



DNA metabarcoding reveals diet composition of invasive rats and mice in Hawaiian forests

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Abstract Rodents are among the most widespread and problematic invasive animals on islands worldwide contributing to declining endemic island biota through predation and disruption of mutualisms. Identifying what rodents eat is critically important to understanding their effects on ecosystems. We used DNA metabarcoding to identify the diets of three invasive rodents in Hawaiian forests: house mouse (*Mus musculus*), black rat (*Rattus rattus*), and Pacific rat (*Rattus exulans*). These rodents primarily eat invertebrates and plants, but previous diet studies have provided only a limited understanding of the diet breadth by relying on morphological identification

methods. We opportunistically collected fecal samples from rodents trapped at seven forest sites across O‘ahu, Hawai‘i for two years. Plant and invertebrate diet items were identified from DNA extracted from fecal samples using *rbcL* and COI primers, respectively. Intact seeds were identified using a dissecting microscope to quantify potential contributions to seed dispersal. All rodent species ate primarily plants and invertebrates of introduced species. However, some native taxa of conservation importance were identified. Neither the rodent species nor the sites drove patterns of diet composition, suggesting that diet variation may be determined by opportunistic foraging or intraspecific variation. Black rat fecal samples contained intact seeds more frequently than house mouse samples, but surprisingly, when samples contained seeds, black rats and house mice both defecated

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hundreds of introduced seeds, likely contributing to seed dispersal. Conservation efforts targeting invasive rodent control should specifically include house mice and should monitor introduced prey items to prevent predation release of unwanted introduced species.

Keywords Invasive rodents · Next-generation sequencing · Hawaiian Islands · Seed dispersal · Seed predation · Invasion biology

Introduction

Rats and mice are some of the most ubiquitous, successful, and destructive invasive species globally, having been introduced and established on all continents, except mainland Antarctica and occupying >80% of all major islands worldwide (Atkinson 1985). The house mouse (*Mus musculus*) and three rat species (black rat, *Rattus rattus*; Norway rat, *Rattus norvegicus*; Pacific rat, *Rattus exulans*) are particularly abundant on islands, where they often have detrimental effects on native biota (Townsend et al. 2006; Drake and Hunt 2009; Harper and Bunbury 2015). Rodents impact ecosystems both directly through predation and herbivory of native species and indirectly through disruption of mutualisms, such as pollination and seed dispersal (Traveset and Richardson 2006; Drake and Hunt 2009; Aslan et al. 2013; Liang et al. 2022), ultimately resulting in extinctions of native species on islands worldwide (Townsend et al. 2006; Jones et al. 2008; Drake and Hunt 2009; Harper and Bunbury 2015). Owing to their omnivorous diet, agile climbing, burrowing, and their prominent incisor teeth, rats and mice consume a wide variety of plants and animals and readily adapt to new environments (Landry 1970; Shiels et al. 2013). In forested ecosystems, plants (fruits, seeds, leaves, and stems) and arthropods are most commonly consumed, but rodents also eat birds, lizards, snails, and other invertebrates (Sugihara 1997; Shiels et al. 2013; Ceia et al. 2017).

Native forest composition has been altered through invasive rodent predation of native plants (stems, leaves, bark, seedlings), predation of native seed dispersers, and predation of seeds from fruiting plants and the soil seed bank (Harper and Bunbury 2015). The stems and bark of plants are often consumed in higher quantities during times of drought when other resources may be more scarce (Meyer and Butaud 2009). Fruits and seeds are particularly nutritious

and therefore targeted by rodents for consumption. On Pacific islands, fruits and seeds from many common large-seeded families (e.g., Arecaceae, Elaeocarpaceae, Oleaceae, Rubiaceae, Santalaceae, and Sapotaceae) are readily depredated by invasive rodents, destroying up to 99% of reproductive structures (Meyer and Butaud 2009; Shiels and Drake 2011; Harper and Bunbury 2015). In addition to suppressing native species, rodents may facilitate the spread of small-seeded invasive plants because some seeds bypass the teeth and then remain intact while passing through the digestive tract (Shiels and Drake 2011).

Rodents also disrupt native plant communities by altering patterns of seed dispersal, but rodents are only part of pervasive changes to island seed dispersal. Avian and other mammalian native seed dispersers have declined to the point of extinction or functional extinction on many Pacific islands (Farwig and Berens 2012; Carpenter et al. 2020; Fernández-Palacios et al. 2021). Identifying how introduced birds and mammals function in the role of seed disperser is crucial to conserve native plant dispersal. Previous work has shown that introduced birds disperse some native plants, but that they primarily disperse introduced plants (Mandon-Dalger et al. 2004; Kelly et al. 2006; Culliney et al. 2012; Vizentin-Bugoni et al. 2019). Determining the role that invasive rodents play as potential seed dispersers will expand our understanding of these novel interactions and will highlight where birds and rodents may be affecting seed dispersal similarly or complementarily to each other.

Rats eat fruit and seeds and are sometimes seed dispersers (passing intact seeds or hoarding and discarding larger seeds), but rats can also be seed predators (eating or destroying the seed embryo), depending on the ecosystem and plant species (Traveset and Richardson 2006; Drake and Hunt 2009; Shiels and Drake 2011). Most studies on rodent seed dispersal have focused on black rats, given their wide distribution, high consumption of fruits and seeds, and large body size (Williams et al. 2000; Traveset et al. 2009; Shiels and Drake 2011). The house mouse has primarily been considered a seed predator or uninvolved in seed dispersal due to its small size and inability to consume larger intact fruits and seeds (Williams et al. 2000; Traveset et al. 2009). However, intact seeds have been found in house mouse stomach contents, indicating the possibility of seed dispersal of small-seeded plant species (Shiels et al. 2013).

Native invertebrate populations have been suppressed by invasive rodents and some species have likely been driven to extinction on islands globally, though explicitly linking invertebrate extinctions with rodent predation is often difficult (St Clair 2011). Arthropods in the orders Coleoptera and Orthoptera and terrestrial snails are the primary taxa known to have been suppressed and extirpated by invasive rodents, and larger bodied invertebrates are particularly vulnerable (Gibbs 2009; St Clair 2011). Limited information on island invertebrate communities exists from before rodents were introduced; however, several studies show dramatic increases in abundance of invertebrates after rodent eradication and through comparisons of islands with and without rodents (Gibbs 2009; St Clair 2011; St Clair et al. 2011; Jones et al. 2016). This suggests that rodent predation can have a large negative impact on island invertebrates, particularly on island communities that evolved without terrestrial mammalian predators (e.g. New Zealand, Hawai'i, Seychelles, Balearic, and Canary Islands, Gibbs 2009; Traveset et al. 2009; St Clair 2011).

Given the negative effects of invasive rodents on native plant and invertebrate species, rodent control and eradication are being implemented on islands worldwide to assist in ecological restoration and recovery of native species (Jones et al. 2016; Spatz et al. 2022). Often rodent control and eradication have the intended effect of releasing native species from direct predation pressures, but species interactions can lead to unintended outcomes (Zavaleta et al. 2001; Caut et al. 2007). Predation release of other invasive species such as predatory snails (Meyer and Shiels 2009), or invasive plants (Miller-ter Kuile et al. 2021) may have unintended negative consequences for native species. Additionally, complex population fluctuations may occur due to interspecific competition between rodent species that promote one rodent species while controlling another (e.g., "competitor release effect"), (Caut et al. 2007). These population fluctuations can lead to additional unforeseen conservation concerns particularly when species diet impacts are unidentified. Identifying the composition of invasive rodent diets can clarify complex interactions with native and introduced species and help conservation practitioners to avoid unintended negative outcomes (Zavaleta et al. 2001).

In the past, diets of invasive rodents have been assessed using captive-feeding trials, identification of stomach contents, and stable isotope analysis (Shiels et al. 2013). Diet studies from wild-caught rodents that visually examine stomach contents or feces can usually identify plant material by general categories (fruits, seed, leaves, and stems) and identify arthropods to the ordinal rank (Holechek et al. 1982; Moreby 1988). At these coarse-level taxonomic ranks, it is difficult to assess alpha diversity and diet composition and nearly impossible to identify soft bodied, or closely related species (Holechek et al. 1982; Moreby 1988). DNA metabarcoding analyses can now identify the genera and species of plants and invertebrates from fecal samples, describing diet composition at a much finer resolution (Chen et al. 2010; De Barba et al. 2014; de Sousa et al. 2019; Jusino et al. 2019). Identifying taxa consumed by rodents will shed new light on the impact invasive rodents have on native ecosystems.

We used DNA metabarcoding on fecal samples to assess the diets of three rodent species (house mouse, black rat, and Pacific rat) present at seven forested sites across the island of O'ahu, Hawai'i. The sites were mesic to wet forest and differed in plant composition and proportions of native/introduced species (Vizentin-Bugoni et al. 2019). While invasive rodents sometimes prey upon vertebrates to varying degrees, invertebrates and plants comprise larger proportions and higher frequencies of their diets on islands globally (Shiels et al. 2014; Pomeda-Gutiérrez et al. 2021). Particularly in forested ecosystems on Hawai'i, rodent diet studies examining stomach contents have found that only 0-0.03% of stomachs contained evidence of birds or lizards (Sugihara 1997; Cole et al. 2000; Shiels et al. 2013). Therefore, our study focused on plants and invertebrates as the dominant components of invasive rodent diets although we note that vertebrates are regularly detected using the primers we use for invertebrates (JTF, unpublished data). The primary aim of this study was to identify introduced and native plant and invertebrate diet items of rodents at a fine taxonomic scale. The following questions were investigated: Do diets differ by rodent species, by site, or by both? Do rodents defecate intact seeds (potential seed dispersal) of native and introduced plant species and if so, which rodents are responsible for defecating the most seeds per fecal sample? We

hypothesized that diet composition would differ by rodent species due to dietary niche partitioning that has been established using microscopic identification of stomach contents (Shiels et al. 2013) and would differ by site, due to the generalist, omnivorous nature of rats and mice taking advantage of site-specific resources. We also hypothesized that rodents would defecate intact invasive seeds, with black rat fecal samples containing the most intact seeds, based on previous research that found black rats readily defecated intact seeds while house mice primarily destroyed seeds (Williams et al. 2000; Shiels et al. 2013). Thus, we sought a more complete picture of the diets, potential resource overlap, and effects of rodents on the plants and invertebrates in these forests, while also providing a new tool in our understanding of the pervasive effects of invasive rodents on food webs globally. We anticipate this information on rodent diets will support conservation

managers in identifying potential impacts on plants and invertebrates after rodent control and eradication to ensure desired results.

Materials and methods

Study sites

Rodent fecal samples were collected at seven study sites on O‘ahu, Hawai‘i (Fig. 1, additional site details provided in Vizentin-Bugoni et al. 2019). All sites were mesic to wet montane forests, with three sites in the Ko‘olau mountain range (Moanalua Valley, Mt. Tantalus, and upper Waimea Valley) and four sites in the Wai‘anae range (‘Ēkahanui Gulch, Kahanahāiki Management Area, Mt. Ka‘ala Natural Area Reserve, and Pahole Natural Area Reserve). Hereafter the sites will be referred to as Moanalua,

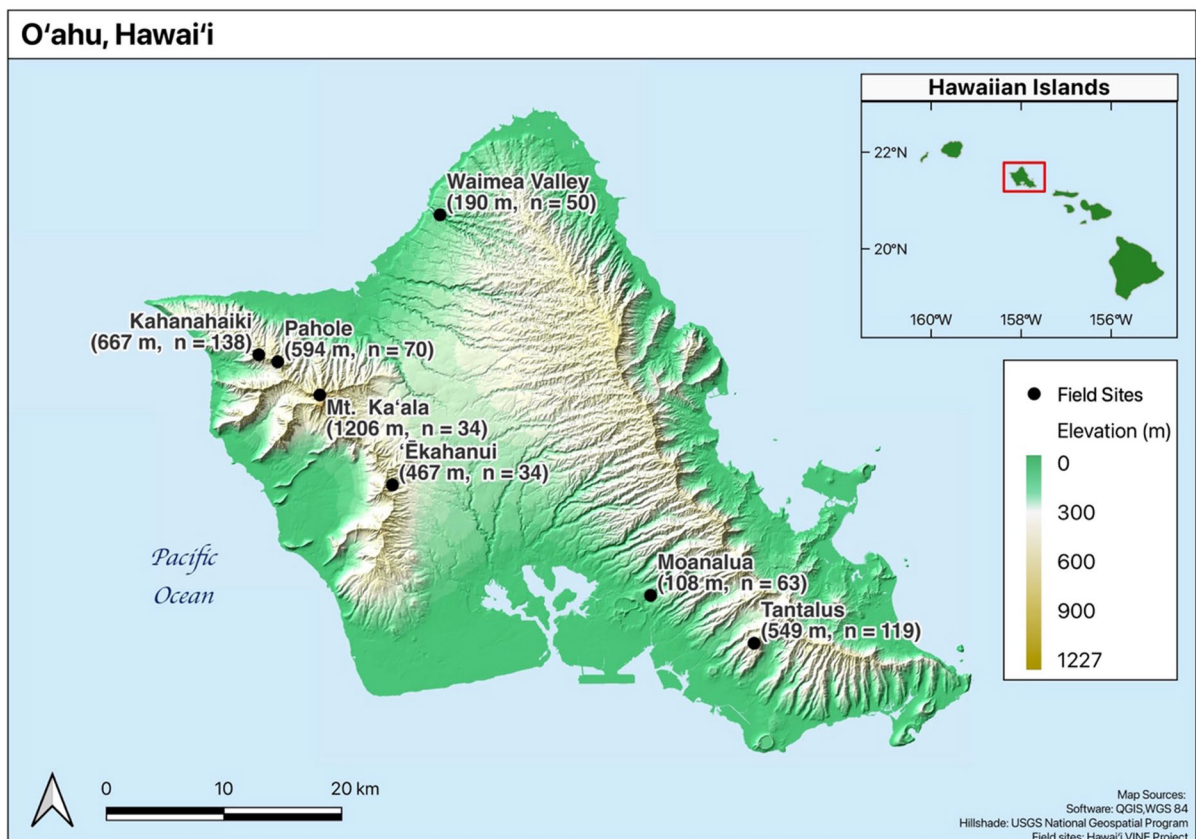


Fig. 1 Rodent fecal sampling locations on O‘ahu, Hawai‘i, 2014–2016. Site elevations (m) and rodent fecal sample sizes (n) are included on the map

Tantalus, Waimea Valley, 'Ēkahanui, Kahanahāiki, Mt. Ka'ala, and Pahole. Sites were managed by the Hawai'i Department of Land and Natural Resources, Army Natural Resources Program of O'ahu, and Waimea Valley Hi'ipaka LLC. The sites varied in extent of plant invasion and ranged in elevation from 108 to 1206 m (Fig. 1). Each forest contained a mix of native and introduced grass, herbaceous, shrub, and tree species. Flowering plant species richness (where graminoids were lumped into a single taxon) ranged from 21 to 46 species with 9–93% of species introduced ('Ēkahanui 92.9% introduced, Kahanahāiki 42.4%, Moanalua 92.0%, Mt. Ka'ala 9.1%, Pahole 30.4%, Tantalus 65.7%, and Waimea Valley 81.0%, Vizentin-Bugoni et al. 2019).

Hawai'i lacks native rodents, thus all rodents at these sites are introduced including the house mouse, black rat, and Pacific rat. Norway rats occur in low elevation populated areas of O'ahu but were not captured nor seen at any of the seven montane forested sites. Body size differs among the rodents present at the sites typically ranging from black rat at 85–165 g, Pacific rats 23–60 g, and house mouse 12–39 g (Wilson et al. 2017). Shiels et al. (2013) found rodent body size at Kahanahāiki was 124 ± 5 g (mean \pm SE) for black rats, 52 ± 4 g for Pacific rats, and 12 ± 1 g for house mice. Rodent control using various traps and baits targeting rats occurred intermittently at all sites except for 'Ēkahanui and Waimea Valley but the extent and effect of these efforts were not consistently quantified. Other introduced mammals present in some sites include feral pigs (*Sus scrofa*), Indian mongoose (*Herpestes javanicus auro-punctatus*), feral dogs (*Canis lupus familiaris*) and feral cats (*Felis catus*).

Field collection

Rodents were live trapped from November 2014 to December 2016 across all sites, rotating among sites once every seven weeks. House mice were trapped using Sherman live traps (7.6 \times 8.9 \times 22.9 cm) and black and Pacific rats were trapped using Tomahawk single door live traps (12.7 \times 12.7 \times 38.1 cm). Fifty traps of each type were deployed on an approximately 25 m grid depending on topography and accessibility at each site. Traps were baited with peanut butter and coconut chunks at dusk and checked the following morning. This study overlapped with a rodent

population study using mark-recapture technique; therefore, rodents were released after identification. Fecal samples were collected from traps using sterile cotton swabs and stored inside sterile plastic sample tubes or bags. Throughout processing and analysis, one fecal sample was treated as all fecal particulates left by a rodent individual during one trap night. Fecal samples were frozen until analyzed. A total of 508 fecal samples from rodents were collected: house mice (n=371), black rats (n=108), and Pacific rats (n=29).

DNA metabarcoding

DNA extraction

DNA was extracted from fecal samples using a Qiagen DNeasy PowerSoil DNA extraction kit (Qiagen, Hilden, Germany). Extractions followed the manufacturer's instructions with a modification of 20 μ L of proteinase K (20 mg/mL) added to each sample and incubated for ~30 min at 65 °C following the sample homogenization step to aid in the breakdown of diet items.

Plant diet sequencing prep

Samples were prepped for metabarcoding for plant diet analysis using a two-step PCR method. An initial PCR was performed to amplify a 379 bp region of the *rbcl* gene using the following primer pair F: 5'-CTTACCAGYCTTGATCGTTACAAAGG-3'; R: 5'-GTAAATCAAGTCCACCRCG-3' (Erickson et al. 2017). Universal tails (UT) were added to these primers to allow for the use of universal barcodes in a second PCR reaction to dual index each sample (Colman et al. 2015). The master mix for the initial PCR contained 1X Platinum Taq II Green Master Mix, 0.3 μ M of each forward and reverse primers, and 2 μ L of template DNA. The PCR was performed in a 15 reaction under the following conditions: 1 cycle of 95 °C for 4 min, followed by 35 cycles of 94 °C for 20 s, 55 °C for 30 s and 72 °C for 1 min, with a final extension of 72 °C for 5 min. Products were visualized on a 1% agarose gel using a SybrSafe DNA gel stain (Invitrogen).

A second PCR was then performed to add unique indexes to each sample. Each 25 μ L reaction contained 2 μ L of template (amplicons from initial PCR),

1X Platinum Taq II Green Master Mix, and 0.4 μM of each forward and reverse universal tail primer containing a unique 8 bp index, as well as the Illumina P5/P7 flow cell adapters. The thermocycling program was as follows: initial denature at 98 °C for 2 min, followed by 10 cycles of 98 °C for 30 s, 60 °C for 20 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. Products were visualized on a 1% agarose gel using a SybrSafe DNA gel stain. Amplicons were purified using AMPure XP SPRI paramagnetic beads (Beckman Coulter, Brea, CA) and normalized with the SequalPrep Normalization Plate Kit (Invitrogen). After pooling equal concentrations of each sample, the library was sequenced on an Illumina MiSeq instrument with a v2 500 cycle kit (2 \times 250 bp reads).

Demultiplexed sequencing reads were returned from the sequencing center and were processed and analyzed in QIIME2 (Bolyen et al. 2019). The ‘cutadapt’ command was used to trim off primers from the sequences, followed by a denoising step using the ‘dada2’ command. A Naïve Bayesian classification method was used to assign taxonomy to sequences using the ‘classify-sklearn’ command in QIIME2 (Bokulich et al. 2018). Taxonomy was assigned to sequences using a reference database downloaded from Bell et al. (2017).

Invertebrate diet sequencing prep

Samples were prepared for metabarcoding for invertebrate diet analysis using a similar two-step PCR process as described for plant diets but using primers targeting a 180 bp region of the COI gene (LCO11490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and COI-CFMRa: 5'-GGWACTAATCAATTTCCA AATCC-3'; Jusino et al. 2019). The master mix for the initial PCR contained 1X Platinum Taq II Green Master Mix, 0.16 $\mu\text{g } \mu\text{L}^{-1}$ Bovine Serum Albumen, and 0.2 μM of each the UT-LCO11490 and UT-CO1-CMFRa primers (Souza-Cole et al. 2022). The PCR was performed in a 15 μL reaction, containing 2 μL of DNA template under the following conditions: initial denature at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 90 s, and 72 °C for 1 min, with a final extension cycle of 72 °C for 10 min. The master mix and PCR cycling conditions of the second PCR to add the dual-indexes and the Illumina flow cell adapters were identical to the methods described

above. After visualizing, purifying, normalizing, and pooling amplicons as described previously, samples were sequenced on the MiSeq Illumina platform with a v2 300 cycle kit (2 \times 150 bp reads; Souza-Cole et al. 2022). Demultiplexed sequencing reads were processed using QIIME2 as described above, with the exception that the reference database used to assign taxonomy to these sequences was created by downloading all animal sequences from the Barcode of Life (BOLD) database (boldsystems.org; January 2019), filtering out sequences that did not have a “COI-5P” markercode, and then removing gap characters and sequences < 100 bp (O’Rourke et al. 2020, 2022). Sequences were then dereplicated using a least common ancestor consensus taxonomy method (Bolyen et al. 2019).

Taxonomic classification

Taxonomic names assigned at all ranks were compared to established Hawaiian species lists maintained by the Bishop Museum (Nishida 2002) and the Smithsonian Institution (Wagner et al. 2005). Additionally, experts in Hawaiian botany (D. Drake pers. comm.) and entomology (P. Krushelnycky pers. comm.) familiar with the site locations reviewed the taxonomy. If only one species of an identified genus occurred at a given locality, the full species name was assigned. Taxa that did not occur at the site location were conservatively classified at a higher taxonomic level to ensure names for all included taxa were accurate. Our approach was thus conservative and may miss novel species introductions.

Seed sorting

Of the total 508 fecal samples collected, 435 samples (298 from house mice, 108 from black rats, 29 from Pacific rats) were sorted and assessed for the presence of intact seeds. A subset of the house mice samples (298/371) was sorted while all black and Pacific rat samples were sorted. Fecal samples were softened with ethanol, dissected in sterile petri dishes, and examined for the presence of seeds under a dissecting microscope. A new dish and sterilized tools were used for each sample. Any intact seeds (embryo of seed undamaged) were counted and identified to species through morphological identification and/or

Sanger sequencing of the *rbcL* gene using the primers described above. Seeds that were not identified after these methods were exhausted were categorized as unknown.

Statistical analyses

R software version 4.0.0 was used for all data analyses in this study (R Core Team 2020). Data used in diversity analyses was first rarefied to 2000 and 3000 sequences for invertebrate and plant diets, respectively, to standardize sequencing depth of samples (Bolyen et al. 2019). To assess differences in diet alpha diversity by rodent species, Species Accumulation Curves (SACs) were produced using vegan package (specaccum function) for plant and invertebrate diets (Oksanen et al. 2020). To determine if the Permutational multivariate analyses of variance (PERMANOVA) test was appropriate, an analysis of multivariate homogeneity of variances was performed (betadisper function). PERMANOVAs were performed using the vegan package (ADONIS function) to identify compositional differences in plant and invertebrate diets by rodent species and site (Oksanen et al. 2020). To parse out differences in these comparisons, pairwise multilevel comparisons were performed (pairwise.adonis function). All p-values for multilevel comparisons were Benjamini-Hochberg corrected and an alpha of 0.05 was used to determine significance. To reduce the dimensionality of diet composition for plants and invertebrates, we used Principal Coordinate Analyses (PCoA) comparing rodent species and sites produced using base R (cmdscale function, (R Core Team 2020). Ordinations were based on Jaccard dissimilarity index using taxa occurrence data (presence or absence of each assigned taxon; Anderson et al. 2011).

The effects of rodent species (factor, three level) were modeled on seed count per fecal samples using two models – a binomial model (with a clog-log link function) and a negative binomial model (with a log link function), similar to a hurdle model (glmmTMB; (Brooks et al. 2017). We chose to break this analysis up into two models given that 69% of the data were zeros and there was overdispersion in the non-zero count data (Zuur et al. 2010). The binomial portion of the model addresses the probability of having seeds or no seeds within a sample, while the count portion addresses the number of seeds within a sample,

given you have at least one seed in the sample (Zuur et al. 2010). We included location (binomial variance=1.285, count variance=0.359) as a random effect in the models to control for spatial variation. The conditional and marginal coefficients of determination for the binomial and negative binomial generalized mixed-effect models were calculated using the MuMIn package (r.squaredGLMM function).

Results

Plants in diets

Species composition

We identified 173 unique plant taxa in 465 rodent fecal samples across all seven sites (Table 1 in Appendix). Only 13% of these taxa were native to Hawai'i, whereas 45% were introduced and 42% were of unknown origin. Fifty plant taxa were assigned to species rank, 61 were identified only to genus, 45 to family, 14 to order, 2 to class, and one only to phylum rank. The five most frequently occurring families for all rodent species were Melastomataceae (n=327), Fabaceae (n=259), Myrtaceae (n=259), Arecaceae (n=223), and Rosaceae (n=187; Fig. 2). Melastomaceae was found in 72% (253/353) house mouse samples, 70% (60/86) of the black rat samples, and 54% (14/26) of the Pacific rat samples. We identified 23 plant taxa that were assigned to native Hawaiian plants including one family (Campanulaceae), 12 genera (*Antidesma*, *Cyrtandra*, *Diospyros*, *Korthalsella*, *Melicope*, *Myrsine*, *Peperomia*, *Pittosporum*, *Pritchardia*, *Psychotria*, *Santalum*, and *Scaevola*), and 10 species (*Alyxia stellata*, *Dianella sandwicensis*, *Erythrina sandwicensis*, *Labordia tinifolia*, *Nertera granadensis*, *Pipturus albidus*, *Planchonella sandwicensis*, *Smilax melastomifolia*, *Vaccinium calycinum*, and *Waltheria indica*). Three of the taxa are indigenous, while the rest are endemic. Substantially more introduced plant taxa were present in rodent diets; 78 plant taxa were assigned to plants introduced to Hawai'i including 9 families, 29 genera, and 40 species. Sequences from the remaining 72 plant taxa could only be assigned to taxonomic rank higher than family so could not be classified as native or introduced.

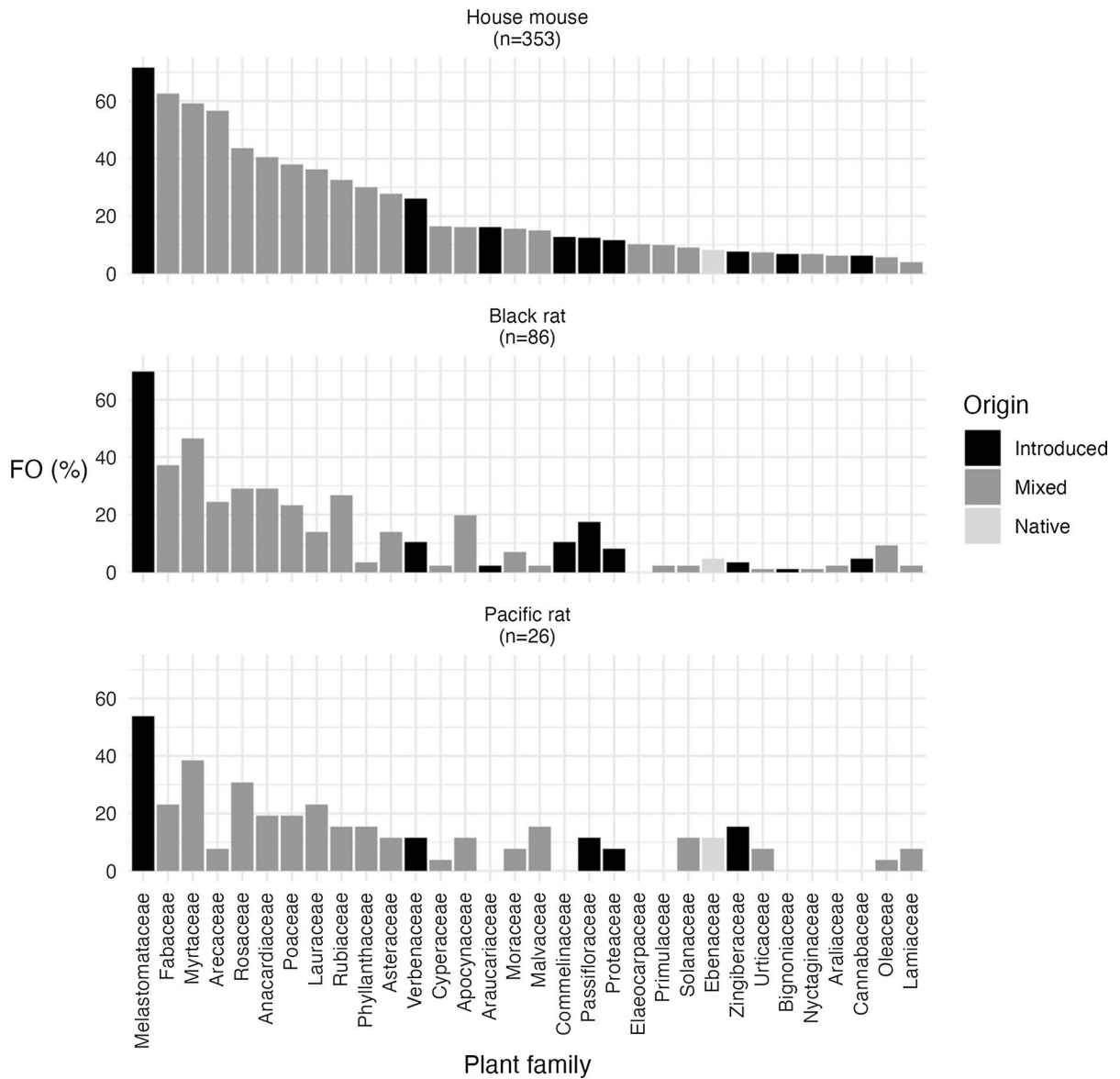


Fig. 2 Frequency of occurrence (FO in percentage) of fecal samples containing plant families ($n=32$ families) from house mouse (top), black rat (middle), and Pacific rat (bottom) at all site locations on O'ahu, Hawai'i from 2014 to 2016. The origin of plant taxa within each family is identified as introduced (black), mixed (dark gray) which includes families that

contained both native and introduced taxa or taxa of unknown status, and native (light gray). Data include non-rarefied taxonomic identification. Families that were identified in less than 5% of all rodent species' samples ($n=47$), and taxa identified at taxonomic rank above family were excluded

Alpha diversity

Mean taxa richness for plant species was 11.27 ± 0.28 SE per house mouse fecal sample, 8.13 ± 0.62 SE per black rat sample, and 6.35 ± 1.06 SE per Pacific rat

sample. However, the sample sizes were limited for black rats ($n=68$) and Pacific rats ($n=23$). Even for the larger sample size of 331 house mice, the species accumulation curve using plant taxa did not reach a clear asymptote, indicating the high richness of plant taxa in these rodent diets and that substantially more

sampling is needed to capture the full diversity of plants consumed (Supp. Figure 1).

Beta diversity

Plants in rodent diets had compositional differences, with significant main effects of rodent species (Jaccard; ADONIS: $R^2=0.01$; $P=0.001$) and site location (Jaccard; ADONIS: $R^2=0.08$; $P=0.001$). Samples did not distinctly visually cluster in the PCoA analysis (Supp. Figure 2a); however, there were significant differences between the diets of the house mouse and black rat (Jaccard; pairwise ADONIS: $R^2=0.01$; $P=0.0001$), and between the house mouse and Pacific rat (Jaccard; pairwise ADONIS: $R^2=0.01$; $P=0.0001$). The Pacific rat plant diet was not different from the diet of black rat (Jaccard; pairwise ADONIS: $R^2=0.01$; $P=0.240$). Plant diet samples for site location were distinctly clustered in the PCoA (Supp. Figure 2b). All 21 pairwise comparisons reflected significant diet differences by site location (Jaccard; pairwise ADONIS: $R^2 \geq 0.02$, all $P < 0.004$; Supp. Table 1). The interaction between location and rodent species was also significant, indicating a pattern of rodent diet differentiation within locations in addition to differentiation overall

(Jaccard; ADONIS: $R^2=0.03$; $P=0.002$). It must be noted, however, that the first two principal coordinate axes for both species comparisons and site comparisons explained less than 14% of the total variation.

Seed sorting

We sorted 435 fecal samples and counted 55,750 intact seeds, 98.8% of which were from *Miconia crenata* (syn. *Clidemia hirta*) and found primarily in house mouse and black rat fecal samples (Fig. 3). Rodent fecal samples contained from 0 to 10,915 intact *M. crenata* seeds, averaging 257.3 seeds per sample in the black rats (Fig. 3). Māmaki (*Pipturus albidus*) was the only native Hawaiian seed identified in the fecal samples and a total of 34 seeds were found in two black rat samples. The probability that black rat fecal samples would contain seeds was 0.49 (95% confidence interval (CI)=0.23–0.81; Fig. 4a) significantly higher ($P < 0.001$; Supp. Table 2) than the probability that house mouse fecal samples would contain seeds at 0.18 (CI=0.08–0.39; Fig. 4a). The probability that Pacific rat fecal samples would contain seeds was 0.30 (CI=0.11–0.67) which was not different compared to the black rat or

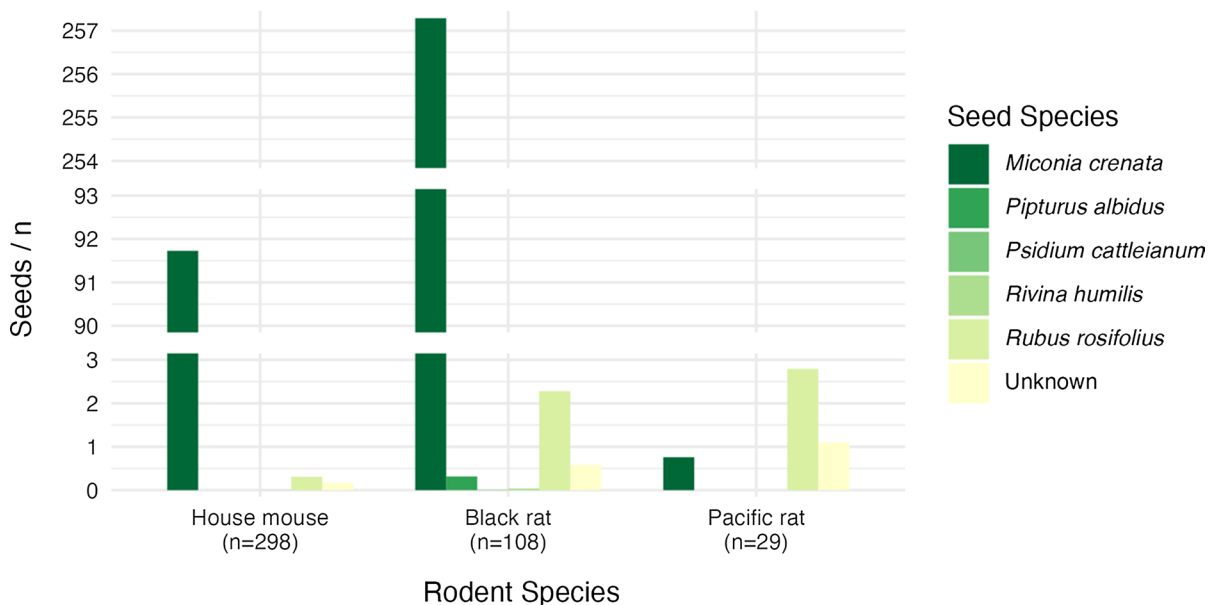


Fig. 3 Seed count per sample (seeds/n) for all intact plant species found in rodent fecal samples (note axis breaks). *Pipturus albidus* was the only native species identified

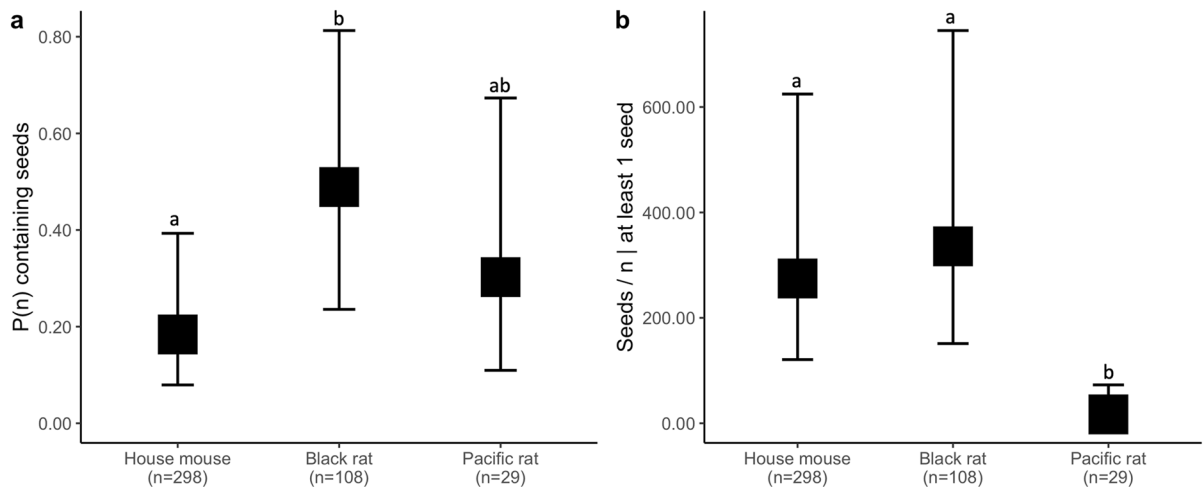


Fig. 4 The difference among rodent species across all sites in O'ahu in **a** the probability of a fecal sample ($P(n)$) containing at least one seed and **b** the mean number of seeds in a fecal sample (seeds/ n), given at least one seed was found. Results

are based on modeled output from generalized linear mixed models and 95% confidence intervals are shown. Rodent species that share the same letter are not significantly different ($P < 0.001$, see Supp. Table 2 for details)

house mouse (Supp. Table 2). Contrastingly, when comparing only samples that contained at least one seed, there was no statistical difference in the number of seeds per fecal sample between the house mouse (275 seeds, CI=121–643) and black rat (336 seeds, CI=151–745; Fig. 4b, Supp. Table 2). Samples from Pacific rats contained very few seeds (17 seeds, CI=4–73) which was significantly different when compared to both black rat and house mouse samples ($P < 0.001$; Supp. Table 2).

Invertebrates in diets

Species composition

We identified 60 unique invertebrate taxa in 216 rodent fecal samples from the seven sites (Table 2 in Appendix). Only 10% of the taxa were native to Hawai'i, whereas 43% were introduced and 47% were of unknown origin. Of the 60 taxa, 11 invertebrates were identified to species rank, 19 were identified only to genus, 15 to family, 8 to order, 5 to class, 1 to phylum, and 1 only to kingdom rank. Lepidoptera (butterflies and moths) was by far the most frequently occurring order, present in 73% (137/188) house mouse samples, 73% (16/22) of the black rat samples,

and 100% (6/6) of the Pacific rat samples (Fig. 5). Diptera (flies) was the second most frequently occurring invertebrate order, present in 36% (68/188) house mouse samples and 36% (8/22) of black rat samples. Six invertebrate taxa, one of which was identified to family and five that were identified to genus came from taxonomic groups native to Hawai'i, including two genera of Lepidoptera (*Schrankia*, *Carposina*), one family (Mycetophilidae) and one genus (*Dicranomyia*) of Diptera, and two genera of Achatinellidae (the Hawaiian land snails, *Auriculella* and *Elasmias*). Twenty-six taxa were assigned to invertebrate groups that were introduced to Hawai'i including 11 assigned to species rank, 12 only to genus rank, and 3 to family rank. The remaining 28 invertebrate taxa had unknown origin due to classification that was too broad to allow for categorization as native or introduced.

Alpha diversity

Mean invertebrate taxa richness per sample was 4.10 ± 0.13 for house mice, 3.15 ± 0.38 for black rats, and 4.00 ± 0.85 for Pacific rats. However, similar to the plants, the species accumulation curve using invertebrate taxa did not show a clear asymptote, indicating

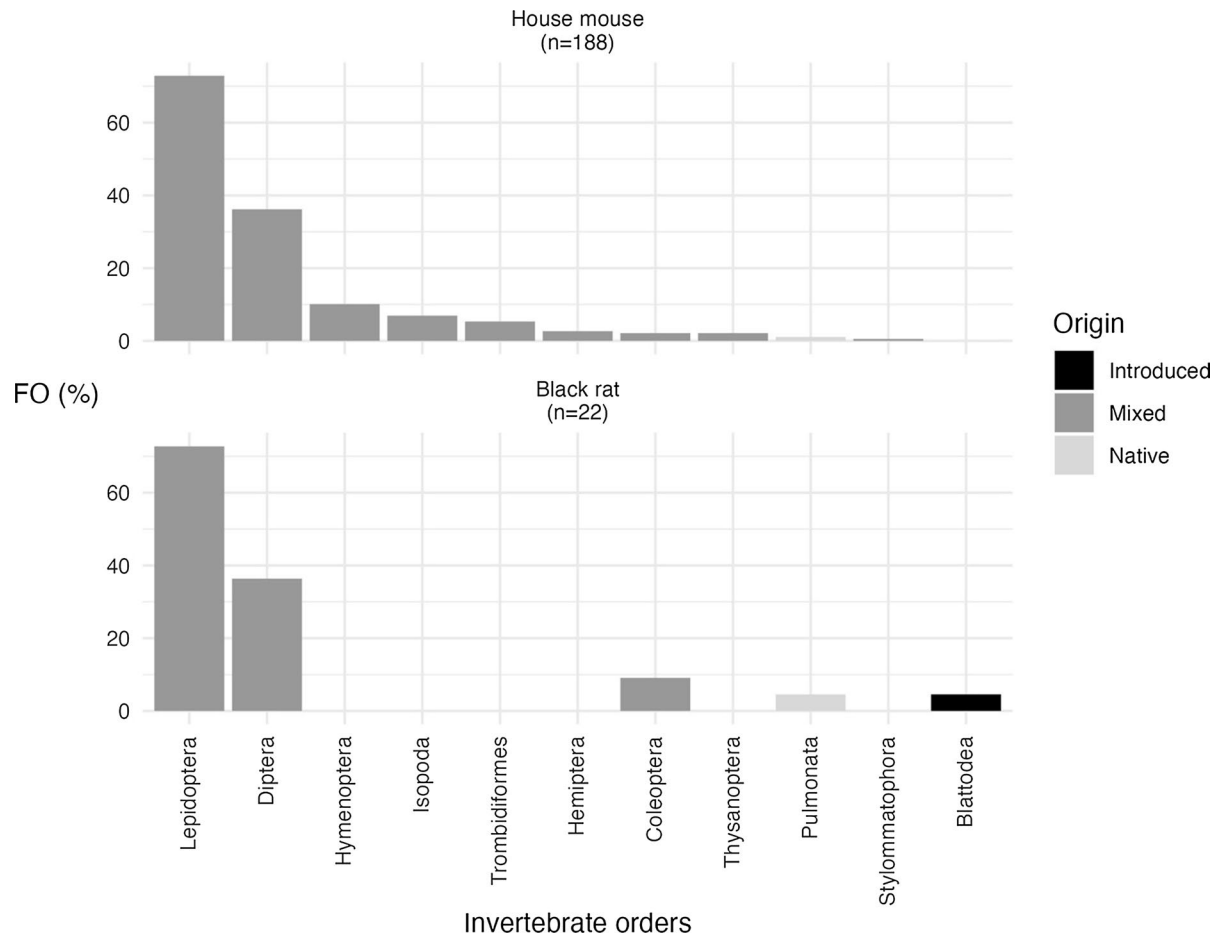


Fig. 5 Frequency of occurrence (FO in percentage) of fecal samples containing invertebrate orders ($n=11$) from house mouse (top) and black rat (bottom) at all sites on O'ahu, Hawai'i 2014–2016. The origin of invertebrate taxa within each order is identified as introduced (black), mixed (dark gray) which includes orders that contained both native and

introduced taxa or taxa of unknown status, and native (light gray). All invertebrate taxa identified to ranks above order were excluded from this figure. Data include non-rarefied taxonomic identification. All the six Pacific rat samples (100%) contained only diet items of the order Lepidoptera

the high variety of invertebrates in these rodent diets and that substantially more sampling is needed to capture the full diversity of prey taxa (Supp. Figure 1).

Beta diversity

Invertebrate taxa in the diets of rodents significantly differed by rodent species (Jaccard; ADONIS: $R^2=0.03$; $P=0.002$) and site location (Jaccard; ADONIS: $R^2=0.08$; $P=0.001$). Although samples did not distinctly cluster in the PCoA analysis (Supp. Figure 3a), the diets containing invertebrates were

significantly different between the house mouse and black rat (Jaccard; pairwise: $R^2=0.02$; $P=0.003$). The Pacific rat diet was not different from either the house mouse or black rat, however, there were four Pacific rat samples. Samples did not cluster in the PCoA for site location comparisons (Supp. Figure 3b). Most sites were different from one another with 13 of 21 pairwise comparisons revealing significant diet differences (Jaccard; pairwise ADONIS: $R^2 \geq 0.02$, all $P < 0.04$; Supp. Table 1) and there was no significant interaction between location and rodent species.

Discussion

Our study provides an in-depth assessment of the diets of invasive rodents in O'ahu forests and suggests key conservation considerations for rodent management. Our taxonomically detailed analyses provide critical missing information to investigate the potential impacts of rodents on Pacific islands and beyond. DNA metabarcoding revealed that the majority of taxa of plants and invertebrates consumed by rodents were introduced as opposed to native species; however, some native taxa of conservation importance were depredated by the rodents. This is starkly different compared to other metabarcoding diet analyses for house mice and black rats on Atlantic islands which found only 5–19% of diet items were introduced (Pomeda-Gutiérrez et al. 2021; Pinho et al. 2022), but aligns with previous morphological studies in Hawai'i (Shiels et al. 2013). We found only weak support for diet differentiation among rodent species and site locations, contrary to our hypothesis of niche and geographic differences based upon non-molecular studies (Shiels et al. 2013). However, our sample sizes prevented rigorous examination of these effects. The seeds of small seeded invasive plants are readily defecated by black rats, consistent with previous studies, and by house mice, contrary to previous studies, with notable implications for seed dispersal (Williams et al. 2000; Shiels 2010; Shiels et al. 2013). Given the potential for house mice to disperse invasive plants and the high numbers of house mice captured, it is important to consider the negative conservation impacts of house mice when identifying rodent control and eradication goals. Due to the considerable component of diet items from introduced taxa in all rodent species' diets, we recommend careful monitoring of introduced species after rodent control to ensure non-target introduced species are not released from predation pressure and causing additional detriment to native species (Caut et al. 2007).

Plants in rodent diets

Invasive rodents are eating a wide diversity of plant diet items primarily comprised of introduced species. Of the 80 plant families identified, more

than half were found in less than 5% of samples from all rodent species' fecal samples. The most frequently occurring plant families in each rodent species' diet include prominent invasive plants that in some cases account for the majority of occurrences of that taxon (see *Rubus rosifolius* in Rosaceae and *Cinnamomum burmanni* in Lauraceae). Melastomataceae was molecularly identified in 70% of all samples and was the plant taxon most frequently occurring in samples from each rodent species. Given the high species prevalence of *M. crenata* at our sites and visual detection of this species in most fecal samples, it is likely *M. crenata* accounts for most of the Melastomaceae molecular detections. There are no native Hawaiian Melastomataceae species, and species in the family, notably *M. crenata* and *M. calvescens*, are some of the most invasive and high priority species for management across the islands (Baruch et al. 2000; O'ahu Invasive Species Committee 2022). All of the most frequently consumed families, with the exception of Fabaceae, include fleshy-fruited introduced plant species that are known to be eaten by invasive rodents (Shiels 2010; Shiels and Drake 2015; Hays et al. 2018). Native plant taxa of note include the family Campanulaceae, genera *Cyrtandra* and *Melicope*, and the species *Labordia tinifolia* each found in 1–2 fecal samples. While these occurrences are low, each of these taxa include federally threatened and endangered species and subspecies in Hawai'i (U.S. Fish and Wildlife Service 2022). Further investigation to pinpoint the species is required to identify if the consumed taxa are federally protected.

Contrary to our hypothesis, rodent species and location accounted for only a small portion of diet variation. Based on previous rodent diet research on O'ahu, we anticipated diet differentiation by rodent species, reflecting possible niche partitioning for these sympatric invasive rodents (Shiels et al. 2013). Metabarcoding can identify differentiation based on the diet items, but this method cannot accurately identify the proportion of diet material contributed by each diet item, which was partially the basis for niche differentiation in Shiels et al. (2013). Using methods that combine diet item identification with the

proportion of each item in the diet, would allow for more robust testing of differentiation by rodent species, site location, and the interaction between these two factors (Stapleton et al. 2022). A weak interaction was found in our data between rodent species and site location, suggesting that dietary niche partitioning by species may be occurring at a given site, but that the rodents are also taking advantage of the resources available within that site based on opportunistic foraging. Further research and extensive sampling are necessary to tease apart these factors.

Overwhelmingly, invasive rodents are defecating intact seeds of small-seeded invasive plants, primarily *M. crenata*. This single species accounted for almost all intact seeds counted. Shiels et al. (2013) identified intact *M. crenata* seeds in 6.4%, 30.5%, and 25% of house mice, black rat, and Pacific rat samples, while our study found *M. crenata* seeds in 18.9%, 40.7%, and 3.4% of samples, respectively. Differences in fruiting phenology, seasonality, and plant composition may explain the variation between these studies in *M. crenata* seeds identified in each rodent species. *Rubus rosifolius* seeds were visually found intact in samples from all three rodent species, and the species was molecularly identified in nearly half of samples. Māmaki was the only intact native seed species identified within the fecal samples. Māmaki possesses traits often considered “weedy”, small widely-dispersed seeds and early successional growth in disturbed sites (Drake 1998; Cordell et al. 2009). This species was found in samples of all three rodent species molecularly, but intact seeds were identified only in black rat samples, possibly indicating that the Pacific rats and house mice are depredate māmaki seeds. All species found intact in the invasive rodent samples (invasive *M. crenata*, *R. rosifolius*, *Psidium cattleianum*, and *Rivina humilis* and native māmaki), are the same seeds commonly found intact within bird seed dispersers on O‘ahu (Vizentin-Bugoni et al. 2019). Similar to the rodents, the main avian seed dispersers in Hawai‘i are introduced species contributing to the spread of invasive plants and compounding challenges to control these plants (Foster and Robinson 2007; Pejchar 2015; Vizentin-Bugoni et al. 2019). That we identified these invasive seeds passing through the rodents intact aligns with previous work which found that *M. crenata*, *Miconia calvescens*, and

R. rosifolius seeds all successfully passed through rats intact (Medeiros 2004; Shiels 2010; Shiels and Drake 2011).

Historically, invasive rats and mice have been regarded primarily as seed predators (Clark 1982; Campbell and Atkinson 2002; Towns et al. 2006; Traveset et al. 2009); but research has identified some contributions to seed dispersal through transport to husking stations (McConkey et al. 2003; Hays et al. 2018), endozoochory (internal consumption and intact seed deposition; (Williams et al. 2000; Shiels 2010; Shiels and Drake 2011), and discarding diaspores containing intact seeds (Drake and McConkey 2021). This seed dispersal has been primarily limited to *Rattus* spp., with Williams et al. (2000) finding that house mice generally destroyed seeds upon consumption, though intact *M. crenata* seeds were identified within 6.4% of house mice stomach samples by Shiels et al. (2013). Surprisingly, our data show that while house mouse fecal samples are less likely to contain intact seeds, when samples did contain seeds, the number of seeds was similar to the number of seeds in black rat samples, likely contributing to the spread of invasive plants. No native seeds were visually identified within house mouse fecal samples, but native plants were identified in house mouse fecal samples molecularly. This suggests that any native seeds ingested were depredated, aligning with previous studies using molecular and non-molecular methods that have identified house mouse seed predation on native plants (Angel et al. 2009; Pinho et al. 2022). These data, coupled with the high number of house mice captured, indicate that mice may be having important and negative impacts on native ecosystems through the likely depredate of native seeds and dispersal of invasive plant seeds, similar to observations from other island systems (e.g., Angel et al. 2009; Pinho et al. 2022).

Invertebrates in rodent diets

The invertebrate taxa found in diets of invasive rodents on O‘ahu are primarily comprised of two orders, Lepidoptera and Diptera. All rodent species are eating Lepidoptera and this order includes the highest diversity of genera and species identified

in the fecal samples. Using visual identification of prey in stomachs, Shiels et al. (2013) also identified Lepidoptera with high frequency of samples from house mice and Pacific rats. In contrast, we found a higher frequency of Lepidoptera in black rat fecal samples (73%) compared to the 33.7% found by Shiels et al. (2013) possibly because soft-bodied Lepidoptera larvae can be difficult to identify through stomach contents analysis. However, Pomeda-Gutiérrez et al. (2021) found 10% frequency of occurrence for Lepidoptera in black rat fecal samples and 30% frequency of occurrence for Diptera using similar molecular approaches in the Canary Islands. This climate and habitat are very different from Hawai'i, which may contribute to the different invertebrate diets. Our frequencies of fecal samples containing Lepidoptera fall at the high end of the range of frequencies identified by Cole et al. (2000) for both house mice (37–78%) and black rat (38–100%) stomachs on Maui. We identified Diptera as the second most frequently occurring order in house mouse and black rat samples, but it did not occur in the few samples of Pacific rat we were able to analyze for the invertebrates. Shiels et al. (2013) found very low frequencies of rodent stomachs containing Diptera 8.5%, 2.1%, and 0%, for house mice, black rats, and Pacific rats respectively, that also may be due to the challenges of Diptera identification in stomach contents. However, Cole et al. (2000) found a range of 0–37.5% of stomach samples contained identifiable Diptera. The majority of the samples containing molecularly identified Diptera contain species of *Drosophila* (fruit flies), which may be consumed directly but are likely incidentally consumed, possibly in egg or larval life stages, as rodents consume fruits. Surprisingly, few samples containing Coleoptera and no samples containing Orthoptera were identified, which may be due to degradation through digestion. This is contrary to other studies examining stomachs and intestines that have found rodent diets to readily include both orders (Cole et al. 2000; Shiels et al. 2014; Ceia et al. 2017; Pinho et al. 2022). Pinho et al. (2022) identified degradation of invertebrate DNA through digestion, which decreased detection in the intestines compared to stomachs. Since our study examined rodent feces,

further degradation may lead to decreasing detection, particularly for invertebrates.

We identified 26 invertebrate taxa that were classified as introduced invertebrates and only six classified as native invertebrates. Many species of Hawaiian tree snails, including the entire *Achatinella* genus, are threatened or endangered and precipitous population declines have been linked in part to rat predation (Hadfield and Sauffer 2009). Of the six taxa classified as native invertebrates, two were genera of Hawaiian Achatinellid tree snails. Our data align with morphologically identified trends of invasive rodents eating introduced species previously reported on O'ahu (Shiels et al. 2013), but are at odds with studies on Maui (Cole et al. 2000) and recent molecular studies of the house mouse on other volcanic islands (Pinho et al. 2022). The omnivorous and opportunistic nature of invasive rodents likely take advantage of the resources available and, therefore, these differences in nativity of diet items may reflect the invertebrate community at a given site. None of these studies, ours included, surveyed the invertebrate community to identify the potential source of this trend. Future diet studies should include site surveys of possible food resources to compare diet composition and resource availability.

We found differences in the invertebrate composition among rodent species and sites, but these factors did not account for a large portion of the variation in rodent diet composition. Again this finding was contrary to our hypothesis and previous studies (Shiels et al. 2013). This lack of diet differentiation may be due to the generalist omnivorous diets of successful invasive rodents or it could be reflective of a high degree of intraspecific diet variation (Landry 1970; Bolnick et al. 2011). The strength of these comparisons was limited by variation in samples at site locations and among rodent species. In addition, the high species richness we identified in each rodent diet, suggests that future studies examining natural diet variation of such omnivorous species may require very large sample sizes to tease apart interspecific trends as well as temporal effects.

Limitations

Working with wild populations is key to establishing the ecological impact of these invasive rodents. Using diet data from free-ranging rodents increases variation and introduces limitations. The uneven sample sizes of rodent species across each site location makes diet comparisons challenging. Particularly the limited number of Pacific rat fecal samples may have contributed to the lack of invertebrate and plant diet differentiation. Past research, but also with small sample sizes, suggests that the Pacific rat occupies a dietary niche between the black rat and the house mouse, potentially explaining these results (Shiels et al. 2013). Nonetheless, we were surprised not to see more differentiation between the Pacific rat diets and those of the other species. Future research should target Pacific rats to ensure robust comparisons among rodent species. On the other hand, the limited number of Pacific rats in our sampling suggests that they play a much smaller role in these ecosystems than black rats or house mice.

The use of DNA metabarcoding has greatly expanded our ability to identify diet items at fine scale taxonomic levels. However, we encountered some limitations using this technique. We were not able to assign all sequences to species, and therefore some of our analyses compare diet items identified at broader taxonomic ranks. As similar studies contribute to fine-tuning both methodology (i.e., target genes and primers) and expanding genetic reference libraries, these limitations will diminish (Browett et al. 2020). Additionally, sequencing reads were not used to estimate abundance or proportion of diet material in samples because primer mismatches and DNA degradation during gut passage make sequencing reads an unreliable measure of abundance (Stapleton et al. 2022). We limited this potential for bias by using conservative presence/absence measures (e.g., frequency of taxa in samples, Jaccard similarity index) as opposed to read-based quantitative measures. While rats have been associated with declines in island vertebrates (Atkinson 1985; Towns et al. 2006), invertebrates and plants comprise the majority of invasive rodent diets on most islands and particularly in forested ecosystems (Shiels et al. 2014; Pomeda-Gutiérrez et al. 2021). Since this work

focused on identifying the main components of rodent diets, a primer set specifically targeting vertebrates was outside the scope of the study. However, our COI primers readily detect DNA from vertebrate taxa (JTF, unpublished data), such as a rat depredating or scavenging a bird, but none were found in the rodent diets in our study. Muletz-Wolz et al. (2021) detected bird DNA in rodent diets, suggesting that rodents in our study were not eating vertebrates as a primary food source. Future studies could use primers that specifically target vertebrates to identify the degree to which invasive rodents are scavenging or predated vertebrate species.

Conservation implications

The conservation implications of this work include: (1) house mice can defecate hundreds of intact invasive seeds, similar to black rats, (2) invasive rodents are largely eating introduced plants and invertebrates, and (3) invasive rodents are consuming some native Hawaiian species of conservation concern including endangered snails. While house mice are at least as ubiquitous on islands as other invasive mammals, only a fraction of conservation resources have been focused on mice compared to rats, cats, and ungulates (Angel et al. 2009, but see Polito et al. 2022). Particularly in areas with low or non-existent rat populations, house mice can have damaging and ecosystem-altering effects on islands comparable to rats (Angel et al. 2009). Our study suggests that both black rats and the house mice are potentially dispersing the seeds of invasive plants, likely contributing to the spread of problematic species such as *M. crenata*. In many restoration areas on O'ahu and more broadly islands globally, resources have been directed towards rat control, not house mouse control (Howald et al. 2007). In fact, at some of the sites included in this study, rat trapping to reduce populations is employed. Anecdotally mice are sometimes caught in the rat traps, but are not specifically targeted for control and may in fact increase in abundance when rats are removed (Barney et al. 2021). Our research shows that house mice have a surprising potential to disperse invasive plants, emphasizing the need to control both mice and rats.

Seed dispersal was historically accomplished by native birds but, with native frugivores largely extinct, we need to understand how introduced species function in this novel ecosystem (Foster and Robinson 2007). Previous work has shown that introduced birds partially fulfill the seed dispersal role by dispersing small seeded native plants, but that most dispersal is of introduced plants (Mandon-Dalger et al. 2004; Kelly et al. 2006; Culliney et al. 2012; Vizontin-Bugoni et al. 2019). Our work shows that many of the most commonly detected introduced (*M. crenata*, *R. rosifolius*) and native (māmaki) plants in the bird diets are the same species found as intact seeds in rodent fecal samples (Vizontin-Bugoni et al. 2019). These results further highlight the lack of dispersal of large-seeded species and the relatively small number of native species included in introduced frugivore diets.

In light of ongoing rodent control work in Hawai'i and on other islands globally, increasing our understanding of invasive rodent diets will help managers target beneficial results of rodent control and mitigate the potential for undesired outcomes. While our study was able to identify native species consumed by invasive rodents, we also found a need for more extensive sequence databases in Hawai'i for metabarcoding of native invertebrate and plant taxa. Metabarcoding reference library construction and management is an essential component (Robeson et al. 2021). Moreover, investing in the sampling and taxonomic expertise required to build these databases is paramount. We include here molecular evidence that rats and mice are eating endangered Hawaiian snails along with a handful of other native invertebrates and plants. Rodent population control and eradication are important conservation tools used to reduce predation and herbivory pressure on native species (St Clair et al. 2011; Spatz et al. 2022). However, rodent control and eradication may create undesired outcomes if complex trophic relationships among rodents and introduced plants and invertebrates are not accounted for, leading to predator release of additional introduced species (Zavaleta et al. 2001; Meyer and Shiels 2009; Millerter Kuile et al. 2021). Specifically, identifying trophic interactions and the functional roles that invasive

species play before control or eradication has been recommended to reduce undesired ecological changes (Zavaleta et al. 2001). This research provides taxonomic details of rodent diets previously undescribed and clarifies the functional role of house mouse seed dispersal. By using information on diet and functional role and monitoring impacts after rodent control or eradication, desired outcomes can be better targeted.

Author contributions JTF, DRD, JHS, JPK, and CET conceived the study. All authors contributed to data collection. SMEG and RLM analyzed data. SMEG wrote the first drafts of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. The sequences generated during and/or analyzed during the current study will be available in GenBank.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

See Table 1.

Table 1 List of plant taxa found in fecal samples of all rodents at all sites on O‘ahu, Hawai‘i, from 2014 to 2016. The number of samples (N) within which each taxon was identified, species of rodent and site (EKA, ‘EkaHanui; KAH, Kahanahāiki; MOA, Moanahā Valley; MTK, Mt. Ka‘ala; PAH, Pahole; TAN, Tantalus; WAI, Waimea Valley) where the sample was identified are noted. Nativity of the taxon is noted if taxon was identified to a level at which its status can be concluded, otherwise unknown (unk). Whether the species produces fleshy fruit (FF) and if the seeds of that species were found intact in any samples are noted. Sequences identified as phylum Magnoliophyta were found in 379 samples in all rodent species at all sites

Order	Family	Genus	Species	N/465	House mouse	Black rat	Pacific rat	Site	Native FF species	Intact seeds
<i>Class: Liliopsida</i> (unassigned at family rank)										
Arecales	Arecaceae			34	X	X	X	ALL	unk	
				210	X	X		EKA, KAH, MOA, PAH, TAN, WAI	unk	
Arecales	Arecaceae	<i>Pritchardia</i>		13	X	X		MTK	Yes	No
Asparagales	Asparagaceae	<i>Asparagus</i>		9	X	X		KAH, MTK, TAN	unk	
Asparagales	Asphodelaceae	<i>Dianella</i>	<i>sandwicensis</i>	1	X			KAH	unk	
Asparagales	Iridaceae			12	X	X	X	KAH, MTK, PAH, TAN	Yes	No
Asparagales	Orchidaceae			1	X			MTK	No	
Commelinales	Commelinaceae			1	X			WAI	No	
Commelinales	Commelinaceae	<i>Commelina</i>		2	X			PAH	No	
Commelinales	Commelinaceae			54	X	X		ALL	No	
Dioscoreales	Dioscoreaceae	<i>Dioscorea</i>		3	X	X		MOA	No	
Liliales	Smilacaceae	<i>Smilax</i>	<i>melastomifolia</i>	1	X	X		MTK	Yes	Yes
Pandanales	Pandanaceae			12	X	X		KAH, MTK, TAN	unk	
Poales	Cyperaceae			2	X	X		KAH, PAH	unk	
Poales	Cyperaceae	<i>Carex</i>		4	X	X	X	KAH, MTK, TAN	unk	
Poales	Cyperaceae			16	X	X		KAH	unk	
Poales	Cyperaceae	<i>Cyperus</i>		43	X			EKA, KAH, MOA, MTK, TAN	unk	
Poales	Juncaceae	<i>Juncus</i>		12	X	X		KAH, MTK, PAH, TAN	unk	
Poales	Juncaceae	<i>Juncus</i>	<i>bufonius</i>	2	X			MTK	No	No
Poales	Poaceae			97	X	X	X	ALL	unk	
Poales	Poaceae	<i>Andropogon</i>	<i>virginicus</i>	9	X	X		KAH, MOA, MTK, WAI	No	No
Poales	Poaceae	<i>Axonopus</i>	<i>fissifolius</i>	7	X	X	X	MOA, MTK, PAH	No	No
Poales	Poaceae	<i>Lolium</i>		1		X		KAH	No	
Poales	Poaceae	<i>Oplismenus</i>		1	X			TAN	No	
Poales	Poaceae	<i>Paspalum</i>		66	X	X	X	ALL	No	
Poales	Poaceae	<i>Paspalum</i>	<i>conjugatum</i>	6	X			KAH, PAH, TAN	No	No
Poales	Poaceae	<i>Poa</i>		2	X			MTK	unk	
Poales	Poaceae	<i>Setaria</i>	<i>palmifolia</i>	32	X	X		ALL	No	No
Poales	Poaceae	<i>Urochloa</i>	<i>mutica</i>	6	X	X	X	EKA, KAH	No	No

Table 1 (continued)

Order	Family	Genus	Species	N/465	House mouse	Black rat	Pacific rat	Site	Native	FF species	Intact seeds
Zingiberales	Heliconiaceae	<i>Heliconia</i>		1	X			MOA	No		
Zingiberales	Musaceae	<i>Musa</i>		13	X	X	X	KAH, MOA, MTK, PAH, TAN, WAI	No		
Zingiberales	Zingiberaceae			14	X	X	X	MOA, TAN	No		
Zingiberales	Zingiberaceae	<i>Alpinia</i>		32	X	X	X	EKA, KAH, MOA, TAN	No		
Class: <i>Magnoliopsida</i> (unassigned at family rank)											
Apiales				314	X	X	X	ALL	unk		
Apiales	Apiaceae			1		X	X	MOA	unk		
Apiales	Apiaceae	<i>Centella</i>	<i>asiatica</i>	4	X	X	X	KAH, MTK, WAI	unk		
Apiales	Araliaceae			1	X			WAI	No	No	
Apiales	Pittosporaceae	<i>Pittosporum</i>		24	X	X	X	EKA, MOA, MTK, TAN	unk		
Aquifoliales	Aquifoliaceae	<i>Ilex</i>		9	X	X	X	KAH, PAH, TAN	Yes	Yes	No
Asterales				3	X			TAN	unk		
Asterales				1	X			KAH	unk		
Asterales	Asteraceae			78	X	X	X	KAH, MOA, MTK, PAH, TAN, WAI	unk		
Asterales	Asteraceae	<i>Achillea</i>	<i>millefolium</i>	1	X			MTK	No	No	
Asterales	Asteraceae	<i>Bidens</i>		45	X	X	X	EKA, KAH, PAH, TAN, WAI	unk		
Asterales	Asteraceae	<i>Emilia</i>		1		X		WAI	No		
Asterales	Asteraceae	<i>Emilia</i>	<i>fosbergii</i>	6	X	X	X	KAH, WAI	No	No	
Asterales	Asteraceae	<i>Erigeron</i>		19	X	X	X	KAH, MTK	No		
Asterales	Asteraceae	<i>Montanoa</i>	<i>hibiscifolia</i>	2	X			KAH	No	No	
Asterales	Campanulaceae			1	X			TAN	Yes		
Asterales	Goodeniaceae	<i>Scaveola</i>		4	X			KAH	Yes	Yes	No
Brassicales	Brassicaceae			4	X			MOA, TAN	unk		
Brassicales	Brassicaceae	<i>Cardamine</i>	<i>flexuosa</i>	3	X	X	X	TAN	No	No	
Brassicales	Caricaceae	<i>Carica</i>	<i>papaya</i>	4	X			KAH	No	Yes	No
Caryophyllales				5	X			EKA	unk		
Caryophyllales	Amaranthaceae	<i>Chenopodium</i>		2	X			KAH	unk		
Caryophyllales	Caryophyllaceae			2		X		KAH	unk		
Caryophyllales	Caryophyllaceae	<i>Cerastium</i>	<i>fontanum</i>	2	X			MTK	No	No	
Caryophyllales	Nyctaginaceae			25	X	X	X	KAH, MTK, PAH, TAN, WAI	unk		

Table 1 (continued)

Order	Family	Genus	Species	N/465	House mouse	Black rat	Pacific rat	Site	Native FF species	Intact seeds
Caryophyllales	Phytolaccaceae	<i>Rivina</i>	<i>humilis</i>	15	X	X	X	EKA, KAH, TAN	No	Yes
Cucurbitales	Cucurbitaceae			2	X			KAH, MOA	unk	
Cucurbitales	Cucurbitaceae			6	X	X	X	MOA, MTK, PAH, TAN	unk	
Ericales	Ebenaceae	<i>Diospyros</i>		1	X			KAH	unk	
Ericales	Ericaceae	<i>Vaccinium</i>	<i>calycinum</i>	36	X	X	X	KAH, MOA, PAH, TAN	Yes	No
Ericales	Primulaceae			6	X			MTK	Yes	No
Ericales	Primulaceae	<i>Ardisia</i>		1	X			TAN	unk	
Ericales	Primulaceae			36	X	X	X	KAH, MOA, MTK, TAN, WAI	No	No
Ericales	Primulaceae	<i>Myrsine</i>		1	X			KAH	Yes	No
Ericales	Sapotaceae	<i>Planchonella</i>	<i>sandwicensis</i>	20	X	X	X	KAH, PAH, WAI	Yes	No
Fabales	Fabaceae			169	X	X	X	ALL	unk	
Fabales	Fabaceae	<i>Chamaecrista</i>	<i>nicitans</i>	10	X	X		WAI	No	No
Fabales	Fabaceae	<i>Crotalaria</i>		5	X	X	X	MOA, MTK, TAN, WAI	No	
Fabales	Fabaceae	<i>Desmodium</i>		14	X	X		EKA, KAH, MTK, PAH, WAI	No	
Fabales	Fabaceae	<i>Erythrina</i>	<i>sandwicensis</i>	6	X	X		EKA, KAH, WAI	Yes	No
Fabales	Fabaceae	<i>Leucaena</i>	<i>leucocephala</i>	27	X	X	X	EKA, KAH, MOA, TAN, WAI	No	No
Fabales	Fabaceae	<i>Medicago</i>		2	X	X		MTK, PAH	No	
Fabales	Fabaceae	<i>Mimosa</i>	<i>pudica</i>	8	X	X		MOA, WAI	No	No
Fabales	Fabaceae	<i>Stylosanthes</i>	<i>viscosa</i>	119	X	X	X	ALL	No	No
Fagales	Casuarinaceae	<i>Casuarina</i>		10	X	X	X	KAH, TAN, WAI	No	No
Gentianales	Apocynaceae			1		X		KAH	unk	
Gentianales	Apocynaceae	<i>Alyxia</i>	<i>stellata</i>	2	X	X		KAH, PAH	unk	
Gentianales	Apocynaceae			77	X	X	X	EKA, KAH, MTK, PAH, TAN	Yes	No
Gentianales	Loganiaceae	<i>Labordia</i>	<i>tinifolia</i>	1	X			TAN	Yes	No
Gentianales	Rubiaceae			86	X	X	X	ALL	unk	
Gentianales	Rubiaceae	<i>Coffea</i>	<i>arabica</i>	25	X	X	X	EKA, KAH, PAH, TAN, WAI	No	No
Gentianales	Rubiaceae	<i>Nertera</i>	<i>granadensis</i>	62	X	X	X	KAH, MOA, MTK, PAH, WAI	Yes	No

Table 1 (continued)

Order	Family	Genus	Species	N/465	House mouse	Black rat	Pacific rat	Site	Native	FF species	Intact seeds
Gentianales	Rubiaceae	<i>Paederia</i>	<i>foetida</i>	8	X	X		KAH, MOA, MTK, TAN	No	Yes	No
Gentianales	Rubiaceae	<i>Psychotria</i>		16	X	X		KAH, PAH, TAN	Yes	Yes	No
Geraniales	Geraniaceae	<i>Geranium</i>		1	X			TAN	unk		
Lamiales				26	X	X		EKA, KAH, MOA, PAH, TAN, WAI	unk		
Lamiales	Bignoniaceae			25	X	X		EKA, KAH, PAH, TAN	No		
Lamiales	Gesneriaceae	<i>Cyrtandra</i>		1	X			TAN	Yes	Yes	No
Lamiales	Lamiaceae			1	X			MTK	unk		
Lamiales	Lamiaceae	<i>Hyptis</i>	<i>pectinata</i>	15	X	X		EKA, KAH, TAN	No	No	
Lamiales	Lamiaceae	<i>Ocimum</i>		2	X	X		EKA, TAN	No		
Lamiales	Oleaceae			29	X	X		EKA, KAH, PAH	unk		
Lamiales	Orobanchaceae	<i>Castilleja</i>	<i>arvensis</i>	18	X	X		KAH	No	No	
Lamiales	Plantaginaceae			2	X			KAH, TAN	unk		
Lamiales	Plantaginaceae	<i>Plantago</i>		5	X			KAH, PAH, TAN	unk		
Lamiales	Plantaginaceae	<i>Plantago</i>	<i>lanceolata</i>	18	X	X		KAH, PAH, TAN, WAI	No	No	
Lamiales	Scrophulariaceae			1	X			PAH	unk		
Lamiales	Verbenaceae			2	X			KAH, MOA	unk		
Lamiales	Verbenaceae	<i>Citharexylum</i>	<i>caudatum</i>	39	X	X		KAH, MOA, PAH, TAN	No	Yes	No
Lamiales	Verbenaceae	<i>Stachytarpheta</i>		67	X	X		KAH, MOA, MTK, PAH, WAI	No		
Lamiales	Verbenaceae	<i>Stachytarpheta</i>	<i>caymensis</i>	3	X			MOA, WAI	No	No	
Laurales	Lauraceae			37	X	X		EKA, KAH, MOA, PAH, TAN	unk		
Laurales	Lauraceae	<i>Cinnamomum</i>	<i>burmanni</i>	135	X	X		ALL	No	Yes	No
Magnoliales	Annonaceae	<i>Annona</i>		1	X			TAN	No		
Malpighiales	Euphorbiaceae			2	X	X		KAH	unk		
Malpighiales	Euphorbiaceae	<i>Euphorbia</i>		5	X	X		EKA, KAH, PAH	unk		
Malpighiales	Euphorbiaceae	<i>Macaranga</i>		2	X			KAH	unk		
Malpighiales	Euphorbiaceae	<i>Manihot</i>		3	X	X		EKA, KAH, WAI	No		
Malpighiales	Hypericaceae	<i>Hypericum</i>		2	X			EKA, MOA	No		
Malpighiales	Ochnaceae	<i>Ochna</i>		2	X	X		TAN, WAI	No		
Malpighiales	Ochnaceae			2	X	X		MOA	unk		

Table 1 (continued)

Order	Family	Genus	Species	N/465	House mouse	Black rat	Pacific rat	Site	Native FF species	Intact seeds
Malpighiales	Passifloraceae	<i>Passiflora</i>		62	X	X	X	EKA, KAH, MTK, PAH, TAN, WAI	No	No
Malpighiales	Phyllanthaceae			8	X			KAH, TAN	unk	
Malpighiales	Phyllanthaceae	<i>Antidesma</i>		9	X			KAH, PAH, TAN	Yes	No
Malpighiales	Phyllanthaceae	<i>Bischofia javanica</i>		109	X	X	X	ALL	No	No
Malpighiales	Salicaceae			2	X			MOA, PAH	unk	
Malvales	Malvaceae			21	X	X	X	EKA, KAH, MOA, MTK, PAH, TAN	unk	
Malvales	Malvaceae	<i>Hibiscus</i>		30	X			TAN	unk	
Malvales	Malvaceae	<i>Sida</i>		11	X			KAH, PAH	unk	
Malvales	Malvaceae	<i>Waltheria indica</i>		3	X			WAI	Yes	No
Myrtales	Combretaceae			1	X			KAH	unk	
Myrtales	Combretaceae			1	X			TAN	No	
Myrtales	Combretaceae	<i>Terminalia</i>		6	X			TAN	No	
Myrtales	Lythraceae	<i>Cuphea carthagenensis</i>		10	X			MTK, TAN	No	No
Myrtales	Melastomataceae			327	X	X	X	ALL	No	
Myrtales	Melastomataceae	<i>Miconia</i>		12	X	X		KAH, PAH, TAN, WAI	No	No
Myrtales	Melastomataceae	<i>Miconia crenata</i>		1		X		WAI	No	Yes
Myrtales	Myrtaceae			244	X	X	X	ALL	unk	
Myrtales	Myrtaceae	<i>Melaleuca quinquinervia</i>		3	X			EKA, KAH	No	No
Myrtales	Myrtaceae	<i>Syzygium</i>		24	X	X	X	MOA, MTK, PAH, TAN, WAI	unk	
Oxalidales				1	X			PAH	unk	
Oxalidales	Elaeocarpaceae	<i>Elaeocarpus</i>		36	X			KAH, TAN	unk	
Oxalidales	Oxalidaceae	<i>Oxalis</i>		4	X			KAH, MTK, PAH	No	
Piperales	Piperaceae	<i>Peperomia</i>		2	X	X		MTK	Yes	No
Proteales	Proteaceae			4	X	X		KAH, PAH, WAI	No	
Proteales	Proteaceae	<i>Grevillea robusta</i>		48	X	X	X	ALL	No	No
Proteales	Proteaceae	<i>Macadamia integrifolia</i>		1		X	X	EKA	No	No
Rosales				2	X			MOA, TAN	unk	
Rosales	Cannabaceae			3	X			MOA	No	
Rosales	Cannabaceae	<i>Trema orientalis</i>		26	X	X		EKA, KAH, MOA, MTK, TAN, WAI	No	Yes

Table 1 (continued)

Order	Family	Genus	Species	N/465	House mouse	Black rat	Pacific rat	Site	Native FF species	Intact seeds	
Rosales	Moraceae	<i>Ficus</i>		14	X	X	X	EKA, KAH, TAN, WAI	unk		
Rosales	Moraceae	<i>Ficus</i>		50	X	X	X	KAH, MOA, MTK, PAH, TAN, WAI	No	No	
Rosales	Rosaceae			2	X			KAH	No		
Rosales	Rosaceae	<i>Prunus</i>		1	X			KAH	unk		
Rosales	Rosaceae	<i>Rubus</i>		9	X	X	X	MTK	No	Yes	
Rosales	Rosaceae	<i>Rubus</i>	<i>rosifolius</i>	178	X	X	X	ALL	No	Yes	
Rosales	Urticaceae			1	X			TAN	unk		
Rosales	Urticaceae	<i>Cecropia</i>	<i>obtusifolia</i>	3	X	X	X	TAN	No	No	
Rosales	Urticaceae	<i>Pipturus</i>	<i>albidus</i>	27	X	X	X	KAH, PAH, TAN	Yes	Yes	
Santalales	Santalaceae	<i>Korhalsella</i>		4	X			KAH	Yes		
Santalales	Santalaceae	<i>Santalum</i>		14	X	X	X	KAH, PAH	Yes	No	
Sapindales				5	X			EKA, KAH, PAH	unk		
Sapindales	Anacardiaceae			170	X	X	X	ALL	unk		
Sapindales	Anacardiaceae	<i>Mangifera</i>	<i>indica</i>	5	X	X	X	MOA, TAN	No	No	
Sapindales	Meliaceae			1	X	X	X	KAH	unk		
Sapindales	Rutaceae	<i>Melicope</i>		2	X	X	X	MTK	Yes		
Sapindales	Sapindaceae			1	X	X	X	WAI	unk		
Solanales	Convolvulaceae			10	X	X	X	MOA, TAN	unk		
Solanales	Convolvulaceae	<i>Ipomoea</i>		1	X	X	X	EKA	unk		
Solanales	Convolvulaceae	<i>Ipomoea</i>	<i>alba</i>	8	X	X	X	TAN	No	No	
Solanales	Solanaceae			5	X	X	X	KAH, TAN	unk		
Solanales	Solanaceae	<i>Capsicum</i>	<i>frutescens</i>	5	X	X	X	MOA, MTK, PAH, WAI	No	No	
Solanales	Solanaceae	<i>Cestrum</i>		2	X	X	X	KAH	No	No	
Solanales	Solanaceae	<i>Cestrum</i>	<i>nocturnum</i>	25	X	X	X	TAN	No	No	
Solanales	Solanaceae	<i>Solanum</i>		2	X	X	X	KAH, WAI	unk		
Vitales	Vitaceae			2	X	X	X	KAH	unk		
		Class: <i>Pinidae</i> (unassigned at family rank)									
Pinales				75	X	X	X	ALL	unk		
Pinales	Araucariaceae	<i>Araucaria</i>	<i>columnaris</i>	59	X	X	X	ALL	No	No	
Pinales	Cupressaceae	<i>Juniperus</i>	<i>bermudiana</i>	1	X	X	X	EKA	No	No	

Table 2 List of invertebrate taxa in fecal samples of all rodents at all sites on O'ahu, Hawai'i from 2014 to 2016. The number of samples (N) within which each invertebrate taxa was identified, species of rodent and site (EKA, 'Ekahanui; KAH, Kahanahāiki; MOA, Moanalua Valley; MTK, Mt. Ka'ala; PAH, Pahole; TAN, Tantalus; WAI, Waimea Valley) where the sample was identified are noted. Nativity of the taxon is noted if identified to a level at which its status can be concluded, otherwise unknown (unk). Sequences assigned to kingdom Animalia were found in 16 samples in all rodent species at six of the seven sites

Class	Order	Family	Genus	Species	N/216	House mouse	Black rat	Pacific rat	Sites	Native
<i>Phylum: Arthropoda</i>										
Arachnida					150	X	X	X	ALL	unk
Arachnida		Trombidiformes			12	X	X	X	EKA, KAH, MOA, PAH, TAN	unk
Arachnida		Trombidiformes			4	X			EKA, TAN, WAI	unk
Arachnida		Trombidiformes			1	X			EKA	No
Arachnida		Trombidiformes			5	X			EKA, KAH, TAN	No
Collembola					2	X		X	MTK, TAN	unk
Insecta					66	X	X	X	ALL	unk
Insecta	Blattodea	Ectobiidae	<i>Balta</i>	<i>notulata</i>	1		X		WAI	No
Insecta	Coleoptera				2	X			KA	unk
Insecta	Coleoptera	Nitidulidae			4	X	X		WAI	unk
Insecta	Diptera				27	X	X	X	EKA, KAH, MOA, PAH, TAN, WAI	unk
Insecta	Diptera	Drosophilidae	<i>Drosophila</i>		59	X	X		ALL	unk
Insecta	Diptera	Drosophilidae	<i>Drosophila</i>	<i>kikkawai</i>	5	X	X		MOA, TAN, WAI	No
Insecta	Diptera	Drosophilidae	<i>Drosophila</i>	<i>simulans</i>	3	X	X		KAH, PAH	No
Insecta	Diptera	Drosophilidae	<i>Drosophila</i>	<i>suzukii</i>	1	X			WAI	No
Insecta	Diptera	Limoniidae	<i>Dicranomyia</i>		1	X			WAI	Yes
Insecta	Diptera	Mycetophilidae			1	X			KAH	Yes
Insecta	Diptera	Psychodidae			2	X			TAN, WAI	unk
Insecta	Diptera	Psychodidae	<i>Psychoda</i>		1	X			TAN	unk
Insecta	Diptera	Tachinidae	<i>Arehytas</i>		1	X			MTK	No
Insecta	Diptera	Tephritidae	<i>Bactrocera</i>		10	X	X		KAH, PAH, WAI	No
Insecta	Hemiptera				2	X			KAH, MTK	unk
Insecta	Hemiptera	Cydnidae			2	X			WAI	No
Insecta	Hemiptera	Delphacidae			2	X			TAN	unk
Insecta	Hymenoptera				1	X			EKA	unk
Insecta	Hymenoptera	Braconidae			10	X			KAH, PAH, TAN	unk
Insecta	Hymenoptera	Braconidae	<i>Meteorus</i>		9	X			KAH, MTK, PAH, TAN	No
Insecta	Lepidoptera				121	X	X	X	ALL	unk
Insecta	Lepidoptera	Carposinidae	<i>Carpocostana</i>		13	X	X		KAH, MOA, WAI	Yes
Insecta	Lepidoptera	Dryadulidae	<i>Dryadula</i>	<i>terpsichorella</i>	1	X			KAH	No

Table 2 (continued)

Class	Order	Family	Genus	Species	N/216	House mouse	Black rat	Pacific rat	Sites	Native
Insecta	Lepidoptera	Erebidae			6	X			KAH, TAN	unk
Insecta	Lepidoptera	Erebidae	<i>Ascalapha</i>	<i>odorata</i>	2	X			KAH	No
Insecta	Lepidoptera	Erebidae	<i>Eublemma</i>		1	X			WAI	No
Insecta	Lepidoptera	Erebidae	<i>Eublemma</i>	<i>accedens</i>	1	X			WAI	No
Insecta	Lepidoptera	Erebidae	<i>Schrankia</i>		18	X	X		KAH, MTK, PAH, TAN	Yes
Insecta	Lepidoptera	Geometridae			2	X			MTK, TAN	unk
Insecta	Lepidoptera	Lycanidae			2	X	X		KAH, WAI	unk
Insecta	Lepidoptera	Noctuidae			7	X			EKA, MTK, TAN	unk
Insecta	Lepidoptera	Noctuidae	<i>Athetis</i>		1	X			WAI	No
Insecta	Lepidoptera	Noctuidae	<i>Athetis</i>	<i>thoracica</i>	18	X	X	X	EKA, KAH, MOA, PAH, TAN, WAI	No
Insecta	Lepidoptera	Noctuidae	<i>Callopietria</i>		3	X			PAH, WAI	No
Insecta	Lepidoptera	Noctuidae	<i>Peridroma</i>	<i>saucia</i>	2	X			KAH	No
Insecta	Lepidoptera	Sphingidae			1		X		WAI	unk
Insecta	Lepidoptera	Sphingidae	<i>Psilogramma</i>		1	X			KAH	No
Insecta	Lepidoptera	Tineidae	<i>Opogona</i>	<i>omoscopa</i>	41	X	X	X	KAH, MTK, PAH, TAN	No
Insecta	Lepidoptera	Tortricidae			1		X		TAN	unk
Insecta	Lepidoptera	Tortricidae	<i>Amorbia</i>		4	X	X		EKA, KAH, PAH	No
Insecta	Lepidoptera	Tortricidae	<i>Archips</i>		1	X			WAI	No
Insecta	Lepidoptera	Tortricidae	<i>Platynota</i>		2	X			KAH	No
Insecta	Thysanoptera				2	X			KAH, TAN	unk
Insecta	Thysanoptera	Phlaeothripidae			1	X			TAN	unk
Insecta	Thysanoptera	Phlaeothripidae	<i>Haplothrips</i>		1	X			TAN	No
Insecta	Thysanoptera	Thripidae	<i>Frankliniella</i>		1	X			KAH	No
Malacostraca	Isopoda				154	X	X	X	ALL	unk
Malacostraca	Isopoda	Philosciidae	<i>Burmoniscus</i>	<i>okinawaensis</i>	3	X			MTK, TAN	unk
Malacostraca	Isopoda	Philosciidae	<i>Burmoniscus</i>	<i>okinawaensis</i>	10	X			EKA, KAH, PAH	No
<i>Phylum: Mollusca</i>					0					
Gastropoda					2	X			EKA, MOA	unk
Gastropoda	Pulmonata	Achatinellidae	<i>Auriculella</i>		2	X	X		PAH, MTK	Yes
Gastropoda	Pulmonata	Achatinellidae	<i>Elasmias</i>		1	X			TAN	Yes
Gastropoda	Stylommatophora				1	X			EKA	unk

References

- Anderson MJ, Crist TO, Chase JM et al (2011) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecol Lett* 14:19–28. <https://doi.org/10.1111/j.1461-0248.2010.01552.x>
- Angel A, Wanless RM, Cooper J (2009) Review of impacts of the introduced house mouse on islands in the Southern Ocean: are mice equivalent to rats? *Biol Invasions* 11:1743–1754. <https://doi.org/10.1007/s10530-008-9401-4>
- Aslan CE, Zavaleta ES, Tershy B, Croll D (2013) Mutualism disruption threatens global plant biodiversity: a systematic review. *PLoS ONE* 8:e66993. <https://doi.org/10.1371/journal.pone.0066993>
- Atkinson IAE (1985) The spread of commensal species of *Rattus* to oceanic islands and their effects on island avifauna. In: Conservation of island birds. pp 35–81
- Barney SK, Leopold DR, Francisco K et al (2021) Successful management of invasive rats across a fragmented landscape. *Environ Conserv* 48:200–207. <https://doi.org/10.1017/S0376892921000205>
- Baruch Z, Pattison RR, Goldsteint G (2000) Responses to light and water availability of four invasive Melastomataceae in the Hawaiian Islands. *Int J Plant Sci* 161:107–118. <https://doi.org/10.1086/314233>
- Bell KL, Loeffler VM, Brosi BJ (2017) An *rbcL* reference library to aid in the identification of plant species mixtures by DNA metabarcoding. *Appl Plant Sci* 5:1600110. <https://doi.org/10.3732/apps.1600110>
- Bokulich NA, Kaehler BD, Rideout JR et al (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6:90. <https://doi.org/10.1186/s40168-018-0470-z>
- Bolnick DI, Amarasekare P, Araújo MS et al (2011) Why intraspecific trait variation matters in community ecology. *Trends Ecol Evol* 26:183–192
- Bolyen E, Rideout JR, Dillon MR et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 37:852–857. <https://doi.org/10.1038/s41587-019-0209-9>
- Brooks ME, Kristensen K, van Benthem KJ et al (2017) glmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 9:378–400. <https://doi.org/10.32614/rj-2017-066>
- Browett SS, O'Meara DB, McDevitt AD (2020) Genetic tools in the management of invasive mammals: recent trends and future perspectives. *Mammal Rev* 50:200–210. <https://doi.org/10.1111/mam.12189>
- Campbell DJ, Atkinson IAE (2002) Depression of tree recruitment by the Pacific rat (*Rattus exulans* Peale) on New Zealand's northern offshore islands. *Biol Conserv* 107:19–35. [https://doi.org/10.1016/S0006-3207\(02\)00039-3](https://doi.org/10.1016/S0006-3207(02)00039-3)
- Carpenter JK, Wilmshurst JM, McConkey KR et al (2020) The forgotten fauna: native vertebrate seed predators on islands. *Funct Ecol* 34:1802–1813. <https://doi.org/10.1111/1365-2435.13629>
- Caut S, Casanovas JG, Virgos E et al (2007) Rats dying for mice: modelling the competitor release effect. *Austral Ecol* 32:858–868. <https://doi.org/10.1111/j.1442-9993.2007.01770.x>
- Ceia RS, Sanches S, Ramos JA (2017) Foraging Ecology of Introduced rodents in the threatened macaronesian Laurel Forest of São Miguel Island (Azores) and contiguous exotic forests. *Mammal Study* 42:141–151. <https://doi.org/10.3106/041.042.0304>
- Chen S, Yao H, Han J et al (2010) Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE* 5:e8613. <https://doi.org/10.1371/journal.pone.0008613>
- Clark DA (1982) Foraging behavior of a vertebrate omnivore (*Rattus rattus*): Meal structure, sampling, and diet breadth. *Ecology* 63:763–772
- Cole FR, Loope LL, Medeiros AC et al (2000) Food habits of introduced rodents in high-elevation shrubland of Haleakalā National Park, Maui, Hawai'i. *Pac Sci* 54:313–329
- Colman RE, Schupp JM, Hicks ND et al (2015) Detection of low-level Mixed-Population Drug Resistance in Mycobacterium tuberculosis using High Fidelity Amplicon sequencing. *PLoS ONE* 10:e0126626. <https://doi.org/10.1371/journal.pone.0126626>
- Cordell S, Ostertag R, Rowe B et al (2009) Evaluating barriers to native seedling establishment in an invaded hawaiian lowland wet forest. *Biol Conserv* 142:2997–3004. <https://doi.org/10.1016/j.biocon.2009.07.033>
- Culliney S, Pejchar L, Switzer R, Ruiz-Gutierrez V (2012) Seed dispersal by a crow that persists only in captivity: the potential role of the Alala (*Corvus hawaiiensis*) in shaping Hawaii's plant communities. *Ecol Appl* 22:1718–1732
- De Barba M, Miquel C, Boyer F et al (2014) DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. *Mol Ecol Resour* 14:306–323. <https://doi.org/10.1111/1755-0998.12188>
- de Sousa LL, Silva SM, Xavier R (2019) DNA metabarcoding in diet studies: unveiling ecological aspects in aquatic and terrestrial ecosystems. *Environ DNA* 1:199–214. <https://doi.org/10.1002/edn3.27>
- Drake DR (1998) Relationships among the seed rain, seed bank and vegetation of a hawaiian forest. *J Veg Sci* 9:103–112. <https://doi.org/10.2307/3237228>
- Drake DR, Hunt TL (2009) Invasive rodents on islands: integrating historical and contemporary ecology. *Biol Invasions* 11:1483–1487. <https://doi.org/10.1007/s10530-008-9392-1>
- Drake DR, McConkey KR (2021) Novel diplochory: native bats and non-native rats disperse seeds of an island tree. *Acta Oecol* 111:103719. <https://doi.org/10.1016/j.actao.2021.103719>
- Erickson DL, Reed E, Ramachandran P, et al (2017) Reconstructing a herbivore's diet using a novel *rbcL* DNA mini-barcode for plants. *AoB PLANTS* 9:plx015. <https://doi.org/10.1093/aobpla/plx015>
- Farwig N, Berens DG (2012) Imagine a world without seed dispersers: a review of threats, consequences and future directions. *Basic Appl Ecol* 13:109–115. <https://doi.org/10.1016/j.baae.2012.02.006>
- Fernández-Palacios JM, Kreft H, Irl SDH et al (2021) Scientists' warning – the outstanding biodiversity of islands is

- in peril. *Glob Ecol Conserv* 31:e01847. <https://doi.org/10.1016/j.gecco.2021.e01847>
- Foster JT, Robinson SK (2007) Introduced birds and the fate of hawaiian rainforests. *Conserv Biol* 21:1248–1257. <https://doi.org/10.1111/j.1523-1739.2007.00781.x>
- Gibbs GW (2009) The end of an 80-million year experiment: a review of evidence describing the impact of introduced rodents on New Zealand's mammal-free invertebrate fauna. *Biol Invasions* 11:1587–1593. <https://doi.org/10.1007/s10530-008-9408-x>
- Hadfield MG, Sautler JE (2009) The demographics of destruction: isolated populations of arboreal snails and sustained predation by rats on the island of moloka'i 1982–2006. *Biol Invasions* 11:1595–1609. <https://doi.org/10.1007/s10530-008-9409-9>
- Harper GA, Bunbury N (2015) Invasive rats on tropical islands: their population biology and impacts on native species. *Glob Ecol Conserv* 3:607–627. <https://doi.org/10.1016/j.gecco.2015.02.010>
- Hays BR, Sperry J, Drake DR, Hruska AM (2018) Husking stations provide insight into diet of nonnative rodents on O'ahu, Hawai'i. *Pac Sci* 72:335–344. <https://doi.org/10.2984/72.3.5>
- Holechek JL, Vavra M, Pieper RD (1982) Botanical Composition determination of Range Herbivore Diets: a review. *J Range Manag* 35:309–315. <https://doi.org/10.2307/3898308>
- Howald G, Donlan CJ, Galván JP et al (2007) Invasive rodent eradication on islands. *Conserv Biol* 21:1258–1268. <https://doi.org/10.1111/j.1523-1739.2007.00755.x>
- Jones HP, Tershy BR, Zavaleta ES et al (2008) Severity of the effects of invasive rats on seabirds: a global review. *Conserv Biol* 22:16–26. <https://doi.org/10.1111/j.1523-1739.2007.00859.x>
- Jones HP, Holmes ND, Butchart SHM et al (2016) Invasive mammal eradication on islands results in substantial conservation gains. *Proc Natl Acad Sci U S A* 113:4033–4038. <https://doi.org/10.1073/pnas.1521179113>
- Jusino MA, Banik MT, Palmer JM et al (2019) An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. *Mol Ecol Resour* 19:176–190. <https://doi.org/10.1111/1755-0998.12951>
- Kelly D, Robertson AW, Ladley JJ et al (2006) Relative (un)importance of introduced animals as pollinators and dispersers of native plants. *Biol Invasions N Z* 186:227–245. <https://doi.org/10.1007/s10023-006-15>
- Landry SO (1970) The Rodentia as Omnivores. *Q Rev Biol* 45:351–372
- Liang CT, Shiels AB, Haines WP et al (2022) Invasive predators affect community-wide pollinator visitation. *Ecol Appl* <https://doi.org/10.1002/eap.2522>
- Mandon-Dalger I, Clergeau P, Tassin J et al (2004) Relationships between alien plants and an alien bird species on Reunion Island. *J Trop Ecol* 20:635–642
- McConkey KR, Drake DR, Meehan HJ, Parsons N (2003) Husking stations provide evidence of seed predation by introduced rodents in Tongan rain forests. *Biol Conserv* 109:221–225. [https://doi.org/10.1016/S0006-3207\(02\)00149-0](https://doi.org/10.1016/S0006-3207(02)00149-0)
- Medeiros AC (2004) Phenology, reproductive potential, seed dispersal and predation, and seedling establishment of three invasive plant species in a Hawaiian rain forest
- Meyer JY, Butaud JF (2009) The impacts of rats on the endangered native flora of french polynesia (Pacific Islands): drivers of plant extinction or coup de grâce species? *Biol Invasions* 11:1569–1585. <https://doi.org/10.1007/s10530-008-9407-y>
- Meyer WM, Shiels AB (2009) Black rat (*Rattus rattus*) predation on nonindigenous snails in Hawai'i: Complex management implications. *Pac Sci* 63:339–347. <https://doi.org/10.2984/049.063.0304>
- Miller-ter Kuile A, Orr D, Bui A et al (2021) Impacts of rodent eradication on seed predation and plant community biomass on a tropical atoll. *Biotropica* 53:232–242. <https://doi.org/10.1111/btp.12864>
- Moreby SJ (1988) An aid to the identification of arthropod fragments in the faeces of gamebird chicks (Galliformes). *Ibis* 130:519–526. <https://doi.org/10.1111/j.1474-919X.1988.tb02717.x>
- Muletz-Wolz CR, Wilson Rankin E, McGrath-Blaser S et al (2021) Identification of novel bacterial biomarkers to detect bird scavenging by invasive rats. *Ecol Evol* 11:1814–1828. <https://doi.org/10.1002/ece3.7171>
- Nishida GM (2002) Hawaiian terrestrial arthropod checklist. Hawaiian Biological Survey Bishop Museum
- O'Rourke DR, Bokulich NA, Jusino MA et al (2020) A total crapshoot? Evaluating bioinformatic decisions in animal diet metabarcoding analyses. *Ecol Evol* 10:9721–9739. <https://doi.org/10.1002/ece3.6594>
- O'Rourke D, Rouillard NP, Parise KL, Foster JT (2022) Spatial and temporal variation in New Hampshire bat diets. *Sci Rep* 12:14334. <https://doi.org/10.1038/s41598-022-17631-z>
- O'ahu Invasive Species Committee (2022) Target Pests. <https://www.oahuisc.org/target-pests/>. Accessed 5 Oct 2022
- Oksanen J, Blanchet FG, Friendly M et al (2020) Vegan: Community ecology package. R package version 2.5-7
- Pejchar L (2015) Introduced birds incompletely replace seed dispersal by a native frugivore. *AoB PLANTS*, <https://doi.org/10.1093/aobpla/plv072>
- Pinho CJ, Lopes EP, Paupério J et al (2022) Trust your guts? The effect of gut section on diet composition and impact of *Mus musculus* on islands using metabarcoding. *Ecol Evol* 12:1–20. <https://doi.org/10.1002/ece3.8638>
- Polito MJ, Robinson B, Warzybok P, Bradley RW (2022) Population dynamics and resource availability drive seasonal shifts in the consumptive and competitive impacts of introduced house mice (*Mus musculus*) on an island ecosystem. *BioRxiv* 1–26
- Pomeda-Gutiérrez F, Medina FM, Nogales M, Vargas P (2021) Diet of the black rat (*Rattus rattus*) in a Canary laurel forest: species identification based on morphological markers and DNA sequences. *J Nat Hist* 55:629–648. <https://doi.org/10.1080/00222933.2021.1915400>
- R Core Team (2020) R: A language and environment for statistical computing
- Robeson MSI, O'Rourke DR, Kaehler BD et al (2021) RESCRIPt: reproducible sequence taxonomy reference database management. *PLOS Comput Biol*

- 17:e1009581. <https://doi.org/10.1371/journal.pcbi.1009581>
- Shiels AB (2010) Frugivory by introduced black rats (*Rattus rattus*) promotes dispersal of invasive plant seeds. *Biol Invasions* 13:781–792. <https://doi.org/10.1007/s10530-010-9868-7>
- Shiels AB, Drake DR (2011) Are introduced rats (*Rattus rattus*) both seed predators and dispersers in Hawaii? *Biol Invasions* 13:883–894. <https://doi.org/10.1007/s10530-010-9876-7>
- Shiels AB, Drake DR (2015) Barriers to seed and seedling survival of once-common hawaiian palms: the role of invasive rats and ungulates. *AoB PLANTS* 7:1–10. <https://doi.org/10.1093/aobpla/plv057>
- Shiels AB, Flores CA, Khamasing A et al (2013) Dietary niche differentiation among three species of invasive rodents (*Rattus rattus*, *R. exulans*, *Mus musculus*). *Biol Invasions* 15:1037–1048. <https://doi.org/10.1007/s10530-012-0348-0>
- Shiels AB, Pitt WC, Sugihara RT, Witmer GW (2014) Biology and Impacts of Pacific Island Invasive Species. 11. *Rattus rattus*, the black rat (Rodentia: Muridae). *Pac Sci* 68:145–184. <https://doi.org/10.2984/68.2.1>
- Souza-Cole I, Ward MP, Mau RL et al (2022) Eastern whip-poor-will abundance declines with urban land cover and increases with moth abundance in the american Midwest. *Ornithol Appl* 124:duac032. <https://doi.org/10.1093/ornithapp/duac032>
- Spatz DR, Holmes ND, Will DJ et al (2022) The global contribution of invasive vertebrate eradication as a key island restoration tool. *Sci Rep*. <https://doi.org/10.1038/s41598-022-14982-5>
- St Clair JJH (2011) The impacts of invasive rodents on island invertebrates. *Biol Conserv* 144:68–81. <https://doi.org/10.1016/j.biocon.2010.10.006>
- St Clair JJH, Poncet S, Sheehan DK et al (2011) Responses of an island endemic invertebrate to rodent invasion and eradication. *Anim Conserv* 14:66–73. <https://doi.org/10.1111/j.1469-1795.2010.00391.x>
- Stapleton TE, Weinstein SB, Greenhalgh R, Dearing MD (2022) Successes and limitations of quantitative diet metabarcoding in a small, herbivorous mammal. *Mol Ecol Resour*. <https://doi.org/10.1111/1755-0998.13643>
- Sugihara RT (1997) Abundance and diets of rats in two native hawaiian forests. *Pac Sci* 51:189–198
- Towns DR, Atkinson IAE, Daugherty CH (2006) Have the harmful effects of introduced rats on islands been exaggerated? *Biol Invasions* 8:863–891. <https://doi.org/10.1007/s10530-005-0421-z>
- Traveset A, Richardson DM (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends Ecol Evol* 21:208–216. <https://doi.org/10.1016/j.tree.2006.01.006>
- Traveset A, Nogales M, Alcover JA et al (2009) A review on the effects of alien rodents in the Balearic (western Mediterranean sea) and Canary islands (eastern Atlantic ocean). *Biol Invasions* 11:1653–1670. <https://doi.org/10.1007/s10530-008-9395-y>
- U.S. Fish and Wildlife Service (2022) Listed Plants - Environmental Conservation Online System. <https://ecos.fws.gov/ecp0/reports/ad-hoc-species-report?kingdom=P&status=E&status=T&status=EmE&status=EmT&status=EXPE&status=EXPN&status=SAE&status=SAT&mapstatus=3&fcrithab=on&fstatus=on&fspecrule=on&finvop=on&fgroup=on&ffamily=>. Accessed 12 Oct 2022
- Vizentin-Bugoni J, Tarwater CE, Foster JT et al (2019) Structure, spatial dynamics, and stability of novel mutualistic networks in Hawai‘i. *Science* 364:78–82. <https://doi.org/10.1126/science.aau8751>
- Wagner WL, Herbst DR, Lorence DH (2005) Flora of the Hawaiian Islands website. <http://botany.si.edu/pacificislandbiodiversity/hawaiianflora/index.htm>
- Williams PA, Karl BJ, Bannister P, Lee WG (2000) Small mammals as potential seed dispersers in New Zealand. *Austral Ecol* 25:523–532. <https://doi.org/10.1111/j.1442-9993.2000.tb00057.x>
- Wilson DE, Lacher TEJ, Mittermeier RA (eds) (2017) Handbook of the Mammals of the World. Lynx Edicions, Barcelona
- Zavaleta ES, Hobbs RJ, A.Mooney H (2001) Viewing invasive species removal in a whole-ecosystem context. *Trends Ecol Evol* 16:454–459
- Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. *Methods Ecol Evol* 1:3–14. <https://doi.org/10.1111/j.2041-210x.2009.00001.x>

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