Journal of Insect Physiology 56 (2010) 1877–1882

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Isotopic fractionation in a large herbivorous insect, the Auckland tree weta

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ARTICLE INFO

Article history:
Received 17 April 2010
Received in revised form 5 August 2010
Accepted 5 August 2010

Keywords:
Discrimination factor
· Diet
· Food web
· Herbivorous insect

ABSTRACT

Determining diet and trophic position of species with stable isotopes requires appropriate trophic enrichment estimates between an animal and its potential foods. These estimates are particularly important for cryptic foragers where there is little comparative dietary information. Nonetheless, many trophic enrichment estimates are based on related taxa, without confirmation of accuracy using laboratory trials. We used stable isotope analysis to investigate diet and to resolve trophic relationships in a large endemic insect, the Auckland tree weta (*Hemideina thoracica* White). Comparisons of isotopes in plant foods fed to captive wetas with isotope ratios in their frass provided variable results, so frass isotope values had limited usefulness as a proxy indicator of trophic level. Isotopic values varied between different tissues, with trophic depletion of ^{15}N highest in body fat and testes. Tissue fractionation was consistent in captive and wild caught wetas, and isotopic values were not significantly different between the two groups, suggesting that this weta species is primarily herbivorous. Whole-body values in captive wetas demonstrated trophic depletion ($\Delta\delta$) for $\delta^{15}N$ of about −0.77‰ and trophic enrichment of 4.28‰ for $\delta^{13}C$. These values differ from commonly estimated trophic enrichments for both insects and herbivores and indicate the importance of laboratory trials to determine trophic enrichment. Isotopic values for femur muscles from a number of local wild weta populations did not vary consistently with body weight or size, suggesting that juveniles eat the same foods as adults. Considerable variation among individuals within and between populations suggests that isotopic values are strongly influenced by food availability and individual foraging traits.

1. Introduction

Stable isotope analysis can provide a useful reconstruction of diet and trophic position because of biases in conventional diet studies (Vanderklift and Ponsard, 2003), and is thus used increasingly to probe the food sources and trophic position of consumers (e.g. Davidson et al., 2003; Tillberg and Breed, 2004). Use of stable isotope techniques to study animal diets and trophic levels, however, requires *a priori* estimates of fractionation (i.e., the differences between an animal and its diet). Stable isotopes reflect a time–averaged assimilation of different foods by a consumer and indicate what a consumer has actually assimilated rather than ingested. The discrimination by enzymes and the process of diffusion between common (^{12}C and ^{14}N) and rare isotopes (^{13}C and ^{15}N) within tissues means that a consumer will always have more of the rare isotope than its food because of preferential respiration of ^{12}C and excretion of ^{14}N. Because C changes minimally between trophic levels, we can thus identify the origin of C, which acts as a tracer, while stable isotope values for N, on the other hand, change in a small but predictable way through food chains and hence are useful to resolve trophic interactions in food webs. Organisms are often assumed to have an isotopic signature similar to that of their diets, with predictable trophic shifts. For instance, a terrestrial poikilothermic animal that excretes uric acid and eats vascular plants should have a trophic enrichment for $\delta^{13}C$ of about 0.4-0.5‰ compared to its diet, and a trophic enrichment for $\delta^{15}N$ of 2.3-2.4‰ greater than its food (McCutchan et al., 2003). Species–specific trophic enrichment estimates are important to accurately calculate the contribution of different foods in a consumer's diet, as errors can substantially affect the results of mixing models and trophic level estimation (McCutchan et al., 2003; Vanderklift and Ponsard, 2003; Caut et al., 2008; Caut et al., 2009; Wolf et al., 2009).

Invertebrate stable isotope studies have generally presented whole-body values for use in mixing models because of mechanical difficulties in separating tissues (but see Gratton and Forbes, 2006). Alternatively, a single insect body part has been used without first ascertaining whether it provides an adequate approximation of the effect of diet. A large ratio of lipids to muscle or chitin may affect carbon isotope values, as lipid synthesis is accompanied by a depletion in ^{13}C (de Niro and Epstein, 1978; de Niro and Epstein,

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doi:10.1016/j.jinsphys.2010.08.005

1981). Physiological stress such as starvation may also affect isotope values (Oelbermann and Scheu, 2002; Vanderklift and Ponsard, 2003) as animals feeding on poor diets with high C:N ratios may recycle internal N stores, leading to increased enrichment values. Additionally, juveniles may also have different nutritional requirements and physiological processes from adults including metamorphosis, leading to different tissue isotopic signatures (Patt et al., 2003; Spence and Rosenheim, 2005). However, there is little consensus on whether juveniles in different taxa or functional groups feed at the same trophic level as adults.

Dietary fractionation, tissue type, trophic position, and N metabolism have been identified as factors that may lead to variation in trophic fractionation (Post, 2002; McCutchan et al., 2003; Vanderklift and Ponsard, 2003; Spence and Rosenheim, 2005; Caut et al., 2009). For example, tissues may accumulate carbon isotopes at different rates, particularly following a diet shift (e.g. Gratton and Forbes, 2006). These authors, who analysed insect tissues separately, demonstrated that stable isotope turnover rates vary between tissues. Dietary isotopic values affect trophic enrichment values, so obtaining species-specific enrichment factors using controlled diets leads to much greater accuracy in trophic level estimation (Gannes et al., 1997; Caut et al., 2009).

We investigated stable isotope values in a large endemic terrestrial insect, the Auckland tree weta (*Hemideina thoracica* White, 1842, Orthoptera: Anostostomatidae, Fig. 1). This tree weta is one of seven in the *Hemideina* genus that survive on mainland New Zealand. Although common throughout the northern half of the North Island of New Zealand (in contrast to many giant and tusked wetas with severely reduced ranges), tree wetas are cryptic nocturnal animals in which foraging is difficult to observe. In New Zealand, a scarcity of native mammals has led to the popular perception that large Orthopteran insects, or wetas (anostostomatidae), occupy a similar trophic position to mice (Ramsay, 1978; Daugherty et al., 1993; Duthie et al., 2006). However, although more than 60 species of wetas have been identified, there have been few diet studies (Little, 1980; Cary, 1983; Wilson and Jamieson, 2005); many wetas are now rare; and their trophic position remains poorly understood with only scattered observations of their foraging. In short, very little is known about their diet or foraging habits, there are few records of seeds, fruits or invertebrates in their diet in the wild in any of the tree weta species and quantitative data are extremely limited. To date, researchers have assumed that tree wetas feed principally on the leaves of woody and herbaceous plants (Trewick and Morgan–Richards, 1995). Nonetheless, the Wellington tree weta *Hemideina crassidens* readily eats the flesh of a variety of native fruits in captivity, with positive benefits for seed germination (Duthie et al., 2006), thus potentially performing a seed dispersal role similar to small mammals elsewhere in the world. It is therefore possible that tree wetas may have a broader omnivorous niche than currently thought.

Fig. 1. Female Auckland tree weta (*Hemideina thoracica*). Photo: David Wheadon.

We began by analysing fractionation in multiple weta tissues for $\delta^{13}C$ and $\delta^{15}N$ in both captive and wild individuals to determine whether tissues had different isotopic signatures. We estimated trophic enrichment in captive Auckland tree wetas that had been fed a known diet, and then compared these with values from wild caught wetas, to confirm whether herbivory is the dominant feeding mode. We compared tissue values with those for whole bodies to discover whether analysis of one tissue might be a useful alternative to whole body analysis, and investigated whether isotope values from frass could provide useful dietary information. Finally, we analysed femur muscle from wild caught wetas that had fed on a natural diet, and tested for differences between sex, body weight and ontogeny to determine whether wetas of different sizes and sexes feed at the same trophic level in the wild.

2. Materials and methods

2.1. Animal collection and containment

Adult Auckland tree wetas caught in late spring and early summer 2008 were assigned randomly to either a 'captive' or 'wild' group. Five wild individuals were frozen within 48 h of capture, for later dissection of tissues. Seven captive animals were housed individually in containers measuring 175 × 175 × 90 mm, under conditions that suit tree weta survival in captivity (Barrett, 1991) and fed fresh leaves every second day. Animals only ate a portion of leaf material before it was replaced. Water was provided *ad libitum*.

2.2. Dietary isotopic ratios and trophic enrichment

To investigate whether Auckland tree wetas can thrive on an all-plant diet, captive wetas were fed a diet of fresh *Corynocarpus laevigatus* (karaka) leaves for at least 6 weeks. For analysis, we dried samples of these leaves at 50 °C for 48 h, before ball grinding for stable isotope analysis. We used these data to estimate trophic enrichment (the discrimination factor) in conjunction with femur muscle data from the captive trials. To determine how closely C:N ratios in frass matched those in plant foods ingested by wetas, we ran six trials for four days each in which we fed one individual weta leaves *ad libitum* from one of the following plant species: *Hebe stricta* (koromiko); *Coprosma robusta* (karamu); *Dacrydium dacrydioides* (kahikatea); *Agathis australis* (kauri); *Podocarpus totara* (totara); and *Melicytus ramiflorus* (mahoe). We then collected frass from the last day of the trial. Plant species were chosen because they had previously been reported as weta plant food, or were suspected to be so.

2.3. Animal dissection for tissue comparisons

All wetas were frozen whole prior to dissection and drying of tissue for stable isotope analysis. Tissue was prepared for analysis by thawing and dissection. Body fat, reproductive tissue, femur muscle, head muscle, and cuticular integument (exoskeleton) from the head were separated for stable isotope analysis. Tissue was oven–dried at 50 °C for 48 h, or until dry, before being ground.

2.4. Population comparisons of matching frass and tissue in wild caught wetas

We removed adult individuals from three urban forest sites with robust populations that were regularly monitored as part of a wider study on tree weta populations in urban forest fragments. All wetas were caught by spotlighting at night, and kept overnight in captivity in individual containers. In this way, frass collected could

be assigned to specific individuals. Weight, sex, and right femur length were recorded for each individual. Frass for stable isotope analysis was oven dried at 50 °C as above for analysis. Individuals were then frozen at −20 °C to compare matched frass and femur muscle isotope values. Muscles from each right-hand femur were later thawed, extruded and dried for isotope analysis. Femur muscle should represent long–term diet because legs were most enriched for ^{13}C compared to reproductive organs and fat that had most rapid C turnover (Gratton and Forbes, 2006).

To compare adult and subadult stable isotope values, we sampled a further six adults and five juvenile wetas from Hillcrest Park, an urban forest fragment in Hamilton in summer 2008. We recorded body weight, femur length, and cerci and ovipositor length for these wetas. All of these individuals produced frass samples before being frozen and their femur muscle extruded for isotope analysis. Additionally, reference samples were taken from adults and juveniles collected in a similar manner from Hillcrest Park and other urban forest and urban garden sites for comparison across different environments. We examined isotope values in the weta femurs, along with frass from the same individuals, to identify whether similar patterns of isotope enrichment might occur in wild populations, and whether ontogeny, age or sex might influence isotope values. After transformation of morphological data using natural logs, we used regression analysis to examine the relationship between morphological characteristics such as femur length, body weight and isotope ratios and thus investigate whether body condition might predict C and N isotope values.

2.5. Stable isotope analyses

Plant samples and femurs and frass from tree wetas were thawed from frozen, oven dried at 50 °C for up to 48 h, then ground to a fine powder. Small subsamples (3–6 mg) of dried, ground material were weighed with a 5–place balance and then oxidised and reduced at high temperature in the furnaces of an ANCA SL analyzer. The resultant CO_2 or N_2 stream was then analysed with a PDZ Europa continuous flow 20/20 isotope ratio mass spectrometer with a triple ion–collector by the IAEC–accredited Waikato Stable Isotope Laboratory, University of Waikato, Hamilton, New Zealand to a precision of about 0.1‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$. The precision was maintained with reference samples of calibrated Australia National University (ANU) cane sucrose for $^{13}C/^{12}C$, and N_2 in air for $^{15}N/^{14}N$. The ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were expressed as relative difference per mil (‰) using the equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where $X = {}^{13}C$ or ^{15}N, and $R = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$. The ratios of ^{13}C to ^{12}C are expressed relative to PDB (Pee Dee Belemnite), for which $R_{standard} = 1.1237$ atom % ^{13}C (Craig, 1957). The ratios of ^{15}N to ^{14}N are expressed relative to N_2 in air, for which $R_{standard} = 0.3663$ atom % ^{15}N (Mariotti, 1983). Trophic enrichment between a food resource (X) and a consumer (Y) was described in terms of the difference in δ values using the Δ notation, where $\Delta = \delta Y - \delta X$.

2.6. Statistical analyses

All the data were statistically analysed and expressed as mean and standard error of the mean. Isotopic values were compared using ANOVA with individual animals used as randomised blocks to reduce variability for between-tissue and between-sex comparisons. This allowed the comparison in a single model:

$$Y_{ijk} = \mu + S_k + S_k * I_i + T_j + T_j * S_k + \varepsilon_{ijk},$$

where $Y_{ijk} = \delta^{13}C$ or $\delta^{15}N$ (response variable), μ = mean, I_i = individual animals (blocking effect), T_j = tissue (fixed effect), S_k captive or wild status (fixed effect), and ε_{ijk} = error term. The operator * represents an interaction and the status*individual interaction is used as error in testing for the status effect. This analysis was carried out with GenStat 12th edition, 2009 (VSN International Ltd). Pair-wise comparisons between tissue means were conducted with a Tukey's multiple comparison test.

3. Results

3.1. Isotopic values of weta tissues

We investigated isotopic variability between tissues and captive or wild status using a randomised block ANOVA design (see Materials and methods) in seven captive wetas fed a known diet and five wild wetas. The diet for captive wetas consisted of leaf material from *Corynocarpus laevigatus* ($\delta^{13}C = -30.4$‰, $\delta^{15}N = 2.0$‰). In this model, there were no differences between wild and captive wetas for either $\delta^{13}C$ or $\delta^{15}N$ ($P = 0.769$ and $P = 0.533$), suggesting that in the wild these wetas are usually herbivorous. However, $\delta^{13}C$ and $\delta^{15}N$ were different among the four testable tissue types, body fat, femur muscle, head muscle, and exoskeleton ($P < 0.001$ for both). The interactions between tissue type and wild or captive status were not significant for either $\delta^{13}C$ or $\delta^{15}N$ ($P \geq 0.361$). Ovaries and testes could not be tested in this model because sex reduced sample sizes.

For $\delta^{15}N$, ovaries, head and femur muscle, and exoskeleton tissues were similar, whereas testes and body fat were about 4‰ lower (Fig. 2; $P < 0.05$, Tukey's multiple comparison). For $\delta^{13}C$, exoskeleton had significantly lower values than body fat (Fig. 2; $P < 0.05$, Tukey's multiple comparison). There was no difference between femur and head muscle.

We also compared isotope values for whole bodies for seven captive and three wild adult wetas. We detected no differences between captive and wild weta bodies for $\delta^{13}C$ (ANOVA, $P = 0.206$). However, $\delta^{15}N$ was greater on average in whole wild weta bodies (5.15 ± 1.1‰) compared to captive (1.21 ± 0.8‰; ANOVA, $P = 0.023$). Whole bodies had $\delta^{15}N$ and $\delta^{13}C$ values most similar to exoskeleton and head and femur muscle (Fig. 2), suggesting that these tissues comprised most of the weta biomass. No significant differences were detected by sex for the whole bodies of seven females and three males for either $\delta^{13}C$ (ANOVA, $P = 0.483$) or $\delta^{15}N$ (ANOVA, $P = 0.883$).

3.2. Trophic enrichment

To determine trophic enrichment, we investigated the relationship between isotopic values of food, frass, and weta tissues.

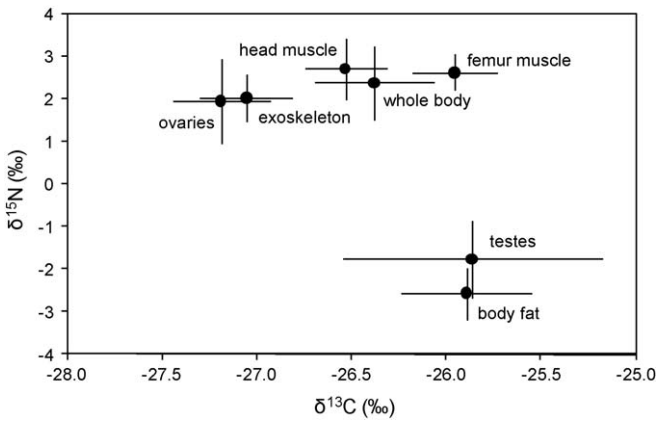

Fig. 2. Mean $\delta^{13}C$ and $\delta^{15}N$ values in body tissues of both captive and wild–caught wetas. Ovaries includes all stages of female reproductive tissue. Error bars are one standard error of the mean.

Table 1
Comparison of N content, and $\delta^{13}C$ and $\delta^{15}N$ values in leaves and frass from wetas fed on different plant species.

Plant species	%N		$\delta^{13}C$		$\delta^{15}N$		Frass minus leaf	
	Leaf	Frass	Leaf	Frass	Leaf	Frass	$\delta^{13}C$	$\delta^{15}N$
Melicytus ramiflorus (mahoe)	2.20	1.57	−30.9	−31.3	−0.9	5.1	−0.4	6.0
Corynocarpus laevigatus (karaka)	1.92	1.11	−30.4	−30.1	2.0	1.9	0.3	−0.1
Hebe stricta (koromiko)	1.64	2.21	−27.2	−27.9	3.6	−0.4	−0.7	−4.0
Coprosma robusta (karamu)	1.60	3.19	−31.0	−30.7	1.9	2.5	0.3	0.6
Dacrydium dacrydioides (kahikatea)	1.34	3.02	−30.8	−30.0	5.3	3.1	0.8	−2.2
Podocarpus totara (totara)	1.16	1.74	−28.3	−27.8	3.8	4.3	0.5	0.5
Agathis australis (kauri)	0.93	1.19	−26.9	−29.4	4.1	3.1	−2.5	−1.0

Leaf $\delta^{13}C$ values for a selection of likely food plants for wild wetas varied between −31.0‰ and −26.9‰ (Table 1). Ratios for $\delta^{15}N$ in these leaves varied between −0.9‰ for *Melicytus ramiflorus* and 5.3‰ for *Dacrydium dacrydioides*. We then carried out individual short–term trials to compare these foods to frass isotope values obtained after 3–4 days on a leaf diet of only that species. Initial trials with carrot demonstrated that plant food is passed through a weta gut within this period, so that frass obtained on day 5 should reflect the specific plant food. Frass was within 1‰ of $\delta^{13}C$ leaf values in these trials with the exception of *Agathis australis*, and $\delta^{13}C$ values of frass were higher in four trials and less in three. $\delta^{15}N$ in frass varied from −0.4‰ to 5.1‰, but did not show a consistent pattern of increase or decrease relative to leaf values. Frass $\delta^{15}N$ was 6.0‰ greater than *M. ramiflorus* as a food, whereas frass $\delta^{15}N$ was 4.0‰ less than *Hebe stricta* as a food (Table 1). This unpredictability suggests a complex interaction between nitrogen metabolism and nitrogen content of food.

For captive wetas, trophic enrichment ($\Delta\delta$) for $\delta^{13}C$ was highly positive in all tissues when $\delta^{13}C$ values for karaka leaves (−30.4‰) were considered in relation to body tissues (Table 2). Similarly, whole-body $\Delta\delta^{13}C$ values were high (4.28 ± 0.41). Body fat and reproductive tissues were highly depleted in ^{15}N relative to the food, leading to a strongly negative trophic enrichment factor for $\delta^{15}N$ (Table 2). Whole–body $\Delta\delta^{15}N$ was also negative (-0.77 ± 0.80), whereas muscle and ovarian tissues had slightly positive $\delta^{15}N$.

3.3. Population level variation

Femur length, a good size indicator in tree wetas, was closely related to body weight in individuals from a number of sites ($r^2 = 0.93$, $P < 0.05$, Fig. 3). There was no relationship between $\delta^{15}N$ and weta body weight ($r^2 = 0.01$, $P = 0.689$), nor between $\delta^{13}C$ and weta body weight ($r^2 = 0.03$, $P = 0.268$), based on isotopic values of femur muscle for different sized wetas ($n = 37$). Similarly, isotopic analysis of femur muscle showed no difference between the sexes (26 males and 17 females) for either $\delta^{15}N$ (ANOVA, $P = 0.115$) or $\delta^{13}C$ (ANOVA, $P = 0.064$).

Analysis of matched frass and femur samples (paired t tests) from 35 individuals from wild populations showed that percent N in femur muscle exceeded that found in the frass of all animals ($13.1 \pm 0.07\%$, and $2.7 \pm 0.18\%$ respectively, $P < 0.001$). $\delta^{15}N$ was similar in femur and frass (2.7 to 2.9‰, $P = 0.446$). $\delta^{13}C$ values of frass (-29.2 ± 0.23 ‰) were within the range of C_3 plant values, but were on average 4.0‰ less than femur $\delta^{13}C$ values (-25.7 ± 0.18 ‰) from the same individuals ($P < 0.001$). This difference was consistent across weta femur muscle and matched frass samples in populations at a range of urban sites (Fig. 4). However, $\delta^{15}N$ values were not consistently higher in femurs compared to frass at all sites, differences were relatively small compared to $\delta^{13}C$, and there was large variation in individuals at sites where femur muscles appeared to be depleted in ^{13}C compared to frass (Fig. 4).

Individual variability was high within populations for $\delta^{15}N$ and $\delta^{13}C$ (Fig. 5), but $\delta^{15}N$ showed greater differences between populations than $\delta^{13}C$ values. Urban sites had a more restricted range of $\delta^{15}N$ values than other sites.

Table 2
Mean tissue–specific trophic enrichment ($\Delta\delta$) values (± 1 SE) of captive wetas fed on *Corynocarpus laevigatus* (karaka) leaves ($\delta^{13}C = -30.4$‰, $\delta^{15}N = 2.0$‰). *C. laevigatus* leaf values were based on leaves taken from the same individual tree that wetas were fed from.

Tissue type	N	$\Delta\delta^{15}N$ (‰)		$\Delta\delta^{13}C$ (‰)	
		Mean	SE	Mean	SE
Body fat	8	−4.9	0.91	4.6	0.35
Testis	4	−3.8	1.17	3.9	0.30
Whole body	7	−0.8	0.80	4.3	0.41
Exoskeleton	9	−0.4	0.67	3.4	0.26
Head muscle	7	0.4	0.85	3.7	0.21
Ovary	3	0.4	0.29	2.7	0.40
Femur muscle	15	0.8	0.51	4.7	0.23

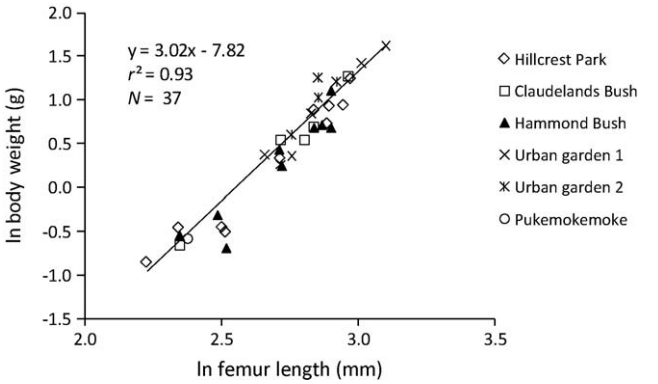

Fig. 3. Relationship of natural log (ln) femur length to ln body weight for wetas from six Waikato localities.

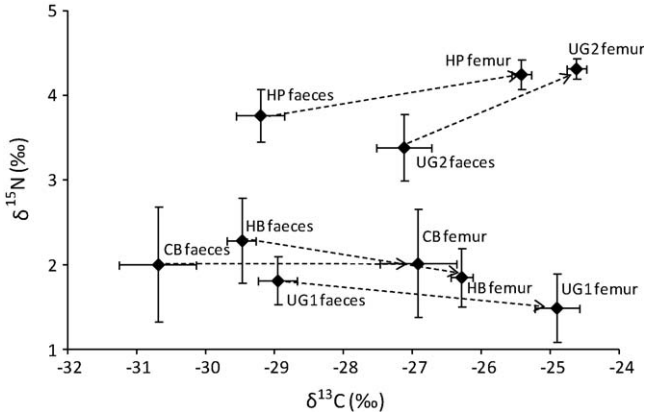

Fig. 4. Relationship between isotopic values of frass and femur muscle for wild–caught wetas from five populations ($n \geq 5$). Populations are located at five urban sites: Claudelands Bush (CB), Hillcrest Park (HP), Hammond Bush (HB) Urban garden 1 (UG1), Urban garden 2 (UG2).

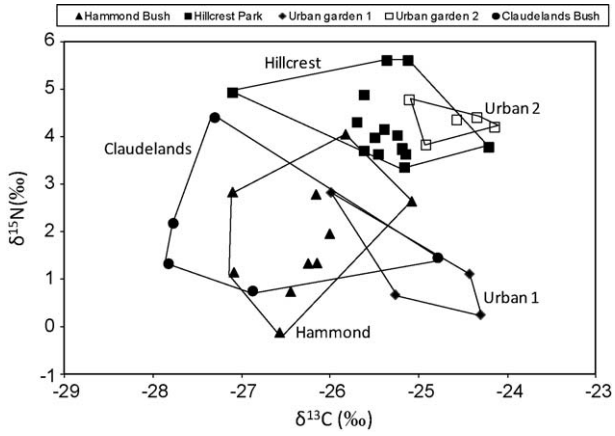

Fig. 5. Stable isotope values of femur muscle from individual tree wetas from native forest remnants (Claudelands Bush, Hammond Bush and Hillcrest Park) and urban gardens in Hamilton City.

4. Discussion

4.1. Isotopic fractionation

Our analyses of stable C and N isotope values from captive and wild Auckland tree wetas suggest that in the wild this species is predominantly or exclusively herbivorous. The wetas showed differential fractionation between tissues, with body fat depleted in ^{15}N, but the most surprising result was the lack of trophic enrichment shown by $\delta^{15}N$ values and the extreme trophic enrichment shown by $\delta^{13}C$ values. These results emphasise the importance of species-specific trophic enrichment estimations. Estimated trophic enrichment values were greater than the usual trophic enrichment averages estimated for $\delta^{13}C$ for insects (Caut et al., 2009). Mean trophic enrichments have been estimated as +0.5 to +1‰ for $\delta^{13}C$ (de Niro and Epstein, 1978; McCutchan et al., 2003), with a greater enrichment in ^{13}C for muscle tissue.

In contrast, values for trophic enrichment of $\delta^{15}N$ were lower than expected from reviews in the recent literature (Post, 2002; McCutchan et al., 2003; Caut et al., 2009); previous $\Delta\delta^{15}N$ enrichment estimates for herbivores of 2.98‰ (Vanderklift and Ponsard, 2003) are higher than those estimated here. However, trophic enrichment in $\delta^{15}N$ for *Melanoplus sanguinipes* grasshoppers was about 1.0‰ (de Niro and Epstein, 1981), similar to our estimates, suggesting that these related orthopterans may metabolise N in a similar way. Chitin from grasshoppers was especially depleted in ^{15}N compared to the diet (−6.6 to −8.6‰, de Niro and Epstein, 1981). Our $\Delta\delta^{15}N$ enrichment estimates for wetas are close to estimates of 0.53‰ for detritivores (Vanderklift and Ponsard, 2003), but tree wetas do not appear to feed on detritus. Rather, the low trophic enrichment for wetas might reflect N limitation of their diets. Leaves have lower nitrogen content (0.9–2.2%; Table 1) than terrestrial invertebrate prey (7.9–9.2%; Hicks et al., 2005), and dietary nitrogen limitation for wetas, a large, herbivorous insect, might be one reason for the limited trophic fractionation for nitrogen that we observed. Other New Zealand tree species have similarly low nitrogen content (0.5–2.3%, Hicks and Laboyrie, 1999).

Meta–analyses suggest that $\Delta\delta^{13}C$ might be greater for animals raised on vascular plant diets, and for omnivores (McCutchan et al., 2003), but considerable variation in the trophic enrichment factor among taxa has led to the calculation of a diet–dependent discrimination factor (DDF) for invertebrates (Caut et al., 2009). Our carbon enrichment values for Auckland tree wetas are greater than these estimates. Nonetheless, we note that *Melanoplus sanguinipes* grasshoppers raised on wheat seedlings showed average trophic enrichment of 2.7‰ for $\delta^{13}C$, with a range of 1.8‰ (de Niro and Epstein, 1978), which is greater than the average figures quoted by many authors, but more similar to our values.

It is difficult to accurately determine the relationship between plant food and consumer isotopic composition in the wild, but we would generally expect fractionation of carbon in herbivores to closely reflect their diet (e.g. de Niro and Epstein, 1978). In field studies, factors such as seasonal variation in the isotopic composition of the diet may influence isotope values. Moreover, animals do not forage randomly, although data that indicate how their preferences might vary according to nutritional needs, predation risk, habitat and other factors are often lacking. Despite variation in isotope values at different sites, the $\delta^{13}C$ values seen in wild wetas provide support for the trophic enrichment value estimated from the captive trials. Carbon is unlikely to be limiting in food, but conversely we would not expect assimilation of $\delta^{13}C$ and excretion of $\delta^{12}C$ to the extent demonstrated in both captive and wild wetas. It appears that wetas may have digestion or excretion processes that allow increased enrichment of $\delta^{13}C$, perhaps related to their ecology and behaviour. Wetas are known to enlarge their refuge cavities by chewing on wood (Field and Sandlant, 2001) in a similar way to termites. Enhanced $\delta^{13}C$ enrichment has also been reported in a parasitoid (Langellotto et al., 2005).

For $\delta^{15}N$, invertebrates have yielded lower estimates of enrichment (2.08‰) than for vertebrates (2.88‰; Vanderklift and Ponsard, 2003), compared with whole body $\delta^{15}N$ values of 0.4‰ in our study. Our mean $\delta^{13}C$ signature for whole bodies of Auckland tree wetas (−26.4‰; $N = 10$) are lower than the −23.5‰ previously reported for Wellington tree wetas (*Hemideina crassidens*) living in a forested area on Stephens Island (Cree et al., 1999). Although the sample size for the latter value is unclear and possibly based on a single individual, both environmental effects and species differences between *H. thoracica* and *H. crassidens* have yet to be clarified.

4.2. Tissue-specific isotope ratios

Carbon Isotope ratios from frass were very similar to those of selected plant foods tested. Isotopic signatures in captive wetas on a known diet showed significant variation between tissues, with body fat and male reproductive tissue particularly depleted in $\delta^{15}N$. However, isotope signatures of exoskeleton, head and femur muscles in captive wetas were relatively similar to the food provided. We were unable to detect differences in isotope values between wild and captive wetas, and suspect that the captive food was most likely similar to food in wild populations, providing support for a herbivorous diet. Metabolically active tissues such as fat and reproductive tissues should show a more rapid change in $\delta^{13}C$ signature towards a new diet when compared against slow turnover tissues such as the exoskeleton (de Niro and Epstein, 1978; Gratton and Forbes, 2006). It should thus be possible to distinguish when consumers have switched from one diet to another as the tissues will be isotopically different. This difference was not, however, evident here when captive and wild wetas were compared. Further trials using both C_4 and C_3 foods to determine turnover rates will allow more exact calculation of isotope assimilation time in different tissues. However, weta femur muscle is probably a good representation of long term diet, with similar values to whole body isotope values.

4.3. Age and population-level diet

In our study, we found no clear effects between life stages or sex in this species. Differences between $\delta^{13}C$ signatures of juveniles and adults were small, indicating no substantial changes in the

energy source utilised for growth associated with age. Variation in enrichment values was evident both among wild caught individuals and populations, indicating that environmental factors may influence isotope composition of food. For example, seasonal variation in isotope values at primary consumer level can be significant and could influence relative trophic position estimates (de Niro and Epstein, 1978).

As yet it is unclear how the Auckland tree weta metabolises C and N, and whether it utilises resources from more than one trophic level. However, although omnivory is typical of the family as a whole (Trewick and Morgan–Richards, 2005), the isotope values reported here suggest that plants contribute most of the wetas' energy requirements, and do not offer support for the contention that wetas occupy an onmivorous niche that is functionally similar to mice in New Zealand. Nonetheless, it is possible that wetas may function as herbivores by processing plant material, but also be opportunistic predators or scavengers to subsidise their N requirements. Some Australian king crickets that feed on the forest floor seem to be generalised scavengers that eat dead and decaying matter (Monteith and Field, 2001), and scavenging in tree wetas has also been reported although it is not clear on what basis (Trewick and Morgan–Richards, 2005).

Tree wetas may forage more extensively on the forest floor than currently recognised (e.g. Mirams, 1957), perhaps when mammalian predation pressure is low (Rufaut and Gibbs, 2003), and this might include scavenging of detrital material. Cannibalism may also occur in wild tree wetas, with eaten out bodies observed within their refuge cavities (P. Wehi, unpublished data). The similarity of faeces and food $\delta^{13}C$ and $\delta^{15}N$, and the observed difference in femur muscle $\delta^{15}N$ in individuals from a number of wild populations raises the possibility that a wholly plant diet is not sufficient for maximum potential growth and reproduction and that trophic position may therefore differ from functional position. The individual variability in $\delta^{13}C$ and $\delta^{15}N$ within forest populations indicate individuals may use a range of foraging strategies. Nonetheless, the isotope values reported here indicate that tree weta are primarily herbivorous, with leaves the most likely food source, and as such may be important primary consumers in New Zealand forests. The intra- and interpopulation variability in $\delta^{15}N$ is clearly derived from the variability in the plant diet rather than from omnivory.

Acknowledgments

This research was funded by Foundation for Research Science and Technology contract UOWX0501 at the University of Waikato, New Zealand. Barry O'Brien provided helpful advice on insect dissection. Isotope analyses were performed by Anjana Rajendram at the Waikato Stable Isotope Unit, University of Waikato. We especially thank Ray Littler, Senior Statistician with the Waikato Centre for Applied Statistics, Faculty of Computing and Mathematical Sciences, University of Waikato, for discussion and assistance with statistical analyses. The Hamilton City Council permitted us to collect tree weta from urban forest sites. We thank William Wehi and Toni Cornes for field assistance. All experiments and procedures comply with the current laws in New Zealand.

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