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The diet of tree weta: natural and captive folivory preferences of *Hemideina crassidens* and *Hemideina thoracica*

A thesis presented in partial fulfilment of the requirements for the degree of
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Abstract

Tree weta are a well-known orthopteran group with a widespread distribution in New Zealand. Basic knowledge of tree weta diet was sought by examining wild diet, and dietary preferences, of two widespread tree weta species. This base line data should help improve our understanding of where tree weta fit into New Zealand forest ecosystems. In the present study I examined the natural (wild) diet of two tree weta species *Hemideina crassidens* and *H.thoracica* and some aspects of dietary preference. The frass of thirty-three wild tree weta indicated that tree weta do not eat at random. It was also of note that a favoured plant species present in the frass was an exotic legume, known to have a high nitrogen content. In captivity tree weta appeared to favour plant species with high nitrogen content, however, nitrogen is not the only factor affecting their dietary preferences. Plants produce feeding inhibitory compounds and by examining three selected plant species, inhibitory effects were identified in two plants species. These inhibitory effects such, as poisoning or affecting nutrient absorption, likely led to the tree weta to developing behavioural adaptations to limit plant toxins. Weta in this study ate on average only two plant species in a single night, however they increased the number of plant species they ate over two nights. By limiting the number of plant species eaten in a single night, tree weta may be allowing themselves time to deal with toxic compounds in the plant. Additional research into tree weta use of natural resources and dietary requirements would help further our knowledge of tree weta ecology.

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Chapter One: The dietary needs of tree weta

Introduction and Thesis Outline



Adult Male *Hemideina thoracica*
Photo courtesy of Andrew Blayney

1.1 Feeding ecology

Food is the energy of life, it provides the fuel to run the engine so organisms can grow and reproduce. Gaining the right nutrition is essential to ensure an individual has the best chance of reaching its optimum and successfully reproducing (Bernays, Bright, Howard, Raubenheimer, & Champagne, 1992; Griffin, 2011; Joern & Behmer, 1997). Optimal foraging theory tells us that an organism will select its food based on a variety of factors, but will do so to select the optimum nutritional or energy gain for the minimum expenditure of resources or risk (Pyke, 1984).

Food provides the nutrients, but those nutrients must be ingested in the correct quantities; insufficient amounts or excessive amounts of certain nutrients can be detrimental to an individual's health. This can be overcome by the correct selection of nutrients (House, 1969). If an animal selects a food source based on its nutrient content, then it can regulate its diet and optimise its fitness. An animal that is only able to select a single food source may vary its rate of consumption based on its own nutritional state (Mayntz, Raubenheimer, Salomon, Toft, & Simpson, 2005). Some organisms may also be able to regulate their nutritional state once the food has been ingested by limiting what is absorbed (Simpson, Sibly, Lee, Behmer, & Raubenheimer, 2004). Generalist herbivores tend to be more tolerant of nutritionally unbalanced food sources, compared to specialist herbivores, due to their increased likelihood of encountering a nutritionally adequate food source compared with a specialist herbivore (Raubenheimer & Jones, 2006). It is also important that the nutritional needs of an individual may change at different stages of its life history (Raubenheimer & Simpson, 1993).

Nitrogen is an essential nutrient for the growth and reproduction of all plant and animal species (Mattson, 1980; White, 1978). However nitrogen is often considered the limiting factor in the diet of many organisms (Joern & Behmer, 1997, 1998; White, 1978). While nitrogen is an abundant element on Earth, it is often in a form that is inaccessible to animals (White, 1978). Nitrogen is at higher levels, and is more accessible, in amino acids and proteins than in carbohydrates (Campbell & Reece, 2005). Thus, protein and nitrogen have often been used interchangeably in the

nutrient literature. If nitrogen is the limiting dietary factor for an organism, it is assumed that all other nutritional needs are met by consuming sufficient quantities of nitrogen (House, 1969). One way for an animal to increase the amount of nitrogen in its diet is to consume non-plant organisms, which have a high protein concentration; plant leaves have comparatively low levels of nitrogen.

1.2 Diet self-selection

Herbivory can result in an arms race (Mello & Silva-Filho, 2002), with plants evolving defences to reduce the impact of insects and other herbivores through means such as physical defences, e.g. thorns and divaricating branches, being tough and unpalatable. Plants, also defend themselves with secondary metabolites which may poison or deter a herbivore. Herbivores that have ways to circumvent plant defences will be more successful. This creates an on-going competition for the plants to defend themselves and the herbivores to find ways around theses defences. Insects use methods such as sequestering the toxic compounds for their own defences, detoxifying compounds, using alternative portions of the plant that are not toxic and avoiding the physical defences produced by a plant (Mello & Silva-Filho, 2002). Circumventing plant defences is more evident in specialist animal species which eat a single plant species; generalist herbivores however are also adapted to detoxifying and using a range of plant species requiring them to be able to detoxify a large range of different compounds (Mello & Silva-Filho, 2002).

Generalist herbivores are able to eat a range of plant species, and may do so in a non-random manner. Diet self-selection is the way in which individuals regulate their nutritional intake on a daily basis by switching between food sources. It has been theorised that this allows animals to balance their nutritional needs while minimising intake of toxic compounds which are also present in their food. This is particularly important for generalist herbivores that use a range of host plant species, as they encounter a range of plants with different nutritional values and toxins. Although also known as diet switching, it is preferably known as diet self-selection as diet switching can refer to the changing of food sources in different stages of an animal's life cycle e.g. a caterpillar eating foliage while the adult butterfly is a nectar feeder (Mello &

Silva-Filho, 2002). Diet self-selection has been demonstrated in a variety of animals, including ungulates (Berteaux, Crête, Huot, Maltais, & Ouellet, 1998; Provenza & Balph, 1987), grasshoppers (Bernays, et al., 1992) and, even a predatory ladybird (*Harmonia axyridis*) (Soares, Coderre, & Schanderl, 2004).

It is the balancing of nutrients that is important for insects, as achieving the right balance can maximise growth (Griffin, 2011), and even egg production (Joern & Behmer, 1998). Taking in too much of a nutrient can be detrimental for an individual, as many nutrients which are required at low level can become toxic at higher levels (Simpson, et al., 2004).

Optimal foraging theory suggests that an activity will be continued as long as its benefits outweigh the cost (MacArthur & Pianka, 1966). With this in mind, optimal foraging theory predicts that every species will attempt to acquire a diet which will give them the optimal growth and reproduction rates. However, it is possible that not all behaviours we observe are optimal (Pyke, 1984), for example tree wetas' ability to balance their diet has been called into question. Griffin (2011) demonstrated that while captive *Hemideina crassidens* grew faster on a high protein diet this did not result in an increased fecundity, with females producing few and poorer quality eggs. When Griffin (2011) used an artificial diet, *H. crassidens* failed to correct any imbalance and 38% of her study animals died. In contrast, other insect species, including orthopterans demonstrate an ability to correct imbalances by selecting complementary artificial diets, to give them the optimal nutrition (Bernays, Angel, & Augner, 1997; Joern & Behmer, 1997).

1.3 Tree weta

Tree weta (*Hemideina*) are common nocturnal orthoptera endemic to New Zealand and can be found in most New Zealand forest and suburban areas. There are seven species of *Hemideina*, these are *Hemideina crassidens* (Blanchard), the Wellington tree weta; *H. thoracica* (White), the Auckland tree weta; *H. trewicki* Morgan-Richards, the Hawke's Bay tree weta; *H. femorata* Hutton, the Canterbury tree weta; *H. ricta* Hutton,

the Bank's Peninsula tree weta; *H.maori* (Pictet and Saussure), the alpine tree weta and *H. broughi* (Buller) the west coast bush weta (Gibbs, 2001).

Tree weta are nocturnal orthoptera that spend the day in hollow branch cavities, except *H. maori* which lives in alpine scree fields (Kelly, 2006). Adult tree weta often live in a harem of a single male and multiple adult females (during the summer and autumn (per communication Cilla Wehi)). Tree weta are sexually dimorphic with the adult males having enlarged mandibles up to 40% of their body length (O'Brien & Field, 2001), which they use to defend their harems from other males. Both tree weta (*Hemideina*) and giant weta (*Deinacrida*) have been described as being unusual as they are predominantely herbivorous in a family, Anostostomatidae, that is generally predatory or scavengers (Cary, 1983; McIntyre, 1998). Wehi and Hicks (2010) used isotope analysis to determine that *Hemideina thoracica* was generally consuming a herbivorous diet and that these tree weta were likely to be nitrogen limited. The diet of wild alpine tree weta (*Hemideina maori*) was predominately herbivorous although insect remains were found when examining their frass (Lodge, 2000; Wilson, 2004).

Tree weta preference for particular plant food species was examined using six plant species, including the exotic eucalyptus, and clear discrimination was observed (Wyman, 2009). Unexpectedly, the tree weta preferred to eat species known to be poisonous to mammals (Tutu and eucalyptus), rather than the soft leafed fuchsia. Diet preferences in *H. crassidens* in regards to difference types of foods (insects, fruit, seeds and leaves) have also been examined and it was concluded that weta are polyphagous, with most weta meals consisting of more than one food source, when given a choice (Griffin, Morgan-Richards, & Trewick, 2011). The diet of *Hemideina maori* studied using frass, consisted of an average of 5.2 plant species per frass, and the weta exhibited a preference for certain plant species over other available plant species (Wilson & Jamieson, 2005). These studies have raised a number of questions about weta diet, in particular what weta are doing in the wild and how that compares to what they eat in captivity.

Tree weta have been noted moving up to 12m in a single night (Kelly, 2006) and although tree weta eat seeds, they are also capable of being seed dispersers. Duthie,

Gibbs and Burns (2006) showed that two New Zealand plants species exhibited a slightly increased germination rate after their seeds passed through a tree weta gut. However Wyman (2009) and King et al (2011) suggested that weta are predominately seed predators rather than seed dispersers, due to a net loss of seeds. To be an effective seed disperser, tree weta should favour fruit over other non-dispersible food types, Wyman (2009) demonstrated that weta showed no preference for fruits of *Fuchsia extorticata* over mahoe (*Melicytus ramiflorus*) leaves, indicating that tree weta are not choosing dispersible food types over non dispersible food types.

Tree weta have been referred to as "invertebrate mice" since 1964, because of their general ecology (daytime refuges, large frass which persists in the environment and polygamous reproduction) (Fleming, 1973; King & Knox, 1974; Ramsay, 1978). Griffin, Trewick , Wehi, and Morgan-Richards (2011) argued that this metaphor was unhelpful, highlighting the differences in weta and mice, and concluding that weta do not impact the ecosystem in the way mice do. Griffin *et al*. (2011) estimated that tree weta consumed between 20g and 500g of leaf material a night per hectare depending on their density, and with that estimate tree weta could be consuming between 65.7 kg and 1825 kg per hectare per year, thus potentially having quite an impact on forest ecosytems. It is due to lack of knowledge that the invertebrate mouse cliché persists. Therefore more studies on weta diets, nutrient optimisation and resource partitioning among weta taxa are needed to correct this cliché and gain a better understanding of how tree weta utilize the natural environment.

The two species of weta used in this study were *H. crassidens* the Wellington tree weta and *H. thoracica* the Auckland tree weta. These are the two tree weta species with large ranges within the North Island of New Zealand and *H. crassidens* extends into the northern tip and west coast of the South Island of New Zealand. They are easy to distinguish as the Wellington tree weta is stripped black and yellow on the abdomen while the Auckland tree weta has a uniformly golden brown abdomen. The two species are almost entirely non-overlapping in distribution suggesting competitive exclusion is operating (Trewick & Morgan-Richards, 1995) although no difference in their ecology has yet been identified (Minnards, 2012). In a few narrow regions of their distributions they overlap, where they are known to share refuge holes.

Thesis outline:

To help understand tree weta and their dietary preference, this thesis has been split into three parts:

Chapter 2: Do tree weta prefer to eat leaves with a higher nitrogen content?

This chapter uses captive tree weta to study diet preferences and compare the leaves of eight native plants as potential weta food. The role of carbon and nitrogen in leaves is examined as possible factors driving weta feeding preferences.

Chapter 3: Tree weta diet in the wild.

I examine tree weta diet by study of the frass of wild animals. The plants species identified in the frass are compared to the plant species present in the environment in which the weta was collected.

Chapter 4: Diet self-selection in captive tree weta.

This chapter further examines weta choice in a captive environment by looking at the number of different plant species that weta consumed, as well as the amount of plant material the tree weta consumed.

Chapter Two: Do tree weta prefer to eat leaves with a higher nitrogen content?



Adult Male *Hemideina thoracica*

2.1 Introduction

Starting to understand how species evolve requires good information about how they interact with their environment. Obtaining basic information on any animal species requires intense work and consequently knowledge of small, non-model invertebrate taxa is generally poor. Poor information can lead to general misconceptions based on minimal evidence that can influence assumptions about evolution and ecosystem function. For instance it has been stated that tree weta are predominately herbivores, which is unusual for their family (Wyman, 2009). However this appears not to be the case as, Griffin (2011) showed that the tree weta *H. crassidens* will readily eat dead insects. Thus, tree weta may be more opportunistic feeders than true herbivores, taking advantage of a protein rich food source when available. Tree weta can nevertheless survive and develop on an herbivorous diet consisting of only leaves (Barrett, 1991; Morgan-Richards 2000).

The correct nutrients are essential for life, as the nutritional balance ingested by an individual affects growth, fecundity and survival of that individual (McDonald, Greenhalgh, & Morgan, 2002). Nutrients are the building blocks of life with carbon and nitrogen being major components in many essential compounds in the body. Nutrients that are essential for life, do not come in a balanced package and if over eaten can become detrimental and even toxic (Raubenheimer, Simpson, & Mayntz, 2009).

In general, organisms are expected to balance their nutritional intake, so that potentially toxic levels of nutrients do not build up and growth is not limited by a lack of a compound (McDonald, et al., 2002). This has been demonstrated to be true in many animals including the grasshopper *Schistocerca americana* (Bernays, Bright, Gonzalez, & Angel, 1994). In tree weta however this has not been demonstrated. When Griffin (2011) provided Wellington tree weta, *Hemideina crassidens* with two artificial diets, the tree weta did not appear to regulate their consumption of either protein or carbohydrate rich foods to balance their intake and 38% of the weta on the high protein diet died during the experiment (over 11 days). It is possible that the weta are not actively regulating these nutrients because their natural environment regulates

them. This does not mean however that weta do not regulate any part of their diet. Weta take about 12 months to reach maturity making them relatively long lived slow developing insects (Gibbs, 1998). This slow growth may mean that tree weta regulate their nutritional needs over a longer period of time than shorter lived insect species that have been studied. For example the grasshopper, *Taeniopoda eques,* has an 8 month generation time, (Bernays, et al., 1992), and the tobacco worm *Heliothis virescens*, has a generation time of 28-30 days (Lee, Behmer, & Simpson, 2006). It is also possible that although consumption of the nutrients manipulated by Griffin (2011) were not actively regulated by tree weta in the time frame of that study, tree weta might regulate different nutrients at different rates.

Nitrogen is generally considered to be an important but usually limiting resource for most herbivorous invertebrate species (Mattson, 1980; Ritchie, 2000; Wehi & Hicks, 2010). From this we can infer that tree weta should eat foods in quantities that achieve their nitrogen needs. Thus one would predict that tree weta would select high nitrogen foods over low nitrogen foods, showing a dietary preference. This would be the case if nitrogen was more important than carbon for tree weta. Food preferences have been looked at in many species (Bernays, et al., 1994; Huseynov, Cross, & Jackson, 2005) and Wyman *et al* (2011), initiated studies in tree weta by performing paired preference tests. Wyman *et al* (2011) showed that tree weta did have a preference for leaves of some plant species over other plant species.

In the present study tree weta feeding preferences were examined further. I repeated some of the preference tests and included two additional native plant species karamū, (*Coprosma robusta*) and putaputaweta, (*Carpodetus serratus*). I used the paired preference approach to test the prediction that weta preferentially eat leaves with high nitrogen over leaves with lower nitrogen. I also compared the carbon uptake by tree weta from the plant species used in the preference trial to seek evidence that tree weta prefer plant species from which they can more readily assimilate carbon.

2.2 Methods:

2.2.1 Nitrogen Content

Ten leaves of each of the eight plant species were collected; mahoe (*Melicytus ramiflorus),* tutu *(Coriaria arborea),* Eucalyptus *(Eucalyptus ficifolia),* tree fuchsia *(Fuchsia excorticata)*, puka *(Griselinia littoralis)*, northern rata *(Metrosideros robusta)*, putaputaweta, (*Carpodetus serratus*) and karamū (*Coprosma robusta)*. Leaves from the same trees were also harvested for the weta in the feeding preference trial. Leaves were dried at 55 ^{o}C until they reached a constant weight. The relative nitrogen content of these leaves was estimated using the Waikato University stable isotope facility (http://bio.waikato.ac.nz/isotope/). This facility uses a fully automated Europa Scientific 20/20 isotope analysis which combusts samples and uses gas chromatography to separate the gases and continuous-flow mass spectrometry to calculate the percentage of ^{15}N to a precision of ± 1%. The relative nitrogen content of each plant species was used to generate an expected preference rank for the weta.

This rank order was then compared to the rank order obtained from the paired preference testing using a Spearman's rank correlation, to determine if the tree weta were solely picking their preference based on nitrogen content of the leaves.

2.2.2 Carbon absorption

Ten tree weta (*H. crassidens* and *H. thoracica*) were fed on carrot (*Daucus carota sativus*, tap root) until their frass (faeces from a plant eating insect) changed to an orange colour (approximately five to seven days) indicating a complete flushing of leaf material from the digestive tract. Individual tree weta were then provided with a known amount of leaf food for two consecutive nights; leaf area eaten was recorded by scanning leaves before and after feeding and converted into dry weight as described below. Ten weta were used for trials of each of the 8 plant species. Leaf remains were removed and the tree weta were returned to the carrot diet until their frass returned to an orange colour. All frass produced after leaf feeding was collected and dried at 80°C until it reached a constant weight (dry weight). Frass samples from

each trial were burned at 500°C for 6 hours and the weight of the remains recorded (ash weight). Frass from the 10 weta were pooled for each plant species.

To convert leaf area eaten into leaf mass (dry weight and ash weight), twenty leaves of each plant species were scanned fresh and their leaf surface areas computed using Compu Eye (Bakr, 2005). The leaves were dried, weighed and then burnt at 500°C for 6 hours and the weight was recorded (ash weight).

Data was analysed using a Kruskal-Wallis chi-squared test to test for a change in ash weight after weta consumption. My null hypothesis was that there would be no difference between the estimated leaf ash weight (based on the leaf area eaten), and the frass ash weight (e.g. no carbon absorption). The Kruskal-Wallis test was used because the data was not normally distributed and were performed in SAS 4.2. The ash-free dry weight was determined for the frass, this is the dry weight (organic plus mineral content) minus the ash weight (mineral content) and can be interpreted as any carbon remaining in the frass which was removed by ashing. This was compared to the estimated quantity of carbon eaten by the tree weta with a regression analysis in minitab (State College, 2010).

2.2.3 Paired Preference Testing

Tree weta were kept in the standard enclosures as described in the general methods and provided with a mixed leaf diet of mahoe (*Melicytus ramiflorus*), *Coprosma repens* and miro (*Prunmopitys ferrunigea*) prior to the feeding trial.

Six plant species fed to tree weta by Wyman (2009) were mahoe (*Melicytus ramiflorus*), tutu (*Coriaria arborea*), Eucalyptus (*E. ficifolia*), tree fuchsia (*Fuchsia excorticata*), puka (*Griselinia littoralis*), northern rata (*Metrosideros robusta*). In addition to these six species, putaputaweta, (*Carpodetus serratus*) and karamū (*Coprosma robusta*) were added. Putaputaweta was chosen as it is a tree species that tends to have a high number of holes in its trunks and branches that make excellent daytime refuges for tree weta (C.Wehi unpublished data). Karamū was chosen because

Griffin (2011) found it to be the least preferred food compared to alternative food sources (moth, fruit, and seeds), and karamū leaves have not been compared to other plant species. These two species were tested against all those previously studied (Wyman, 2009). A comparison of mahoe with all species was repeated as a control to ensure that the data was comparable to that of Wyman (2009). Data from these eight species were used to complete a matrix in which each species was tested against every other species, resulting in 28 different combinations, in order to generate a preference rank. Each trial used at least 30 weta with approximately equal numbers of *H. crassidens* and *H. thoracica*.

One leaf of each of two different plant species was placed in each individual weta's enclosure as a choice of food every second night. It was known from initial feeding trials that both *H. crassidens* and *H. thoracica* readily consume carrot (tap root). Therefore, on alternative nights between the leaf choice tests weta were given a slice of carrot which acted to partition each choice experiment by 'cleansing the pallet' and provide some nutrition if weta had chosen not to eat the night before. The night during which plant leaves where provided to the weta was preselected to ensure that the tree weta were not exposed to an accumulation of potentially toxic secondary compounds from any one plant species e.g. the tree weta were not presented with the same plant species on two consecutive feeding nights.

The software program Compu Eye Leaf and Symptom Area (Bakr, 2005) was used to determine the surface area of the leaves before and after tree weta feeding. Leaves were digitally scanned before and after each food choice test and the programme Compu Eye was used to calculate the surface area of the leaves. The difference between these values showed the amount eaten. This software uses scanned colour images to determine the surface area of a green image against a black or white background.

In order to better quantify the amount of each plant species eaten, surface area estimates were converted to dry weight per mm^2 using an average dry weight to surface area index previously calculated for each plant species. These indices were obtained by collecting ten fresh leaves of each species, calculating surface area and

drying the leaves at 55°C (dry weight). Dry weight was divided by surface area for each leaf and the results were averaged for each species.

Weta that did not consume any leaf material in a particular trial were excluded from analyses of that trial, but in all trials between 30 and 40 weta ate some leaf material. Each paired preference test was analysed using a Kruskal-Wallis test (null hypothesis: no significant variation between plant species). A Spearman's rank correlation test was performed to compare all the rank orders.

2.3 Results:

2.3.1 Nutrient content

Nitrogen levels in leaves ranged from 0.76% to 2.31 % ± 1%. The species with the highest nitrogen value was tutu, which was three times that of the species with the lowest value, puka (Table 2.1). The second highest in the nitrogen ranking was putaputaweta which was 0.22 % lower than the highest. These two species were the only species with over 2% nitrogen. Rata and puka were the only two species under 1% nitrogen content at 0.96% and 0.76% respectively. The other four species fell between 1% and 2%.

Table 2.1 The rank order for the carbon assimilation (average for each plant species), the nitrogen percentage, and the paired preference tests, (values with significant *p*-values are in bold).

Plant Species (Carbon)	Ash free dry weight (per mm^2)	Plant Species (Nitrogen)	Nitrogen % (± 0.1%)	Plant species (Weta Preference)
Mahoe	**0.0000110**	tutu	2.31	tutu
Karamū	**0.0000109**	mahoe	2.09	putaputaweta
Tutu	**0.0000100**	putaputaweta	1.59	karamū
Putaputaweta	**0.0000099**	karamū	1.46	rata
Puka	**0.0000074**	fuchsia	1.33	eucalyptus
Fuchsia	0.0000130	eucalyptus	1.21	mahoe
Rata	0.0000069	rata	0.96	puka
Eucalyptus	0.0000063	puka	0.76	fuchsia

To determine the carbon availability, the estimated quantity of leaf material eaten (ash weight) by the weta was compared to the quantity of their frass (ash weight). It was predicted that if the weta had absorbed a significant fraction of the leaf material then their frass (ash weight) would be less than the expected ash weight for the amount of leaf eaten. Interestingly, for three of the eight plant species investigated, the was not evidence that the weta had been able extracted measurable amounts of carbon from the leaves; the ash weight of their frass was similar to that of the leaves consumed. The three plant species that were the poorest source of carbon for weta were fuchsia, rata, and Eucalyptus. In contrast, five plant species in my analysis showed a significant reduction in mass of the plant material after passage through the tree weta gut, and this was attributable to removal of carbon by the tree weta. Mahoe, tutu, karamū, putaputaweta, and puka all provided significant carbon resource absorbed by the tree weta.

All plant species exhibited an average decrease in carbon from plant to frass (Figure 2.1) except tutu. When the amount of carbon consumed was compared to the ash-free dry weight (Figure 2.2) there was a positive correlation for 7 out of the 8 plant species, the only negative correlation was fuchsia. Fuchsia exhibited no relationship between carbon eaten and the ash-free dry weight of the frass (R^2 = 0.006, F= 0.02, and *p*-value = 0.88). Eucalyptus had a weak positive relationship between the ash-free dry weight of the frass and the carbon eaten (R^2 = 0.109, F= 0.61, and *p*-value = 0.47). Putaputaweta also had a weak positive relationship between the ash-free dry weight of the frass and the carbon eaten (R^2 = 0.12, F= 0.81, and *p*-value = 0.40). Karamū had a moderate positive relationship between the carbon eaten and the ash-free dry weight of the frass (R^2 = 0.589, F= 7.17, and *p*-value = 0.04), as did puka (R^2 = 0.677, F= 8.4, and *p*-value = 0.04). Tutu (R^2 = 0.808, F= 20.98, and *p*-value = 0.006), mahoe (R^2 = 0.805, F= 20.65, and *p*-value = 0.008), and rata (R^2 = 0.856, F=23.75, and *p*-value = 0.04) all had strong positive relationships between the amount of carbon eaten and the ash-free dry weight of the frass.

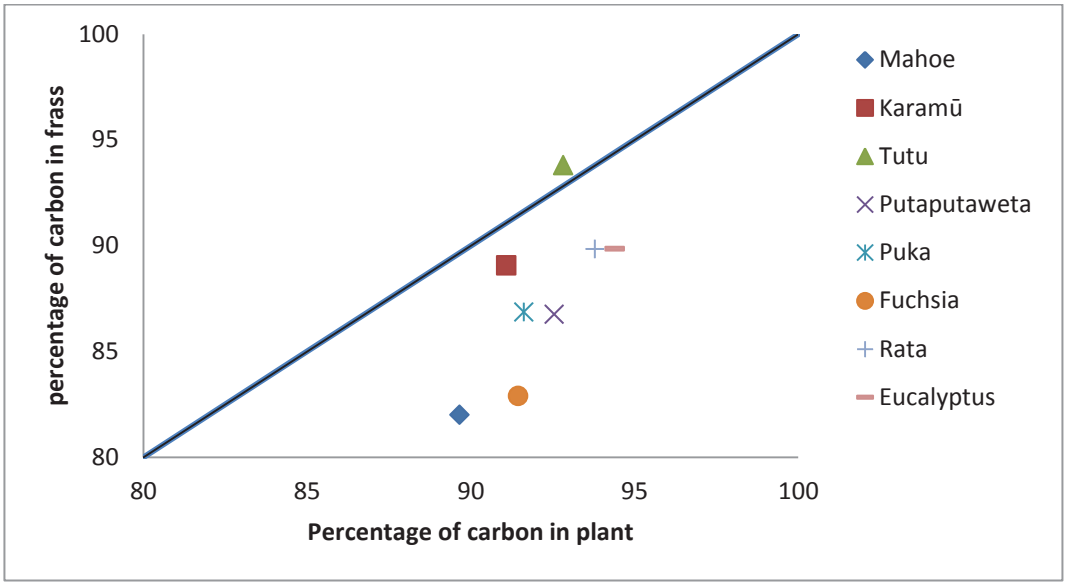


Figure 2.1 The average percentages of carbon in the frass of the tree weta and plant species, with a line at the 1:1 ratio which would be expected if no carbon was removed by the tree weta.

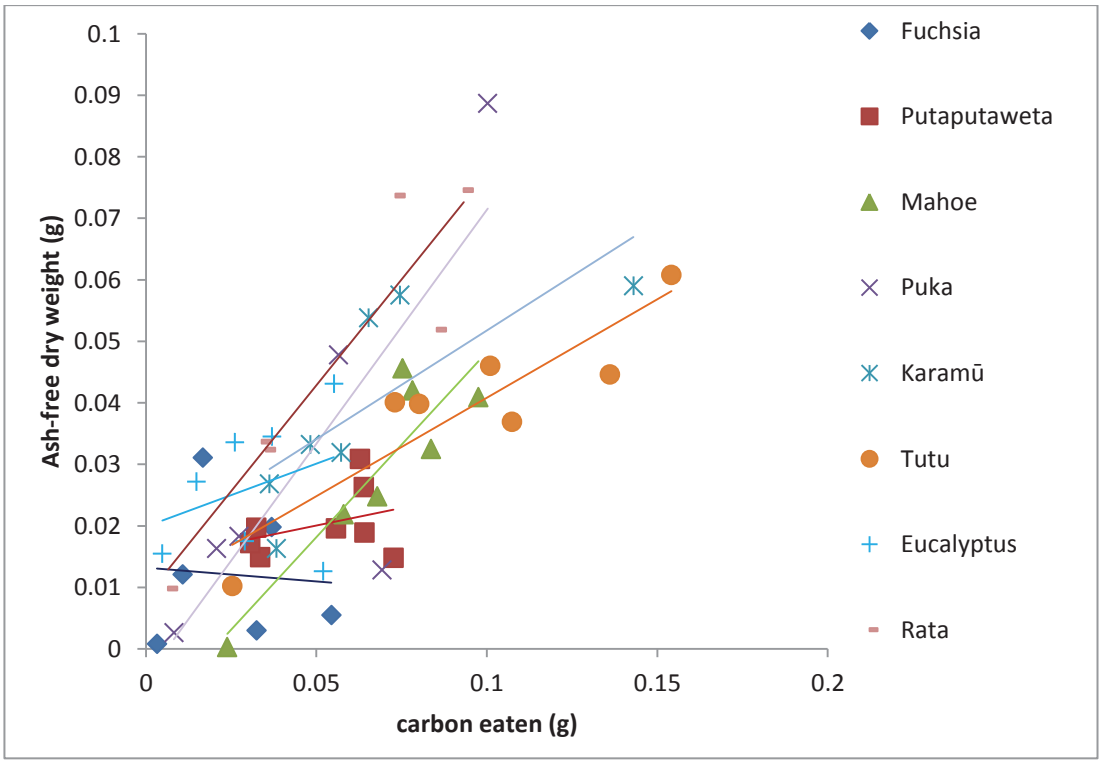


Figure 2.2 The amount of carbon present in the plant species versus the ash-free dry weight of weta frass, with trend lines.

The Spearman's rank correlation for the nitrogen and paired preference was 0 (*p*-value 0.512), indicating no correlation at all. The Spearman's rank correlation for the carbon assimilation and paired preference was 0.55 (*p*-value 0.085), indicating a moderate positive correlation. The Spearman's rank correlation for the nitrogen and carbon was -0.19 (*p*-value 0.69), indicating a weak negative correlation.

2.3.2 Paired Preference testing:

Tree weta ate all the plant species they were given but not in the same quantities. Nineteen of the twenty eight pairings revealed significant difference in the quantity of the two plant species eaten (Table 2.2). The data from the two weta species were grouped together because although they differed on occasion as to preference, the different preferences were limited to eight plant pairings. These pairings comprised 7 of the 8 plants used in the experiment, and resulted in fewer significant plant preferences but did not alter the final rankings.

Tutu was the tree weta's most preferred plant species, but was not the plant which was eaten in the highest amount. Karamū was the plant species which had the highest average consumption but ranked third on the tree weta's preference ranking. Puka and fuchsia were the two least preferred plant species and also the plant species that were eaten the least. Mahoe was ranked sixth in the preference ranking but was the species with the third highest amount eaten.

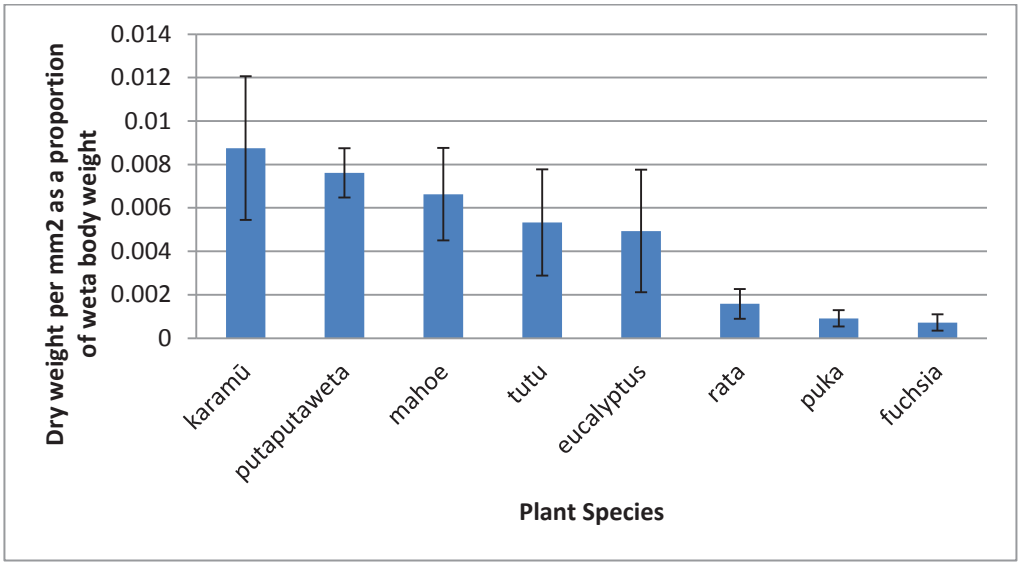


Figure 2.3 The average amount of each plant species eaten by the tree weta over all 28 paired preference tests, as a dry weight proportion of the weta body weight and SE.

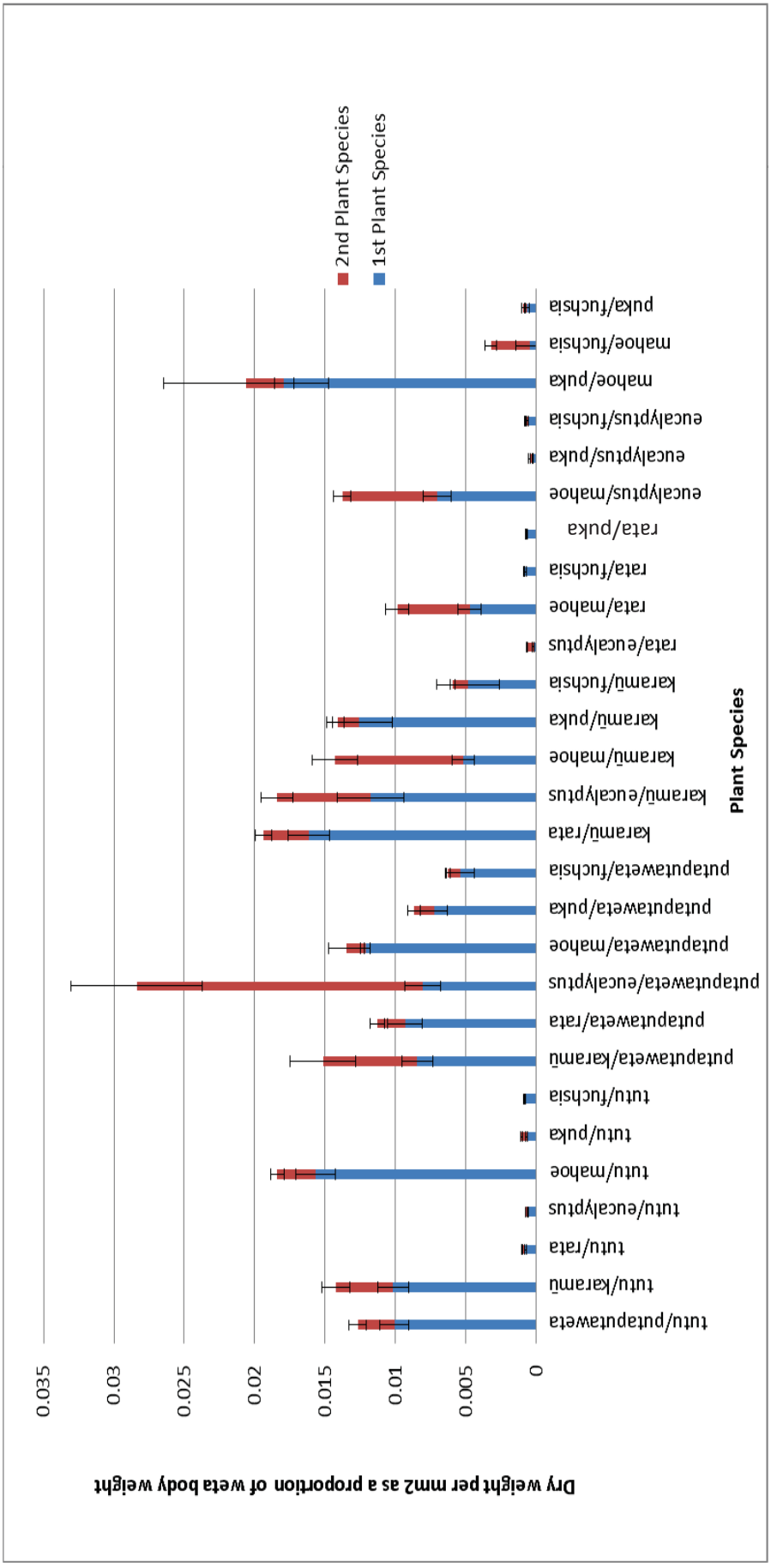


Figure 2.4 The average amount eaten for each of the 28 paired preference tests and SE.

Table 2.2 Results from the 28 plant paired preference tests for eight species of plants eaten by tree weta. *P*-values obtained from the Kruskal-Wallis chi-squared test significant values are in bold.

	Tutu	Putaputaweta	Karamu	Rata	Eucalyptus	Mahoe	Puka	Fuchsia
Tutu								
Putaputaweta	**0.0001**							
Karamu	**0.0001**	**0.0145**						
Rata	**0.0001**	**0.0001**	**0.0005**					
Eucalyptus	**0.0015**	0.1422	0.3654	0.1101				
Mahoe	**0.0001**	**0.0001**	0.5758	0.7407	**0.0368**			
Puka	**0.0006**	**0.0001**	**0.0001**	**0.0001**	0.4021	**0.0002**		
Fuchsia	**0.0001**	**0.0017**	0.7316	**0.0001**	**0.0074**	**0.0368**	0.0844	

2.4 Discussion

Like all living things, insects must manage their nutrient intake to maximise their growth and fecundity (Bernays, 2001). Many invertebrate species have diets that are nitrogen limited in their diet (Joern & Behmer, 1997; White, 1978), so they should eat all other nutrients up to the point where their nitrogen requirements are met. Thus I predicted that the tree weta used in this trial would prefer to eat the foods with relatively high nitrogen content. Carbon is also an important nutrient and the essential building block of all life (Hart, Hart, Craine, & Hadad, 2011). Carbon is abundant in nature but it is often inaccessible for use by animals (McDonald, et al., 2002). So a plant from which weta can get high levels of carbon easily would be expected to be preferred over plants with less accessible carbon. Thus I expected tree weta to prefer the plant species from which carbon was most readily assimilated and to see this reflected in the paired preference experiment.

It was hypothesised that weta choose their food on the basis of its nutrient quality either preferring high nitrogen content or high carbon accessibility. The analysis of preference from the paired preference test indicates tutu as the most preferred of all eight species in this study (Table 2.2). When the average amount eaten was compared for all eight plant species it was also interesting to note that while tutu was the preferred plant and the highest nitrogen content, the plant which had the highest

average amount eaten was karamū, followed by putaputaweta, mahoe, then tutu, eucalyptus, rata, puka and lastly fuchsia. The paired preference order is not the same as the order for either nitrogen content or carbon availability (Table 2.2).

Tree weta used in this trial were observed to have a clear preference among the eight plant species used in this trial. While, this was different to the two possible expected rankings it contained elements of them both. For example, tutu was the species with the highest nitrogen content probably because tutu has an associated bacteria that aids in nitrogen fixation (Stevenson, 1958). It is most likely that tree weta need to regulate a range of nutrients within their diet so they take cues from more than one factor to determine their diet.

It also appears that potential carbon absorption is limited to only a certain percentage of the carbon present in the plant species (Figure 2.2). This may mean that only an increase consumption of plant material will result in an increase in carbon absorption and that the tree weta cannot regulate the adsorption of carbon once it has entered their digestive system. This may have an impact on the tree weta especially if carbon blocks the absorption of other nutrients or becomes toxic at higher levels.

Plants however do not contain only nutrients but also a range of other chemicals including secondary compounds which may contribute to plant defence mechanisms for deterring herbivory (Guglielmo, Karasov, & Jakubas, 1996). This may help explain the position of mahoe in the preference ranking (sixth), despite its high nitrogen ranking (second) and carbon assimilation ranking (first). Perhaps mahoe has evolved chemical defences to limit herbivory by tree weta and other animals and this may be in the form of a secondary metabolite as seen in many other plant species (Agrawal, 2006). Wyman (2009) observed that mahoe was always attractive to tree weta in captive studies but that its' contribution to the diet was intermediate. Wyman (2009) hypothesised that toxic loading may limit the quantity of mahoe eaten on consecutive nights.

As polyphagous herbivores, tree weta are expected to choose not to feed on a single plant species but a range of plant species (Bernays, et al., 1992). Tree weta would be expected to switch between food sources in the wild, however individual tree weta

may have a preference for a particular plant species, a natural diet for a tree weta would need to consist of more than one plant species. Being a generalist also means that tree weta would have to deal with a large range of secondary compounds and plant defences (see chapter 4).

Putaputaweta is a plant noted for the abundance of holes in the plant stem resulting from the activity of Puriri moths caterpillars (Stevenson, 1982), the maori name means many many weta. These holes make attractive daytime refuges for tree weta and other arboreal invertebrate species in the North Island. Putaputaweta as a food was found to be a favourite of the tree weta, coming second in the preference ranking, despite being fourth on the carbon assimilation rank. As putaputaweta was favoured by the tree weta, there is a high probability that it may contain a nutrient not looked at in this study that is essential for tree weta or that putaputaweta lacks an effective chemical defence against the tree weta. On the other hand, tree weta may have adapted to utilize putaputaweta, as it provides both a refuge and a food source minimising their exposure to predators (Rufaut & Gibbs, 2003).

There are a variety of factors which were not considered in this study that might also affect the palatability of a plant species, such as leaf tensile strength (Cornelissen et al., 1999) composition, internal structure (e.g. the number of veins) and the age of the leaf (Basset, 1994). For example, puka has a physical defence of a thick waxy cuticle on their leaves along with low nitrogen which together might make the species unattractive to weta, puka also had a low carbon assimilation ranking. Wyman (2009) did not find any difference between tree weta species while doing the paired preferences testing in her study. The current study did find a preference difference between the two tree weta species however this was limited to a minority of plant pairings and was not considered a significant factor in determining plant rankings. However, the minor differences observed among species may be of interest in comparative studies of these two weta species.

Wyman (2009), found *Eucalyptus* was high in the tree wetas' preferences; however in this study it was fifth. This change in the preference is partly due to the new plant species used and suggests that *Eucalyptus* is not a preferred species for tree weta to

eat when plants such as putaputaweta and *Coprosma* are available. This is likely because it is low in nitrogen (sixth in the ranking) and high in essential oils and phenolic compounds, both of which are believed to have a role in the palatability of foliage and are possible barriers against herbivory (Wyman, 2009). Few insect or mammal species eat eucalyptus to any great extent (Landsberg & Cork, 1997) and the insects that do feed on eucalyptus have developed a variety of mechanisms for dealing with the secondary compounds in the foliage (see chapter 4).

Plant preference is a complicated topic involving a number of confounding factors, but how and why a species chooses its diet are questions that must be asked. The tree weta used in this study showed a clear preference for tutu, a species high in nitrogen, which suggests that tree weta are nitrogen limited. However, the rest of the preference ranking suggests that there is a range of other factors which are affecting how the weta chooses to divide its diet. These factors are likely to include the presence of secondary compounds and other nutrients. Secondary compounds are often used as plant defence mechanisms. It is only with continued research that the full picture of why tree weta choose to eat particular plant species at higher rates than others may be fully understood.

Chapter Three: Tree weta diet in the wild.



Weta frass

3.1 Introduction

Foraging animals must weigh a variety of factors to ensure their dietary needs are met. Maintaining vigilance for predators and minimising handling time are two major factors. (Molles, 2002; Ricklefs, 1980; Rothley, Schmitz, & Cohon, 1997). This maximises energy gains and minimises energy lost. For a tree weta, this means that an ideal food source would provide good nutrition, a day time refuge (with protection from predation) and be easy to manipulate.

As polyphagous insects, tree weta are capable of eating a wide range of foods, with a wide variety of plant species making up the majority of their diet (Gibbs, 1998; Griffin, Morgan-Richards, et al., 2011). But how individual weta choose their diet is unknown. Diet self-selection theory suggests that a polyphagous species should regulate its diet by switching species often, which will maximise nutrient intake while minimising the toxic effects of plants' secondary compounds (Waldbauer & Friedman, 1991). Basic information about the number of plant species eaten in one meal or one night by tree weta will help understand their diet switching decisions.

Although weta have been used in a number of diet and feeding studies (Duthie, et al., 2006; Fadzly & Burns, 2010; Wyman, et al., 2011) little has been published on the diet of wild individuals. Griffin et al. (2011) summarised what was known about plant species eaten by four species of tree weta. This included had 59 plants that were fed to captive tree weta but only 17 that were known from a wild diet. Therefore, basic information on the plant species eaten in the wild, as well as plant species not eaten, is of general interest. Such basic information can be derived from cuticle studies of the frass from wild caught tree weta (Wilson & Jamieson, 2005). Cuticle studies of wild animals will also provide estimates on the number of plant species in an average tree weta meal and any differences among the sexes is of interest for diet switching models.

Weta use daytime refuges in dry wood and living trees, with small entrance holes, making it difficult for birds and rats and other predators to access the tree weta during

the day. Tree weta are almost entirely arboreal (Gibbs, 1998) but it is not known how far weta travel to find their food at night, and this is especially interesting in regards to the presence of predators. To get their full nutritional needs, weta may need to travel some distance away from their refuge, but doing so would potentially expose them to longer periods when they would be vulnerable to predation. Due to this and the potential limitation of finding new refuges it is assumed that tree weta return to the same refuge hole everyday (Field, & Sandlant, 2001; pers.com M. Morgan-Richards and P.Wehi). The wellington tree weta (*Hemideina crassidens*) spends more time during the evening/night at the entrance of its refuge being "vigilant" when in the presence of mammalian predators than on mammal free islands (Rufaut & Gibbs, 2003). In addition, it has been suggested that the refuge hole can be a limiting resource (Field & Sandlant, 2001) which the weta would want to retain access to and thus optimal foraging might require a speedy return to the refuge if weta densities are high.

The optimal strategy for tree weta might be to feed only on plant species adjacent to their day time refuge hole. I set out to test this hypothesis by collecting data on the available food plants and compared this to the plant species in the diet of wild caught tree weta using cuticle analysis of their frass. Because the digestibility of plant material is species dependent, I used a presence/absence method of detection with the null hypothesis that all plant species in the weta frass would also be close (< 2.5m) to their refuge hole. Balancing nutrients and possible plant toxins might result in weta eating more than one plant species per night and such basic data will be sought from the cuticle study.

3.2 Methods

All tree weta (11 *Hemideina thoracica* and 22 *Hemideina crassidens*) used in this study were randomly collected during the day from three sites in the Western foot hills of the Tararuas; the Back Track, Palmerston North (40˚47'148''N, 175˚60'943''E), Kimberly Reserve, Levin (40˚66'592''N, 175˚30'664''E), and Nga Manu (40˚86'196''N,

175˚06’098’’E). Nga Manu is 63.31km south of the Back Track and 29.92km south of Kimberley reserve. Kimberley reserve is 33.49km south of the Back Track (Figure 3.1). At the time the weta were collected, the plant species in which their daytime refuge was located, as well as the first plant foliage that would be contacted upon exiting their refuge, was recorded. A 5 x 5 m quadrat was established, with the tree weta refuge as the centre point, and all plant species inside the quadrat were recorded. The random sampling resulted in weta that varied in age from 4^{th} – 10^{th} instar (adult) and 17 of the 33 weta used were female.

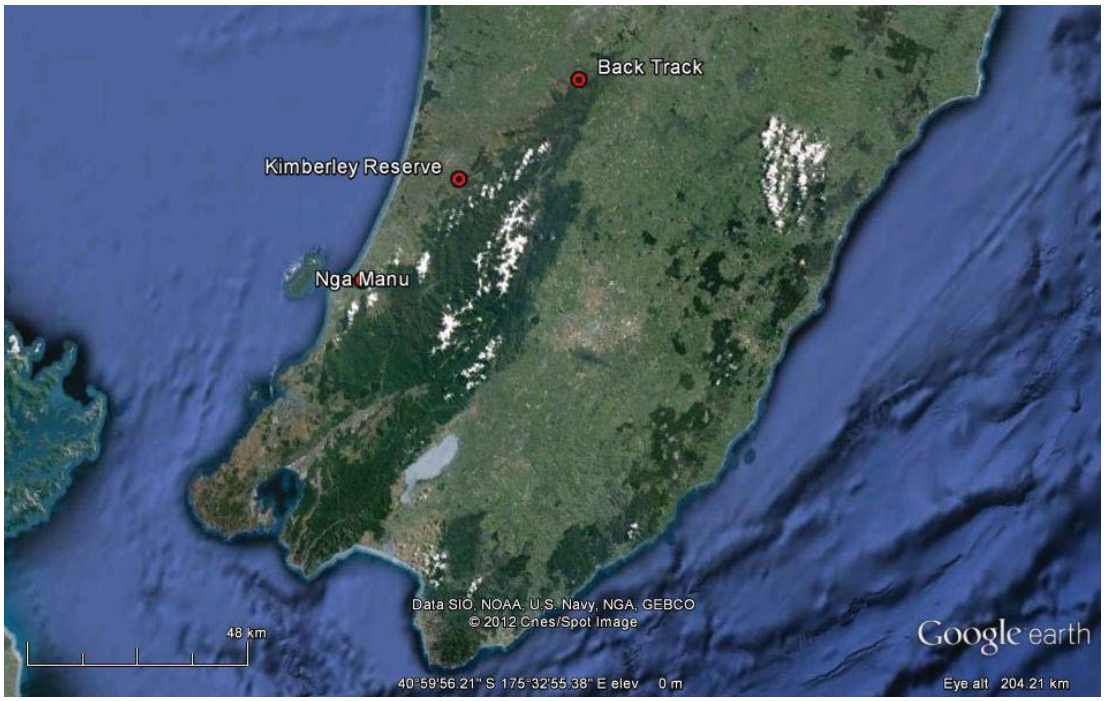


Figure 3.1 Tree weta collection sites in the lower North Island of New Zealand.

The first frass that each weta produced in captivity was collected to study their natural diet. The frass was broken up and bleached in 15% sodium hypochlorite until at least 80% of particles were transparent. The frass was washed with water using a microfilter, stained with basic fuchsin, and mounted on a glass microscope slide with glycerol and coverslip. The frass slides were examined under a microscope at low magnification and plant species were identified using diagnostic cuticle characteristics (usually the arrangement of guard cells around stomata) until 10 identifiable plant fragments were found. Because the ‘fragmentability’ of plant material is species dependent (Wilson, 2004), I used a presence/absence method of detection and did not

try to estimate relative abundance. In order to determine whether 10 identifiable plant fragments were enough to detect the majority of species present, I scanned material from the whole frass of 5 tree weta. No additional plant species were identified when this approach was used.

A plant cuticle reference collection had already been partially created by Wyman (2009), and was supplimented to in this study. To create the plant cuticle collection, captive tree weta were fed carrot until their frass turned orange, indicating a complete cleaning of the digestive tract. Single plant species of interest was then fed to individual tree weta. Once the weta passed black/green frass a reference slide was made, in the same way as above. The plant cuticle collection was completed by ensuring that all plant species recorded from quadrats were included and that may have been in the frass had reference specimens. There was one noticeable exception, kawakawa (*Macropiper excelsum*), which the tree weta would not eat during a two weeks period, even though no other food sources were supplied. A reference slide for kawakawa was made by stripping the cuticle layer off the leaf by hand and then processing it in the same method as above.

The plant species at different sites were compared using a single factor Permutation Multivariate Analysis of Variance (PERMANOVA) and a similarity percentage analysis (SIMPER) was used to highlight the differences. A single factor PERMANOVA was performed on the plant species present in the quadrat and also on the plants present in the frass comparing the sexes of the tree weta. A two-way PERMANOVA was performed on the plant species in the frass comparing the two tree weta species and the site differences simultaneously; this was done so that collection site, which varies in species composition, could be eliminated as a confounding factor. These tests were performed in PRIMER (Clarke, 1993).

I compared the presence of plant species in the weta's frass with the occurrence of that plant species in the 5 x 5m quadrat around the weta refuge. If the weta had eaten a plant species which was not adjacent to its refuge then the weta must have travelled outside the quadrat to find that food. If a plant species was present both inside and outside the quadrat then it was assumed that the weta had eaten the plant closest to

its refuge and this was considered to be eating inside the quadrat. A two-tailed Fisher's exact test was performed on each plant species comparing the number of times a plant species was eaten inside a quadrat to the number of times it was eaten from outside a quadrat, (null hypothesis = no difference between the number of times a plant was eaten inside a quadrat to the number of times it was eaten outside).

3.3 Results

Frass from thirty-three (22 *Hemideina crassidens* and 11 *Hemideina thoracica*) tree weta were studied and 29 plant species were identified (Table 3.2). The number of plant species identified per frass ranged from zero to six. Two tree weta, both *H. crassidens,* had frass containing only arthropod remains (identified by tarsal claws and mouthparts). Although interesting, these weta were excluded from further analysis. On average 2.65 ± 0.26 plants were observed per frass. In contrast, on average each weta had 6.58 ± 0.43 plant species within the environment surrounding its refuge (5 x 5m quadrat). *Hemideina crassidens* had an average of 2.25 ± 0.32 plant species in the frass and *H. thoracica* had an average of 3.36 ± 0.39, this difference is not significant. The tree weta from the Back Track and Kimberley Reserve on average had 3.5 ± 0.5 and 3.0 ± 0.53 plant species in the frass respectively, while the tree weta from Nga Manu only had an average of 1.86 ± 0.27 plant species. The two sexes of tree weta ate similar numbers of plant species: average of 2.75 for ± 0.44 females and 2.53± 0.29 for male tree weta. Six tree weta which ate from only inside the quadrat and had an average of 1.33 ± 0.21 plant species in their frass, while the ten tree weta that had eaten exclusively outside had an average of 1.56 ± 0.18 plant species per frass. The fifteen tree weta that ate both inside and outside the quadrat had an average of 3.75 ± 0.30 plant species per frass.

Table 3.1 The average number and standard error of plant species present in the quadrats and frass of the tree weta.

Variable	Average	Standard Error
both inside and outside the quadrat	3.75	0.30
inside the quadrat	1.33	0.21
outside the quadrat	1.56	0.18
Female	2.75	0.44
Male	2.53	0.29
Tree weta	2.65	0.26
Wellington tree weta	2.25	0.32
Auckland tree weta	3.36	0.39
Back Track	3.50	0.50
Kimberley Reserve	3.00	0.53
Nga Manu	1.86	0.27
Plants in quadrats	6.58	0.43

Kawakawa (*Macropiper excelsum*) was recorded in every quadrat from which a tree weta was collected, but it was never observed in the weta frass. Likewise, four fern species (black tree fern, *Cyathea medullaris*, Hen and Chicken fern, *Asplenium bulbiferum*, Shining speenwort, *Asplenium oblongifolium* and *Blechum sp*.) were common in the areas adjacent to the tree weta refuge holes but evidence of ferns (cuticle or spores) were rarely observed in weta frass (Table 3.2). Three plant species that were recorded in the frass were not identified as any of the plant species present in the quadrats, these were radiata pine (*Pinus radiata*), and makomako (*Aristotelia serrata*), while the third plant species is unidentified and labelled here as unknown (it was not represented in the reference slides in the Massey University collection).

Table 3.2 The total number of times each plant species was recorded for the quadrat and the frass. The number of times the plant was recorded in a frass but was not present in the quadrat the weta was caught in (corresponding percentage in brackets). The p-values are from a two-tail Fisher's exact test comparing the number of times a plant species was eaten inside a quadrat to the number of times it was eaten outside a quadrat. Significant values are in bold.

Plant Species (d.f. 1)	Total number quadrats with this plant species	Total number of frass with this plant species	Number of times plant eaten outside quadrat	*p*-value
Kawakawa (*Macropiper excelsum*)	31	0	0 (0%)	1
Mahoe (*Melicytus ramiflorus*)	29	13	0 (0%)	1
Hen and Chicken fern (*Asplenium bulbiferum*)	11	0	0 (0%)	1
Coprosma spp.	10	3	1 (33%)	0.27
Blechnum sp.	9	1	1 (100%)	**0.01**
HangeHange (*Geniostoma ruprestre*)	9	3	0 (0%)	1
Hounds Tongue (*Microsorum pustulatum*)	8	3	2 (66%)	0.06
Tawa (*Beilschmeida tawa*)	7	2	1 (50%)	0.25
Mamaku (*Cyathea medullaris*)	7	0	0 (0%)	1
Parataniwha (*Elatostema rugosum*)	7	1	0 (0%)	1
Pigeonwood (*Hedycarya arborea*)	7	1	0 (0%)	1
Rewarewa (*Knightia exelsa*)	7	7	4 (57%)	0.19
Lancewood (*Pseudopanax crassifolius*)	7	1	1 (100%)	0.13
Tradescantia (*Tradescantia fluminensis*)	7	6	2 (33%)	0.4286
Titoki (*Alectryon excelsus*)	6	0	0 (0%)	1
Rangiora (*Brachyglottis repanda*)	6	3	1 (33%)	0.43
Kahikatea (*Dacrycarpus dacrydioides*)	6	0	0 (0%)	1
Lemonwood (*Pittosporum eugenioides*)	6	3	2 (66%)	0.11
Pseudopanax laetus	6	0	0 (0%)	1
Pate (*Schefflera digitata*)	5	4	1 (25%)	1
Tree Lucerne(*Chamaecytisus palmensis*)	4	12	8 (66%)	1
Puriri (*Vitex lucens*)	4	1	0 (0%)	1
Shining Spleenwort (*Asplenium oblongifolium*)	2	1	1 (100%)	0.33
Climbing Rata (*Metrosideros perforate*)	1	2	2 (100%)	0.33
Totara (*Podocarpus totara*)	1	0	0 (0%)	1
Supplejack (*Ripoganum scandens*)	1	1	2 (0%)	1
Radiata Pine (*Pinus radiata*)	0	1	1 (100%)	1
Makomako (*Aristotelia serrata*)	0	2	2 (100%)	1
Unknown	0	11	11 (100%)	1

All the wild tree weta were collected from three low altitude sites within 65 km of one another but the sites have different plant species compositions. A single factor Permutation Multivariate Analysis of Variance (PERMANOVA) revealed that the plant species present in the quadrats of the three sites differ significantly (*pseudo*-F = 23.119, d.f.= 2, *p*= 0.001). A similarity percentages analysis (SIMPER) showed that the major differences between the Back Track and Nga Manu sites was the presence of shining spleenwort (*Asplenium oblongifolium*), hangehange (*Geniostoma ruprestre*), and the *Blechnum* species, which were all present at the Back Track but not at Nga Manu. *Blechnum* spp. and hangehange were also part of the difference between the Back Track and Kimberly Reserve sites, as they were present at Back Track but not Kimberly reserve, but Kimberly Reserve had rewarewa (*Knightia excelsa*) and *Tradescantia fluminensis* which were not present at the Back Track. The rewarewa, *Tradescantia* and titoki (*Alectryon excelsus*) were the major differences between Nga Manu and Kimberly Reserve, all three species were present at Kimberly Reserve and not at Nga Manu.

The environmental differences appeared to affect the plant species eaten by wild tree weta. A single factor PERMANOVA revealed that the tree weta collected from the three sites also had significantly different plant species present in their frass (*pseudo*-F = 7.408, d.f.= 2, *p*= 0.001). Male and female tree weta were eating the same plant species as revealed by a single factor PERMANOVA (*pseudo*-F = 1.066, d.f.= 1, *p*= 0.374). The two tree weta species were not eating a different set of plant species as revealed by a two-way factor PERMANOVA (*pseudo*-F = 0.25, d.f.= 1, *p*= 0.892) but collection site did influence what was available to them.

I compared the plant species eaten by weta that had been selected from either close plants (inside quadrat), or more distant plants (outside quadrat) or from both inside and outside their quadrat. A two-way factor PERMANOVA revealed that the location of plant species eaten did differ significantly (*pseudo*-F = 02.53, d.f.= 2, *p*= 0.011) but that weta collection site was the significant factor. A similarity percentages analysis (SIMPER) showed that the major differences between eating outside the quadrat and eating both inside and outside was the presence of mahoe (*Melicytus ramiflorus*), makomako, and tree lucerne (*Charaecytisus palmensis*). All of these species were

present in the frass of tree weta that ate both close and more distant plants. Tree lucerne, the makomako and rewarewa were also part of the difference between eating outside the quadrat and inside the quadrat, with tree lucerne in both groups and rewarewa and makomako eaten only outside the quadrat. Tree lucerne, mahoe and makomako were also the major difference between eating inside the quadrat and both inside and outside with tree lucerne and mahoe being present in both groups but makomako only being in the combination group (Table 3.3).

Table 3.3 Presence/absence data for the plant species that contributed most to the tree weta feeding either close to their refuge or more than 2.5m from their refuge or both. 1 represents presence and 0 represents absence.

	In	out	both
mahoe	1	0	1
Tree lucerne	1	1	1
makomako	0	1	1
rewarewa	0	1	1

Of the 31 tree weta, 20 (64.5%) had eaten plant species from both within and outside the quadrat, while 7 (22.6%) tree weta had only eaten plant species found within their quadrat. 4 (12.9%) weta ate plant species found only outside their retrospective quadrats.

The plant species closest to the refuge and therefore the most likely to be encountered first by weta was kawakawa (68%). I have shown here that tree weta will not eat kawakawa, and this species was excluded from further analysis. It was rare for these wild caught weta to have eaten the first plant encountered when they left their daytime refuge (1/10), the exception was when mahoe, (*Melicytus ramiflorus*), was the closest plant species. Most of the weta in this study were removed from dead branches of unidentifiable origin, but five weta had their refuge in an identifiable living plant species identified. Only the weta living in mahoe had eaten its host species.

3.4 Discussion

The two species of tree weta studied here (*H. crassidens, H. thoracica*) were found to eat at least 24 different plant species in the wild. On average each weta ate 2.65 species of plant per frass, and generally these were not the plants most likely to be first encountered, nor their refuge tree species. Thus tree weta appear to be non-randomly selecting from the available food sources, and frequently travel more than 3.13 metres from their home refuge.

The two tree weta that produced frass containing only arthropod fragments offered further support to the theory that tree weta are opportunistic omnivores and not pure herbivores (Griffin, Morgan-Richards, et al., 2011).

There was no evidence that the two tree weta species or the two sexes differed in the plant species they consumed. The sampling sites differed significantly with respect to their plant compositions, with the main contributors being the presence or absence of the trees hangehange, rewarewa, titoki and the ground cover plants shining spleenwort, *Blechnum* spp., and *Tradescantia*. The tree weta were collected from lowland native forest in the southern North Island New Zealand (McGlone, 1989) and they are not identical as all three collection sites are at varying stages of regeneration. Nga Manu is noted for its large collection of around 700 native plant species some of which do not naturally occur in the area (Nga Manu Ecological Trust, 2012), while the Back Track, bordered by a pine plantation, is a relatively young regenerating forest. Kimberley reserve has some large native trees but lacks a dense understory due to the abundance of the invasive *Tradescantia*. These environmental and physical characteristics of the sites are what lead to the varying plant compositions and which probably therefore created the difference in the tree weta diet.

One plant species (Kawakawa) was common in the weta habitat but never found in the frass. This plant species was also rejected by captive weta. Kawakawa leaves are known to contain toxic compounds (Hodge et al., 1998), yet the leaves commonly show sign of feeding by the specialist Kawakawa Looper caterpillar (*Cleora scriptaria*). Other animals have been known to eat kawakawa including as the Brownheaded

leafroller moth (*Ctenopseustis obliquana;* (Spiller & Wise, 1982) and goats (M. Morgan-Richards, personal communication, June 5 2012).

The daytime refuge is one of the tree weta's primary defences against predation (Field & Glasgow, 2001; Field & Sandlant, 2001), but tree weta must leave the safety of their day time refuges to feed, and in doing so expose themselves to the risk of predation (Rufaut & Gibbs, 2003). It might be assumed that, tree weta would stay close to their refuge in order to minimise the amount of time travelling that exposes them to predation. This could be achieved by feeding on plant species close by. However, over 50% of the tree weta in this study ate plants which were at least 3.13m from their refuge hole. Generally the tree weta in this study had not eaten their refuge plant species or the first plant encountered outside their refuge. Ferns may have been avoided as they are forest floor species and the forest floor is associated with an increased predation risk (Field, & Sandlant, 2001).

The collection method, which was designed to minimise plant destruction by not removing weta from live plants where possible, resulted in a small proportion of weta coming from living trees. This in combination with the abundance of the unpalatable kawakawa at all three collection sites meant that few weta in this study could eat the first plant encountered. Further exploration into the use of immediate resources is needed to determine if tree weta do consume their refuge species on a regular basis. For example tree weta are known to eat the leaves of the tree putaputaweta (see Chapter 2) and to use empty caterpillar tunnels in the trunk as refuge holes (Cilla Wehi pers. comm.). Does the tree weta therefore bite the hand that protects them?

I have shown here that tree weta selectively eat from outside their immediate environment (5 x 5m quadrat) indicating that there is the possibility of a trade-off between predation risk and getting sufficient nutrition (Molles, 2002). If the tree weta could not get sufficient nutrition close to their refuges, then travelling outside the quadrat would be necessary. This might be especially true for juvenile weta (the majority of tree weta used in this study) as they would need higher levels of essential nutrients for growth (Bernays, et al., 1994).

Tree weta may maximise their feeding time, but minimise the time exposed to predators, by limiting the number of plant species they eat in a night. Multiple feeding events can result in a single frass (personal observation), so it was not possible in this experiment to determine the number of plant species eaten in a single feeding event (Chapter 4). But these results suggest that nutrition is a greater driving force for movement than risk of predation, at least outside the quadrat.

The availability of daytime refuges has been described as a limited resource for tree weta, with loss of a refuge resulting in a large search time for a new one, and greater exposure to predators (Field & Sandlant, 2001). This may be a reason for tree weta to not travel far from their refuges. However, in this study, over 50% of the tree weta must have travelled beyond the $25m^2$ area adjacent to their refuges. Potential weta refuge holes vary in abundance but usually there are many empty holes in a forest. In Kaikoura, kanuka forest, 62% of tree holes contained tree weta (*H. femorata*) and in Westland broadleaf forest 41.6% of tree holes contained tree weta (*H crassidens*; (Field & Sandlant, 2001), whereas only 3% of suitable holes examined on Banks Peninsula were occupied by weta (Townsend, Brown, Stringer, & Potter, 1997). This indicates that there should be an excess of holes for the tree weta to reside in.

Plant species were recorded as either present or absent in the tree weta frass, in this study because the rate and frequency of plant cuticle fragments in the frass of the weta had not been quantified against the rate at which the plant was eaten. Tree weta, like many exothermic invertebrate species exhibit temperature dependant digestion, which could also be a factor of the number of plant cuticle fragments seen in the frass (Fitzgerald & Waddington, 1979). Despite this, the presence of a plant species in the frass is clear evidence of consumption of that species, and combined with data on the local environment this allows strong inference on weta self-selection and foraging strategies.

The tree weta used in this study appear to be selective in their choice of plant species in their diet, evident by the fact that the tree weta did not eat all the plant species available to them within the $25m^2$ quadrat. Rather, weta only had 2 or 3 plant species on average represented in their frass. This evidence indicates a form of self-selection

or foraging preference to select plants on either a nutritional basis or another basis which is yet to be determined. For example, weta that ate plant species from outside their immediate refuge environment were selecting both tree lucerne and the unknown species non-randomly, clear evidence of self-selection. Tree lucerne is an introduced legume, high in nitrogen and known to be palatable to a range of vertebrates and invertebrates (Shelton, 2005; Unkovich, Pate, Lefroy, & Arthur, 2000). Fifteen other plant species were eaten when the weta moved outside their quadrat, strengthening the picture of an animal that is foraging to carefully regulate its diet. This selection informs us that tree weta make choices on what to include in their diet; which would suggest that tree weta select a diet that maximises their nutritional balance and enhances their growth and fecundity.

Chapter four: Diet self-selection in captive tree weta



Tutu leaf and a piece of stem

4.1 Introduction

Food is essentially energy and nutrients, but gaining the right nutrients is essential for ensuring that an organism reaches its full potential. If an organism fails to optimise its diet, the result could be a lowered growth rate, or death (Bernays, et al., 1992; Griffin, 2011; Joern & Behmer, 1997). Generalist herbivores can optimise their diets by switching between food sources to maximise nutritional benefits and reduce the impact of plant secondary compounds (Bernays, et al., 1992). This is called diet self-selection (Waldbauer & Friedman, 1991) and has been observed in many animal species. It has also been called diet mixing (Bernays, et al., 1994) and diet switching.

Diet self-selection has been demonstrated in a range of organisms across multiple kingdoms and phyla (Waldbauer & Friedman, 1991). This includes several orthoptera species such as the grasshoppers; *Taeniopoda eques* (Bernays, et al., 1992) and *Schistocerca americana* (Bernays, et al., 1994). *Taeniopoda eques* exhibit diet self-selection between plant species in the field. *Schistocerca americana* was demonstrated to have a faster growth rate and a lower mortality rate when on a mixed diet than when they were fed solely individual plant species. This indicates that the grasshopper species was practicing diet self-selection among the supplied plant species, which aided in nutrient regulation, allowing for the increased growth rate.

Diet self-selection may have evolved as a way to combat the effects of plant secondary compounds. These plant secondary compounds are under selection in plants by herbivores. Plant secondary compounds are involved in plant defences against herbivores; acting as repellents, feeding inhibitors, and/or containing toxins that can be specific to a plant family or species (Pichersky & Gang, 2000).

Plants produce defensive chemicals that are selected for and have evolved to produce inhibitory effects in herbivorous animal species (Pichersky & Gang, 2000). Compounds which cause inhibitory effects are secondary metabolites, but can also be the nutrients which are required by the animals (Simpson, et al., 2004). Many feeding inhibitory compounds produced by plants have yet to be studied; this is particularly true for native plants in New Zealand. However, one plant included in the present study that

has had its toxic compounds examined is tutu (*Coriaria arborea*) which produces a mammalian neurotoxin called tutin. Tutin effectively acts as a feeding inhibitor in mammals by causing convulsions (Anderson, 1968) or even death (Graham & Cartridge, 1961). However for most New Zealand native plant species there is still a lack of knowledge as to their potential inhibitory compounds and how they affect herbivores.

Plant secondary compounds can accumulate to toxic levels in herbivores. The toxic effect of these compounds can be ameliorated in a variety of ways such as switching food sources, metabolizing the secondary metabolites, and even via mutualistic relationships with micro-organisms. Generalist herbivores have, paradoxically, been described as more specialised than host-specific herbivores, as they have to deal with a large range of secondary metabolites and other plant defences (Mello & Silva-Filho, 2002).

Some generalist herbivores will add novel food items to their diet, even if they have been provided with a nutritionally sufficient food source (Bernays, et al., 1992). This behaviour has been shown to improve their performance relative to a diet consisting of any one single food type and minimises accumulation of particular toxins (Bernays, et al., 1994). For this reason generalist herbivores are expected to optimise their diet by switching between plant species to optimise their diet and meet all their nutritional needs. I have shown in chapters two and three that tree weta (*Hemideina crassidens* and *H. thoracica*) are generalist herbivores that eat the leaves of a wide range of plant species, but that the tree weta do not choose their food at random. If tree weta are presented with a range of plant species, they may choose to eat them at varying rates dependant on their nutritional or energetic needs. If the tree weta choose to eat more than one species they still may not eat all of the available plant species.

While it has been shown that tree weta are omnivorous rather than purely herbivorous, the amount of protein tree weta need in their diet has not yet been determined. While plant material makes up the bulk of the tree weta diet, it probably does not meet all their nitrogen needs, and tree weta, therefore, probably opportunistically supplement their diet with another protein source (Chapter 3; Wehi

and Hicks, 2010). Isotope data suggest that adult tree weta consume the same food sources as juveniles (Wehi & Hicks, 2010). As protein acquisition would be opportunistic, and plants make up the bulk of the diet, switching between plant species would be advantageous and is the focus of this study.

The aim of the two experiments in this study was to test whether tree weta have an upper limit on the amount of a particular plant species that they consume; either in response to secondary plant compounds or other toxic compounds present in the plant species. How many plant species do tree weta eat each night? In a second experiment tree weta are presented with a variety of food options. Do they choose to just eat one plant species or do they choose to eat a range of plant food sources? Is weta size a limitation on how much they eat or how many species they eat?

It was hypothesised that feeding tree weta on a single plants species would result in an inhibitory effect on tree weta feeding, and this would be demonstrated by a negative correlation between nights one and two, when offered the same species in successive nights. This would result if weta were gorging themselves on a novel plant species the first night it was available and then eating a lesser amount on the second night. It was hypothesised that the tree weta would then display a positive correlation between all subsequent consecutive nights (e.g. night two with three, and night three with night four etc.) that would indicate the tree weta are consuming leaves to a limited level, determined by the accumulation of toxin (the limiting factor).

The evidence from wild weta (chapter 3) suggested that tree weta select only a small number of plant species to eat each meal/night. To explore this further I used captive experiments to investigate the diet selection allowing weta, of varying size, access to five different plant species each night. Based on the natural diet I hypothesised that tree weta would not eat all species available but limit their consumption to a few plant species per night.

4.2 Methods

4.2.1 Trial 1: Feeding inhibitory effects of plants

In captive conditions (appendix A), twenty Auckland tree weta (*Hemideina thoracica*) and twenty Wellington tree weta (*Hemideina crassidens*) were given a diet of carrot for one week to allow time for any toxins ingested from their normal diet to be metabolised, sequestered or removed via frass. Each weta was then fed a single plant species, at a rate of one fresh leaf per night, for ten consecutive nights. Three plant species were fed in separate trials; mahoe (*Melicytus ramiflorus*), tutu (*Coriaria arborea*) and putaputaweta (*Carpodetus serratus*) in that order. The mahoe trial was conducted first, directly followed by the tutu trial. Only ten tree weta were used for the last trial with putaputaweta leaves, and with fresh animals. Tutu and putaputaweta were chosen for this trial due to the tree wetas preference for them. Mahoe was included because although high in both carbon and nitrogen, mahoe was not favoured by the tree weta in previous studies (Wyman, 2009), and it is possible that mahoe contains inhibitory compounds. The quantity of foliage eaten each night by the individual weta was determined by scanning leaves before and after feeding (see below). Leaf area was converted into dry weight using a previously calibrated species specific index (see chapter 2).

4.2.2 Trial 2: Multiple plant species feeding trial

Individual weta were housed in separate 42 x 42 x 26 cm Perspex tanks for two nights in complete darkness. Each weta was provided with a single fresh leaf from each of the five different plant species: putaputaweta (*Carpodetus serratus*), mahoe (*Melicytus ramiflorus*), rata (*Metrosideros robusta*), puka (*Griselinia littoralis*), and tutu (*Coriaria arborea*). The leaves were positioned in each corner, and the top centre of the base of the tank and the weta refuge was placed at the bottom centre with the weta emerging backwards into the centre of the tank (as depicted in Figure 4.1). The position of the

different plant species in the enclosure was randomised. Leaves were replaced each day. For half the tree weta, the position of the different plant species was kept the same for both nights, while for the others the position of the plant species was changed at random. A grid underneath the tank was used to ensure the standardised placement of leaves and refuges.

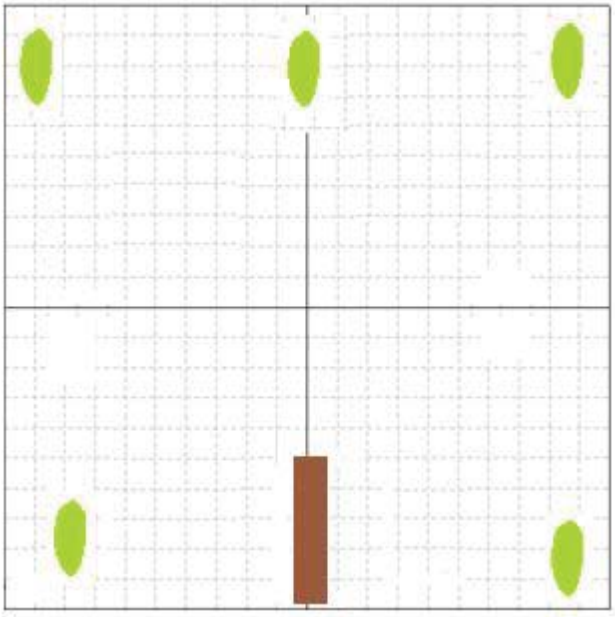


Figure 4.1. Diagram of the Perspex trial arenas, with leaves in each corner and top centre of the base of the arena, and the refuge in the middle on the bottom.

4.2.3 Analysis of eaten plant material

The amount of leaf material eaten by each tree weta was determined using the software program "Compu Eye Leaf and Symptom Area" (Bakr, 2005). This software uses scanned colour images to determine the surface area of a green image against a black or white background. Leaves were digitally scanned before and after feeding. The surface area of each leaf was determined before and after being offered to the tree weta, and the difference between these values gave an estimate of the amount eaten.

To allow comparison between plants species with differing leaf characteristics, the dry weight of the amount of plant material eaten was determined using the method described in Chapter two, expressed in grams per mm^2. In trial 1, this measure was then converted into a proportion of each weta's body weight to correct for variation in weta size. The data was analysed using a partial correlation matrix. A positive correlation suggests that if the weta ate little on day one it would also eat little on day two, or that if the weta ate a large amount on day one it would also eat a large amount on day two. A negative correlation suggests for example, that if a weta ate lots on day one, it would eat little on day two or vice versa. The null hypothesis for a correlation is that there is no relationship between events. It is the significance of that relationship which is important; this is measure by the correlation's p-value and the strength of the correlation.

In trial 2, a linear logistic model was used to test the effects of leaf repositioning, the night, and the plant species. A chi-squared analysis was used to test the frequencies at which different plant species were eaten with the null hypothesis that there was no difference between the frequencies of plant species eaten among nights. The amount of plant material eaten over the two nights and the number of different plant species were both compared to weta mass and a regression was performed for each in Minitab(State College, 2010).

4.3 Results

4.3.1 Trial 1: Feeding inhibitory effects of plants

The amount of leaf material of the three plant species eaten by each tree weta differed among days and plant species. Tutu appeared to be an attractive food on the first night, as a large volume was consumed by most weta ($521mm^2 \pm 226\ mm^2$ on average per weta per day). A clear decline in the amount of tutu eaten by the tree weta was observed from night one to all subsequent nights of the experiment (Figure 4.2). There was a moderately positive significant correlation between feeding events

on night one and night two. Night two and night three had a significant moderately positive correlation, as did nights three and four and nights nine and ten.

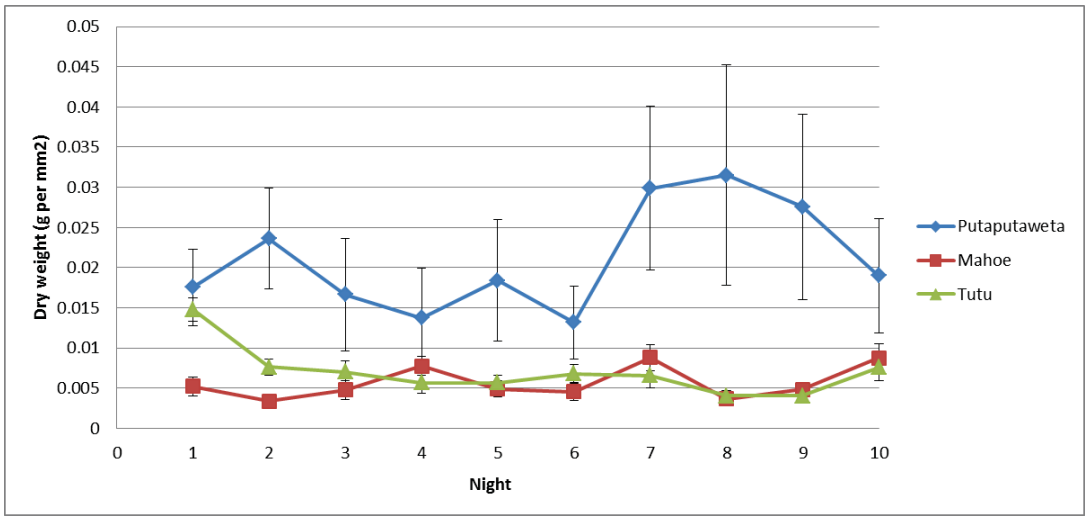


Figure 4.2 Average amount of leaf material eaten, as a dry weight proportion of the weta body weights, for each of the three different plant species. This covers ten consecutive nights by the tree weta in the trial, with standard error bars.

Mahoe consumption displayed a pattern with multiple peaks and dips, which is cyclic, in 3 day cycles. The correlation between night one and night two was positive but not significant. Nights eight and nine had the only chronological significant correlation for mahoe and it was moderately positive. Overall, the amount of putaputaweta leaves eaten was higher than the other two plant species. However, putaputaweta consumption had larger standard errors (due to fewer weta in the experiment), with a peak towards the latter part of the experiment (see Figure 4.2). Putaputaweta exhibited a significant strong positive correlation, between nights one and two and also between nights six and seven (Figure 4.3).

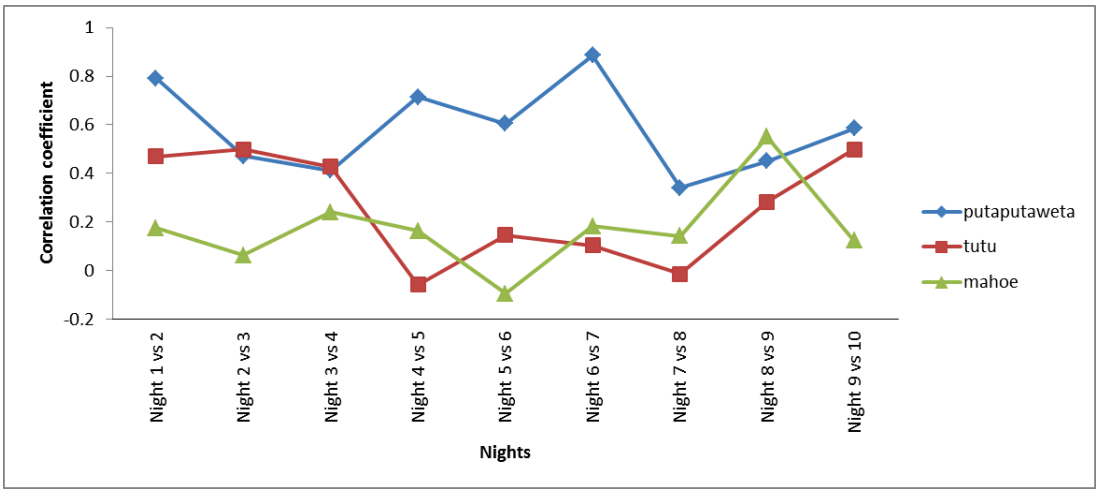


Figure 4.3 Correlation coefficient between consecutive nights for the feeding inhibition trial, for the three plant species.

Table 4.1 Mahoe partial correlation matrix, with correlation values (lower left) and p-values (top right). Significant p-values are in bold (d.f.7).

	Night 1	Night 2	Night 3	Night 4	Night 5	Night 6	Night 7	Night 8	Night 9	Night 10
Night 1		0.3088	**<0.0001**	0.4269	**0.024**	0.0611	**0.0072**	**0.0493**	**0.004**	0.9791
Night 2	0.1745		0.7156	**0.0279**	0.5032	0.5335	0.2876	0.9304	0.2507	0.1514
Night 3	0.6468	0.0629		0.1592	**0.0218**	0.109	**0.0279**	**0.0226**	**0.0128**	0.8841
Night 4	0.1366	0.3666	0.2397		0.3433	0.2617	0.0986	**0.0284**	0.1195	0.8363
Night 5	0.3754	-0.1153	0.3811	0.1626		0.5828	0.2432	0.1316	0.0987	0.9152
Night 6	0.3153	0.1073	0.2717	0.1921	-0.0947		0.2856	0.1868	0.1823	0.6402
Night 7	0.4406	0.1822	0.3666	0.2796	0.1996	0.1829		0.4073	0.0891	0.1275
Night 8	0.3301	-0.0151	0.487	0.3655	0.2561	0.2251	0.1424		**0.0005**	0.3693
Night 9	0.4677	-0.1965	0.4111	0.2642	0.2795	0.2274	0.2875	0.5515		0.4761
Night 10	0.0045	-0.2441	0.0252	0.0357	0.0184	-0.8062	0.2588	0.1542	0.1226	

Table 4.2 Tutu partial correlation matrix, the correlation values (lower left) and p-values (top right). Significant p-values are in bold (d.f.7).

	Night 1	Night 2	Night 3	Night 4	Night 5	Night 6	Night 7	Night 8	Night 9	Night 10
Night 1		**0.0038**	**0.001**	**0.0071**	**0.005**	**0.0168**	**0.0125**	0.547	0.0565	**0.0168**
Night 2	0.4706		**0.002**	**0.0032**	0.2146	**0.008**	0.3147	0.2032	0.1805	**0.0046**
Night 3	0.5251	0.4988		**0.0095**	**0.0016**	**0.0039**	0.0617	**0.0173**	**0.0013**	**0.0129**
Night 4	0.4413	0.4782	0.4265		0.7339	**<0.0001**	0.0946	0.2407	**0.0012**	**<0.0001**
Night 5	0.458	0.2119	0.5062	-0.0587		0.3949	**0.0017**	0.1165	0.2128	0.4711
Night 6	0.3958	0.4348	0.469	0.7176	0.1462		0.0546	0.0587	**0.0018**	0.0003
Night 7	0.412	0.1724	0.3145	0.2829	0.5051	0.104		0.9388	0.4925	**0.0011**
Night 8	0.1038	0.2172	0.3943	0.2006	0.2663	0.318	-0.0133		0.9041	0.2601
Night 9	0.3207	0.2283	0.5159	0.5181	0.2128	0.5012	0.1182	0.2832		**0.002**
Night 10	0.3959	0.4615	0.4106	0.7973	0.124	0.565	0.523	0.1927	0.4982	

Table 4.3 Putaputaweta partial correlation matrix, with correlation values (lower left) and p-values (top right). Significant p-values are in bold (d.f.7).

	Night 1	Night 2	Night 3	Night 4	Night 5	Night 6	Night 7	Night 8	Night 9	Night 10
Night 1		**0.0193**	0.1341	0.3444	**0.0137**	0.4189	0.564	0.1431	0.2656	0.7443
Night 2	0.7912		0.2385	0.6107	0.2726	0.4095	0.591	0.1586	0.3115	0.8415
Night 3	0.5772	0.4712		0.3116	0.1776	0.1988	0.5538	**0.0013**	0.4603	0.2841
Night 4	0.3863	0.2141	0.4111		0.0464	**0.0009**	**0.0122**	0.1434	**0.0067**	**0.0329**
Night 5	0.815	0.4422	0.529	0.7145		0.1126	0.178	0.1595	0.987	0.2058
Night 6	0.3339	0.34	0.5079	0.9287	0.6043		**0.0034**	0.656	**0.003**	0.123
Night 7	0.2418	0.2257	0.2479	0.8222	0.5286	0.8862		0.4085	**0.0003**	0.125
Night 8	0.5566	0.5492	0.9179	0.5663	0.5481	0.6762	0.3409		0.2626	0.0707
Night 9	0.448	0.4112	0.3065	0.8559	0.6233	0.8906	0.9496	0.4505		0.1276
Night 10	0.1381	0.0849	0.4328	0.7478	0.5012	0.8218	0.5883	0.6672	0.5851	

4.3.2 Multiple plant species feeding trial

When tree weta were given a choice of leaves from five different plant species to eat, their feeding was not influenced by leaf location, the night on which the leaf was fed nor the plant species. Thus, all of the five plant species were eaten, without any strong preference shown for any one type in particular. A comparison of trial night one and trial night two for the number of plant species eaten using chi-squared tests also showed no significant difference (Table 4.4). Two or three were the most common number of plant species eaten per night occurring 30 and 22, times respectively. The frequency at which different numbers of plant species were eaten was not random as there were significant differences between the number of tree weta that ate two, four and five plant species. The tree weta never ate all five plant species on one night. When data from the two nights were combined, four tree weta had eaten all five plant species, and these tree weta had eaten a greater than average amount of leaf material. The average for the individual night was 2.075 plant species per night, while the average for the combined nights was 3.025 plant species per night. So over two nights the tree weta ate on average more plant species than they did on just one night.

Table 4.4 The results from the linear logistic model comparing leaf place, night and plant species with the amount eaten by the tree weta.

	d.f.	Wald chi-squared value	*p*-value
location of leaves	1	0.3706	0.5427
Plant Species	4	6.1420	0.1888
Night	1	0.2790	0.5974

The consumption of leaf material was not dependant on body mass of the tree weta. Small tree weta ate slightly less than medium or large sized tree weta but this was not significant (df= 1, F-stat, 1.95, p-value 0.17). The total number of different plant species eaten by the tree weta over the two nights did not depend on weight (df= 1, F-stat, 0.39, p-value 0.54), Although there was a slight decrease in the number of plant species eaten by larger tree weta..

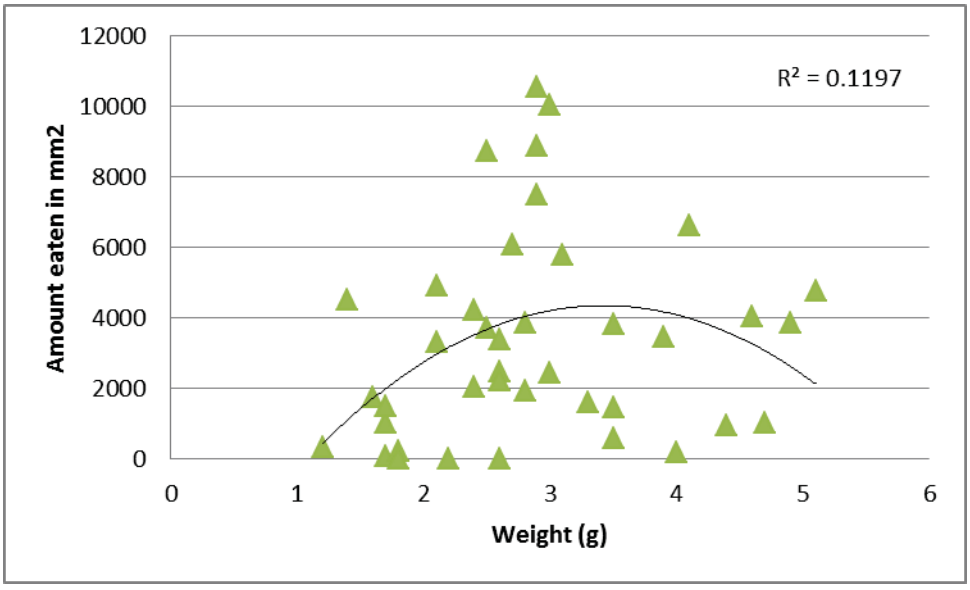


Figure 4.4 Total amount of plant material eaten compared to the tree weta's body weight.

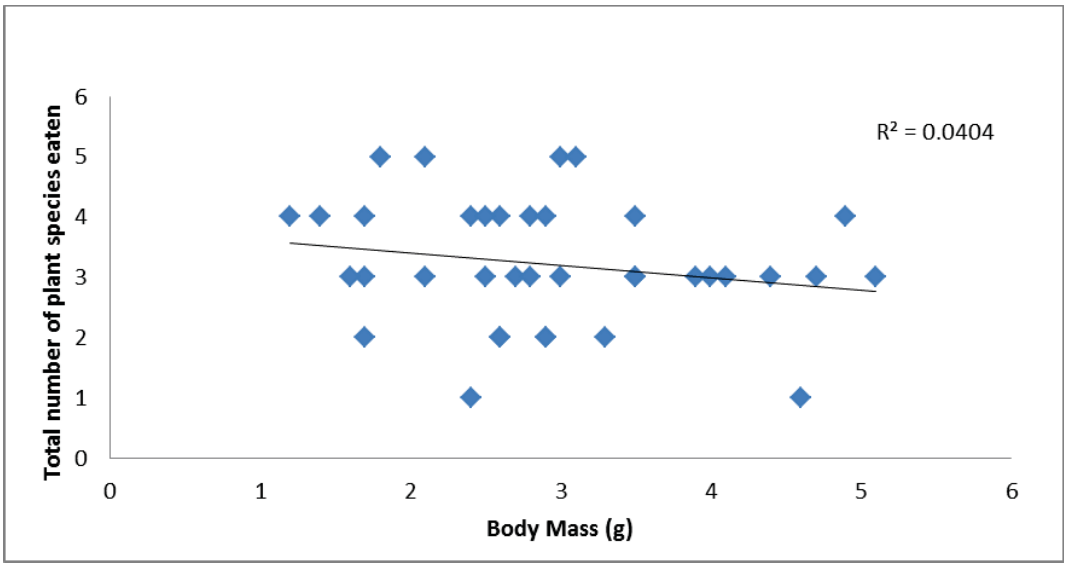


Figure 4.5 Total number of different plant species eaten compared to the tree weta's body weight.

Table 4.5 The number of plant species eaten per night by the tree weta, their relative frequencies, and expected values if the tree weta were eating a random number of plants. The chi-squared values and the p-values for the number of plants consumed are also shown, with significant p-values in bold.

Number of plant species eaten	Frequencies of plant species being eaten	Expected	Chi-squared value	*p*-value
0	12	13	0.0829	0.7733
1	8	13	1.5367	0.2151
2	30	13	8.7952	**0.003**
3	22	13	2.7306	0.0984
4	8	13	1.5367	0.2151
5	0	13	14.5415	**0.0001**

Table 4.6 The values for the individual night and the combined nights, for the number of different plant species eaten by the tree weta. Significant p-values are shown in bold.

Number of plant species eaten (d.f. 1)	Individual nights	Combined nights	chi-squared value	*p*-value
0	12	2	2.5876	0.1077
1	18	2	0.8727	0.3502
2	30	7	5.0016	**0.0253**
3	22	15	1.2504	0.2635
4	8	10	4.706	**0.0301**
5	0	4	8.2759	**0.0040**

4.4 Discussion

The results of the feeding inhibition trials showed that mahoe and putaputaweta did not appear to have an inhibitory effect on the average feeding rate of the weta, but that tutu did. This is evident by the sudden drop, and then levelling out, in the volume of leaf material consumed for this species (Figure 4.2). This pattern was not seen in the amount eaten for the other two plant species. However, this pattern may not be significant as the correlation between night one and night two was not negative but a positive correlation suggesting that individual weta that ate a great deal of tutu on the first night also ate more than average on the second night, even if the average was low. However, both tutu and mahoe were eaten at a lower rate than putaputaweta on 8 of the 10 nights, suggesting that both tutu and mahoe might be limiting tree weta consumption with chemicals in their leaves.

Plants produce chemicals that have evolved to produce inhibitory effects in herbivorous animal species (Pichersky & Gang, 2000). Tutu (*Coriaria arborea*) produces a mammalian neurotoxin called tutin, which effectively acts as a feeding inhibitor in mammals by causing convulsions (Anderson, 1968) or even death (Graham & Cartridge, 1961). The results of the present study suggest that tutu induces some sort of feeding inhibitory effect in tree weta, although at the level of individual weta it is subtle. However, herbivorous insects are often nitrogen-limited in their diet (Mattson, 1980; Slansky Jr & Feeny, 1977; Wehi & Hicks, 2010), therefore eating tutu may be nutritionally advantageous to the tree weta, as tutu had the highest nitrogen content, at 2.31% of those plant species assayed (see Chapter two).

It is advantageous for a plant species to minimise the amount of feeding on its foliage, as every feeding event destroys part of the plant, and creates open wounds which may allow pathogens or diseases to enter (Edwards & Wratten, 1983). As tutu has been shown to be a particular favourite of the two tree weta species used in this trial, some sort of defence by tutu would be an adaptive advantage. This is particularly likely, as tutu has a preference for 'edge' habitats (Clarkson & Clarkson, 1995), and edges

(Murcia, 1995), which are often trimmed and result in holes in woody vegetation which are suitable for weta, appear to also provide a good habitat for tree weta. This, combined with the fact that broken tutu stems provide day time refuges, brings the tutu and the tree weta into close proximity, increasing the regularity of interaction.

Animals can break down inhibitory compounds that plants produce, but may do so at different rates (Bernays, 1991). This rate may depend on a variety of factors, including chemical makeup of the inhibitory chemical, and temperature controlled metabolism in insects (Schmidt-Nielsen, 1997). The mahoe eating pattern, characterised by an alternation of larger and smaller feeding events (see Figure 4.2) suggests that the plants inhibitory effect may be ameliorated by the tree weta, but that this process occurs at variable rates. The tree weta in this study were kept at 14°C - 16°C, which was a temperature deemed to be suitable for maintaining healthy animals. Since the temperature remained relatively constant it is unlikely to be the cause of this pattern. It is possible, however, that the chemical makeup of the mahoe's secondary compound is itself affecting the feeding pattern.

All five species in the multiple species feeding were suitable sources of food, but interestingly no tree weta tried eating all five species in one night. There was no significant variation among nights, plant species eaten or leaf positions. When the frequency of the number of plant species eaten by the tree weta on night one was tested against night two, no significant difference was found. However, the frequency of the number of plant species eaten on a single night did differ significantly, indicating that the number of species eaten by each weta was not random. Further, the weta expanded their diet over time, with the number of plant species eaten increasing from 2.075 on the first night to 3.025 over both nights. The feeding rate did not differ depending on the size of the tree weta (weight in grams).

The results from the multiple species feeding trial suggest that location of a particular plant species in the arena was not important, and neither was the fact that half of the tree weta had their leaf positions switched between nights. The number of plants the tree weta fed on was not random, with few weta eating more than two plant species per night. This result agrees with the findings from wild weta diet studies of frass

(chapter 3) where the average number of plant cuticles per frass was 2.65 although far more plant species were in the immediate environment of the tree weta in the wild. The experiment confirms that tree weta are selective in their feeding and eat a minority of the species available. This may be explained by the fact that weta are a slow growing, slow changing insect, living for up to three years, and spending eighteen months of that as juveniles (Gibbs, 2009).

The plant species the tree weta were presented with may have met the tree weta nutritional requirements by eating just two of the five available plant species. However, if this experiment was run for a longer period of time, it may have found that weta change their food sources more extensively. So it may be that while weta are generalist herbivores, they do not necessarily need to eat a particularly large selection of food in any one, or even two nights, to meet their dietary needs, making the tree weta facultatively polyphagous and not obligate polyphagous. Rather, their nutritional needs may change at a slower rate or be sufficiently sated by eating a smaller range of plant species in a single feeding event (one night), but eating a larger range over a longer time period and this should be investigated in the future.

All the tree weta used in this study were collected from the wild, this means that any feeding experiences prior to capture are unknown, so any learnt aversion cannot be known. Herbivorous insects, like many other animals, such as blue jays (Coppinger, 1969), goats (Ginane, Duncan, Young, Elston, & Gordon, 2005) and the grasshopper *Schistocerca americana* (Lee & Bernays, 1990), may have the capacity to learn from pre-ingestion or post-ingestion cues (Ginane, et al., 2005). It may be that the post-ingestion consequences, prior to capture, which the tree weta may have suffered from eating a plant species has resulted in a lower feeding rate in these experiments.

Both experiments were conducted in complete darkness. This means that any visual cues that the tree weta could have received about the plant species were eliminated. This was done to eliminate a confounding variable, light level, as there is debate as to how much colour a weta can see (Burns, 2006; Fadzly & Burns, 2010; Morgan-Richards, Trewick, & Dunavan, 2008). The colour of a leaf may give clues to its general nutritional quality (Schaefer & Wilkinson, 2004), and without these weta may choose

food with a lower nutritional value, or food that is not necessarily the best option. Further tests on how light levels affect food choice in tree weta would be needed to determine if light plays any role in weta food preference.

Tree weta may be constrained by the amount an individual is able to consume in a single feeding event; these constraints may be the amount of food the gut is able to hold and digestive constraints (Hainsworth, 1978). Organisms may have different nutritional requirements at different life stages (Raubenheimer & Simpson, 1993),and this might be what we are seeing here with the weta being at varying instars. Tree weta in this study exhibited a pattern that indicated that the size of the tree weta was not significant when related to the amount the tree weta ate. However, small weta did consume less than medium or large tree weta. The number of different plant species eaten by the tree weta was also not dependent on the size of the weta. This would indicate that tree weta are not limited by their size as to how much they eat or the number of plant species they ate. This supports the finding of Wehi and Hicks (2010) which determined that juvenile tree weta ate the same food sources as adults.

It appears that tutu has an inhibitory effect on tree weta feeding rates, while any effects of mahoe and putaputaweta need further investigation. This study suggests that the optimum number of plant species a weta consumes in a night is between two and three. This confirms the results from cuticle studies of frass from wild tree weta, which indicate that weta are selecting their diets from a range of possible food sources. It appears that tree weta can gain sufficient nutrients in a single night from eating only a few plants species. However, the choice of plant species may change over a longer time period, which is demonstrated by the fact that the weta ate, on average, more species over two nights than they did on any one night. It is likely that tree weta are selecting food and limiting the volume they eat based on prior exposure to both required nutrients and plant secondary compounds.

Chapter five: General Conclusions



Tree weta in a flax roost

5.1 Introduction

Basic ecological knowledge of many species is lacking and knowledge of diets is no exception. Optimal foraging theory predicts that an individual should chose an optimum diet based on energy input vs. output and predation risk, but there are many exceptions (Pyke, 1984). A generalist herbivore faces many challenges when selecting its diet, including selecting optimal nutrients, while avoiding plant inhibitory compounds (House, 1969). Although, many organisms have been described as nitrogen limited in their diet (Joern & Behmer, 1997, 1998; White, 1978), the driving force of the diet of many organisms is still unclear, due to lack of knowledge. Previous studies on the diet of tree weta have looked at protein and carbohydrate consumption, protein related growth rates (Griffin, 2011), preference (Wyman, 2009), and tree weta as seed dispersers (Wyman *et al*, 2011, King *et al*, 2011). Improving the knowledge on a well-known endemic insect such as the tree weta is important for understanding of the ecology of the species. The two species of tree weta studied here are both common species (*Hemideina crassidens* and *H. thoracica*) and both tree weta are found in native and modified urban environments. Tree weta likely play an important role as herbivores in the New Zealand forest ecosystem, by placing varying levels of selection pressure on selected plant species. By gaining an understanding the feeding preferences and feeding behaviour of these tree weta species, we were able to reduce the data deficiency regarding tree weta diets.

5.2 Results

Chapter Two: Do tree weta prefer to eat leaves with high nitrogen content?

Tree weta in this study exhibited a clear diet preference, however this did not match either of the hypothesised preference ranking which were based on carbon or nitrogen content of the eight plant species. Nitrogen appeared to be a factor in tree weta diet choice but not the only factor. Tutu was the favoured plant, and had the highest nitrogen content. Mahoe was of interest, as it was high in both carbon and nitrogen content, but failed to be ranked highly by the tree weta. Further research into

mahoe's inhibitory compounds, could elucidate why the tree weta did not have a preference for this species.

Chapter Three: Tree weta diet in the wild.

Tree weta in the wild ate a range of species and did not limit themselves to species which were close to their daytime refuges. However, when studying tree weta frass, only a low average number of plant species were present and tree weta did not eat every plant species available to them. This indicated that tree weta were selective about the plant species they ate. Of note was Lucerne, which was found present in many frass and is known to have a high nitrogen content. Mahoe although not always eaten when present in the environment, was still eaten at a relatively high rate, so although it was not ranked highly in the tree weta preference ranking (Chapter 2) it still may contain an essential nutrient that the tree weta require.

Chapter Four: Diet self-selection in captive tree weta

The three plants fed in the feeding inhibition trials showed only two of them had any sort of feeding inhibition, the two species being mahoe and tutu. Putaputaweta did not appear to have this effect. The multiple species feeding trial showed that although all five plant species used were suitable sources of food, no tree weta tried eating all five species in one night. There was no significant variation among nights, plant species eaten, leaf position, or the frequency of the number of plant species eaten by the tree weta on night one against night two. However, the frequency of the number of plant species eaten on a single night did differ significantly, indicating that the number of species eaten by each weta was not random. Further, the weta expanded their diet over time, with the number of plant species eaten increasing from 2.075 on the first night to 3.025 over both nights. The feeding rate did not differ depending on the size (weight in grams) of the tree weta. This result agrees with the findings from wild weta diet studies of frass (Chapter 3) where the average number of plant cuticles per frass was 2.65 although far more plant species were in their immediate environment in the

wild. The experiment confirms that tree weta are selective in their feeding and eat a minority of the species available.

5.3 Discussion

Food Preferences

Previous studies have shown that tree weta have preferences for certain food types (Wyman, 2009; Griffin, 2011) but failed to look at precise values of nutrients in the varying natural food sources. While many studies state that nitrogen should be the driving force behind diet selection in tree weta (Griffin, 2011; Wehi & Hicks, 2010), the tree weta in this study did not conform entirely to the preference ranking predicted based on either the nitrogen or carbon content of the leaves for the eight plant species tested. The tree weta in this study exhibited a clear preference within the plant species used in this study with tutu being the preferred plant species of tree weta in this trial, and tutu was also the plant species with the highest nitrogen content.

Diet Preference Related to Nitrogen and Carbon Content

Nitrogen isoften considered the limiting factor in the diet of many organisms (Joern & Behmer, 1997, 1998; White, 1978). Nitrogen is an abundant element on Earth, but it is often in a form that is inaccessible (White, 1978). Nitrogen is at higher levels, and is more accessible, in amino acids and proteins than in carbohydrates (Campbell & Reece, 2005). Carbohydrates however, contain large amounts of carbon. Carbon is also essential for life and forms an essential part of the diet of organisms.

Tree weta are slow growing, long lived species, which are nocturnal and are opportunistic herbivores, feeding on leaves which are low in nitrogen. Selecting a diet which is high in nitrogen would be advantageous for the tree weta, especially if the tree weta are nitrogen limited in their diet. Tree weta in this study repeatedly exhibited a preference for certain plant species with high nitrogen levels (tutu and tree lucerne). Nitrogen was a factor in weta food choice but not the only factor. This was evident by the feeding inhibition trial as well as the plant preference ranking.

Interestingly, plants with the lowest nitrogen and carbon assimilation rankings were also the least preferred plants by the tree weta, with the exception of mahoe. Mahoe was high in both nitrogen and carbon, but did not rank highly with tree weta. This is likely due to some other compounds in the mahoe affecting the tree wetas ability to consume it. Wild tree weta ate tree lucerne at a higher level than was expected and this was likely due to tree lucerne's high nitrogen content which is a result of its Rhizobium fungal associations.

Other Factors Related to Feeding Preference

Although tree weta do not eat a large quantity of mahoe per night in captivity, wild weta seem to go out of their way to find and eat mahoe. Cuticle analyses of frass revealed that weta that ate plant species not within their immediate $25m^2$ environment were often eating mahoe. The overall ranking from the preference trial indicated that while both carbon and nitrogen may be important, there are other factors influencing the tree weta's feeding preferences. One of these factors could be other essential nutrients which can result in an inferior food source being eaten at higher quantities than would be expected (Pyke, 1984).

Feeding Inhibition

Plants produce chemicals which act to inhibit the feeding of herbivorous insects, these may be secondary metabolites but also can be the very nutrients the tree weta need. The tree weta exhibited a feeding inhibition effect from only one of the three plant species examined in the feeding inhibition trial, this was tutu. Mahoe also exhibited some feeding inhibition but this was on a shorter cycle (3 days). Mahoe's inhibition of feeding begins to explain why a plant species which is high in both nitrogen and carbon was relatively unfavoured by the tree weta. Tutu also exhibited inhibitory properties, and is well known for being a mammalian neurotoxin (Anderson, 1968; Graham & Cartridge, 1961), but the tree weta appear to be able consume tutu with no apparent ill effects (pers. observation). Herbivory is an arms race, and while tree weta appear to be able to eat mahoe and tutu, the plant appear to be able to limit that consumption.

Wild Weta Diets

Wild tree weta diets varied between collection sites, but not between sex or species of tree weta. Tree weta in this study failed to utilise all available plant food sources particularly ferns which were largely not eaten, and that although kawakawa was present in all sites it was never present in the frass. Kawakawa is known for containing inhibitory compounds, (Hodges et al, 1998), but the reason ferns were not favoured is unknown as tree weta would willingly eat ferns in captivity. While tree weta (*Hemideina*) have been described as being unusual in this they are mostly herbivorous in a family, Anostostomatidae, that is generally predatory or scavenging (Cary, 1983; McIntyre, 1998), 2 of the 33 frass examined in this study only had invertebrate remains present, demonstrating the tree weta's omnivory. The alpine tree weta (Hemideina maori) was also found to have insect remains in the frass (Wilson, 2004).

Number of Plant Species Eaten Per Night

The number of plant species that the tree weta ate was not random. This study shows that tree weta appear to limit the number of different plant species they eat in a single night, but eat a larger range over a long time period (multiple nights). By limiting the number of plant species the tree weta ingest in a single night but increasing that number of plant species eaten on subsequent nights, tree weta can help regulate their ingestion of nutrients and plant toxic compounds. The number of plant species found in the frass of *H. maori* was 5.2 (Wilson, 2004) and 5.6 (Lodge, 2000), this is more than the 2.65 plant species per frass found in this study. However, *H. maori* is an alpine scree weta while the two species in this study (*H. crassidens* and *H. thoracica*) are forest dwelling species. This variation in habitat may mean that *H. maori* may have different nutritional needs to the tree weta species used in this study.

Tree weta appear to increase the number of plant species they eat over multiple night. The increase of the number of plant species eaten over subsequent nights indicates that the tree weta are practising a form of diet self-selection by switching to new plant species. Diet self-selection is well demonstrated in orthoptera (Bernays, et al., 1992) and many other organisms (Berteaux, et al., 1998; Provenza & Balph, 1987; Soares, et

al., 2004) but this is the first time it has been recorded in the two tree weta species used in this study.

5.4 Conclusion

While nitrogen seems to be an important factor in weta diet, it is not the only factor affecting diet choice. Plant inhibitory compounds seem to also play an important role in the selection of food sources by tree weta. Tree weta limit the number of plant species eaten in a single night, but increase the number over subsequent nights, this behaviour may be an adaptation to plant inhibitory compounds. Future research into how light levels affect food choice and expanding on the plant preference choices to include more species would add to our knowledge of how weta interact with the natural environment.

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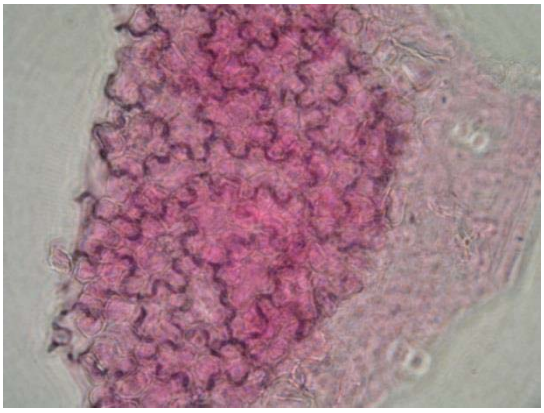
Appendix A:
General Husbandry of Tree Weta

Both species of tree weta (*Hemideina crassidens* and *H. thoracica*) were capture from three location sites described in chapter three. The method of capture was by physical daytime search of trees and dead logs for holes. Each hole was explored for the presence of tree weta by searching with a torch. If a tree weta was located in a tree a peg was used to mark the spot, and the collection site plotted with a global positioning satellite system. The tree weta was then removed by carefully breaking the branch apart. All vegetation inside the 5m by 5m quadrat around each weta collection site was recorded. If tree weta where found in large live branches or trees they were not collected as to limit the destruction of native plants specie. Most tree weta collected in this study were collected from dead branches.

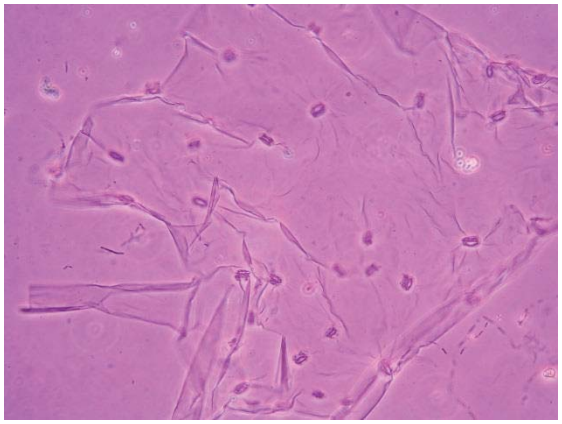
All tree weta in this study were given a unique code which indicated collect site and species of weta. Each tree weta was weighed using a digital scale and their tibia lengths were recorded. Each tree weta was house separately in a 15 x 15 x 9cm plastic container (ice cream container), with a 5 x 5cm hole in the lid, which had stainless steel insect mest (1mm aperture) glued over it. Weta were keep at 14±1°C, in a temperature controlled room, which had a 14-10 day night cycle. All enclosures had a wet paper towel and a refuge. Refuges were made from hollowed out flax (*Phormium tenax*) flower stalks. These were cut into 7cm lengths and split in half longitudinally and placed back together, secured by a rubber band. Containers could be easily accessed during the day as tree weta are nocturnal. The containers also allowed feeding manipulations to occur during the day and while the feeding trials took place at night.

Appendix B

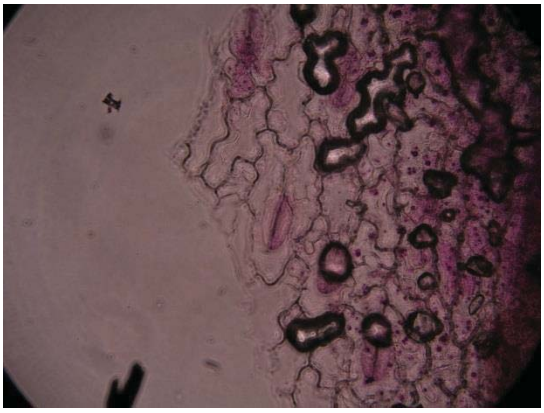
Plant cuticle reference slide collection



i) Titoki (*Alectryon excelsus*)(200x)



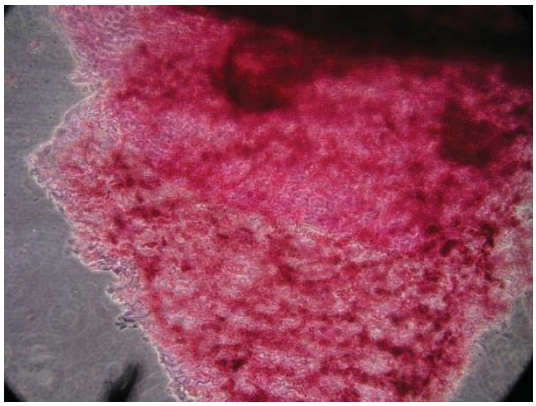
ii)Makomako (*Aristotelia serrata*) (400x)



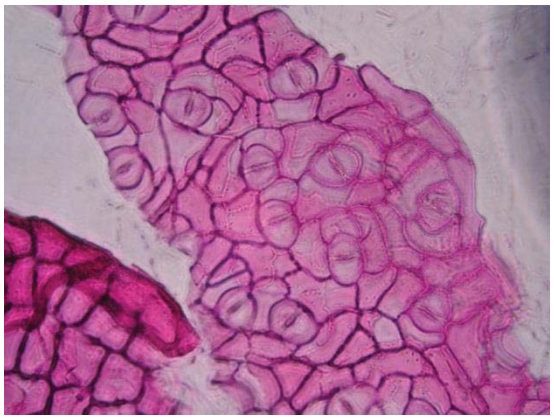
iii)Hen & Chicken fern (*Asplenium bulbiferum*)(400x)



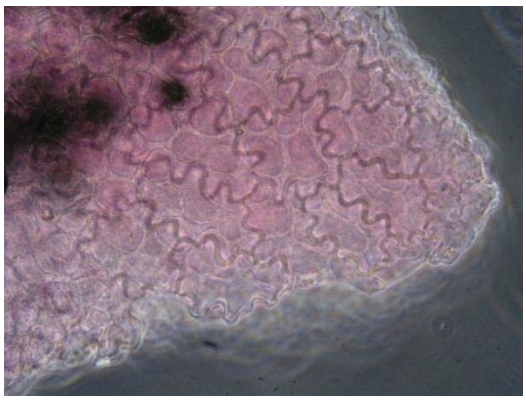
iv) Hen & Chicken fern spores capsules(100x)



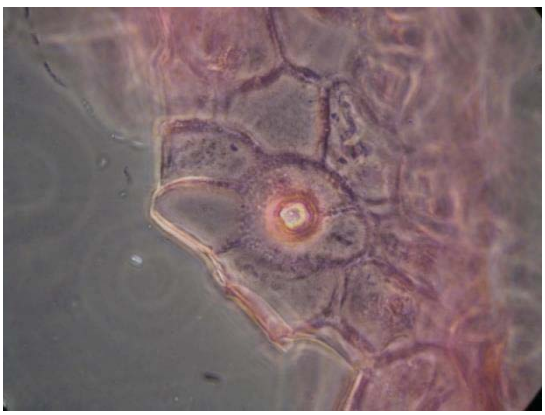
v)Shining Spleenwort (*Asplenium oblongifolium*) (200x)



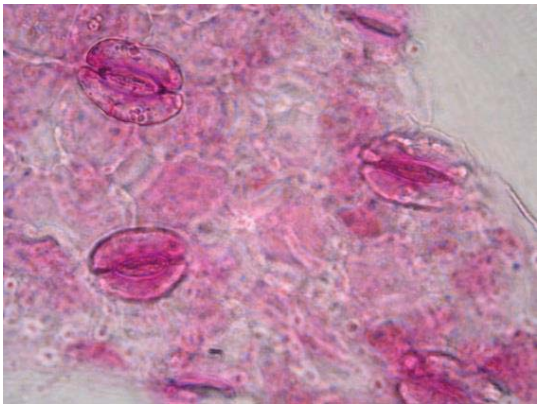
vi)Tawa (*Beilschmeida tawa*) (800x)

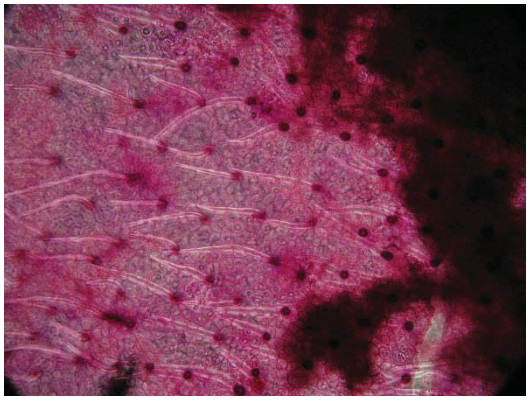


vii) kiwikiwi (*Blechnum fluviatile*) (400x)

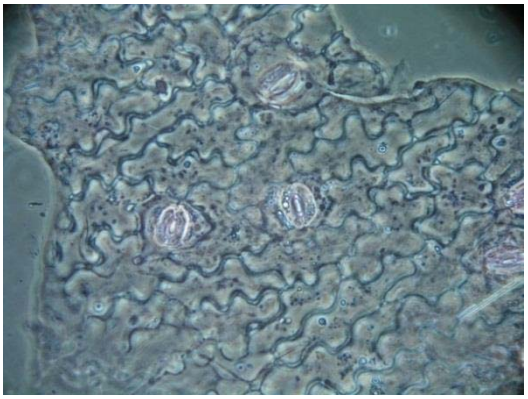


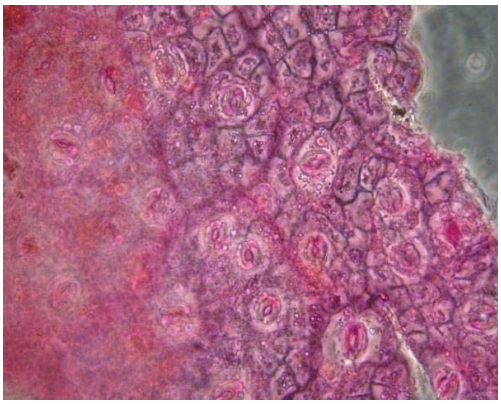
viii)Rangiora (*Brachyglottis repanda*) (400x)



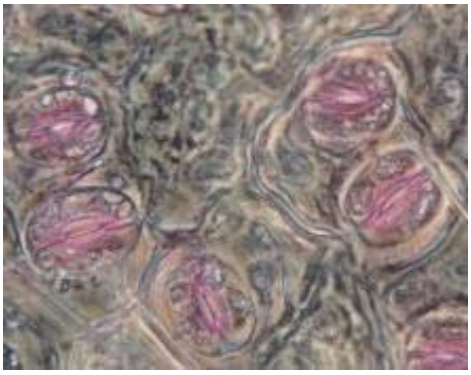


ix) Putaputaweta (*carpodetus serratus*) (400x) **x)Tree Lucerne**(*Chamaecytisus palmensis*)(200x)

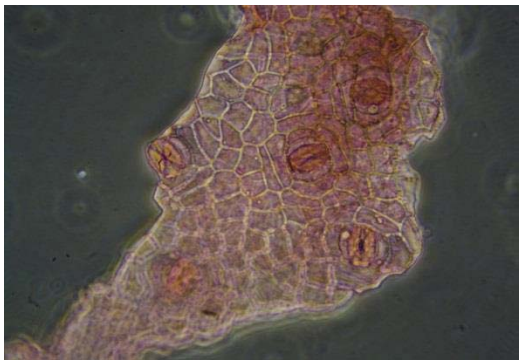




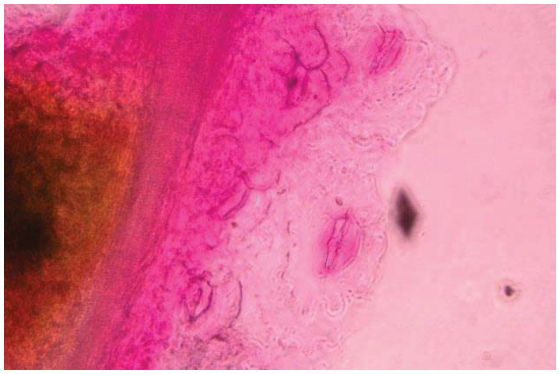
xii) kanono (*Coprosma grandifolia*) (400x)



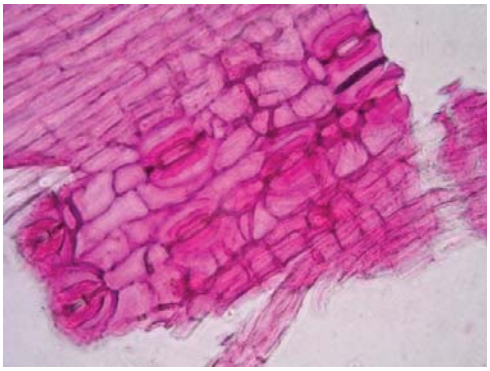
xiii) taupata *Coprosma repens* (400x)



xiv) Karamu *Coprosma robusta* (x800)

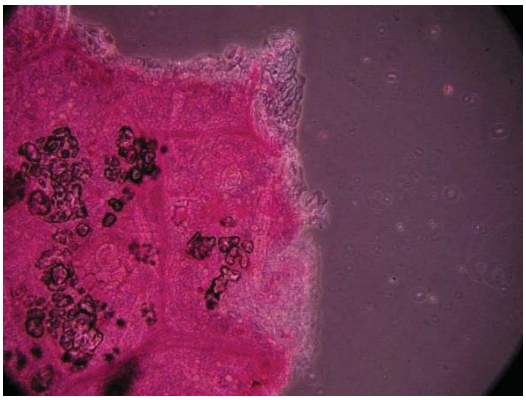


xv) Karaka (*Corynocarpus laevigatus*) (400x)



xvi) Mamaku (*Cyathea medullaris*) (400x)

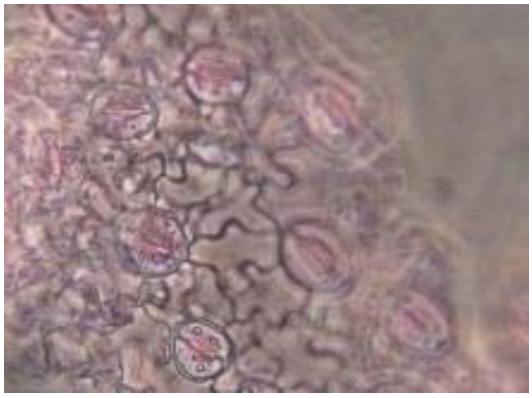
xvii) Kahikatea (*Dacrycarpus dacrydioides*) (200x)



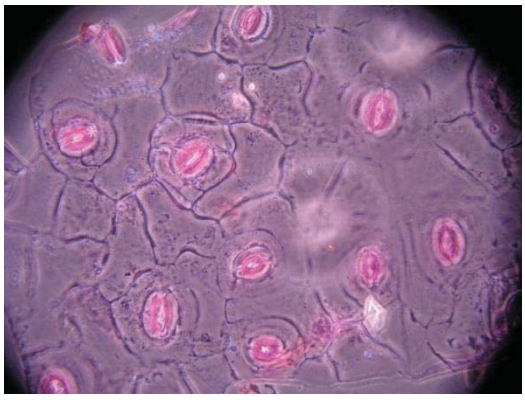
xviii)Parataniwha (*Elatostema rugosum*) (400x)



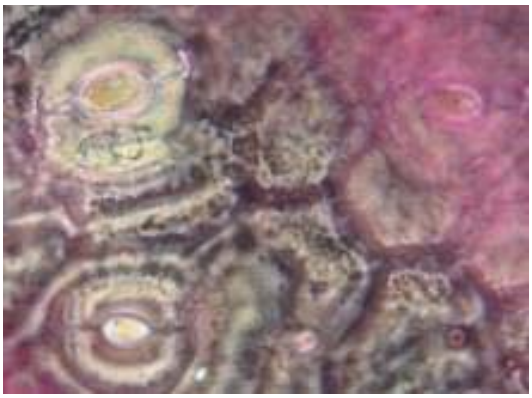
xix) Parataniwha hairs (400x)



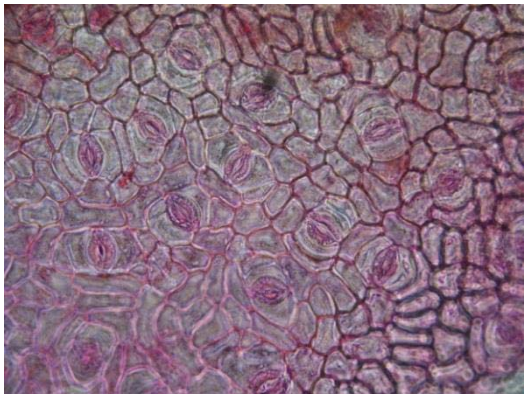
xx) *Fuchsia excorticata* (x400)



xxi) HangeHange (*Geniostoma ruprestre*) (400x)



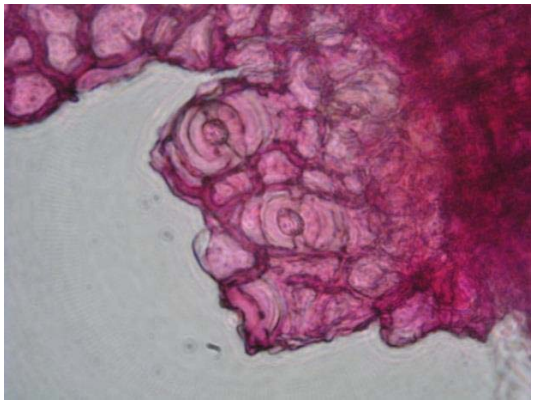
xxii) Puka *Griselinia littoralis* (x800)



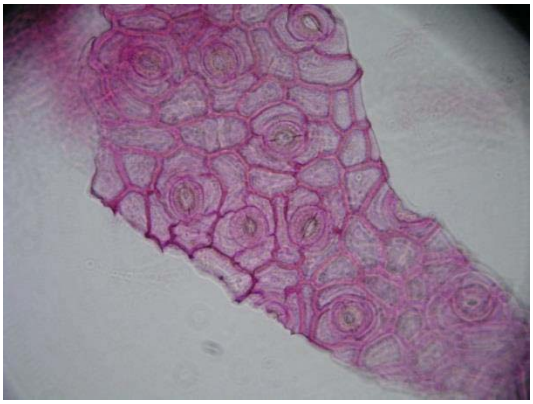
xxiii) Pigeonwood (*Hedycarya arborea*) (400x)



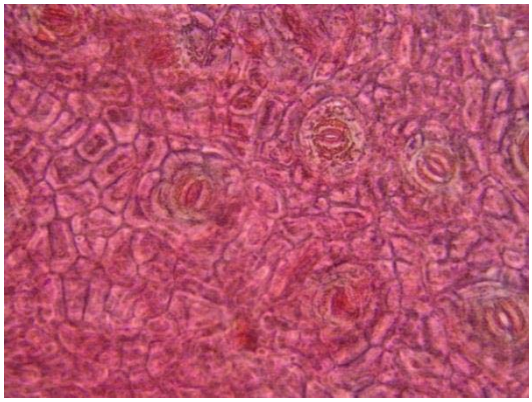
xxiv) Houhere (*Hoheria sexstylosa*) (400x)



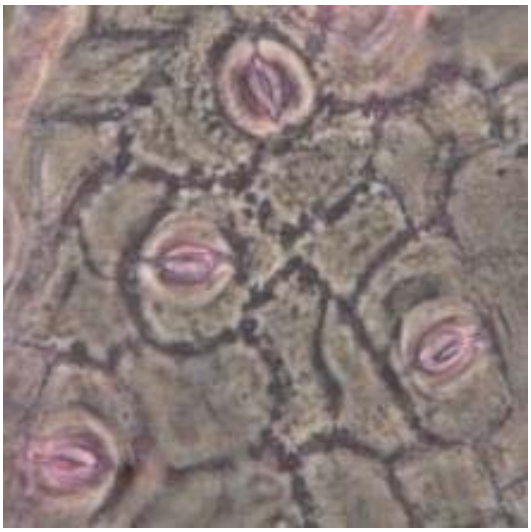
xxv) Rewarewa (*Knightia exelsa*) (800x)



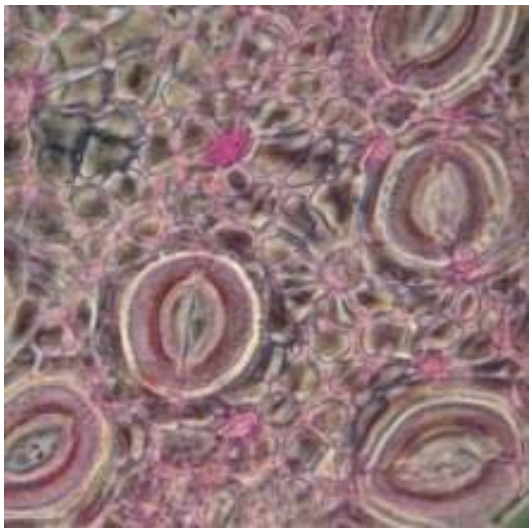
xxvi) pukatea (*Laurelia novae-zelandiae*) (200x)



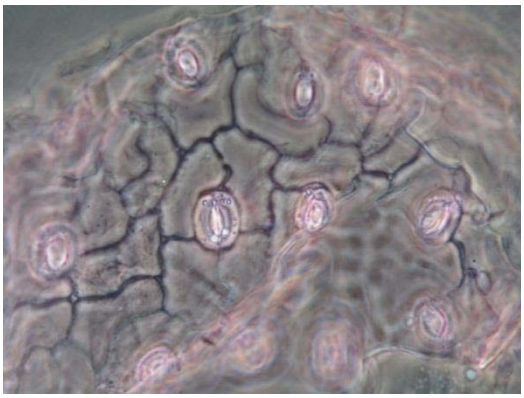
xxvi) Kawakawa (*Macropiper excelsum*) (200x)



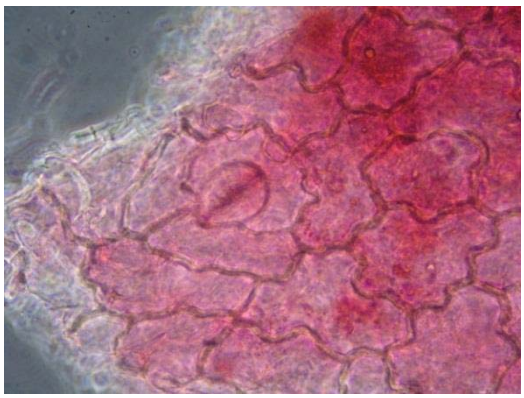
xxvii) mahoe *Melicytus ramiflorus* (x800)



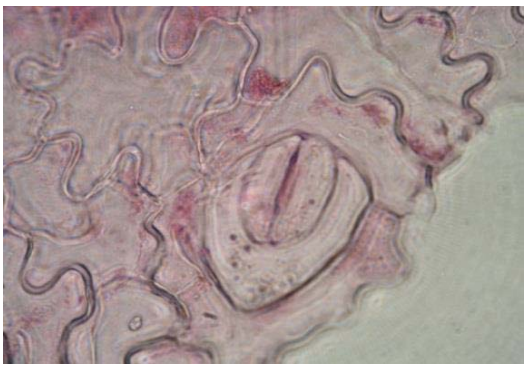
xxviii) Northern rata *Metrosideros excelsa* (x800)



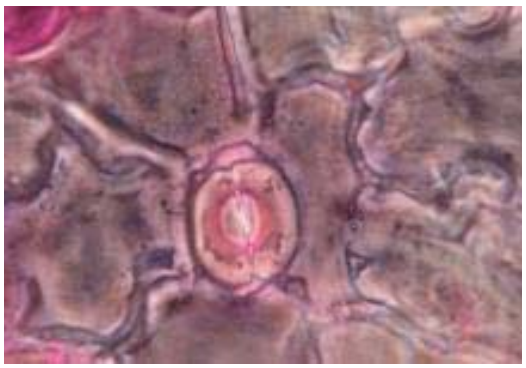
xxix) Climbing Rata(*Metrosideros perforata*) (400x)



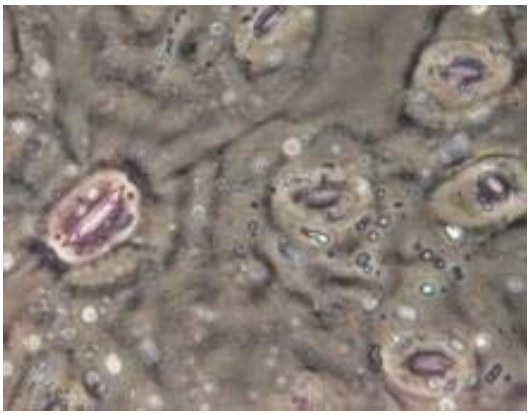
xxx)Hounds Tongue(*Microsorum pustulatum*) (400x)



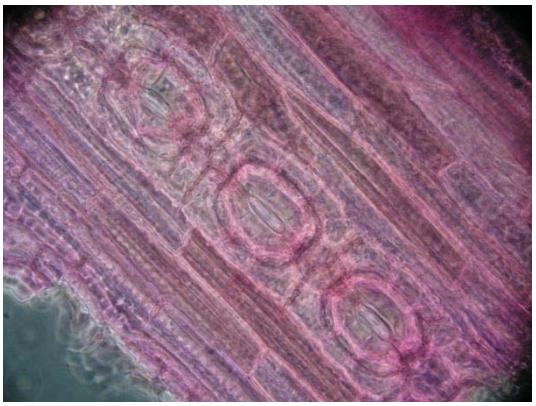
xxxi) mokimoki (*Microsorum scandens*) (400x)



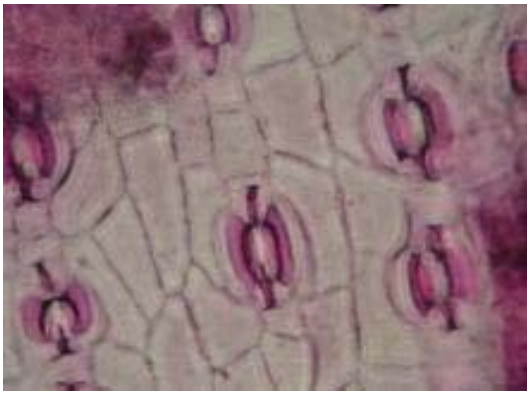
xxxii) mapou *Myrsine australis* (x800)



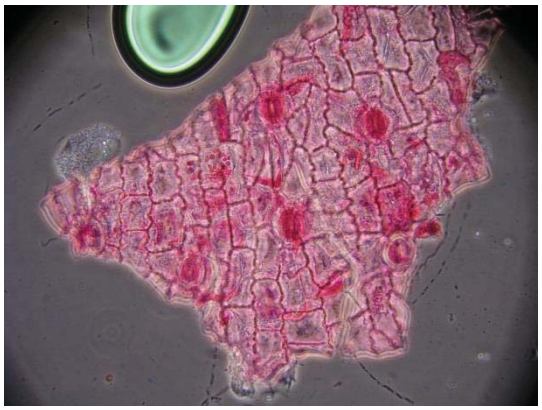
xxxiii) Lemon wood *Pittosporum eugenioides* (x800)



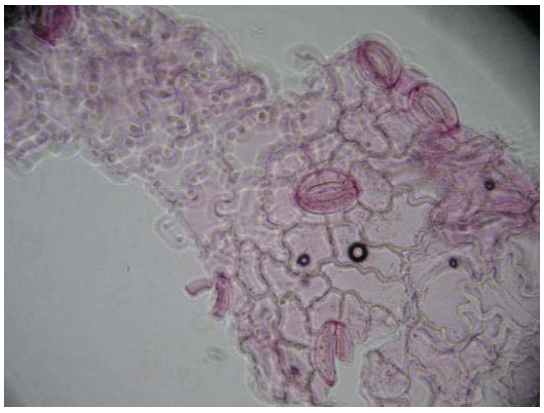
xxxiv) Radiata Pine (*Pinus radiata*) (400x)



xxxv) totara *Podocarpus totara* (x800)



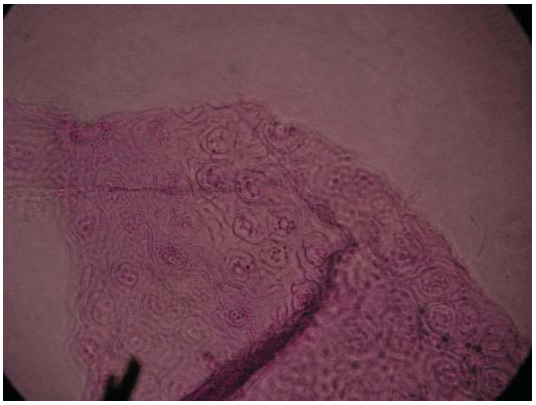
xxxvi)miro (*Prumnopitys ferruginea*) (200x)



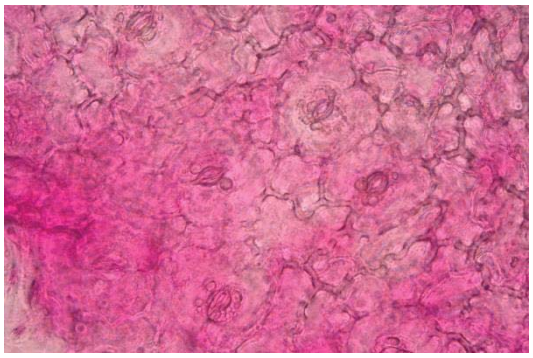
xxxvii)whauwhaupaku(*Pseudopanax arboreus*)(200x)



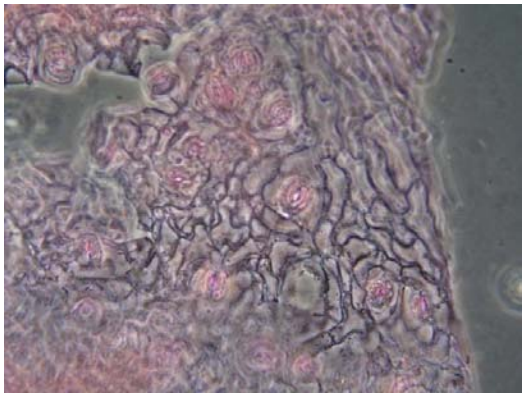
xxxviii)Lance wood(*Psuedopanax crassifolius*) (400x)



xL)*Pseudopanax laetus* (400x)



xLi) Supplejack (*Ripoganum scandens*) (400x)



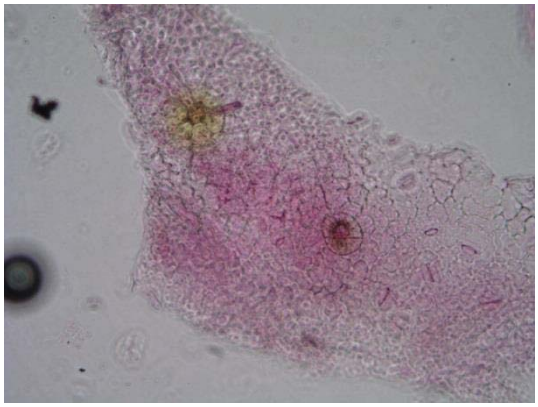
xLii) Pate (*Schefflera digitata*) (400x)



xLiii) Tradescantia (*Tradescantia fluminensis*)

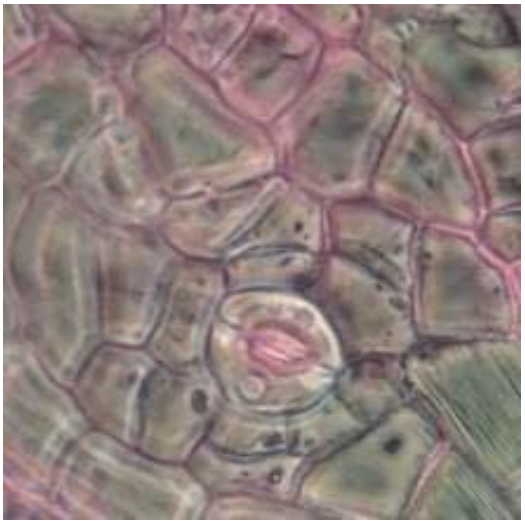
(200x)





xLiv) Gorse *Ulex europaeus* (x800)

xLv) Puriri (*Vitex lucens*) (400x)





xLvi) Kamahi *Weinmannia racemosa* (x800)

xLvii) Unknown (200x)

Appendix C

The tale of a rotting leg.



Photo courtesy of Andrew Blayney

A tree weta *H.thoracica* presented with a missing leg. This is not unusual, but over the proceeding days the stump of the leg became blackened and increasingly shorter. The same symptoms then present in a second tree weta of the same species. An infected leg was then removed from the tree weta originally presenting the symptoms. The leg was then dissected aseptically and the internal muscle, containing the bacteria was plated out on blood agar and incubated over night at 30˚C. This was subsequently plated out again onto two blood agar plate and incubated overnight at 30˚C, one was incubated anaerobically and the other aerobically. Both plates were incubated overnight. Both plates were then re-plated onto two new blood agar plates. The plate which grew aerobically failed to grow anaerobically and the plate which grew anaerobically failed to grow aerobically, indicating there were two different bacteria present. The anaerobic bacteria were gram positive and oxidase positive, but there was no future test preformed and this bacteria was not identified. The aerobic bacteria were identified as gram-negative and oxidase negative. An Api-20E test was performed on the aerobic bacteria and it was identified as *Klebsiella ozaenae*. Unfortunately due to the contagious nature of the bacteria, all tree weta identified as having symptoms were isolated and humanely euthanized to prevent the spread of the disease.