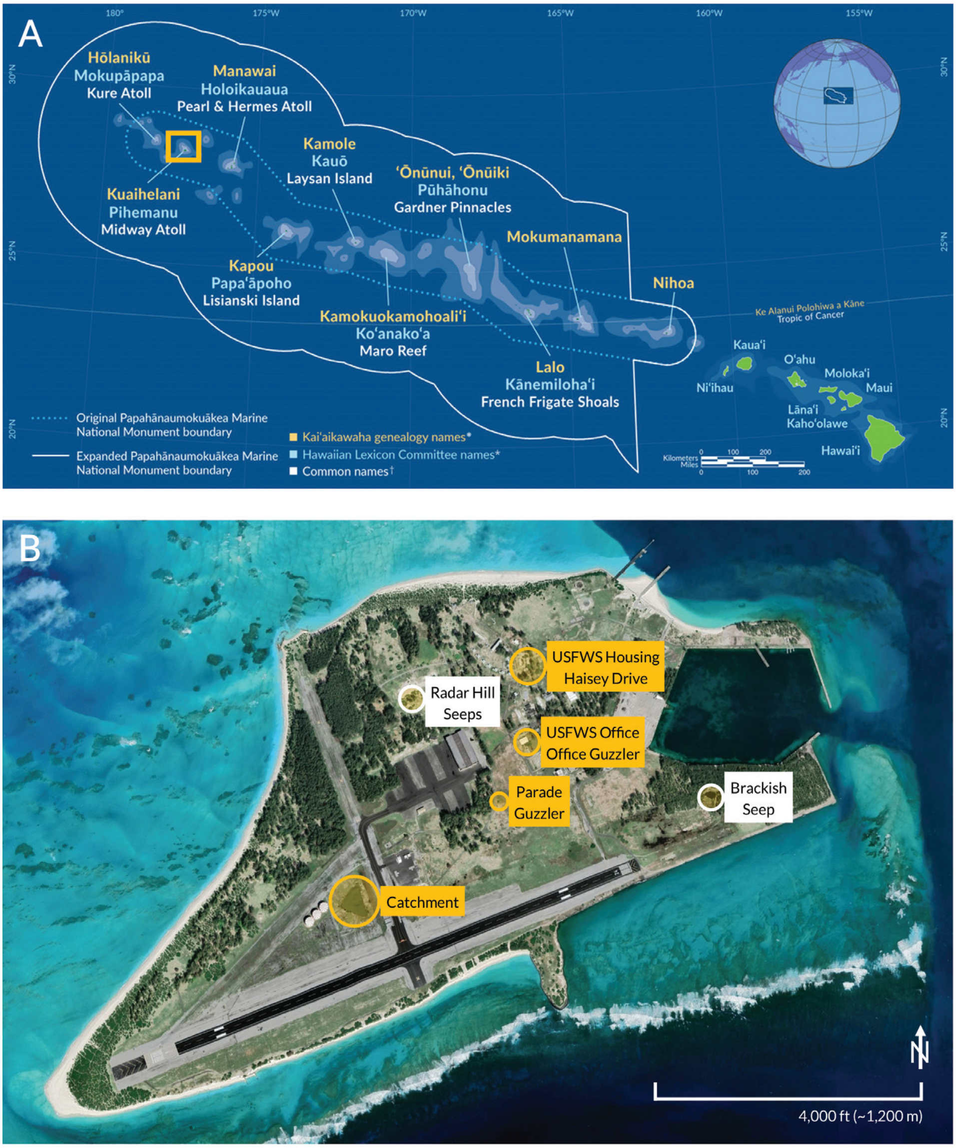

Figure 1. A) Map of the Hawaiian archipelago in the north Pacific Ocean, including the Papahānaumokuākea Marine National Monument. Kuaihelani is outlined in orange (map credit: NOAA). B) Map of Laysan Teal sampling locations on Sand Island, Kuaihelani (from Maxar Technologies 2022). Areas coloured in orange denote locations from which faecal samples were used for next-generation sequencing (NGS).

degraded by soil microorganisms as well as through exposure to oxygen and sunlight (Hamer Environmental 2019). Moreover, it is expected that brodifacoum will degrade faster in warm, sunny environments (such as the Northwestern Hawaiian Islands) than in cold, dark environments (Eason & Wickstrom 2001; Eisemann & Swift 2006; Hamer Environmental 2019). For example, based on bait trials conducted on Lehua Island in Hawaiʻi, brodifacoum was estimated as undetectable in 35–40 days when under vegetation and at *c.* 65 days when on rocks or bare ground (Hamer Environmental 2019; Mazurek 2015).

From August 2019 to February 2020, we collected 71 fresh faecal samples from Laysan Teal across six locations on Sand Island (Fig. 1B). Sample collection occurred weekly and involved 30 min surveys in areas where teal aggregate and faecal samples with minimal contamination could be collected (from concrete or asphalt surfaces with scant vegetation; McInnes *et al.* 2017). Samples were collected immediately after an individual was directly observed defecating. Although many Laysan Teal have US Geological Survey aluminium alloy bands and plastic auxiliary bands, not all teal are banded across Kuaihelani; we therefore were unable to identify the individual teal that we sampled. Faecal samples were individually stored in vials of 70% isopropyl alcohol, labelled, then placed in a –20°C freezer until shipment to the mainland. Our sampling pool consisted of 33 females, 31 males and seven teal whose sex could not be confirmed visually. Of the 71 samples, 55 were from individuals one year or older (AHY = after hatch year, SY = second year, ASY = after second year; 77.5%), 10 from hatch-years (HY; 14.1%), and six from teal of unknown age (8.5%).

Next-generation sequencing (NGS)

For DNA extraction, we used the QIAamp PowerFecal DNA Kit (Qiagen, Carlsbad, California, USA) and modified the second step to remove inhibitors in the faecal samples. Here, we rinsed the samples with water after removing them from storage media and incubated them overnight (instead of the recommended 5 min) during the first refrigeration step. We prepared our DNA library using a two-step polymerase chain reaction (PCR) approach. Faecal DNA samples were first amplified with the ZBJ-ArtF1c/ZBJ-ArtR2c primers that target a 157 bp region of cytochrome c oxidase I (COI) of the *MT-CO1* gene (Zeale *et al.* 2011), modified with adapters for the Illumina MiSeq system (Illumina, San Diego, California, USA). Because we used only arthropod-specific primers, our study should not be understood as a comprehensive summary of Laysan Teal diet. The PCR consisted of reactions of 1X PCR gold buffer, 2.5 mM $MgCl_2$, 0.8 mM dNTP blend, 0.125 µL AmpliTaq Gold (Applied Biosystems, Foster City, California, USA), 5 µg BSA (Sigma-Aldrich, St. Louis, Missouri, USA), 10 µM of each primer (Integrated DNA Technologies, Coralville, Iowa, USA) and 5 µL of faecal DNA. Cycling parameters for PCR consisted of an initial denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 30 s, with a final elongation step of 72°C for 10 min. We confirmed amplification success by running

4 μL of each sample on a 2% agarose gel (Sigma-Aldrich). After this step, we purified PCR products with Agencourt AMPpure beads (Beckman Coulter, Brea, California, USA).

After the first round of PCR, the purified products were amplified in a second PCR containing Nextera XT v2 indexes (Illumina, San Diego, California, USA), with each sample receiving a unique combination of forward and reverse indexes. The indexed samples were purified again with AMPpure beads, and the purified, indexed products were quantified on a nanodrop spectrophotometer (Fisher Scientific, Waltham, Massachusetts, USA) and pooled to approximately equal concentrations. The quality and quantity of the purified, indexed products were confirmed on a Bioanalzyer (Agilent Technologies, Santa Clara, California, USA). The samples were diluted, pooled with 20% PhiX control DNA (Illumina, San Diego, California, USA), and run on a v2 flow cell (Illumina MiSeq) reading 220 nucleotides, paired end, at the University of Tennessee Genomics Core. We included one reaction blank of water (in place of a DNA template) as a negative control through the entire process. Aerosol barrier tips were used to minimise cross-contamination, and all steps were performed in a laminar flow hood.

Analysis

Bioinformatics was performed by the UIC Research Informatics Core in the Research Resources Center using the DADA2 pipeline (Callahan *et al.* 2016) with a 97% identity (sequence similarity) threshold to increase accuracy of taxonomic assignment and exclude chimeric sequences. A nucleotide BLAST search (Altschul *et al.* 1990) was used to match sequences to references through nt, the NCBI Genbank non-redundant nucleotide database (Benson *et al.* 2012). The resulting data were exported into a table of amplicon sequence variants (ASVs) – *i.e.* a table containing taxonomic annotations for all sequences. We filtered out all ASVs assigned to any phylum other than Arthropoda and provided the most precise level of taxonomic identity possible. In addition, we cross-referenced ASVs with arthropod records from Kuaihelani (Holthuijzen 2022; Nishida & Beardsley 2002). However, there are no comprehensive surveys of terrestrial arthropods and invertebrates on Kuaihelani; aquatic and marine invertebrates, in particular, are poorly documented. Thus, we did not exclude ASVs from taxa that had not been previously documented on Kuaihelani, since that would risk eliminating arthropods from our data set that may be important diet items for teal but have not yet been formally surveyed on Kuaihelani. Most arthropods on Kuaihelani are non-native (≥ 80%; Holthuijzen 2022), many of which feature a cosmopolitan range and distribution (and are well represented in GenBank). We provided an evaluation of the status for each ASV detected via NGS in Table 1; here, we noted if the taxon is considered native or non-native on Kuaihelani (based on arthropod surveys and records) or if the taxon has not been previously recorded ("unknown" status).

We then converted ASV sequence counts to occurrence data (*i.e.* presence/absence of arthropod taxa), since the number of sequence reads belonging to each arthropod taxon is not a reliable estimate for relative biomass consumed (Jedlicka *et al.* 2017; Taberlet *et al.* 2018). Biases can occur throughout the extraction, sequencing, and bioinformatics analysis processes that can influence the number of sequence reads. While the presence/absence transformation can introduce additional bias by excluding poorly amplified taxa (Deagle *et al.* 2019), it is a more conservative approach that still provides a semi-quantitative description of consumed taxa. Our goal is not to identify the proportion that arthropods contribute to Laysan Teal diet, but instead to gather information about the presence/absence of arthropods. We used this transformed occurrence data set (or percent frequency of occurrence (%FOO); Deagle *et al.* 2019) to summarise arthropod taxa consumed by Laysan Teal. Arthropod ASV sequence data are available via Dryad (Holthuijzen *et al.* 2023).

Results

We successfully extracted and sequenced DNA from 21 of our 71 samples. These came from nine females, 11 males and one individual of unknown sex. Most samples (19) were from teal that were one or more years old (AHY, SY, ASY; 90.4%), with two from hatch-years (9.5%). Most (18 samples; 85.7%) were collected in the freshwater "Catchment" area on Sand Island (Fig. 1B).

Overall, we detected 27 arthropod ASVs from 11 orders and 18 families, with a mean (± s.d.) of 7.05 ± 1.86 ASVs per faecal sample (range = 4–13). Laysan Teal commonly consumed cockroaches (Blattodea), freshwater ostracods (Cyprididae), midges (Chironomidae) and isopods (Porcellionidae) – all of which were present in ≥ 30% of samples (Table 1, Fig. 2). In particular, two taxa were detected in every faecal sample – an unidentified Blattodea species and a freshwater ostracod the Mussel Shrimp *Cypridopsis vidua* (Table 1). Spiders (Araneae), moths (Lepidoptera), beetles (Coleoptera), springtails (Entomobryomorpha), thrips (Thysanoptera) and crabs (Decapoda) were detected in ≤ 3 samples. A gastropod – the Wandering Snaggletooth Snail *Gastrocopta servilis* – was detected in one sample, but we excluded it from our analysis (along with rotifers and other non-arthropods). Teal appear to eat several introduced taxa; at least 16 arthropod ASVs (59.3%) are considered to be non-native (Table 1). The only native species consumed was the Hide Beetle *Dermestes maculatus*.

Discussion

This is the first study of Laysan Teal arthropod consumption on Sand Island in Kuaihelani. NGS of faecal samples revealed that teal frequently consume cockroaches (Blattodea) and freshwater ostracods (Cyprididae), followed by midges (Chironomidae) and isopods (Porcellionidae). To a lesser degree, teal also eat spiders (Araneae), moths (Lepidoptera), beetles (Coleoptera), springtails (Entomobryomorpha), thrips (Thysanoptera) and crabs (Decapoda). This contrasts with the main arthropods consumed on Kamole by teal, namely flies (Diptera) and brine shrimp (Reynolds *et al.* 2006).

In comparison to Kamole, Sand Island has a highly altered landscape with diverse

Table 1. Arthropod amplicon sequence variants (ASVs) detected in Laysan Teal faecal samples through next-generation sequencing (NGS); $n = 21$ samples. Frequency of occurrence (FOO) is the number of samples containing a given ASV, presented here as per cent (%FOO). Status based on the classification scheme from Nishida & Beardsley (2002), compiled by Holthuijzen (2022); non-native = introduced, native = endemic to the Hawaiian archipelago.

Class (Order)	Family	Genus	Species	Count	% Frequency of Occurrence	Status
Arachnida						
Araneae	Dictynidae	Dictynidae genera	*Dictynidae* sp.	3	14%	Unknown; family not previously observed
Collembola						
Entomobryomorpha	Entomobryidae	*Seira*	*Seira dowlingi*	1	5%	Unknown; genus not previously observed
Insecta						
Blattodea	Blaberidae	Blaberidae genera	*Blaberidae* sp.	3	14%	Non-native; entire order is non-native
Blattodea	Blaberidae	*Pycnoscelus*	*Pycnoscelus* sp.	1	5%	Non-native; entire order is non-native
Blattodea	Blaberidae	*Pycnoscelus*	*Pycnoscelus surinamensis*	13	62%	Non-native; entire order is non-native
Blattodea	Blattodea family	Blattodea genera	*Blattodea* sp.	21	100%	Non-native; entire order is non-native
Blattodea	Ectobiidae	*Blattella*	*Blattella germanica*	18	86%	Non-native; entire order is non-native
Blattodea	Ectobiidae	*Blattella*	*Blattella* sp.	1	5%	Non-native; entire order is non-native
Blattodea	Ectobiidae	Ectobiidae genera	*Ectobiidae* sp.	2	10%	Non-native; entire order is non-native
Coleoptera	Dermestidae	*Dermestes*	*Dermestes maculatus*	3	14%	Native
Coleoptera	Nitidulidae	Nitidulidae genera	*Nitidulidae* sp.	1	5%	Unknown; likely non-native
Coleoptera	Tenebrionidae	*Gonocephalum*	*Gonocephalum adpressiforme*	2	10%	Non-native

Table 1 (*continued*).

Class (Order)	**Family**	**Genus**	**Species**	**Count**	**% Frequency of Occurrence**	**Status**
Diptera	Chironomidae	Chironomidae genera	*Chironomidae* sp.	4	19%	Unknown; native and non-native species of this family present
Diptera	Chironomidae	*Chironomus*	*Chironomus* sp. NIESD0481	16	76%	Unknown; genus not previously observed
Diptera	Chironomidae	*Polypedilum*	*Polypedilum nubifer*	8	38%	Non-native
Diptera	Sarcophagidae	*Sarcophaga*	*Sarcophaga argyrostoma*	3	14%	Non-native
Lepidoptera	Crambidae	*Herpetogramma*	*Herpetogramma licarsisalis*	3	14%	Non-native
Lepidoptera	Noctuidae	*Leucania*	*Leucania* sp.	3	14%	Non-native
Lepidoptera	Noctuidae	*Leucania*	*Leucania stenographa*	1	5%	Non-native
Lepidoptera	Tineidae	*Praeacedes*	*Praeacedes atomosella*	2	10%	Non-native; species not previously observed
Odonata	Libellulidae	Libellulidae genera	Libellulidae sp.	5	24%	Unknown; native and non-native species of this family present
Thysanoptera	Phlaeothripidae	*Haplothrips*	*Haplothrips* sp. BOLD:AAN5799	1	5%	Non-native
Malacostraca						
Decapoda	Portunidae	Portunidae genera	*Portunidae* sp.	1	5%	Unknown; native and non-native species of this family present
Isopoda	Porcellionidae	*Porcellio*	*Porcellio laevis*	7	33%	Non-native
Ostracoda						
Podocopida	Cyprididae	Cyprididae genera	*Cyprididae* sp.	2	10%	Unknown; family not previously observed
Podocopida	Cyprididae	*Cypridopsis*	*Cypridopsis* sp.	2	10%	Unknown; family not previously observed
Podocopida	Cyprididae	*Cypridopsis*	*Cypridopsis vidua*	21	100%	Unknown; family not previously observed

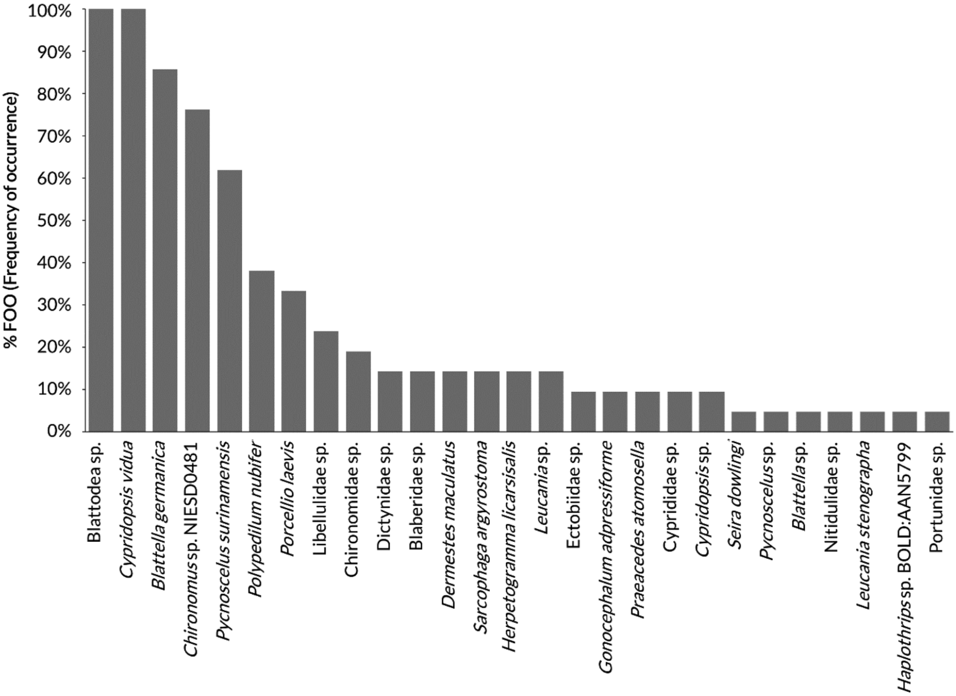

Figure 2. Bar graph showing the most commonly detected to least commonly detected arthropod amplicon sequence variants (based on %FOO) detected in Laysan Teal faecal samples.

arthropod communities dominated by non-native taxa (Nishida & Beardsley 2002). Since many arthropods present on Kamole do not occur on Kuaihelani, we were not surprised to find differences in arthropod consumption on Sand Island. Although Sand Island lacks a hypersaline lake, this island's teal population consumes aquatic arthropods (Cyprididae), which are likely obtained from freshwater at Catchment and other small, human-constructed, seeps and at wildlife drinking stations (*i.e.* "guzzlers"; Reynolds & Klavitter 2006). More broadly, teal prey on arthropod orders that are abundant across Sand Island (Holthuijzen *et al.* 2021).

The prominent differences in arthropod consumption by teal on Sand Island and those on Kamole support Walters & Reynolds (2013), in that phenotypic plasticity allowed teal to exploit novel habitats and food sources on Kuaihelani. Reynolds *et al.* (2006) posited that an opportunistic foraging strategy has helped teal to survive periods of prey and food scarcity on Kamole following the introduction of European Rabbits *Oryctolagus cuniculus* and domestic breeds thereof in *c.* 1903 (Dill & Bryan 1912). The rapid adaptation of teal to Kuaihelani's highly modified landscape and non-native arthropod food sources will likely facilitate conservation of this species. We expect that teal could be translocated successfully to other Northwestern or Main Hawaiian Islands to establish additional self-sustaining populations – an important recovery action (USFWS 2009). For example, Kapou (Lisianski Island; Fig. 1A) is a prospective translocation site for Laysan Teal and has supported a teal population in the past; however, extensive habitat restoration efforts would be required, and there are concerns about the capacity to monitor and manage teal on such a remote island. Other Main Hawaiian Islands (such as Kahoʻolawe) with easier accessibility have also been suggested as translocation sites, although mammalian predators would need to be removed (Reynolds & Kozar 2009; USFWS 2009).

Although our study provides a high degree of taxonomic certainty on arthropod prey, most faecal samples came from teal in the Catchment area. Thus, we may not have comprehensively sampled arthropod consumption across Sand Island. Laysan Teal are highly mobile and nomadic across the atoll (including between different islands); samples collected at one location may not correspond to feeding at that same location. However, arthropod communities on Kuaihelani show a high degree of overlap in community structure and composition, likely due to biotic homogenization via introduced taxa (Holthuijzen *et al.* 2021; Nishida & Beardsley 2002), so we can expect similarities across habitats. Despite our small sample size, the arthropod taxa we detected are likely common food sources for teal across Sand Island. For example, cockroaches and ostracods were observed in all samples, even those from beyond Catchment (Table 1).

We also experienced difficulties in extracting DNA from faecal samples. DNA extraction from faeces can fail for several reasons, including insufficient sample collection, the presence of inhibitors, high bacterial load, variability of DNA concentration, and degradation of DNA (Liu *et al.* 2021). In the case of remote field sites, adequate storage facilities are often unavailable. Thus, we recommend using storage media (such as DNA/RNA Shield Reagent, Zymo Research, Irvine, California, USA) that do not require refrigeration or freezing.

Our study adds to the literature on the ecology of Laysan Teal populations by using advanced techniques to study arthropod consumption with taxonomic precision. Although we focused solely on the arthropods eaten by teal, we urge researchers to complete a comprehensive dietary study of Kuaihelani's population to provide a better understanding of how teal use novel habitats and food sources. In addition, our study serves as a model for risk mitigation during invasive rodent eradications. Knowledge of which arthropod taxa that teal consume can inform non-target mitigation measures for the pending House Mouse eradication (Hamer Environmental 2019). The USFWS and cooperating agencies will use this information to identify focal arthropods for monitoring brodifacoum residue levels (Antaky 2022), helping to guide the safe release of teal following bait deployment.

The eradication of invasive rodents on islands often conflicts with the protection of native, non-target wildlife, requiring mitigation measures to safeguard them. Several rodent eradications have resulted in short-term impacts to native biota, especially birds. With respect to wildfowl, the eradication of the Brown Rat *Rattus norvegicus* and House Mouse from Motuihe Island in the Hauraki Gulf of New Zealand resulted in a 60% mortality of native Paradise Shelducks *Tadorna variegata* (Dowding *et al.* 1999; Eason *et al.* 2002). Mortality was also reported for Pacific Black Ducks *Anas superciliosa* and Mallards *A. platyrhynchos* during a multi-species eradication of House Mice, Black Rats *Rattus rattus* and European Rabbits on Macquarie Island in the southwestern Pacific Ocean (Springer & Carmichael 2012). Knowledge of non-target taxa diet is key to reducing risk to non-target taxa during rodent eradication operations. Specifically, dietary information can inform tactics used to manage non-target wildlife mortality, such as captive holding of wildlife *in situ* or *ex situ*, translocating wildlife off-island, and reducing or eliminating exposure pathways (Castaño *et al.* 2022). With the aid of dietary studies (such as ours) and a range of practical measures, the risk of poisoning to non-target wildlife can be greatly reduced while achieving conservation objectives.

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This work was carried out on Kuaihelani of Papahānaumokuākea (Kūpuna Islands, Northwestern Hawaiian Islands), part of an indigenous space whose original people are today identified as kānaka ʻōiwi or Native Hawaiian. We sought to incorporate ʻōlelo Hawaiʻi (Hawaiian language) appropriately throughout our manuscript and research process, as outlined by the Papahānaumokuākea Marine National Monument. We thank Tristan Luxner for his help in collecting Laysan Teal faecal samples. We are grateful to Daniel Simberloff for his feedback and comments on our manuscript as well as two anonymous peer-review referees. Laysan Teal faecal samples were collected under permit #PMNM-2019-001-L by the US Fish and Wildlife Service. Bioinformatics analysis was performed by the University of Illinois Research Informatics Core, supported in part by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR002003. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

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Photograph: Female Laysan Teal with brood at Catchment, Sand Island, Kuaihelani, by Wieteke Holthuijzen.