GIVING-UP DENSITY AND DIETARY SHIFTS IN THE WHITE-FOOTED MOUSE, *PEROMYSCUS LEUCOPUS*

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Abstract. Dietary shifts are commonly exhibited by omnivorous consumers when foraging from variable food resources. One advantage of dietary shifts for a consumer is the ability to gain complementary resources from different foods. In addition, dietary shifts often affect food-web dynamics. Despite the importance of dietary shifts to organismal, community, and ecosystem ecology, empirical studies of the ecological mechanisms that control dietary shifts are limited in scope. In this study, we tested the effects of complementary resources on dietary shifts of an omnivorous mammal, the white-footed mouse *Peromyscus leucopus*, in the context of depletable food patches in the natural environment. We used two complementary resources: seeds that provide a higher energy gain per unit handling time and mealworms that provide a higher protein gain per unit handling time. Stable isotopes of carbon and nitrogen ($\delta^{13}C$, $\delta^{15}N$) in mouse plasma were used to quantify dietary shifts, and we determined giving-up density (GUD), the food density at which a forager leaves a food patch (for an optimal forager, it should correspond to the quitting harvest rate that balances net fitness gain with various costs of foraging). The results showed that GUD increased most significantly when a mixture of seeds and mealworms was added, compared to when only seeds or mealworms were added. This suggests that, given a similar level of food availability, a patch with a mixture of complementary foods is of higher quality than a patch with only one type of food. Moreover, GUD measured with seeds (GUD_s) correlated positively with seed availability, and GUD measured with mealworms (GUD_{mw}) correlated positively with mealworm availability, indicating that the marginal value of seeds or mealworms decreases with their relatively availability in the environment. As GUD_s increased, *P. leucopus* shifted their diets toward higher trophic levels, and as GUD_{mw} increased, *P. leucopus* shifted their diets toward lower trophic levels, suggesting dietary shifts driven by food complementarity. This study demonstrated that the combination of giving-up density and stable-isotope analysis holds great potential for testing ecological mechanisms underlying dietary shifts.

Key words: complementary resources; dietary shifts; food availability; food quality; foraging behavior; foraging theory; giving-up density, GUD; oak–hickory forest, Virginia, USA; omnivory; Peromyscus leucopus; *stable isotopes.*

INTRODUCTION

One central theme in ecology is how animals shift their diets to cope with variation in food availability, food quality, and associated costs and risk (MacArthur and Pianka 1966, Pulliam 1974, Stephens and Krebs 1986, Bernays et al. 1994, Demott 1995, Schmidt et al. 1998, Agrawal et al. 2000, Cruz-Rivera and Hay 2000, Schmidt 2000, Brown and Kotler 2004, Schmitz et al. 2004, Singer et al. 2004). In foraging theory, it is predicted that a consumer should become more selective in diets when its energy requirement is satisfied (Pulliam 1975). Being selective in diets does not always mean narrower diet width. For omnivorous consumers, it can mean a higher degree of dietary mixing (e.g., a mixture

of food items with complementary quality). Dietary shift is not only a foraging strategy to achieve dietary mixing, it also affects food-web dynamics (Polis and Strong 1996, Fagan 1997, Zanden et al. 1999, Kondoh 2003, Krivan and Schmitz 2003). Despite the importance of dietary shifts to organismal, community, and ecosystem ecology (Tilman 1982, Vincent et al. 1996), empirical studies of the ecological mechanisms that control dietary shifts are limited in scope. For instance, it is exceedingly difficult to attribute dietary shifts to food availability in natural settings. This is not only because each species has a unique set of locomotion and perception abilities (Vasquez 1996, Rosenheim and Corbett 2003, Wall et al. 2003), but also because food availability is often a function of both the abundance of different food items and the interactions among organisms. Therefore, previous studies have relied heavily on theoretical and laboratory experiments to test the complex interactions among energy, nutrients, and risk in determining foraging behavior (Pulliam 1975, Bowen et al. 1995, Demott 1995, Agrawal et al. 2000, Cruz-Rivera and Hay

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2000, Diehl 2003, Brown and Kotler 2004, Singer et al. 2004).

For a generalist consumer, foraging often may be directed toward patches of highest cumulative prey availability rather than any particular prey type (Schmidt et al. 2001, also see "patch-use theory" in Stephens and Krebs [1986]). However, different foods may not be perfectly substitutable. When foods are complementary to a consumer (i.e., the relative value of a food increases as it becomes increasingly rare in the diet; see Tilman [1982] for a detailed discussion on different nutritional relationships, and Schmidt et al. [1998] for a focused test on complementary resources), the preference for patches of highest cumulative food availability may shift to patches of appropriate mixtures of complementary foods. In order to test the effects of food complementarity on patch use and dietary shifts in nature, we applied an experimental approach using a combination of depletable food patches and stable isotopes.

Giving-up density (GUD; Brown 1988, 1992) was developed to quantify different aspects of foraging behavior, such as perceived food availability and predation risk, and is particularly useful in a natural setting. Giving-up density is the food density at which a forager leaves a food patch. For an optimal forager, it should correspond to the quitting harvest rate that balances net fitness gain with various costs of foraging (Schoener 1971, Charnov 1976). Conceptually, when an animal experiences diminishing returns to harvest rates in a food patch to a point that the fitness gain no longer exceeds costs, it should leave the patch. The quitting harvest rate of a food patch is equivalent to the marginal value of that food patch (Schmidt et al. 1998, Schmidt 2000), which is often a function of the availability of the food in the environment. For example, when food A and food B are complementary to a consumer, an increase in the availability of food A in the environment will result in a decrease in the marginal value of food A, and an increase in the quitting harvest rate (or GUD) of food-A patches. In the meantime, the relative availability of food B in the environment decreases as a result of increased availability of food A, leading to an increase in the marginal value of food B, and a decrease in the quitting harvest rate (or GUD) of food-B patches. Energy is commonly used as the currency in a GUD model. However, other types of currency that measure certain aspects of fitness (e.g., survivorship) can also be used. Recent developments of giving-up-density methods have facilitated field experiments on foraging behavior across various animal taxa, including mammals (e.g., Bowers et al. 1993, Davison and Morris 2001, Brown and Kotler 2004), birds (e.g., Olsson et al. 2001), and arthropods (e.g., Kay 2002).

The goals of this study are to determine how relative availability of food items with different nutritional values is perceived by a mammalian omnivore, the white-footed mouse, *Peromyscus leucopus*, and whether it promotes dietary shifts toward foods of complemen-

tary quality. *Peromyscus leucopus* feed on a wide range of food items including plant foods (e.g., fruits, nuts) and animal foods (e.g., arthropods, eggs), and have been found in the laboratory to discriminate among food items based on their protein and energy contents (Lewis et al. 2001). Moreover, the method of giving-up density has been applied successfully to *P. leucopus* in natural settings (e.g., Davison and Morris 2001), which makes *P. leucopus* an ideal species for this study. We hypothesize that *P. leucopus* treat plant and animal foods as complementary resources, and with a seed-vs.-mealworm addition experiment (seed addition, mealworm addition, mixed-food addition of both seeds and mealworms), we tested the following predictions: (1) GUD should increase with food addition, and the increase should be greatest with mixed-food addition; (2) GUD measured with seeds (GUD_s) should correlate positively with relative seed abundance in the environment, and GUD measured with mealworms (GUD_{mw}) should correlate positively with relative mealworm abundance in the environment; and (3) *P. leucopus* should shift their diets toward higher trophic levels (e.g., more insectivorous) with increasing seed availability, and shift their diets toward lower trophic levels (e.g., more granivorous) with increasing mealworm availability. In addition, because consumer density may also influence GUD and confound our ability to use GUD to measure foraging behavior, we also tested the correlation between *P. leucopus* density and GUD.

Traditionally, dietary studies utilize methods such as stomach content analysis or food-choice trials. There are several limitations in these approaches. For example, stomach contents usually reflect only the last meal, and food-choice trials (e.g., cafeteria trials) often involve just a few feeding bouts during which animals are provided with a limited set of food items without diminishing returns. In reality, diets are cumulative over a series of sequential feeding events that may span weeks or seasons, and dietary sources can come from diverse taxa of plant or prey species that have different nutritional values.

In our study, the naturally occurring stable isotopes of nitrogen and carbon were used to quantify trophic positions of free-ranging mice. Stable isotopic compositions provide information on assimilated food and reflect a diet accumulated over a period from days to animals' lifetime, depending on the type of tissues analyzed. Stable nitrogen isotopic composition ($\delta^{15}N$‰) is consistently enriched by 3 to 3.5‰ per trophic level (DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987, Tieszen et. al. 1989, Michener and Schell 1994, Vanderklift and Ponsard 2003, MacAvoy et al. 2005). And while shifts in $\delta^{15}N$ of a forager may not reveal changes in diets occurring within the same trophic level, dietary shifts in *P. leucopus* often involve the inclusion of food items from different trophic levels, particularly plants and arthropods (Whitaker 1966, Wolff et al. 1985). Therefore, dietary shifts as estimated with $\delta^{15}N$ are equivalent to

TABLE 1.   Energy content, protein content, energy gain per unit handling time, and protein gain per unit handling time of millet seeds and mealworms eaten by *Peromyscus leucopus* in a food-addition experiment.

Food type	Energy (KJ/g)	Protein (%)	Water content (%)	Handling time per item (s)†	Dry mass per item (g)	Protein gain per unit handling time (mg/s)	Energy gain per unit handling time (KJ/s)
Mealworm	6.755	0.106	63	77.5 ± 11.08‡	0.061 ± 0.0022§	0.075	0.005
Millet seed	13.360	0.106	10	8.5 ± 0.47‖	0.0047 ± 0.0001¶	0.059	0.007

Notes: Data on energy, protein, and water content of millet seeds and mealworms are from the commercial suppliers (millet seeds: ebirdseed.com, Hawley, Minnesota, USA.; mealworms: Flucker Farm, Port Allen, Louisiana, USA) with the exception of energy content of mealworms, which is based on Allen (1989).

† Data on handling time and dry mass per item were measured in this study. Mice captured during the experimental period were randomly selected for feeding trials. Each trial lasted ~10 minutes during which a mouse was placed in an observation box at the location where it was captured and was provided with abundant seeds or mealworms in a dish; the time a mouse spent on handling a single seed or a mealworm was then recorded. Protein and energy gain per unit handling time were derived from protein content, energy content, handling time, and dry mass data. Data are reported as means (±SE).

‡ Sample size: $n = 9$ samples.

§ Sample size: $n = 40$ samples.

‖ Sample size: $n = 32$ samples.

¶ Sample size: $n = 20$ samples.

changes in trophic levels for *P. leucopus*. Whereas stable carbon isotopic compositions ($\delta^{13}C‰$) show little trophic effect, they are distinct between terrestrial C_3 and C_4 plants (Vogel 1993), which can be used to quantify relative contributions of C_3- vs. C_4-based food sources to the diet of a consumer (e.g., Teeri and Schoeller 1979, West et al. 2004).

Methods

Study site and food-addition experiment

Fieldwork was conducted in two forest sites at the Blandy Experimental Farm, University of Virginia, Clarke County, Virginia, USA (78°00′ W, 39°00′ N). The two sites are 2 km apart and are both composed of an oak–hickory (*Quercus–Carya*) community. Within each site, three trapping grids were established. The adjacent grids of a site were 90 m apart, each comprised of 25 trapping stations in a 5 × 5 array with 10 m between stations. The grid size was 0.25 ha, separated from other grids by an area of 0.5 ha. Adult *Peromyscus leucopus* were known to have small home ranges, usually less than 0.3 ha (Stickel 1968), and *P. leucopus* populations were known to exhibit high demographic heterogeneity within a patch as small as 3 ha (Krohne and Burgin 1990). Therefore, for the purpose of this study, each grid was treated as a population. In July 2004, each of the three grids at a site was supplemented with one of the following food types: millet seeds (*Panicum miliaceum*), mealworms (larvae of *Tenebrio molitor*), or a mixture of seeds and mealworms at equal proportion. Seeds and beetle larvae are common food items for *P. leucopus* in the natural environment, and they have distinct nutritional contents. Specifically, seeds often have higher energy content and larvae have higher protein content. For *P. leucopus*, millet seeds provide a higher energy gain per unit handling time whereas mealworms provide a higher protein gain per unit handling time (Table 1). Therefore, millet seeds and mealworms may be complementary sources of energy and protein for the mice.

The three food-addition treatments were designed to create different levels of relative availability of millet seeds and mealworms. Food was broadcast by hand equally over all the trapping stations in a grid daily. Total cumulative food density for each of the treatment was 18 kg/ha over the 20-d period of the experiment. Because mealworms have 52% more water content than millet seeds, the 18 kg of added food represents 7 kg dry mass of mealworms in mealworm-addition grids, 11 kg dry mass of mealworms and seeds in mixed-food-addition grids, and 16 kg dry mass of millet seeds in seed-addition grids. Even though the dry mass of food added was lower in mealworm-addition grids, it provided an average of 15 g of food per mouse per day (using *P. leucopus* density estimated prior to food addition in these grids), which is much higher than their average daily consumption rate, 2–3 g (e.g., Vickery et al. 1994). Although there was not a "no addition" treatment, the effects of food addition on GUD and dietary shifts were examined by comparing data before and after food addition. The experiment was completed in a relatively short time period (20 days) and it is assumed that the differences before and after food addition largely reflect food-addition effects.

Mouse trapping and giving-up density

Peromyscus leucopus were trapped for 3–6 consecutive nights both before and after food addition. Upon capture, each individual mouse was marked with an ear tag, its sex and age were recorded, and ~0.1 mL of blood sample was taken. All procedures involving mouse trapping and bleeding followed protocols approved by the Animal Use and Care Committee at the University of Virginia. Giving-up density (GUD) measurements were made both immediately prior to and after each trapping session, using enclosed boxes with access holes tailored to the size of mice and filled with 250 mL of fine sand within which 1.25 g of millet seeds and 1.25 g of mealworms were mixed. The weight

of the food remaining in the boxes was checked every morning until it stopped decreasing, determined with a repeated-measurement ANOVA. This usually occurred by day 3. Therefore, GUD was taken as the weight (mass) of each food type remaining on day 3. Giving-up density was measured at the grid level by averaging across five randomly selected stations within a grid. The spatial scale of GUD in this study was similar to the intermediate scale defined in previous studies, an aggregate of patches coupled by a forager's daily movement (Morgan et al. 1997). This is the scale that foragers assess the average quality of their environment (Brown and Alkon 1990, Morgan et al. 1997). Mouse density for each trapping session within each grid was estimated using mark–recapture data with a robust design model implemented in the program MARK (Pollock 1982, White 2000).

Stable-isotope analyses and dietary shifts

Stable isotopic compositions are reported in the conventional form:

$$\delta^x E(‰) = [(^x E/^y E)_{\text{sample}}/(^x E/^y E)_{\text{std}} - 1] \times 1000 \quad (1)$$

where E is the element being measured, $(^x E/^y E)_{\text{sam}}$ is the isotopic composition of the sample, and $(^x E/^y E)_{\text{std}}$ is the isotopic composition of a standard material specific to the element. The standard for stable carbon and nitrogen isotopic composition is Peedee Belemnite (PDB) and atmosphere molecular nitrogen (N_2), respectively. In order to reflect short-term dietary shifts, plasma samples were separated from whole blood for isotopic analysis. Although direct isotopic turnover estimates are not available for $P.\ leucopus$, it has been shown that stable carbon isotopic turnover in American crow (Corvus brachyrhynchos) was almost 10 times faster in plasma than in blood cell (Hobson and Clark 1993). In addition, the half-life of stable carbon isotopes in blood of mice (Mus musculus) was estimated to be 16.9 d (MacAvoy et al. 2005), suggesting that plasma of $P.\ leucopus$ may reflect dietary shifts within days. Samples were kept frozen prior to a drying process, and then were oven-dried at 60°C for 24 h. Stable carbon and nitrogen isotopic compositions of the samples were measured with a G. V. Micromass Optima Isotope Ratio Mass Spectrometer (IRMS; Manchester, UK).

Stable nitrogen isotopic compositions vary across space in terrestrial ecosystems, especially between locations with different land-use histories (e.g., fertilizer application, successional stages). In order to take into account the differences in $\delta^{15}N$ of plants between sites, $\delta^{15}N$ of $P.\ leucopus$ was adjusted with site-specific, baseline $\delta^{15}N$ of plants (Appendix). Individual trophic position was estimated by the difference in $\delta^{15}N$ between $P.\ leucopus$ and the baseline plants (i.e., $\Delta\delta^{15}N$). Foliar tissues were used in this study to maximize the number of plant species included in the baseline data. Although $P.\ leucopus$ mainly feed on seeds or fruits instead of leaves, most plant species vary only about 2‰ in $\delta^{15}N$

between different tissues (Lajtha and Marshall 1994). It is important to note that trophic position as measured by $\Delta\delta^{15}N$ is relative trophic position (e.g., a mouse with a $\Delta\delta^{15}N = 3‰$ feeds at a lower trophic level than a mouse with a $\Delta\delta^{15}N = 6‰$), and should not be used to indicate absolute trophic position (e.g., a mouse with $\Delta\delta^{15}N = 3‰$ feeds 100% on plants). The mean trophic level of a population was estimated by averaging individual $\Delta\delta^{15}N$ within a grid. Because the supplemented millet seeds had an elevated $\delta^{15}N$ relative to the baseline plants, the baseline $\delta^{15}N$ was adjusted based on the fraction of millet seeds in the diets of $P.\ leucopus$. Millet is a C_4 plant and has a distinct $\delta^{13}C$ from most plants at the study site, a C_3-dominanted community (millet $\delta^{13}C = -11 \pm 0.2‰$ vs. baseline $\delta^{13}C = -32 \pm 0.6‰$ and $-29 \pm 1.2‰$; Appendix), which allows us to estimate the fraction of millet seeds in the diets of $P.\ leucopus$ with the following equation (modified from Sanzone et al. 2003):

$$P.\ leucopus_{\text{millet}} = \frac{\delta^{13}C_{\text{mouse},t2} - \delta^{13}C_{\text{mouse},t1}}{\delta^{13}C_{\text{millet}} - \delta^{13}C_{\text{baseline}}} \quad (2)$$

where the subscripts "$t1$" and "$t2$" indicate the isotopic composition of a sample measured "prior to" and "after" food addition, respectively.

The mixing equation used in Sanzone et al. (2003) can be expressed in a more general form:

$P.\ leucopus_{\text{foodtype1}}$

$$= \frac{\delta^{13}C_{\text{consumer}} - \delta^{13}C_{\text{foodtype2}} - \text{trophic effect}}{\delta^{13}C_{\text{foodtype2}} - \delta^{13}C_{\text{foodtype1}}.} \quad (3)$$

With two repeated measurements on the same mouse, and with the assumption of a constant trophic effect (i.e., the isotopic differences between a consumer and its food), Eq. 3 becomes:

$P.\ leucopus_{\text{foodtype1},t1}$

$$= \frac{\delta^{13}C_{\text{consumer},t1} - \delta^{13}C_{\text{foodtype2}} - \text{trophic effect}}{\delta^{13}C_{\text{foodtype2}} - \delta^{13}C_{\text{foodtype1}}} \quad (4)$$

$P.\ leucopus_{\text{foodtype1},t2}$

$$= \frac{\delta^{13}C_{\text{consumer},t2} - \delta^{13}C_{\text{foodtype2}} - \text{trophic effect}}{\delta^{13}C_{\text{foodtype2}} - \delta^{13}C_{\text{foodtype1}}} \quad (5)$$

In our study, foodtype1 $\delta^{13}C$ is the stable carbon isotopic composition of millet seeds and foodtype2 $\delta^{13}C$ is the stable carbon isotopic composition of the baseline plants. $P.\ leucopus_{\text{millet},t1}$ was assigned a zero because no millet seeds and very few C_4 plants were available in the environment prior to food addition. Therefore, Eq. 2 can be derived by subtracting Eq. 4 from Eq. 5.

Statistical analysis

The effects of food addition on GUD, trophic level of $P.\ leucopus$, and population density of $P.\ leucopus$ were

tested using a repeated-measures ANOVA, followed by planned contrast comparisons between pre-augmentation data and post-augmentation data, for each of the food-addition treatments. The repeated-measurement design was used to avoid potential bias that may be introduced from different GUD levels among grids during the pre-augmentation period. For post-augmentation data, post hoc contrast comparisons with Tukey adjustment were used to compare the differences in GUD between mealworm-addition grids, mixed-food-addition grids, and seed-addition grids. The daily consumption rates of seeds and mealworms in the food stations were compared using a paired t test for each of the three food-addition treatments, as well as for data before and after food addition, to test if daily consumption rates of seeds and mealworms remain constant under different cumulative and relative food availability. Associations between GUD and trophic level of $P.$ $leucopus$, and between GUD and population density of $P.$ $leucopus$ were examined with a Spearman correlation.

RESULTS

Giving-up density and food addition

Prior to food addition, the mealworm giving-up density (GUD_{mw}) was the same among all grids, whereas the seed GUD (GUD_s) was lower in mealworm-addition grids than in other grids (ANOVA, GUD_{mw}: $F_{2,26} = 2.28$, $P = 0.12$; GUD_s: $F_{2,26} = 3.98$, $P = 0.03$). When GUD_s and GUD_{mw} were combined ($GUD = GUD_s + GUD_{mw}$), there was no difference in GUD among all grids (ANOVA, GUD: $F_{2,26} = 1.10$, $P = 0.35$). Both GUD_s and GUD_{mw} increased after food addition (Fig. 1; repeated-measures ANOVA, GUD_s: food-type effect: $F_{2,53} = 17.58$, $P < 0.0001$; food-addition effect: $F_{1,53} = 13.79$, $P = 0.0005$; food type × food-addition effect: $F_{2,53} = 0.47$, $P = 0.61$; GUD_{mw}: food-type effect: $F_{2,53} = 8.72$, $P = 0.0005$; food-addition effect: $F_{1,53} = 5.02$, $P = 0.03$; food type × food-addition effect: $F_{2,53} = 1.67$, $P = 0.20$). Contrast comparisons on each of the three food-addition treatments showed that only in mixed-food-addition grids, both GUD_s and GUD_{mw} increased. In seed-addition grids, GUD_s increased but not GUD_{mw}, whereas in mealworm-addition grids, neither increased significantly (Fig. 1A and 1B). When GUD_s and GUD_{mw} were combined, GUD increased more in mixed-food-addition grids after food addition, followed by seed-addition grids and mealworm-addition grids (repeated-measures ANOVA, food-type effect: $F_{2,53} = 8.42$, $P = 0.0007$; food-addition effect: $F_{1,53} = 13.89$, $P = 0.0005$; food type × food-addition effect: $F_{2,53} = 0.67$, $P = 0.52$.) Contrast comparisons on each of the three food-addition treatments showed that GUD increased in mixed-food-addition grids and in seed-addition grids, but not in mealworm-addition grids. With GUD_s and GUD_{mw} examined separately and combined, mixed-food-addition grids showed the most significant increase in GUD, indicating these grids are of higher quality. The lack of changes in GUD, GUD_s, and
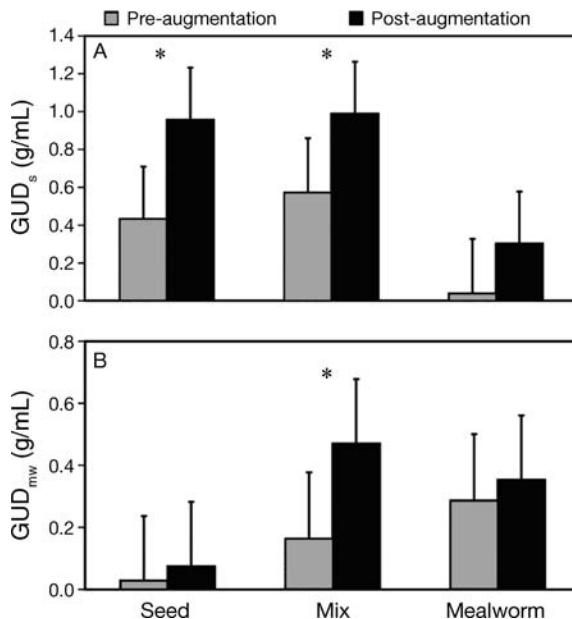

FIG. 1. Giving-up density for (A) seeds (GUD_s) and (B) mealworms (GUD_{mw}) in seed-addition grids, mixed-food-addition grids, and mealworm-addition grids. GUD is a measure of the amount of food in a given volume of materials searched. Asterisks (*) denote a significant difference ($P < 0.05$) between pre- and post-food-augmentation pairs. Error bars represent upper 95% confidence limits averaged across the two grids.

GUD_{mw} in mealworm addition grids was not likely a biased result from the lower GUD levels prior to food addition in these grids. This is because only GUD_s was lower prior to food addition, yet all three GUD measures showed no response to food addition in these grids.

After food addition, GUD_{mw} was higher in mealworm-addition grids and mixed-food-addition grids, and lower in seed-addition grids. By contrast, GUD_s was higher in seed-addition grids and mixed-food-addition grids, and lower in mealworm-addition grids (post hoc comparisons with Tukey adjustment). This indicates that the marginal value of mealworms is lower when mealworm availability in the environment is high, and similarly, the marginal value of seeds is lower when seed availability in the environment is high. Furthermore, after food addition, GUD_{mw} became lower than GUD_s for all grids (paired t test, pre-augmentation: $t = 1$, $P = 0.36$, df = 5; post-augmentation: $t = 2.47$, $P = 0.06$, df = 5), and the daily consumptions of mealworms became higher than that of seeds in seed-addition grids and mixed-addition grids (Fig. 2), suggesting that the mice preferentially consume mealworms over seeds only when food availability is high.

Giving-up density and dietary shifts

Prior to food addition, the trophic level of $P.$ $leucopus$ as measured by stable nitrogen isotopes was positively
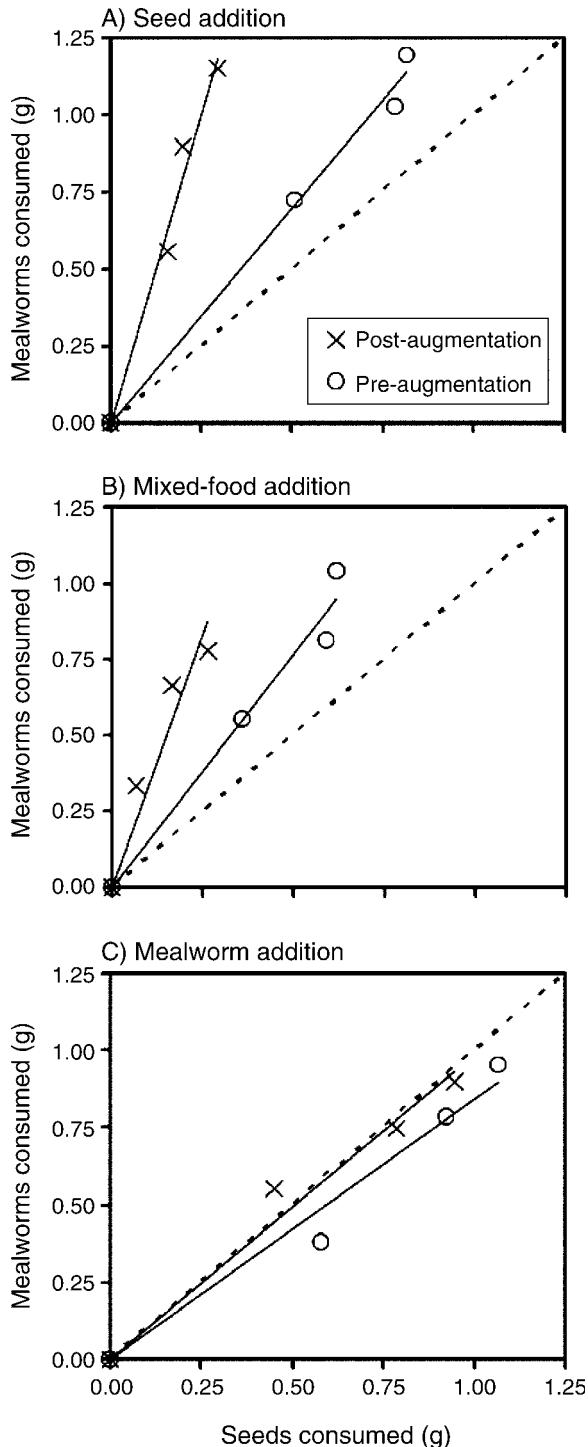
FIG. 2. The daily consumption by *Peromyscus leucopus* of seeds and of mealworms from the food stations in (A) seed-addition grids, (B) mixed-food-addition grids, and (C) mealworm-addition grids. The 1:1 seed vs. mealworm consumption line is plotted for visual comparison purpose. Each data point is a daily average across 10 food stations between the two sites, before and after food addition.

correlated with GUD_s and negatively correlated with GUD_{mw} (Fig. 3A; Spearman correlation, trophic level vs. GUD_s: $r = 0.46$, $P = 0.04$; trophic level vs. GUD_{mw}: $r = -0.69$, $P = 0.0005$, $n = 21$ samples). Because GUD_s reflected seed availability (or availability of other plant foods with similar nutritional quality, e.g., high energy gain per unit handling time), and GUD_{mw} reflected mealworm availability (or availability of other animal foods with similar nutritional quality, e.g., high protein gain per unit handling time), the relationship between GUD and trophic level of *P. leucopus* suggests that plant and animal foods are complementary, and that the mice shifted their trohpic levels in response to relative availability of plant and animal foods. However, contrary to our prediction, trophic level of *P. leucopus* did not correlate with either GUD_s or GUD_{mw} after food addition (Fig. 3B; Spearman correlation, trophic level vs. GUD_s: $r = -0.36$, $P = 0.11$; trophic level vs. GUD_{mw}: $r = -0.19$, $P = 0.41$, $n = 21$ samples; additional correlations were performed using log-transformed GUDs to make sure that the lack of correlations after food addition was not a result of the overall high GUD levels).

Giving-up density and mouse density

Food addition did not influence population density of *P. leucopus* (Fig. 4; repeated-measures ANOVA, food-type effect: $F_{2,5} = 0.84$, $P = 0.49$; food-addition effect:
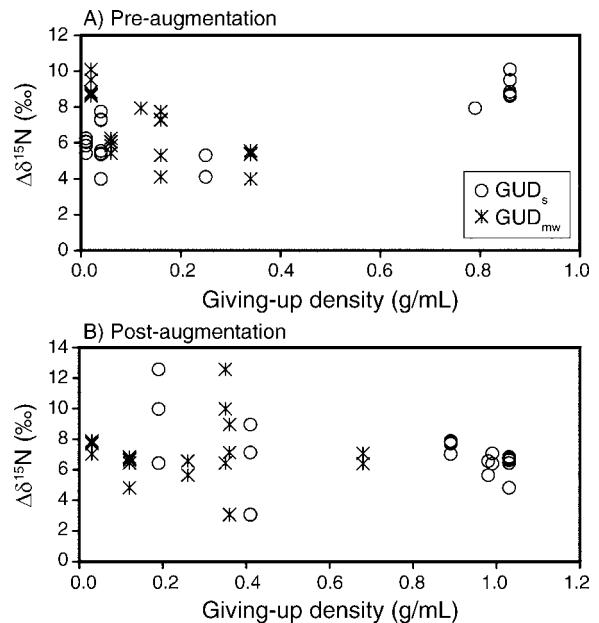

FIG. 3. Correlations between the trophic level of *Peromyscus leucopus* (estimated by the difference in $\delta^{15}N$ between *P. leucopus* and the baseline plants) and the giving-up density (GUD) for seeds, and between the trophic level of *P. leucopus* and the GUD for mealworms, before and after the food-addition experiments. Each data point denotes an individual mouse. Panel (A) shows pre-augmentation data, and panel (B) shows post-augmentation data.

$F_{1,5} = 0.62$, $P = 0.47$; food type × food-addition effect: $F_{2,5} = 1.01$, $P = 0.43$). Neither GUD_s nor GUD_{mw} was correlated with population density (Spearman correlation, before food addition: density vs. GUD_s: $r = -0.62$, $P = 0.19$; density vs. GUD_{mw}: $r = 0.35$, $P = 0.49$, $n = 6$ samples; after food addition: density vs. GUD_s: $r = 0.75$, $P = 0.08$; density vs. GUD_{mw}: $r = 0$, $P = 1$, $n = 6$ samples). These results confirmed that the effects of food availability on foraging behavior of *P. leucopus* were not likely confounded by their density.

DISCUSSION

Our results demonstrated that giving-up density (GUD) can be used to estimate not only cumulative food availability but also relative availability of food items with different nutritional quality. It is not clear, however, at which level a forager assesses relative availability of different types of food (e.g., different plant/prey species, high-energy vs. low-energy foods). In this study, GUD successfully reflected relative availability of millet seeds and mealworms in the environment because of the complementary nature of these two food types to *Peromyscus leucopus*. Nevertheless, GUD may or may not reflect relative availability of different species of seeds, which often provide much similar nutritional value. The potential of GUD method in measuring cumulative and relative availability of foods, although not yet fully tested, can serve as an important tool for understanding plant–animal or predator–prey relationships.

For a generalist consumer such as *P. leucopus*, relative availability of different foods may be more important than cumulative food availability in determining their foraging behavior. Our results demonstrated that GUD increased most significantly in mixed-food-addition grids, despite the fact that more food (on a dry mass basis) was added to seed-addition grids than to mixed-food-addition grids. Therefore, patches with equal or lower cumulative food availability, if having an appropriate mixture of food types, may be perceived by omnivorous consumers as food patches of higher quality.

Using GUD as a surrogate for the quitting harvest rate and the marginal value of a food patch, we demonstrated that omnivores shifted their diets in response to relative availability of complementary foods. Prior to food addition, *P. leucopus* shifted toward higher trophic levels with increased GUD_s (increased availability of foods similar in quality to seeds), and toward lower trophic levels with increased GUD_{mw} (increased availability of foods similar in quality to mealworms), suggesting that seeds and mealworms are complementary foods to the mice. Considering that for *P. leucopus*, seeds are one of the most common food items from lower trophic levels, and larvae are one of the most common food items from higher trophic levels, our results further suggest that trophic shifts in an omnivorous consumer such as *P. leucopus* may be driven by resource complementarity. This helps explain why the degree of mixing between plant and animal foods in *P.*
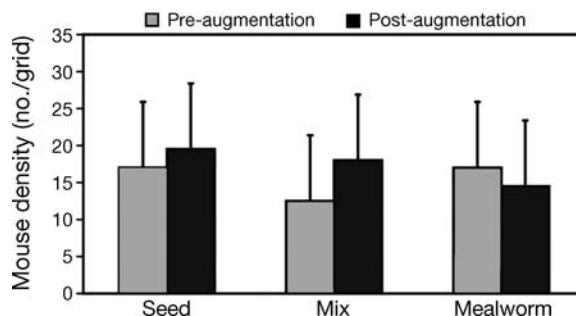

FIG. 4. Population density of *Peromyscus leucopus* in seed-addition grids, mixed-food-addition grids, and mealworm-addition grids. Error bars indicate upper 95% confidence limits averaged across the two grids.

leucopus tends to vary across seasons and between habitats (Whitaker 1966, Wolff et al. 1985, Stapp et al. 1999) that most likely have different food web structures (e.g., different relative availability of plant and animal foods). Surprisingly, after food addition, *P. leucopus* showed little trophic shift in response to relative availability of seeds and mealworms. This suggests that at high food availability, marginal value of any given food type decreases to a level at which complementary foods become perfectly substitutable.

Although energy and protein are complementary nutrients, mice in the genus of *Peromyscus* were shown more selective for protein content than for energy content in foods (Lewis et al. 2001, Vickery et al. 1994). Our results support these findings in that the daily consumption rate of mealworms became higher than that of seeds in seed-addition grids and in mixed-addition grids, whereas daily consumption rate of seeds did not become higher than that of mealworms in mealworm-addition grids. That is, the complementary nature of mealworms and seeds is asymmetrical— mealworms are often preferred over seeds. Furthermore, the marginal rate of substitution (the ratio of marginal values of two food patches; see Schmidt et al. 1998, Schmidt 2000) of mealworms for seeds in *P. leucopus* is likely to vary depending on relative availability of these foods in the environment.

In addition to dietary shifts at the population level, variation in trophic positions of individual *P. leucopus* seemed to increase with decreasing GUD, suggesting (1) stronger complementarity of resources at low food availability, which drives individual mice cycling from herbivore to predator and back depending on their most recent meal; and/or (2) increased intra-population competition for food leading to individual diet specialization. Specifically, as food availability increases (i.e., GUD increases), the trophic levels of individual mice converge, and as food availability decreases (i.e., GUD decreases), the trophic levels of individual mice diverge. This may seem a deviation from the expectation of greater diet selectivity at high food availability predicted in classical optimal-foraging theory. However, consid-

ering that different food items may be complementary to a consumer, greater diet selectivity can mean that individual consumers are converging toward an optimal, mixed diet. These observations also suggest that when food availability is high, omnivore populations may occupy a narrower, omnivory niche, whereas when food availability is low, omnivore populations may occupy a broader, herbivory–omnivory–carnivory niche. The influence of cumulative and relative food availability on individual and population diet specialization in omnivorous consumers warrants more study.

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APPENDIX

A table listing the C and N stable-isotope composition of the plants at the study site (*Ecological Archives* E088-006-A1).