

CHAPTER THREE:  
NICHE PARTITIONING BASED ON DIET ANALYSIS OF THREE INTRODUCED  
RODENTS IN HAWAIIAN MONTANE FOREST

Aaron B. Shiels

Department of Botany

University of Hawaii at Manoa

3190 Maile Way

Honolulu, HI. 96822

## Abstract

Determining the diets of sympatric rodents can uncover patterns of resource partitioning and competitive interactions. Prey items that appear in the stomachs of introduced rodent predators can provide insight into trophic divisions and disruptions and help to assess the vulnerability of native prey. In the Hawaiian Islands, where rodents were absent prior to human arrival ca. 1000 years ago, three rodents (*Rattus rattus* or black rat, *Rattus exulans* or Pacific rat, *Mus musculus* or house mouse) are common to forests and are voracious predators of plants and animals. These three rodents were trapped in mesic montane forest from February 2007 to September 2009 to determine their short-term diet by analyzing stomach contents and their long-term (life-time) diets by extracting bone collagen for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopic analysis. For all three rodents > 75% of individuals had plants and > 90% had arthropods in their stomachs. Mean relative abundance of the major items in stomachs was 81% plant and 14% arthropod for black rats, 60% plant and 38% arthropod for Pacific rats, and 36% plant and 57% arthropod for mice. Rodents may be dispersing some native and non-native seeds, including the highly invasive *Clidemia hirta*, because intact seeds were found in some of the stomachs of all three species. Fruit pulp comprised 55% of the black rat diet, 41% of the Pacific rat diet, but only 11% of the house mouse diet. Caterpillars were particularly common in Pacific rats and mice, and aside from a native spider, a cricket, and two species of beetle, the majority of identifiable arthropods in all three rodents were non-native species. None of the stomachs contained evidence of birds, snails, or lizards, all of which are common at the study site. The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures revealed that the Pacific rat and house mouse occupy the same trophic level, whereas the slightly lower

$\delta^{15}\text{N}$  values of black rats reflect a proportionally more vegetarian diet compared to the two smaller rodents. Therefore, the strongest evidence of food resource partitioning among the three rodents is between the black rat and house mouse; the Pacific rat has an intermediate diet that partly overlaps with the two other rodent species.

## **Introduction**

How closely related species coexist in a community has long intrigued ecologists. Numerous factors influence a species niche, including biotic and abiotic attributes, resource use, and competition (Elton 1927; Hutchinson 1957). Niche partitioning reflects competitive interactions among species and can be determined by diet analysis of sympatric animals (Biró et al. 2005; Purnell et al. 2006). Animals with similar life-history traits and close phylogenetic associations, such as different species of rodents in the same habitat, must partition resources across time and space in order to coexist (Gause 1934).

Introduced animals can disrupt food webs by consuming native prey and by altering the niches of native competitors (Fritts and Rodda 1998; Fukami et al. 2006). Identifying prey consumed by introduced predators provides insight into native species' vulnerability and can help improve strategies of native and non-native species management (Stapp 2002; Caut et al. 2008a). Rodents (*Rattus rattus* or the black rat or ship rat, *R. norvegicus* or the Norway rat, *R. exulans* or the Pacific rat or Polynesian rat, and *Mus musculus* or the house mouse) are omnivorous predators that have been introduced to many ecosystems worldwide and are among the most widespread and problematic invasive animals affecting islands (Townsend et al. 2006; Angel et al. 2009;

Drake and Hunt 2009). Introduced rodents may consume a wide variety of prey, including plants (e.g., fruits, seeds, vegetative material) and animals (e.g., arthropods, mollusks, birds; Sugihara 1997; Campbell and Atkinson 2002; Stapp 2002), and their diets can shift depending upon a number of factors, including the availability of food items, the chemical and nutritional quality of the food items, and the rodents' competitive ability relative to other animals that coexist in the environment (Clark 1982).

Predation by introduced rodents is rarely observed directly, perhaps because they are nocturnal and often forage in the canopy or burrow belowground (Lindsey et al. 1999; Towns 2009). Consequently, field observations of partially consumed prey items (e.g., seeds, mollusks, arthropods; Norman 1970; Campbell et al. 1984; McConkey et al. 2003) or captive-feeding trials (Williams et al. 2000; Pérez et al. 2008; Meyer and Shiels 2009) have been used to determine the species suffering from predation by introduced rodents. Additionally, stomach content analysis has been used to identify rodent's prey and assess their diets (e.g., Clark 1982) despite this method reflecting one to a few meals.

Stable isotopes ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) have been widely used to determine the trophic levels at which animals have fed during tissue development (Peterson and Fry 1987; Lajtha and Michener 1994); however, interpretations of diet using stable isotopes are restricted by the tissue type analyzed and tissue turnover rates. For example, liver tissue has a higher turnover rate than blood cells or muscle, and bone collagen is deposited and reworked during the lifetime of an animal, so its isotopic values represent a long-term average of an animal's diet (Lajtha and Michener 1994; Caut et al. 2008b). The difference in isotopic composition between a predator and its prey (discrimination values) is presumed to average ca. 3‰ and 1‰ for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively; however, the

discrimination values can differ widely depending on the species of prey involved, as demonstrated by Post (2002) where the majority of the  $\delta^{15}\text{N}$  discrimination values in lake organisms were 2-4.5‰, and Caut et al. (2008c) determined from lab trials that the  $\delta^{15}\text{N}$  discrimination values for black rats ranged from -1.46‰ to 4.59‰. Applying isotope mixing models enables quantification of each prey item's contribution to a predator's diet (Phillips and Gregg 2003). However, without isotopic signatures for all prey items consumed by the predator, collected from the specific habitat of the predator (Flaherty and Ben-David 2010), interpretations of mixing models can be problematic (Martínez del Rio et al. 2009). Combining  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  to assign trophic-level feeding of coexisting predators and prey (Peterson and Fry 1987; Lajtha and Michener 1994) with stomach content analysis of predators can provide an understanding of the diet and trophic level occupancy of similar, coexisting predators.

In the Hawaiian Islands the first rodents (the Pacific rat) arrived with humans ca. 1000 years ago (Athens et al. 2002), and later arrival of two additional rats (the Norway rat and the black rat) and the house mouse occurred upon European settlement ca. 230 years ago (Tomich 1986). In contemporary Hawaii, the Norway rat has rarely been found in forests. The black rat appears to be the most common rat in forests, and both black rats and mice are distributed from sea level to nearly 3000 m (Amarasekare 1994; Lindsey et al. 1999; unpublished data). The Pacific rat is the smallest of the three introduced rats and may be outcompeted in many environments in Hawaii and other Pacific islands by the European-introduced rats (Russell and Clout 2004). Similarly, the house mouse may be suppressed by all three introduced rats (Russell and Clout 2004; Angel et al. 2009), such as in New Zealand where mice are rarely known to occur where Pacific rats are

present (Yom-Tov et al. 1999; Ruscoe and Murphy 2005). It has been established that these introduced rodents consume plants and animals in Hawaii, as evidenced by field trials (VanderWerf 2001; Shiels and Drake, in press), captive feeding trials (Pérez et al. 2008; Meyer and Shiels 2009; Shiels, in press), and two high elevation studies that analyzed stomach contents (Sugihara 1997; Cole et al. 2000). However, the short- and long-term diets and potential resource partitioning of three introduced rodent species has not been established in Hawaii or elsewhere. The objectives of this study are to 1) determine the short- and long-term diets of black rats, Pacific rats, and mice where they coexist in mesic montane forest in Hawaii, 2) identify some of the native and non-native prey species that are vulnerable to these three rodents, 3) determine if there is evidence of niche partitioning that may result from differential feeding and trophic level occupancy for each of the three rodent predators relative to their dominant prey.

## **Methods**

### *Study site*

Rodents were obtained from Kahanahaiki Management Unit (21° 32' N, 158° 11' W), a 36 ha segment of mesic forest in highly dissected terrain (500-660 m a.s.l; Gagné and Cuddihy 1999) in the northern Waianae Mountains, on the island of Oahu, Hawaii. Kahanahaiki is managed for native species preservation by the U.S. Army, and the forest was fenced in 1996 to exclude feral goats and pigs. Daily air temperature at the site ranges from 16-24°C (unpublished data) and monthly rainfall varies from 50 mm to 170 mm (cited in Joe and Daehler 2008).

Two species of rats, the black rat ( $133 \pm 3$  g; mean  $\pm$  SE) and Pacific rat ( $47 \pm 2$  g), and the house mouse ( $10 \pm 1$  g) are present at Kahanahaiki (Meyer and Shiels 2009; Shiels and Drake, in press). Average estimated relative abundance measures using bi-monthly mark-and-recapture sampling over 15 months during 2007-2008 were 17 indiv./100 trap nights for black rats and 1 indiv./100 trap nights for Pacific rats. No Norway rats were captured at the study site and the house mouse was captured at ca. 6 indiv./100 trap nights (Shiels and Drake, in press).

#### *Stomach content analysis*

Rodents (black rats, Pacific rats, mice) were collected from kill-traps (Victor<sup>®</sup> rat traps) from February 2007 through September 2009. Traps, each spaced 10-25 m apart, were established along a single 300 m transect and at two 50 x 50 m plots where native snails (*Achatinella mustellina*) were relatively abundant. Each month, 15-32 traps were baited with coconut chunks or peanut butter and set for 2-5 consecutive days and checked each 24 hours. From May-September 2009, approximately 400 kill-traps were added to the site and arranged along multiple transects that circled the core interior and were spaced ca. 25-50 m apart. I used the same bait and trap spacing as described above, and traps were checked each 1-7 days. Only rodents that were freshly (< 24 hours) killed, evidenced by lack of obvious decay, were used in this study. Upon capture, each rodent was weighed and its sex was determined whenever possible. Carcasses were stored in a freezer for later analysis.

Rodent stomach contents were extracted from the stomach cavity, swirled for 5 min in water and mild detergent (Joy<sup>®</sup> brand) to separate contents and dissolve gastric

juices and oils, and then sieved through a No. 35 US Standard sieve (0.4 mm opening; Sugihara 1997). Recovered contents were preserved in 95% ethanol and analyzed for: 1) frequency (percent) of occurrence of each food type, and 2) relative abundance (percent) of each food type for each individual. For each sample, a transparent grid (5 x 5 mm for rats; 3 x 3 mm for mice) was positioned beneath a Petri dish that contained the stomach contents of the sample. Samples were inspected using a dissecting microscope with 10-20x magnification. Rodent stomachs that were more than half-filled with intestinal worms were excluded from this study (Sugihara 1997). Relative abundance of each food type was determined for each individual sample by scoring the number of grid-boxes containing a given food type and dividing by the total number of grid-boxes (i.e., 40 grid-boxes). If more than one food type was in a grid-box, the item closest to the center of the grid-box, or the centermost item on top when overlapping items were present, was recorded (Cole et al. 2000). Plant food types included fruit, seed, and other plant material (including leaves, flowers, stems, wood); whereas arthropod food types included caterpillar, ant, bug, spider, and other arthropod material. Rodent hair and flesh, and other/unknown (i.e., material that did not fit any of the listed categories), represented the final two major food type categories. Food items were classified to the lowest taxonomic level whenever possible using voucher specimens collected from the study site. Frequency of occurrence for each food type was calculated by the presence of each of the food types in a given sample divided by the total number of samples.

### *Stable isotope analysis*

To augment the short-term diet assessment from stomach contents, the long-term diets of each of the rodents was determined using stable isotope analysis. On a subset of the trapped rodents (N = 20 black rats, N = 12 Pacific rats, and N = 11 mice), bone collagen was extracted and analyzed for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. Bone collagen was extracted using methods described in Lajtha and Michener (1994) where the femur bone (plus tibia and fibia bones for mice) of each individual was excised, cleaned of flesh, and soaked in 0.5 M HCl for 48 hours at 4° C; the remaining sample (now collagen) was rinsed with deionized water and allowed to dry at 60° C for 5 days before grinding to a fine dust. Prey items (fruit, seed, arthropod) were collected from the study site and were chosen based on similar species (or life forms) identified in the rodent stomachs and those found to be attractive to rodents at this site using field trials (Shiels and Drake, in press). For plant-prey items (N = 5), one sample from each of the following species was collected for analysis: *Alyxia stellata* (seed), *Clidemia hirta* (fruit + seed), *Diospyros hillebrandii* (seed), *Psidium cattleianum* (seed), and *Pouteria sandwicensis* (fruit). Three samples of each of the three herbivorous arthropods were analyzed: caterpillar, isopod, and amphipod. Three predatory arthropods (spiders) were analyzed, including *Steatoda grossa* and two unknown species. Samples were dried at 60° C, ground to homogenize either multiple individuals of the same species (e.g., plants, herbivorous arthropods) or single individuals (e.g., spiders), and, like the rodent bone collagen, analyzed isotopically using a Carlo Erba elemental analyzer (model NC2500) with an attached mass spectrometer (Finnegan DeltaS with source upgrade). Stable isotope ratios were expressed in  $\delta$  notation as parts per thousand (‰) deviation from international standards.

### *Statistical analysis*

The relative abundances of each of the major food types were compared among the three rodents by parametric and non-parametric ANOVAs. The major food types were grouped into three general categories: 1) Plants, which included fruit, seed, and other plant material, 2) Arthropods, which included caterpillar (i.e., Lepidoptera larvae), ant, burrowing bug, spider, other arthropod material, and 3) Other, which included rodent hair + flesh, and unknown (unidentifiable) items. Because these data were percentages, all data were arc-sin square-root transformed prior to assessment of meeting ANOVA assumptions. Fruit and seed were the two major food types that met parametric assumptions of ANOVA; whereas the remaining comparisons used Kruskal-Wallis tests to assess significant differences among rodents for each major food type. Significance was based on  $P < 0.05$ , and if significant differences occurred when all three rodents were compared, post-hoc Tukey's test (for fruit and seed) or Mann-Whitney U tests were applied to assess significance between rodent species (SPSS 1998).

To test whether the diet of black rats changed during the time period when few (February 2007-April 2009), and the majority (May-September 2009), of Pacific rats and mice were trapped, ANOVAs, after square-root transformations to meet normality assumptions, were used to compare the two time periods for the three dominant food types (fruit, seed, arthropod).

### **Results**

The black rat was the most commonly trapped rodent at the study site and 95 stomachs were analyzed for short-term diets. Forty-seven mouse stomachs were

analyzed, and 12 stomachs were analyzed from the less common rat, Pacific rat. There were roughly equal numbers of males and females analyzed for each rodent, and the sizes of the rodents were  $124 \pm 5$  g (mean  $\pm$  SE) for black rats,  $52 \pm 4$  g for Pacific rats, and  $12 \pm 1$  g for mice.

### *Stomach content analysis*

All major food types were found in stomachs of some individuals of all three rodents (Table 12). There were significant differences among rodents for most major food types found in stomachs, and burrowing bugs were the only major food type that was not significantly different among rodents (Table 12). Plant relative abundance in stomachs significantly differed among rodents ( $P < 0.001$ ;  $\chi^2 = 56.7$ , d.f. = 2), and was highest for black rats, intermediate for Pacific rats, and lowest for mice ( $P < 0.015$  for each post-hoc comparison; Fig. 12). Fruit comprised the majority of the plant material for both rats, but seed was the most abundant plant material in mice (Table 12).

Arthropod mean relative abundance also differed significantly among rodents ( $P < 0.001$ ;  $\chi^2 = 56.7$ , d.f. = 2), and was highest for mice, intermediate for Pacific rats, and lowest for black rats ( $P < 0.035$  for each post-hoc comparison; Fig. 15). Caterpillars (Lepidoptera larvae) comprised the majority of the identifiable arthropods found in each of the rodents, and were  $> 50\%$  of the mouse's arthropod diet (Table 12). Rodent hair, which dominated the 'other' category in Fig. 15, was found in most stomachs of each rodent (69% of black rat individuals, 67% of Pacific rats, and 57% of mice), and mean relative abundance for rodent hair was highest for mice and lowest for black rats (Table 12; Fig. 15). Rodent flesh (with rodent hair attached) was found only in black rats ( $N = 1$ ) and Pacific rats ( $N$

= 1). There was no evidence of birds, reptiles, or other vertebrates in any of the stomachs, and there were no snails or fungi.

Table 12. Mean  $\pm$  SE relative abundances (%) of major food types identified in stomachs of invasive rodents in Hawaiian mesic forest. The P-value reflects the comparison among species, and different letters represent significant ( $P < 0.05$ ) differences from post-hoc comparisons between each rodent species. When no amount of food item was present for a rodent (i.e., unknown), that rodent was not included in the statistical analysis. Rodent flesh was only found in rats, and comprised  $< 5\%$  of rodent hair + flesh.

Food type	Black rat (N = 95)	Pacific rat (N = 12)	House mouse (N = 47)	P-value
<b>Plants</b>				
Fruit	55.1 $\pm$ 2.4 <sup>a</sup>	40.6 $\pm$ 5.7 <sup>a</sup>	10.8 $\pm$ 2.7 <sup>b</sup>	< 0.001
Seed	24.9 $\pm$ 2.2 <sup>a</sup>	15.9 $\pm$ 4.3 <sup>a,b</sup>	19.0 $\pm$ 3.3 <sup>b</sup>	0.037
Other plant material	1.1 $\pm$ 0.4 <sup>a</sup>	2.5 $\pm$ 0.8 <sup>b</sup>	5.8 $\pm$ 1.5 <sup>b,c</sup>	< 0.001
<b>Arthropods</b>				
Caterpillar	3.2 $\pm$ 0.7 <sup>a</sup>	28.8 $\pm$ 3.8 <sup>b</sup>	53.8 $\pm$ 4.9 <sup>c</sup>	< 0.001
Ant	1.7 $\pm$ 0.3 <sup>a</sup>	1.3 $\pm$ 0.8 <sup>a</sup>	0.5 $\pm$ 0.2 <sup>b</sup>	0.007
Burrowing bug	0.9 $\pm$ 0.3	0.2 $\pm$ 0.2	0.7 $\pm$ 0.5	0.110
Spider	0.4 $\pm$ 0.1 <sup>a</sup>	0.2 $\pm$ 0.2 <sup>b</sup>	0.1 $\pm$ 0.1 <sup>b</sup>	0.016
Other arthropod material	7.4 $\pm$ 1.4 <sup>a</sup>	7.4 $\pm$ 2.7 <sup>a</sup>	2.1 $\pm$ 1.0 <sup>b</sup>	0.002
<b>Other</b>				
Rodent hair + flesh	4.9 $\pm$ 0.7 <sup>a</sup>	4.2 $\pm$ 1.4 <sup>b</sup>	7.2 $\pm$ 1.5 <sup>b,c</sup>	< 0.001
Unknown	0.4 $\pm$ 0.2	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	0.528

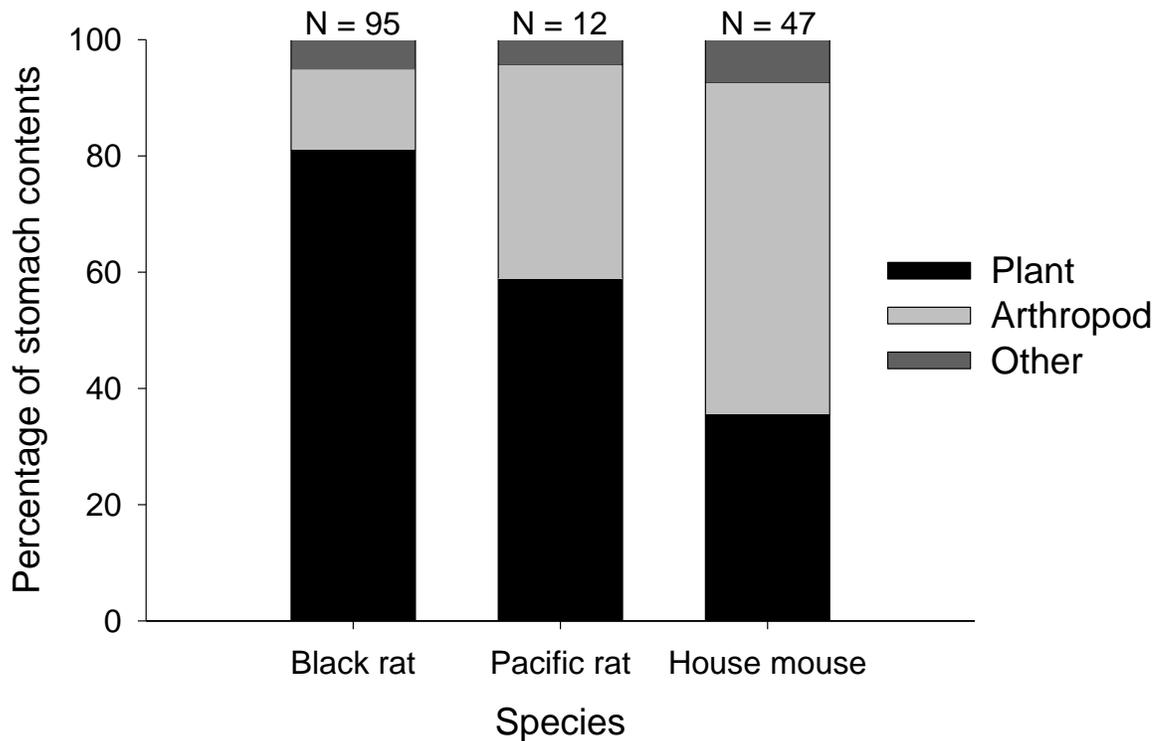


Figure 15. Mean relative abundances (%) of the three major items found in stomachs of black rats, Pacific rats, and house mice in Hawaiian mesic forest. The ‘other’ category is dominated by rodent hair, which was most likely a result of grooming rather than cannibalism (see Table 12 for minimal presence of rodent flesh in stomachs). For each of the three major items, there were significant ( $P < 0.05$ ) differences between each species.

In each rodent species > 75% of individuals had plants and > 90% had arthropods in their stomachs (Table 13). All individuals of black rats and Pacific rats had fruit in their stomachs, and > 90% also had seed. The frequency of mouse stomachs with fruit (40%) tended to be less than those containing seed (64%). The majority of seed in all three rodents appeared chewed and was probably destroyed, but intact seeds of some native and non-native species were found in black rats and all three rodents had intact seeds of the non-native weed *C. hirta*. The frequency of other plant material, which

mostly included stems and leaves, tended to be highest in Pacific rats, intermediate in mice, and lowest in black rats.

Table 13. Frequency (%) of rodent stomachs with identifiable seed, fruit, and arthropod taxa in Hawaiian mesic forest. Both *Cyrtandra dentata* and *Delissea subcordata* are federally endangered species in Hawaii. Many food items were damaged such that they could not be classified more specifically than fruit, seed, plant, or arthropod.

Species or group	Life form	Native or non-native	Black rat (N = 95)	Pacific rat (N = 12)	House mouse (N = 47)
<b>Plants</b>			100	100	76.6
Intact seeds					
<i>Clidemia hirta</i>	Shrub	Non-native	30.5	25.0	6.4
<i>Rubus rosifolius</i>	Shrub	Non-native	7.4	0	0
<i>Paspalum conjugatum</i>	Grass	Non-native	2.1	0	0
<i>Cyrtandra dentata</i>	Shrub	Native	1.1	0	0
<i>Delissea subcordata</i>	Shrub	Native	1.1	0	0
Unknown #1	-	-	1.1	0	0
Unknown #2	-	-	1.1	0	0
Unknown #3	-	-	2.1	0	0
Total intact seeds			42.1	25.0	6.4
Total seed			93.7	91.7	63.8
Fruit fragments					
<i>Clidemia hirta</i>	Shrub	Non-native	33.7	50.0	6.4
<i>Rubus rosifolius</i>	Shrub	Non-native	7.4	8.3	0
<i>Psidium cattleianum</i>	Tree	Non-native	25.3	2.1	0
Total fruit			100	100	40.4
Other plant material			16.8	58.3	34
<b>Arthropods</b>			91.6	100	95.7
<i>Solenopsis papuana</i>	Ant	Non-native	36.8	16.7	10.6
<i>Rhytidoporus indentatus</i>	Burrowing bug	Non-native	16.8	8.3	4.3

Species or group	Life form	Native or non-native	Black rat (N = 95)	Pacific rat (N = 12)	House mouse (N = 47)
<i>Balta</i> spp.	Cockroach	Non-native	6.3	0	0
<i>Platyzosteria sorer</i>	Cockroach	Non-native	1.1	8.3	0
<i>Steatoda grossa</i>	Spider	Non-native	3.2	8.3	0
<i>Mecaphesa</i> sp.	Spider	Native	0	0	1.1
<i>Blackburnia</i> sp.	Beetle	Native	1.1	0	0
<i>Rhyncogonus</i> sp.	Beetle	Native	1.1	0	0
<i>Araecerus fasciculatus</i>	Beetle	Non-native	1.1	0	0
<i>Xylosandrus compactus</i>	Beetle	Non-native	1.1	0	0
<i>Stelidota geminata</i>	Beetle	Non-native	2.1	0	0
<i>Pantomorus cervinus</i>	Beetle	Non-native	0	0	1.1
<i>Banza</i> sp.	Cricket	Native	1.1	0	0
<i>Apis mellifera</i>	Honey bee	Non-native	2.1	0	0
Hemiptera	Aphid	Non-native	1.1	0	0
Phthiraptera	Louse/lice	-	2.1	0	0
Orthoptera	Cricket	-	1.1	0	0
Lepidoptera larvae	Caterpillar	-	33.7	100	83.0
Diptera larvae	Maggot	-	2.1	0	8.5
Araneida	Spider	-	11.6	0	0
Other arthropod material	-	-	9.5	75	23.4

Arthropods, which mostly appeared as fragments rather than intact animals, were found in nearly all (> 90%) of the rodents examined. Only four species of arthropod were found intact in rodent stomachs, and these were *Solenopsis papuana* (Papuan thief ant), *Xylosandrus compactus* (black twig-borer), *Stelidota geminate* (strawberry sap beetle), and Phthiraptera (rat lice) (Table 13). The two arthropod species that were found

in some individuals of all three rodents were *S. papuana* and *Rhytidoporus indentatus* (burrowing bug, Hemiptera). Most identifiable arthropods were non-native species; the only native arthropods found in stomachs were *Banza* sp. (bush cricket/katydid, Orthoptera), *Blackburnia* sp. (beetle, Coleoptera), and *Rhyncogonus* sp. (weevil, Coleoptera) in black rats, and *Mecaphesa* sp. (crab spider, Araneae) in mice. Unknown species of caterpillars were found in all 12 Pacific rats, 83% of mouse individuals, and 34% of black rat individuals (Table 13).

There was no evidence indicating a dietary shift in black rats between seasons when few (February 2007-April 2009), and the majority (May-September 2009), of Pacific rats and mice were trapped ( $P = 0.709$  for fruit;  $P = 0.860$  for seed;  $P = 0.549$  for arthropod; Fig. 16).

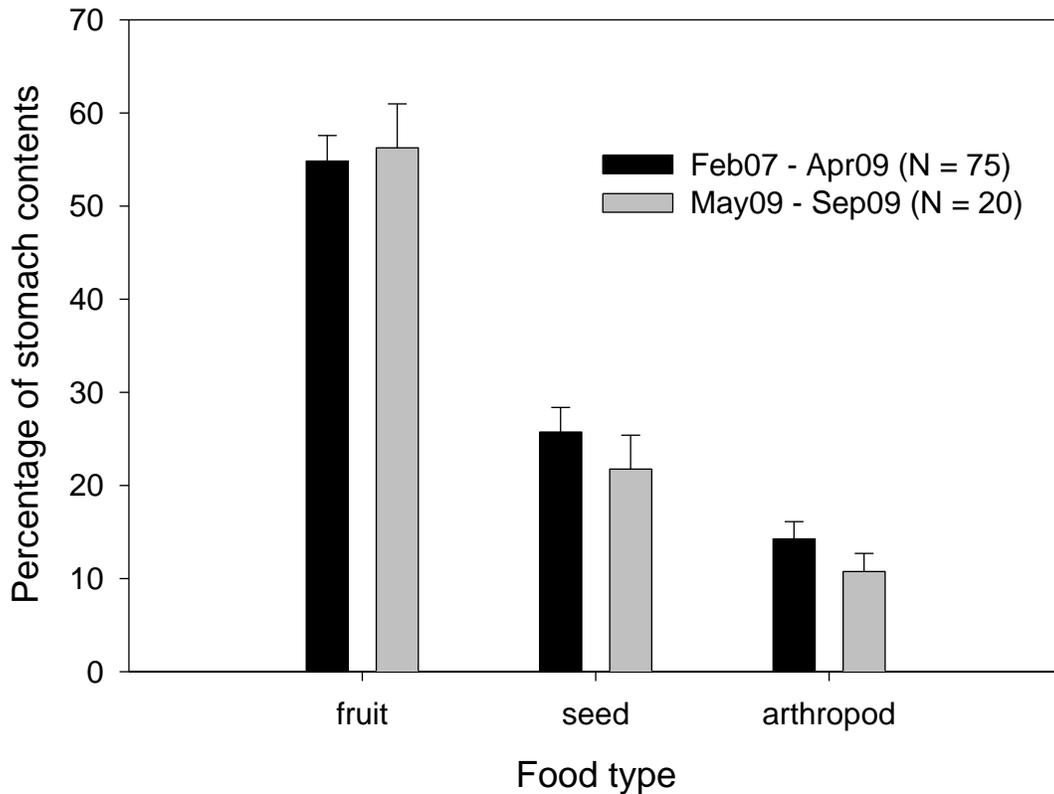


Figure 16. Mean  $\pm$  SE relative abundances (%) of major food items in stomachs of black rats for the time periods when few (February 2007-April 2009), and the majority (May-September 2009), of Pacific rats and mice were trapped. There were no significant differences ( $P > 0.05$ ) between time periods for any of the major food items.

#### *Stable isotope analysis*

Examining long-term diets via stable isotopes shows that black rats have lower  $\delta^{15}\text{N}$  values than Pacific rats and mice (Fig. 17). Assuming that a ca. 2‰ discrimination value in  $\delta^{15}\text{N}$  indicates a difference of one trophic level, black rats appear to be feeding at a lower trophic level than Pacific rats and mice. The Pacific rat and mouse have similar  $\delta^{15}\text{N}$  signatures. Spiders, which are arthropod predators and are not known to consume plants, appear to be feeding at a higher trophic level than all three rodents. While all

three rodents consume plants and animals as part of their diets, the  $\delta^{15}\text{N}$  findings complement the short-term stomach content findings that depict black rats as mainly vegetarian and Pacific rats and mice as slightly more carnivorous.

A difference of ca. 1‰ in  $\delta^{13}\text{C}$  is typically indicative of a difference between organisms in trophic-level, and the three rodents appear to form a distinct grouping from their potential prey (Fig. 17). Although there is a relatively high amount of variability among Pacific rat and mouse samples for  $\delta^{13}\text{C}$ , these two rodents are nearly equal in  $\delta^{13}\text{C}$  and appear slightly higher than the  $\delta^{13}\text{C}$  signature of the black rat (Fig. 17). The herbivorous insects are about 1.5‰ from plants, but surprisingly the spiders are also aligned with the insects for  $\delta^{13}\text{C}$  (Fig. 17).

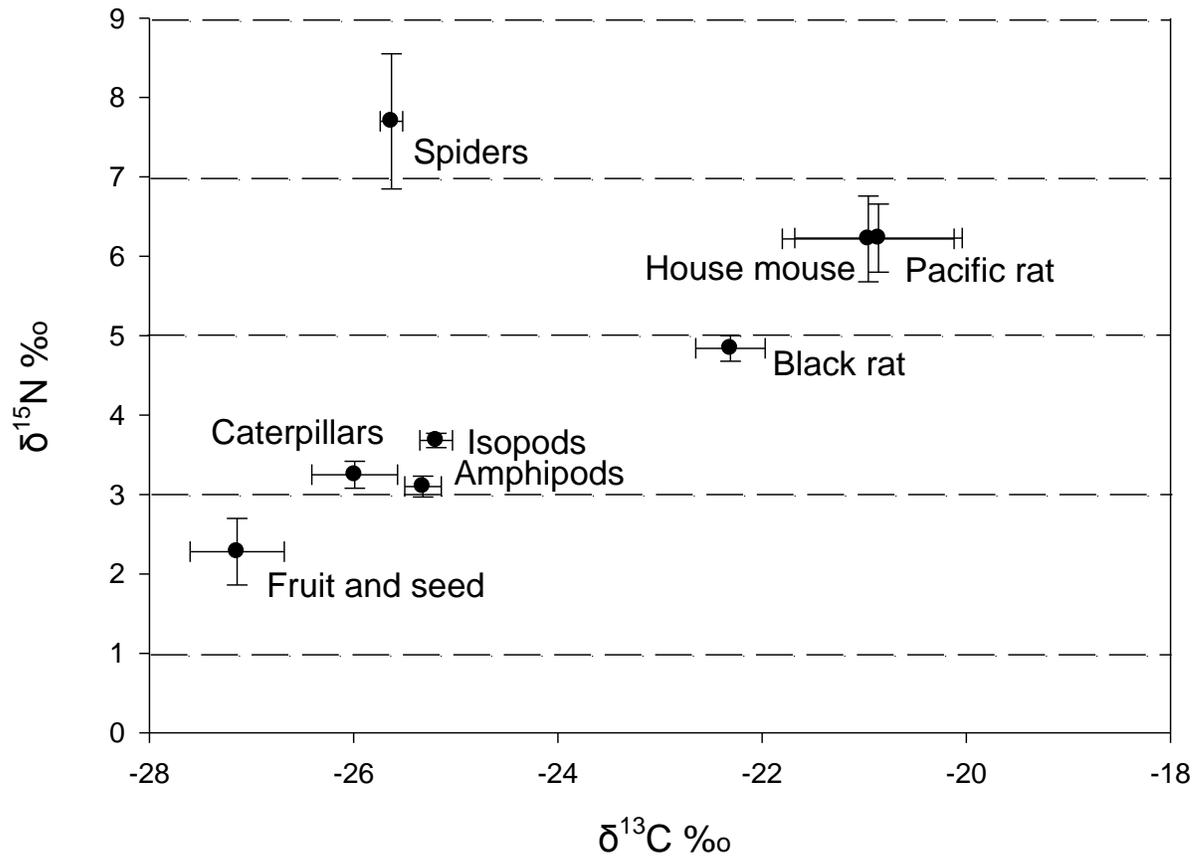


Figure 17. Mean  $\pm$  SE  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values for black rats (N = 20), Pacific rats (N = 12), and the house mouse (N = 11), and some of their potential prey items (spiders N = 3; isopods N = 3; amphipods N = 3; caterpillars N = 3; fruits and seeds N = 5), from Kahanahaiki forest, Oahu. Isotopic values were determined from bone collagen for rodents, whole individuals for arthropods (caterpillars, isopod, amphipod) and spiders, and multiple seeds and/or fruit from five common plant species. Dashed lines divide the proposed trophic levels.

## Discussion

The findings from this study reveal that black rats, Pacific rats, and house mice each consume a variety of plant and animal prey, and that where these three introduced rodents coexist they appear to partition food resources such that the house mouse is primarily carnivorous and feeds mainly on arthropods, the black rat is primarily vegetarian and feeds mainly on fruit and seed, and the Pacific rat has an intermediate diet that, over its life-time, is more closely related to the house mouse than to the black rat. An understanding of the trophic dynamics involving these introduced rodents, as evidenced by stomach contents and stable isotope analysis, should help identify the types of species and trophic levels that may be vulnerable to rodent predation and deserving of conservation attention.

Habitat and resource partitioning are complementary mechanisms for coexistence of different species. While all three rodents consumed the main types of prey (e.g., arthropods, seed, fruit) on Oahu, the relative abundances and identifiable species of prey were different among rodent species. A similar finding occurred with these same three sympatric rodents in gulches adjacent to sugarcane fields on Hawaii island (Kami 1966). There, insects were a small part of both rat diets, but black rats fed heavily on fruit and grass stalks, Pacific rats ate mainly sugar cane stalks, and mice consumed mainly seeds and insects (Kami 1966). Habitat partitioning has been previously observed for these three rodents when sympatric; in New Zealand the Pacific rat is often restricted to grassland or thick ground cover when other introduced rodents are present (Atkinson and Towns 2005), and in Hawaii, Lindsey et al. (1999) used radio tracking to determine that black rats ( $N = 9$ ) nested in trees or treeferns whereas Pacific rats ( $N = 4$ ) nested belowground. In Hungary where diets of three species of cat (*Felis* spp.) were analyzed,

small mammals dominated their diets but the species compositions and microhabitats of the consumed prey differed among cat species (Bíro et al. 2005). Additional evidence of partitioning habitats and feeding were demonstrated by Sala and Ballesteros (1997) where fish in the same genus partitioned the near-shore zone, and by Chapman and Rosenberg (1991) where four species of Amazonian woodcreepers partitioned forest habitats and feeding substrates. Therefore, habitat partitioning by coexisting and closely related species can result in consumption of similar types, but often different species and abundances, of prey.

In addition to resource competition, interference competition can be reduced through habitat partitioning. Experimental evidence of habitat partitioning between black rats and Pacific rats was demonstrated by Strecker and Jackson (1962) where several rats of each species were confined in 3 x 3 m enclosures for 14 days and then examined for signs of conflict and weight loss. The authors concluded that if food and available microsites were present, then these rats could coexist in a confined space. However, when smaller cages limited space and microhabitats to a greater extent, or arrival times of different rat species into the cage were altered, then there was strong evidence of interference competition that resulted in fighting and high death rates (Barnett 1964; Norman 1970). Stokes et al. (2009) in Australia, and Harris and MacDonald (2007) in the Galápagos Islands, demonstrated that native rats (*Rattus fuscipes* and *Nesoryzomys swarthi*, respectively) suffered from interference competition rather than resource competition with non-native black rats. There have not been, to my knowledge, competition trials conducted with the house mouse and *Rattus* spp.; however, increased house mouse abundance following black rat reduction has been recorded in the

Galápagos Islands (Harper and Cabrera 2010) and in New Zealand (Ruscoe and Murphy 2005). If aggressive confrontation occurred among the three rodent species at Kahanahaiki, it apparently did not result in much rodent consumption or cannibalism because there were only two rats with rodent flesh in their diets, and this probably originated from their scavenging from the kill-traps as indicated by past studies (Norman 1970; Clark 1980; Sugihara 1997). The presence of rodent hair in the majority of the stomachs of all three rodents may be explained by grooming, which is a common behavior of these rodents (personal observation). The degree to which competition occurs among the three rodents in Hawaiian forest is unknown, yet it is probable that the larger and more abundant black rat is the most dominant of the three rodents (Yom-tov et al. 1999; Russell and Clout 2004; Chapter 2). Past findings of habitat partitioning between *Rattus* spp., and partitioning of potential prey demonstrated in my study, is evidence that these three rodents adjust their habits and realized niches when sympatric.

The species composition and relative abundances of plants and arthropods in rodent-occupied environments directly affects rodent diets. Based on past studies of rodent stomach contents in Hawaii, the habitats dominated by non-native plants and arthropods have mostly non-native prey in stomachs (Beard and Pitt 2006; this study), whereas native-dominated habitats have mostly native prey in stomachs (Cole et al. 2000). Strong seasonal patterns common to temperate environments can also alter resource availability and perhaps the proportion of animal and plant material consumed by rodents. In parts of New Zealand, wetas, which are large, flightless Orthoptera, were the dominant food found in black rat stomachs, probably because they were available throughout the year and are high in protein (Innes 1979; Gales 1982; Miller and Miller

1995). On Green Island, Tasmania, Norman (1970) found that 58% of the 77% plant material in black rat stomachs was monocotyledons that were present all year and encompassed > 70% of the total plant cover. In general, invasive rodents' diets will in part reflect their surrounding environment, and these rodents seem to survive well in areas with native, non-native, or a mixture of both types of these prey.

Fruit was a large part of both rat diets, and the relative abundance of fruit in black rat stomachs was similar in my study (55%) to that in a wet Hawaiian forest (23-53%; Sugihara 1997) and in an arid Hawaiian shrubland (44%; Cole et al. 2000). However, fruit in Pacific rat stomachs in my study (41%) was greater than in other habitats where Pacific rats were also sympatric with black rats (3-16% seasonal averages; Sugihara 1997). On 37 Tokelau islands where the Pacific rat was the only rodent, the stomach contents of Pacific rats consisted of 88% plant material (mostly coconut, *Cocos nucifera*) and 4% arthropod (Mosby et al. 1973). Similarly, the stomach contents of Pacific rats on Kure Atoll, Hawaii, consisted of 62% plant material and 30% arthropods and there were no other rodents present (Wirtz 1972). Fruit fragments of *C. hirta*, *R. rosifolius*, and *P. cattleianum*, which are all problematic invasive species in Hawaii, were found in both rat species trapped in Kahanahaiki forest, and ripe fruit of all three of these species were available at the site during the May-September time period (unpublished data). Fruit of *Rubus* spp. (Sugihara 1997) and *R. rosifolius* and *C. hirta* (Beard and Pitt 2006) were also found in stomach of black rats and Pacific rats in Hawaiian wet forests. Interestingly, fruit of *R. rosifolius* and *P. cattleianum* were not found in any of mouse stomachs at Kahanahaiki despite the availability of these common fruits during the time period when the bulk of mice were trapped. The amount of fruit (11%) in mouse stomachs was much

less than in both rat species in my study, but was almost identical to the 10% determined by Cole et al. (2000). Fruit was absent from the 25 mouse stomachs analyzed from gulches adjacent to sugar cane fields on Hawaii island (Kami 1966). From studies within and outside Hawaii, it appears that the house mouse consumes relatively small portions of fruit (especially fleshy fruit) compared to seed, vegetative material, and arthropods (Kami 1966; Cole et al. 2000; Angel et al. 2009; this study). It is unclear why fruits and seeds from other species that are present in Kahanahaiki were not observed in any of these rodent stomachs; however, it may be a result of food preference or simply a reflection of the difficulty with microscopically identifying chewed fragments in rodent stomachs.

Most seeds consumed by rodents appeared highly vulnerable to predation as evidenced by chewed and fragmented seed tissues. However, there were several native and non-native seeds that were intact and would possibly be dispersed by these rodents. Because the black rat is the largest of the three rodents studied and body sizes of vertebrates often positively correlate with the sizes of seeds that pass intact through digestive tracts (Wheelright 1985; Vander Wall 1990), black rats pass intact seeds of a greater size range than the smaller rodents in my study (Williams et al. 2000). Intact seeds of eight plant species, including at least two natives, occurred in black rat stomachs in my study. Two of these, *C. hirta* and *R. rosifolius*, are among the most problematic invasive plants in the Hawaiian Islands, and their small seeds ( $\leq 1.5$  mm length) pass intact through the rat's digestive tract and germinate (Chapter 5; Shiels, in press). Some individuals of all three rodents had intact *C. hirta* seeds in their stomachs, suggesting that they may be capable of dispersing this and other small-seeded species.

Arthropods comprise a substantial portion of the diet of introduced rodents, as evidenced by > 90% of all stomachs examined in this study containing arthropods. In a recent review by Angel et al. (2009), arthropods were found to be the prey of choice for the house mouse on islands in the Southern Ocean, and this pattern may be supported at Kahanahaiki where arthropods accounted for an average of 57% of their stomach contents. However, in high elevation (2000-3000 m) shrubland in Hawaii, plant material (mainly seeds) was the dominant food type for mice and arthropods comprised 33% of their diet. Relative to mice, arthropods were a much smaller component of black rat stomach contents in my study (14%) and that of Cole et al. (2000) where stomachs contained 16% arthropods. There were no arthropods in black rat stomachs and only trace amounts of caterpillars in Pacific rat stomachs in lowland wet forest in Hawaii (Beard and Pitt 2006). The arthropod composition of Pacific rats in my study was 37% and was therefore intermediate between black rats and mice. There was no report of Pacific rats captured in the study by Cole et al. (2000), but Sugihara (1997) compared invertebrates, which were mostly arthropods but included slugs, snails, and earthworms, and found that stomachs contained 67-96% and 34-45% invertebrates for Pacific rats and black rats, respectively. Despite the presence of native and non-native snails (Meyer and Shiels 2009) and non-native slugs and earthworms (Joe and Daehler 2008) at Kahanahaiki, there was no evidence of any of these organisms in rodent stomachs.

Several of the same taxonomic groups of arthropods that were consumed by rodents in my study are similar to those found in black rat and mouse stomachs by Cole et al. (2000) from higher elevations in Hawaii, including bugs, spiders, and ants. The non-native ant *Solenosis papuana* is ca. 1 mm in length and was present in at least 10%

of individuals of each rodent species, but it is unlikely, due to its small size, that these ants were consumed intentionally. Although crickets and beetles are common components of both black rats and mice in New Zealand (Innes 2005b; Ruscoe and Murphy 2005), as well as in the study by Cole et al. (2000), they were only found in black rats in my study. Two groups that were found in my study but not that of Cole et al. (2000) were cockroaches (Blattaria) and lice (Phthiraptera). Cole et al. (2000) found that the majority of the arthropods in rodent stomachs were native; however, ca. one-third of the identifiable arthropod groups or species in black rats were native in my study, and only one native species (*Macaphesa* sp.) was found in the house mouse. Caterpillars (larvae of Lepidoptera) appear to be a vulnerable food item to all three rodents studied in Hawaii because some individuals of all species studied had caterpillars in their stomachs in high elevation environments (Sugihara 1997; Cole et al. 2000), and 100%, 83%, and 34% of individuals of Pacific rats, mice, and black rats, respectively, had caterpillars in their stomachs in Kahanahaiki. The proportion of stomach contents that contained caterpillar was greatest in mice (54% in my study; 22% in that of Cole et al. 2000) and least in black rats (3% in my study; 4% in Cole et al. 2000). In New Zealand, caterpillars are generally the most common arthropod eaten by mice (Badan 1986; Ruscoe and Murphy 2005), and one of the most common groups eaten by Pacific rats (Bunn and Craig 1989; Atkinson and Towns 2005).

Although the stomach contents revealed that Pacific rats had an intermediate (short-term) diet between those of black rats and mice, the greater amount of caterpillar and unknown arthropod material that was consumed by Pacific rats and mice relative to black rats may partially account for the slightly higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for the two smaller

rodents compared to black rats. Additionally, Pacific rats may be more similar to mice than to black rats in lifetime average diet because of differences in foraging microsites when the black rat is present (Lindsey et al. 1999; Stapp 2002; Atkinson and Towns 2005; Chapter 2) or assimilation rates of different prey types (Gannes et al. 1997).

Rather than using isotopes to assign trophic levels to introduced rodents that are sympatric in forests, most past isotope studies involving introduced rodents examined effects on seabirds and the proportion of the rodent diet attributable to marine and terrestrial feeding (Strapp 2002; Caut et al. 2008a; Quillfeldt et al. 2008). Isotopic analysis shows that the black rat is a substantial predator of seabirds on some islands (e.g., Strapp 2002; Caut et al. 2008a) but not others (Cassaing et al. 2007). Evidence of bird consumption by rodents was absent at Kahanahaiki despite an abundance of introduced passerines and game birds at the study site (personal observation). Marine inputs to rodent diets are unlikely at Kahanahaiki because the site is > 3 km from the ocean and home-ranges are typically < 4 ha for each rodent (Chapter 2). Therefore,  $\delta^{13}\text{C}$  differences in my study are more likely to involve unequal consumption of  $\text{C}_3$  and  $\text{C}_4$  plants (Gannes et al. 1997), which may help explain the slightly higher  $\delta^{13}\text{C}$  values for Pacific rats and mice relative to black rats. In an interior forest habitat on Stewart Island, New Zealand, Harper (2006) determined  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values and found that the diets of Pacific rats and black rats were similar. The only isotopic study available with wild house mice was by Quillfeldt et al. (2008) in the Falkland Islands where  $\delta^{13}\text{C}$  were similar to those in my study (-21‰ and -24‰); yet the  $\delta^{15}\text{N}$  for mice ranged from 12-31‰ and were indistinguishable from potential prey, including terrestrial plants ( $\delta^{15}\text{N}$  8-35‰), terrestrial invertebrates ( $\delta^{15}\text{N}$  14‰), and upland birds ( $\delta^{15}\text{N}$  16-19‰) (Quillfeldt

et al. 2008). Therefore, using isotopes to study diets and differentiate trophic feeding among predators may not always give a clear depiction of predator-prey interactions without supplemental dietary analysis such as stomach contents (Strapp 2002; Caut et al. 2008a; Quillfeldt et al. 2008; Flaherty and Ben-Davis 2010; this study).

The three rodents in this study appear to occupy two different trophic levels and their dietary patterns provide evidence of niche differentiation resulting from unequal consumption of types and quantities of prey. When these three rodents coexist in Hawaiian forest, the larger-bodied black rat appears to dominate fruit and seed consumption. The house mouse, which eats some seed and even less fruit, feeds primarily on caterpillars. The Pacific rat, which has been in Hawaii for ca. 1000 years but may be outcompeted by black rats because it is sparsely abundant in contemporary Hawaii (Chapter 2), has an intermediate diet between the other two rodent species and its  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures suggest that this small rat has a lifetime diet more like the house mouse than the black rat. The relative effects of each of the rodent species on their prey cannot be determined without further understanding of prey abundances in the presence and absence of each rodent predator (e.g., using removal experiments). Conservationists working in island habitats containing these three introduced rodents should not overlook the range of arthropods that may be at risk to rodent predation, and that all three rodents typically chew, and probably destroy, most consumed seeds > 2 mm in length. The degree to which prey species and trophic levels are exploited by each introduced rodent in Hawaii and elsewhere may depend upon the assortment of rodent species that are present at a given site.

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