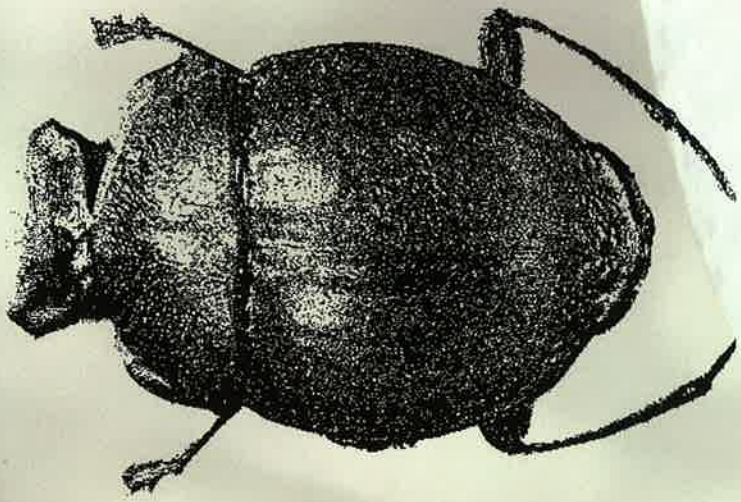
Pilot trials of secondary seed dispersal potential from tree weta frass by the endemic dung beetle, *Saphobius edwardsi* in the Punakaiki Coastal Restoration Project, New Zealand

by

Morgan Shields, Stephane Boyer & Mike Bowie



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Introduction

Restoring ecosystem functions has been recognised over the last 30 years as essential to providing a stable ecosystem that is resistant to perturbations during habitat restoration projects (Longcore 2003). This requires more than just supplying the correct vegetation diversity as ecosystem functions utilise animals to implement functions such as nutrient cycling, pollination and seed dispersal (Grant *et al.* 2007; Majer *et al.* 2007). Arthropods are the most abundant and diverse of these animals, occupying more niches than all others (Longcore 2003), provided the presence of vegetation structural diversity (Southwood *et al.* 1979). Therefore understanding the role these taxa play and implementing them as indicators of restoration success has become common (Hughes & Westoby 1992; Longcore 2003; Wall *et al.* 2005; Majer *et al.* 2007). Ants have been used as indicators of restoration success in gold mining in South America (Ribas *et al.* 2012) and as secondary seed dispersers for restoration projects such as during Australian bauxite mining restoration, (Majer & Nichols 1998; Grant *et al.* 2007; Majer *et al.* 2007). Similarly dung beetles have been used as indicators of disturbance and restoration success (Davis *et al.* 2001; Vulinec 2002; Bowie *et al.* 2012). Dung beetles also provide key ecosystem functions such as secondary seed dispersal, nutrient cycling, and improving soil fertility and porosity (Vulinec 2002; Nichols *et al.* 2008; Brown *et al.* 2010; Santos-Heredia *et al.* 2010). However in a New Zealand context, dung beetle research has focussed on ecosystem services provided by introduced species in pastures such as nutrient recycling during summer and reducing parasitic nematodes, flies and surface run-off (Dymock 1993; Fowler 2012; Forgie *et al.* 2013).

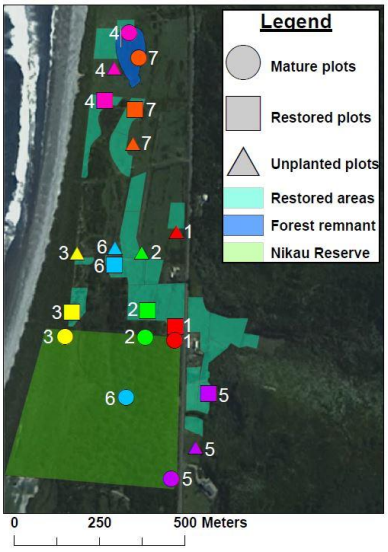
But recent studies have investigated endemic dung beetle diet and behaviour (Jones *et al.* 2012; Stavert *et al.* 2014a**;** Stavert *et al.* 2014b). New Zealand Canthonini dung beetles are unusual as they are flightless and have evolved in an environment predominantly free of large mammals (Stavert *et al.* 2014a). There are 15 endemic dung beetle species in New Zealand, 13 of which are in the *Saphobius* genus (Stavert *et al.* 2014a) with the most widespread being *Saphobius edwardsi* Sharp 1873 (*S. edwardsi*) (3.5-4 mm) which is found from Northland in the North Island to Westland in the South Island (Jones *et al.* 2012). *Saphobius edwardsi* appears to have a generalist diet utilising wetapunga (*Deinacrida heteracantha*) dung, humus, fungi and carrion from squid and invertebrates (Hodge *et al.* 2010; Seldon & Beggs 2010; Jones *et al.* 2012; Stavert *et al.* 2014a). However, *S. edwardsi* prefers avian, mammal and tuatara dung and avian carrion (Jones *et al.* 2012; Stavert *et al.* 2014a). *Saphobius edwardsi* uses an acute olfactory system to differentiate 115 compounds produced by previously stated food types to determine diet preference and the food's location (Stavert *et al.* 2014b). However there has been little research to our knowledge on quantifying ecosystem functions such as potential secondary seed dispersal of endemic dung beetles in New Zealand, especially on the

West Coast. The Punakaiki Coastal Restoration Project (PCRP) site is adjacent to the 20.2 ha Nikau Scenic Reserve which is a rare remnant of lowland coastal forest opposite Paparoa National Park within the Punakaiki Ecological District. The site was cleared and surveyed for mining, then farmed until around 1970. It is now the centre of a restoration project to restore a functioning ecosystem while promoting conservation and tourism (Bowie *et al.* 2012).

As part of the PCRP we investigated the potential of *S. edwardsi* in providing secondary seed dispersal as an ecosystem function. Our objectives were to gain some understanding on whether *S. edwardsi* utilised tree weta frass, quantifying frass utilised per night, the distance *S. edwardsi* travelled per night, seed burial depth and what seeds could be dispersed by tree weta at the site which have potential for secondary seed dispersal by *S. edwardsi*. We hypothesised that *S. edwardsi* would utilise 0.01 g of tree weta frass/night/beetle and move a maximum distance of 5 m/night (Shaun Forgie pers. comm.) while burying dung and seeds a maximum depth of 40 mm which would allow most seeds to germinate. This information would enable the estimation of the population density, recycling of organic and potential secondary seed dispersal of *S. edwardsi* at the restoration site.

Methods & Materials

Punakaiki site



The PCRP site is located at 42˚08’38.00” S, 171˚19’49.94” E and is approximately 4 km south of Punakaiki (Figure 1). The climate in the area is warm/temperate and wet, with mean annual rainfall of 2,000 mm to 4,000 mm; mean annual temperature of 10 - 12˚C; and mean annual sunshine hours of 1,600 – 1,800 (www.niwa.co.nz, 2012).

Figure 1. PCRP site with transect and plot locations. Mature plots (circles) were used in the current study. Adapted from Bowie *et al.* (2012).

Pitfall trapping

Dung beetles were caught at the PCRP site using baited pitfall traps. These were 350 ml plastic cups set in an 80 mm diameter plastic tube that had been previously used for invertebrate monitoring as part of the PCRP (Bowie *et al.* 2012). The pitfall traps were baited with fresh

cow dung, collected from a nearby farm which was tied in a mesh bag. Each mesh dung bag was then tied to the underside of the galvanised 180 mm x 180 mm steel roof of each pitfall trap so the bag was hanging over the pitfall trap. The steel roofs of each pitfall trap were raised by four wire legs which reduced rain and leaves entering the traps and weka interfering with them (Bowie *et al.* 2012). Leaves and humus were added to the plastic cup to prevent beetle desiccation (Jones *et al.* 2012). In each of the six mature forest remnant plots (M) used for this study (M1-M5, M7) there were seven pitfall traps, set 4 m apart in an approximately straight line, of which only one or two in each plot were used during this investigation at any one time. When pitfall traps were not being used, the plastic cups were removed and sticks were added to provide an escape route for any organism that happens to fall into the holes. The live baited pitfall traps were checked for beetles every day until the bait and cup were removed. Pitfall traps were used on a total of 17 trap nights during November 2014 to January 2015.

Keeping in Captivity

The beetles were transported in plastic containers with air holes, soil, leaf litter from the site and sprayed with water every two to four days. Beetles in captivity from November 2014 to February 2015 were kept in a black plastic fish bin (Figure 2) with 100 mm of reasonably undisturbed soil and leaf litter from where they were caught (M4 and M7) with green mesh for a lid to resemble forest canopy shade (Shaun Forgie pers. comm.). This was sprayed with water and had fresh cow dung added twice a week and kept in a temperature controlled (CT) room at 15 ˚C in a 16:8 light:dark cycle. Beetles were collected for experiments from this fish bin using a miniature pitfall trap with cow dung as bait.

Weta frass from *Hemideina crassidens* was collected from sieves attached under already present weta motels at the site and from five *Hemideina crassidens* collected from the site and kept in captivity in wooden weta boxes. Captive conditions were the same as for the beetles but weta were fed fresh leaves and berries from native plants such as wineberry (*Aristotelia serrata*), mahoe (*Melicytus ramiflorus*), kawakawa (*Macropiper excelsum*), *Coprosma robusta* and broadleaf (*Griselinia littoralis*). Frass was collected from captive weta regularly.

Figure 2. Method used for keeping dung beetles in captivity. Photo taken by Morgan Shields.

S. edwardsi behaviour with tree weta frass

Tree weta frass had not been used in experiments with *Saphobius* species before, therefore tree weta frass utilisation by *S. edwardsi* was tested with no choice tests to assess utilization of frass and the beetle's behaviour. Initial trials involved 20 petri dishes with wet 425 mm filter paper, one *Hemideina crassidens* frass pellet, either fresh or dry in the centre of each petri dish and five *S. edwardsi* spread evenly around the edge of the filter paper. These were caught in pitfall traps 5-7 hrs prior to the experiment and were therefore assumed to be hungry. The petri dishes were left for three days, under shade in the field site eco-lab.

Tree weta frass utilization was then tested in a more natural environment with a substrate, involving ten plastic ice cream containers containing a 40 mm layer of soil from the site mixed with sand substrate. Each container was sprayed with water and had one fresh *Hemideina crassidens* frass pellet in the centre of a 425 mm filter paper in the middle of the container with 20 *S. edwardsi* which had been starved for two days and were spread evenly around the filter paper's edge. The containers were left in the CT room as mentioned above for three nights with lids on that had a 100 mm x 100mm square cut out and replaced with mesh to allow more natural conditions to occur. Frass movement and signs of beetle activity were recorded the next morning.

Figure 3. Weta frass pellet after dung beetle utilisation. Photo taken by Morgan Shields.

Frass and dung utilisation trials

Pilot trials to investigate how much dung Punakaiki *S. edwardsi* utilised consisted of 11 plastic containers replicates with the same protocol as above except using than ten *S. edwardsi*, were placed with 1 g of fresh *H. crassidens* frass from the field per replicate. The control consisted of no beetles added to the container. This experiement required around 50 weta frass pellets. The frass was mixed randomly before use in the trial to account for moisture variation. Frass and filter paper were weighed before the beetles had been added and after one night in the CT room using a Sartorius LE225D scale. This protocol was repeated three times with fresh cow dung from the Lincoln University farm due to limited fresh *H. crassidens* frass being available. Modifications to the protocol when using cow dung involved having three controls and exposing the dung to air for one to two nights prior to the experiment to remove moisture.

Distance trials

Determining the distance individual *S. edwardsi* could move in the context of abundance and seed dispersal was attempted in the field using a mark-recapture method. This involved trialling different markers on individual *S. edwardsi*. Beetles marked with Day-glow dye were released at 1 m, 2 m, 5 m and 10 m distances at four release sites per distance in north, south, east, west directions from a baited pitfall trap using cow dung as previously mentioned. Each release site had ten marked beetles which had been starved for two days that were released with different colours for each distance. Pitfall traps were monitored daily for up to two nights with the captured beetles been removed each day. This protocol was later attempted using white marker pens with only 2 m and 5 m distances but with five replicates at mature sites (sites M1, M2, M4, M5 and M7). Measuring the distance *S. edwardsi* was also attempted in the laboratory at 0.25 m, 1 m and 1.25 m in an enclosure made from polystyrene boxes and filled with a 10 mm layer of the soil/sand substrate mix mentioned earlier. A miniature pitfall trap was installed in the arena consisting of a 50 mm diameter petri dish coated with fluon on the vertical walls to prevent beetles from escaping, and baited with cow dung (Figure 4). Beetles were starved for two days prior to the experiment and there were two replicates per distance.

Figure 4. Polystyrene enclosure to test beetle distance. Photo taken by Morgan Shields.

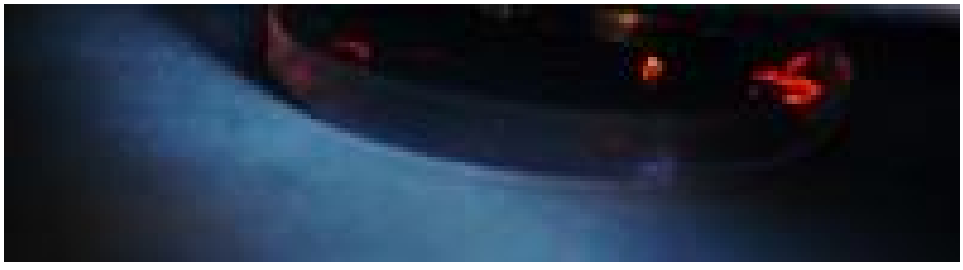


Figure 5. Testing fluorescent DayGlow dye on *S. edwardsi*. Photo taken by Morgan Shields

Burial depth of beetles & seed trials

Beetles burying activity was investigated in 250 mm high x 100 mm wide x 20 mm deep terrariums filled with 100 mm depth of the moistened soil/sand substrate mentioned above and with black plastic covering the glass sides. Ten *S. edwardsi* and one fresh *H. crassidens* frass pellet were added to each terrarium, which were left for three nights at 15 ˚C in a 16:8 light:dark cycle. This experiment was replicated four times. Beetle burrying depth was measured using a ruler and searching 5 mm depth of soil at a time. A second set of experiment miner's lettuce (*Claytonia perfoliata*) seeds (1 mm x 0.5 mm each) inserted into the fresh frass prior to the experiment to test whether *S. edwardsi* buried the seed. This trial was replicated three times and was left for approximately three weeks to observe germination and measure seed burrial depth (Figure 6).

A sample of *Saphobius* beetles caught in the field were identified based on descriptions (Shaun Forgie pers. comm.).

Seed searching & dung beetle seed dispersal

Hemideina crassidens frass collected in the field were searched under a microscope for the presence of whole seeds that had passed through the weta digestive system. Any seeds that were found were identified by seed morphology and were attempted to germinate. A list of candidates plant species present at the site that have potential to pass through the digestive tract of *Hemideina crassidens* was collated mainly based on seed size. Wineberry (*Aristotelia serrata*) berries were fed to *Hemideina crassidens* and their frass was collected and searched with the same protocol as above. Seed sizes that could pass through weta gut were then provided to *S. edwardsi* in the distance and burial depth apparatus used above to indicate potential secondary seed dispersal.

Figure 6. Seed beetle burial by measuring seed depth after germination. Photo taken by Morgan Shields.

Analysis

As most of the pilot trials used actual counts, with limited replicates and protocols did not work or had to be altered due to time and resource constraints, most results could not be statistically analysed and are shown as proportions, measurements, counts and means.

Results

Most *Saphobius* beetles that were caught were in sites M7 (48 %), M4 (23 %) and M1 (15 %) (Table 1). Sixty seven individuals were identified to species level with 11 being confirmed as *S. lesnei*, 56 confirmed as *S. edwardsi* (Figure 11). The remaining beetles (857 individuals) were assumed to be *S. edwardsi* due to their small size, however, it is possible some could be *S. wakefieldi* (Figure 11). The PCRP site is confirmed to contain two species of *Saphobius* dung beetles, *S. edwardsi* and *S. lesnei* with the possibility of a third species, *S. wakefieldi* (Figure 11). This is very similar to *S. edwardsi* but lacks a subapical sinuation along the lateral margins of its elytra and there are no bends, up-lifting or disturbances of the head plate side margins (Shaun Forgie, pers. comm.).

Initial no choice tests in petri dishes indicated that *S. edwardsi* utilised more fresh frass (70 %) of *H. crassidens* then old frass (40 %) and only burrowed into fresh frass (40 %). When on a soil/sand substrate *S. edwardsi* moved the frass 21 mm to the edge of the filter paper during the first night (10 %). Over three nights, the greatest frass movement was 50 mm with some frass pellets being removed or broken down by *S. edwardsi* (30 %). A mean weight of 0.02 g/beetle/night of weta frass was utilised and a mean weight of 0.01 g/beetle/night of cow dung was utilised (Figure 7).

Mark-recapture trials failed in the field with very few marked beetles recaptured. The laboratory distance trial indicated that *S. edwardsi* can move at least 1.25 m over two nights (Figure 8).

In terrariums, *S. edwardsi* were found at a soil depth range of 0 mm - 39 mm after three nights with beetles predominantly occurring at a depth of 10 mm - 19 mm (57 %) (Figure 9). Seeds that were implanted into weta frass were found after germination at a range of 0 mm – 14 mm with most of them at 0 mm – 9 mm (0.72) (Figure 10). No undamaged seeds were found in *H. crassidens* frass collected from the field or from *H. crassidens* in captivity.

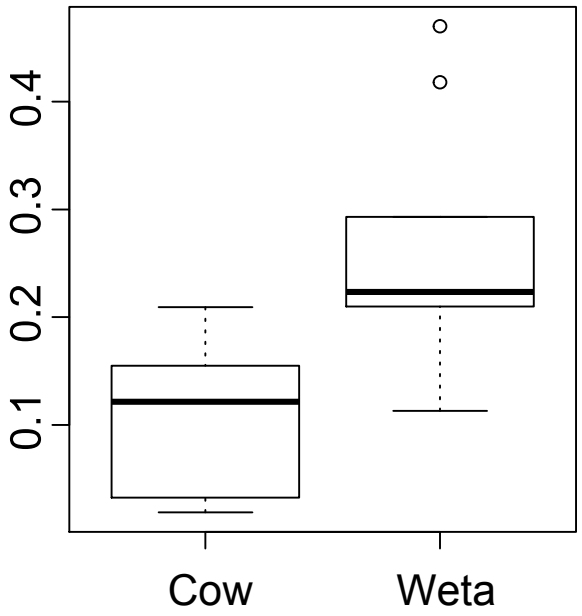


Figure 7. Boxplot showing dung utilisation after one night by ten *S. edwardsi* in grams on y-axis and dung type on x-axis. *S. edwardsi* used an average of 0.01 g/beetle/night of cow dung and 0.02 g/beetle/night of tree weta frass.

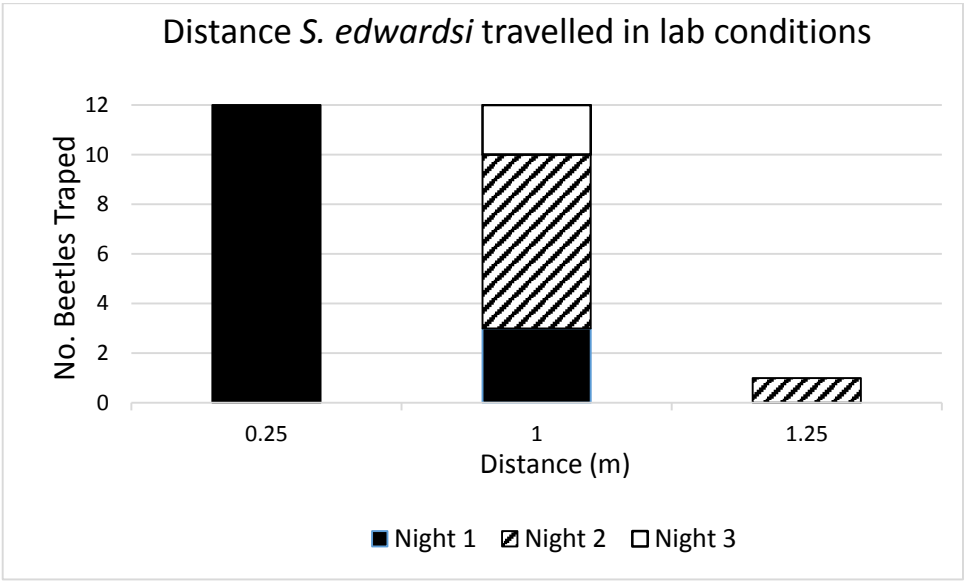


Figure 8. Distance travelled by *S. edwardsi* in polystyrene tunnels with a baited pitfall in laboratory conditions.

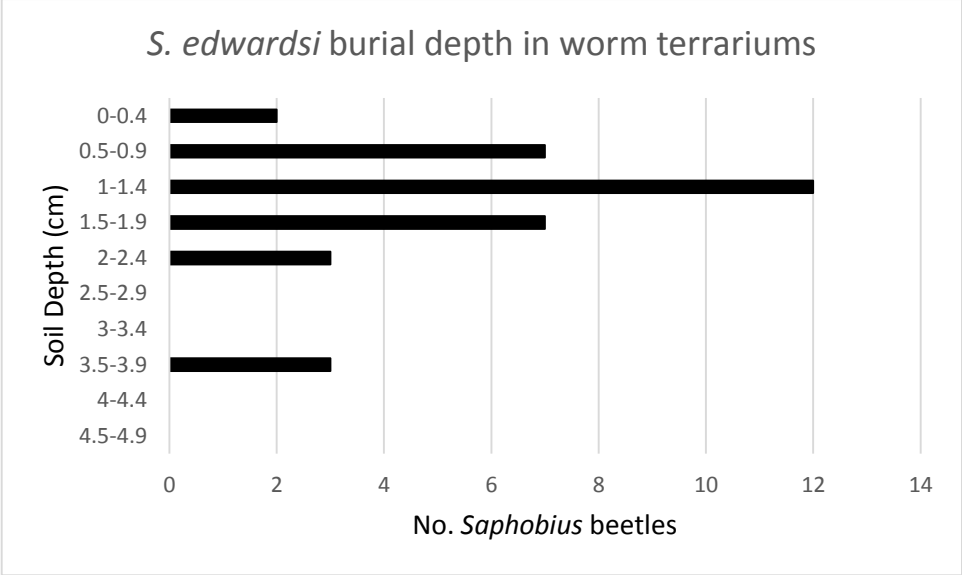


Figure 9. Depth of *S. edwardsi* in soil profile after three nights in worm terrariums under laboratory conditions.

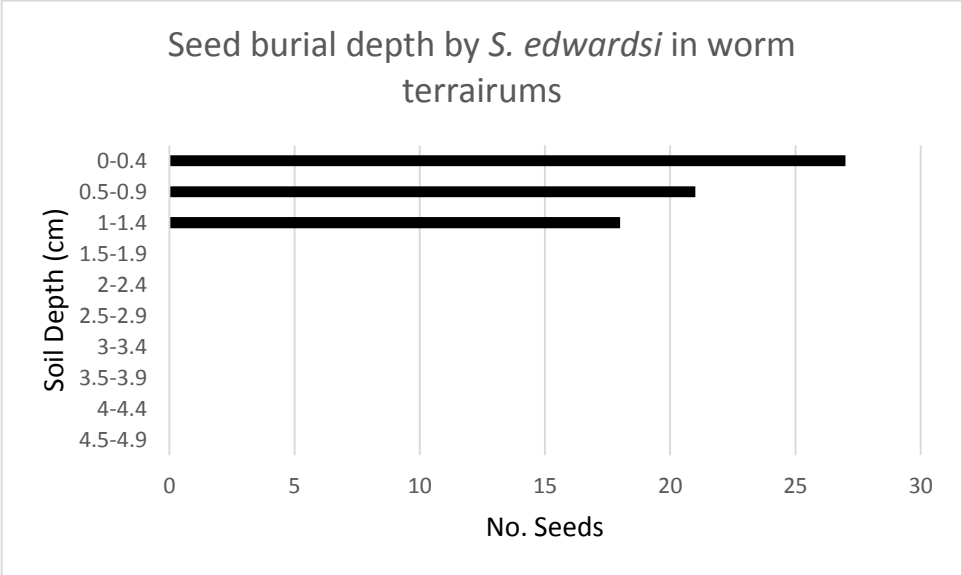


Figure 10. Germinated miner's lettuce seed (1 mm x 0.5 mm) burial depth by *S. edwardsi* after seed was inserted into weta frass.

Table 1. *Saphobius* baited pitfall trap quantities and plot locations, Novemeber 2014 – February 2015.

Plot	Total	Pitfall label
M1	141	E, D
M2	42	G, F, E
M3	0	not labelled
M4	221	G, F, C
M5	72	not labelled
M7	448	not labelled
Total	924	

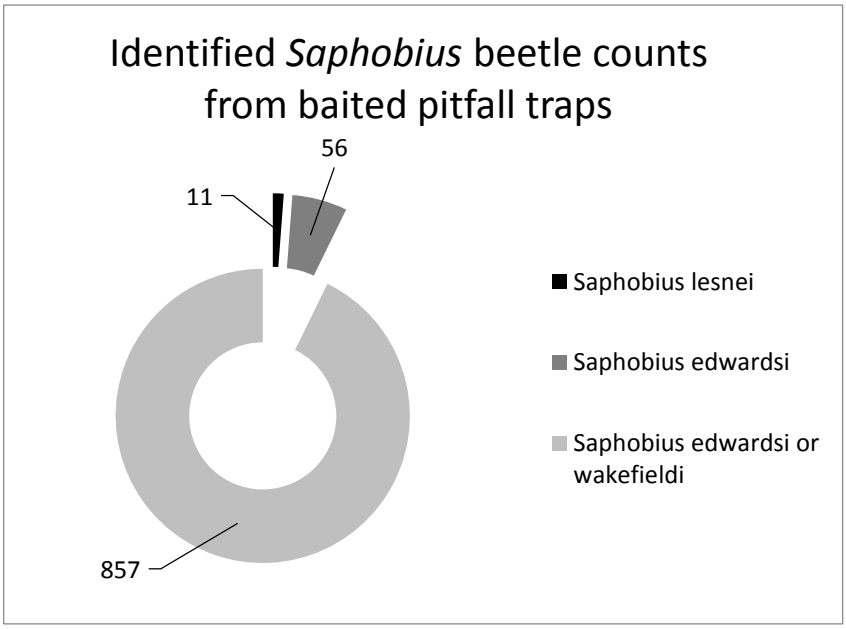


Figure 11. *Saphobius* beetles that were identified to species level compared to total *Saphobius* beetles collected from baited pitfall traps.

Table 2. Plant species that have potential for their seeds to pass through *Hemideina crassidens* digestive tract, that *Saphobius* beetles may utilise.

Plant species fruiting list with small seeds that may pass through a tree weta digestive tract			
Small berry species	**At Punakaiki**	**Seeds known to pass through**	**Fruit timing**
Mistletoe	N		
Gaunera	N		
Nertera	N		
Pratia	N	Y	January-July
Supplejack (*Ripogonum*)	Y		Flowers throughout year
NZ Jasmine (*Parsonia*)	Y		Autumn
Snowberry (*Gautheria*)	N	Y	January-April
Myrsine divaricata	Y (planted)		Feb-May
Neomyrtus pedunculata	N		
Lophomyrtus	N		
Coprosma acerosa	Y		Autumn
Mingimingi (*Cyathodes*)	Y		Autumn
Mahoe (*Melicytus ramiflorus*)	Y (planted)		Feb-April
Fuchsia	Y	Y	summer-autumn
Marbleleaf (*Carpodetus serratus*)	Y		late summer-Autumn
Ngaio (Myoporum laetum)	N		
Wineberry (*Aristotelia serrata*)	Y (planted)		January-March

Discussion

Dung beetles were easily caught at the field site using the pitfall trap method with baited cow dung. They survived in captivity for the duration of the project (90 days) in the plastic fish bin and were relatively simple to maintain. This was similar to Jones *et al.* (2012) where *S. edwardsi* survived in captivity for a maximum of 132 days in a sand/vermiculite substrate in plastic containers. The Initial trials showed that *S. edwardsi* utilised *H. crassidens* frass in petri dishes and ice cream containers with a soil/sand substrate and preferred fresh frass as expected. *S. edwardsi* utilises a mean of 0.02 g/beetle/night of *H. crassidens* tree weta frass (Figure 7) which was more than hypothesised but must be treated with caution as only one trial with one control was conducted due to the limited resource in fresh frass. Beetles rolling the frass whole was also observed. Cow dung was used as a surrogate as it was readily available which had a mean of 0.01 g/beetle/night (Figure 7). This shows that *S. edwardsi* removes very small amounts of frass and dung. However, this dung type was not ideal as there was severe moisture loss resulting in 2/3 trials failing to have a detectable difference with high variability between replicates. Coprinae (tunnelers) from the Onthophagini tribe such *Onthophagus granulatus* (6 mm – 8 mm) which can remove 1 kg of cattle dung over a relatively short period of time if beetle density is >100 beetles/dung pad in optimal conditions (Forgie 2009), that is a maximum of 10 g/beetle. At a density of two beetles/100 grams of dung, *O. gazella* can completely bury a dung pad within 48 hours (Bornemissza 1970). These findings indicate that there is high variability between species which is related to body size, habitat and temperature (Forgie 2009) but there is scarce research on Canthonini dung removal rates to compare with. This investigation needs to be repeated using tree weta frass as it has less moisture loss, but at the field site so fresher frass is available and with 10–20 trials to be accurate. This may also require 20 captive tree weta which could be easily fed if at the field site.

Individual *S.edwardsi* movement per night was expected to be 2-5 m (Shaun Forgie, pers. comm.) but none of the marked beetles were caught in the mark-recapture field trials. Only up to a 1.25 m distance was confirmed (Figure 8) with two replicates at each distance in the laboratory trial due to space and time limitations. It is well known that large (22 mm-47 mm) Canthonini dung beetles can travel 100 m in less than 24 hrs (Kryger *et al.* 2006) however *S. edwardsi* is 3 mm-5 mm and therefore was expected to travel around 5 m with a maximum of 10 m based on preliminary mark-recapture work near Auckland (Shaun Forgie, pers. comm.). In future this experiment could be conducted in the wharehouse at the field site on a seven day duration as it was discovered that beetles could take several days before moving in a new environment. The adjusted polystyrene containers proved effective enclosure for this experiment as more could be added to increase the distance. If conducted at the field site, fresh soil could be used for each replicate. Beetles could be easily replaced for each replicate by pitfall capture at the field site.

S.edwardsi burial depth was within the top 40 mm of the soil as hypothesised with 0.57 of the beetles occurring between 10 mm -19 mm (Figure 9). This is similar to other Canthonini dung beetles as they are telecoprids (ball rollers) rather than 'tunnelers' (Medina *et al.* 2003; Viljanen 2009). Some limitations to this finding is that it was not conducted in the field and there were only four replicates. When small seeds (1mm x 0.5 mm) were inserted into the frass that the beetles utilised under the same conditions, all germinating seeds were located within the first 15 mm of the soil profile (Figure 10). This corresponds to the depth at which most beetles were also found (Figure 9) and indicates some degree of seed burial or vertical secondary seed dispersal occurring. The range of beetle burial depth (0 mm-40 mm) is suitable for most seeds to germinate, provided the appropriate environmental conditions (Burrows 1996). This was however conducted in the artificial environment of a CT room with three replicates over the Christmas break with no controls due to lack of fresh frass. In future it could occur in the nursery or in a fenced off area in the forest itself at the field site with around ten replicates and three controls. Germination should be checked every week as three weeks allowed the plants to grow, making measuring difficult.

Dung beetles had the highest relative abundance in M7, M4, and M1 respectively (Table 1) which could be due to the forest remnant with M7 and M4 (Figure 1), having greater access to cows. This could have provided a large food resource in the form of mammal dung that did not occur elsewhere at the site which sustained a larger the dung beetle population (Mike Bowie pers. com.). This is an interesting result because dung beetles are being used as indicators of restoration success (Bowie *et al.* 2012). However at this site more dung beetles were caught in the disturbed, relatively open M7 and M4 forest remnants compared to the less disturbed, larger M1-M3/M5 forest remnant (Figure 1) in Nikau Reserve (Table 1). This could be to soil types, vegetation and previous removal of beetles. It was found that on very wet nights or during extended dry periods, few beetles were caught which may be reflected in the results.

No whole seeds were found in tree weta frass collected from the field or in the CT room. This is due to the time of year, as most native flowering plants with small seeds do not bear fruit until late summer and autumn (Table 2). Table 2 indicates potential plant species that tree weta could have a role in primary seed dispersal, when they bear fruit and if they are present at the PCRP site for future reference. Research suggests that weta prefer blue berries, over red berries (Fadzly & Burns 2010) and individual tree weta size determines its seed dispersal capabilities as larger weta can pass more seeds and larger seeds (King *et al.* 2011). Wineberry seeds (3 mm x 2 mm) appear to be too large to pass through weta or the berry is too large compared to seed size as no seeds were found in frass collected from captive tree weta and it was observed that the weta consumed the berry around the seed.

This project was heavily restricted by time and resources resulting in many pilot trials but with a low number of replicates. Environmental conditions limited the availability of fresh frass resulting in changes in methodology and a limited number of controls. To mitigate these issues in the future, it is suggested to conduct experiments on site. As a summer scholarship this was a great learning experience in planning, experimental design and problem solving when studying living organisms.

In conclusion the above findings indicate *S. edwardsi* has very restricted secondary seed dispersal because they cover a limited distance each night to find and move tree weta frass. However *S. edwardsi* does potentially play a role in burying small seeds which may increase seed survival and germination. This aspect should be further investigated as an ecosystem function in native forests and restoration projects.

Summary

- *Saphobius* beetles utilise *H. crassidens* frass.
- *S. edwardsi* utilised a mean weight of 0.02 g/beetle/night of tree weta frass and a mean weight of 0.01 g/beetle/night of cow dung was utilised.
- *S. edwardsi* can disperse at least 1.25 m per night.
- *S. edwardsi* tunnel up to 3.9 cm below the soil surface but were predominantly found at a depth of 1-1.4 cm. Seeds were buried to a depth of 1.4 cm.
- *S. edwardsi* is likely to be a poor secondary seed disperser but may encourage seed germination by burying seeds just below the soil surface.

Recommendations

- Repeat experiments with more replicates and at the Punakaiki field site so that tree weta frass, beetles and soil is more available.

Acknowledgements

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