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Project title

The contribution of fruit bats to forest regeneration in Madagascar – do bat-processed seeds do better?

Abstract

1. Germination experiments performed on filter paper revealed that bat-processed seeds of two strangler fig species germinated significantly better than unprocessed seeds.
2. GPS tracking of Madagascan flying foxes *Pteropus rufus* indicated that the bats are very efficient long-distance seed dispersers able to travel long distances over short periods of time.
3. Compositional analysis revealed that bats even though spend significant amount of time feeding on widely available sisal, show preferences toward remaining small forest patches.
4. A gut retention time study confirmed that bats have fast gut transit times for large numbers of seeds (less than 30min) but are able to retain small numbers of seeds in the gut for up to 24h.
5. *Pteropus rufus* is likely to contribute to forest regeneration by enhancing germination of dispersed seeds in isolated forest patches and dispersing them in areas that are not attractive to other frugivores.

Background

Frugivory and seed dispersal by animals (zoochory) have been highlighted as being of major ecological and evolutionary importance (Traveset, 1998). The efficiency of an animal as a successful dispersal agent depends on many aspects of its behaviour, physiology and/or morphology. Food handling, processing, and gut passage time may affect the viability of a seed and the size of the area in which it is deposited. Home range and movement patterns may affect the location where a seed can be deposited in terms of preferable microclimate conditions and distance from parental trees (Izhaki et al., 1995).

In their review Traveset and Verdu (2002) analysed the influence of gut passage in frugivores on seed germination from 351 available experiments. These included 19 experiments on bats, 180 on birds and 113 on non-flying mammals. The results showed that gut passage generally increased seed germination success in all bats and birds studied with similar effect sizes, while in non-flying mammals gut passage increased the germination success of eaten seeds only marginally.

Old World fruit bats (Family Pteropodidae, Order Chiroptera) are phytophagous and feed almost exclusively on fruits, leaves, nectar and pollen (Long and Racey, 2007). They usually have broad diets and eat a wide range of native and introduced/cultivated species of plants (Long and Racey, 2007), and they are able to consume relatively large quantities of fruits (50- 250% of their body mass per night) (Izhaki et al., 1995).

Old World fruit bats are highly mobile and their wing morphology allows them to track resources over large areas (5-40km) and among scattered forest fragments (Richter and Cumming, 2008;

Jenkins et al., 2007; McConkey and Drake, 2007; Thomson et al., 2002). Therefore, they appear to be of fundamental importance in the maintenance and regeneration of tropical forests because they promote the large-scale dispersal of fruit seeds in tropical environments (Muscarella and Fleming, 2007). Indeed, at least 300 plant species are known to rely on Old World fruit bats (Sato et al, 2008; Muscarella and Fleming, 2007; Lobova et al., 2003; Shilton et al., 1999). However, there are some controversies remaining about the effectiveness of seed germination after the passage through the bats' guts with some species showing increased germination success while others do not (Sato et al., 2003).

Bats may provide several advantages to the plants on which they feed (Nyhagen et al., 2005). Bats extract juice and pulp of a fruit by crushing the fruit against the palate, and spit out the remaining dry pellet of pulp, fibres and seeds as 'ejecta' (Entwistle and Corp, 1997). Thus, the separation of pulp from seeds may increase seed survival by reducing microbial attack and the chances that other frugivorous species (that may destroy the seeds) will eat the fruits (Nyhagen et al., 2005). Also dispersal of seeds away from parental trees may reduce predation rate and increase seedling establishment (McConkey and Drake, 2006). Additionally, bats may selectively ingest unparasitized seeds, increasing dispersion of healthy ones (Entwistle and Corp, 1997).

Frugivorous bats exhibit special modifications to their digestive tract (Hansen et al., 2008; Traveset, 1998), hence food transit time is relatively rapid and ranges between 12 and 114min (usually less than 30min) (Nakamaoto et al, 2009; Tang et al., 2007; Traveset, 1998; Fleming and Heithaus, 1981). Such a short passage time lessens the amount of mechanically and chemically induced changes suffered by the seeds (Lobova et al., 2003). Ingestion of seeds may cause an increase in germination success because of partial digestion or mechanical abrasion of impermeable layers, causing breakage of seed coat dormancy. Differential digestion and death of less developed seeds can influence the germination of the remaining, well-developed seeds. However, chemical or mechanical damage or leaching of growth-promoting substances may decrease germination rate or cause death of seeds within the frugivore's digestive tract. Therefore, events causing an increase or decrease of germination are not qualitatively different from each other and the nature of the seed coat, the ability of the seed to survive digestion, and the type of seed dormancy involved may be more important (Lobova et al., 2003; Liberman and Liberman, 1986).

There are three major patterns of seed dispersal by bats. Bats may drop oral and faecal pellets and food remains from a parental tree, they can carry large fruits from the parental tree to feeding trees by mouth (fruit can be dropped before bats reach the feeding tree) and finally small seeds can be transported from the parental tree to other fruiting trees or into open areas in the faeces (Nakamoto et al., 2009; Entwistle and Corp, 1997). Additionally, it has been suggested that bats, rather than birds, have a tendency to disperse seeds in clearings (Muscarella and Fleming, 2007; Gorchov et al., 1993). Thus, fruit bats are likely to be of fundamental importance in the maintenance and regeneration of tropical forests by moving seeds from forest to cleared areas as well as from primary to secondary forests and vice-versa (Sato et al., 2008; Muscarella and Fleming, 2007; Picot et al., 2007; Lobova et al., 2003). Bollen et al. (2004) state that in the littoral forest of Sainte Luce, Madagascar, each of the frugivore species plays its own role in seed dispersal and mobility. Thus, lemurs are important for dispersal of large seeds within the forest boundaries, fruit bats are crucial for long-distance dispersal across fragment boundaries and into the clearings, whereas birds are important for enhancing succession and regeneration of plants in degraded areas as they are more likely to deposit seeds while perching on trees.

Although a small number of earlier studies have investigated seed germination after passage through bats' guts, our understanding of the subject is limited. Previous research does not provide us with clear conclusions about the influence of bats on seed germination rate.

For example, Entwistle and Corp (1997) recorded that the germination of *Ficus lutea* and *F. natalensis* seeds was higher after ingestion by *Pteropus voeltzkowi* compared with that of intact seeds. However, germination of bat-passed *F. sur* seeds was significantly lower than that of seeds from ejecta. Studies on *Eriobotrya japonica* (fruit) and *Rousettus aegyptiacus* (bat) showed that seed passage through the bat's digestive tract did not enhance germination. However, in the case of *E. japonica*, the percentage of seed germination of the ejected seeds was significantly higher compared with the seeds that had not been processed (Izhaki et al., 1995). In south-west China, *Rousettus leschenaulti* and *Cynopterus sphinx* commonly feed on two fig species (*Ficus hispida* and *F. racemosa*). However the seeds of both species after bat-ingestion germinated as well as control seeds (Tang et al., 2007). In north-west Argentina, a study on seed germination of *Solanum riparium* after digestion by a Neotropical phyllostomid bat (*Sturnira lilium*) revealed that neither the speed nor the rate (the number of germinating seeds at the same time) of germination were affected by passage of the seeds through the bat's digestive system (Iudica and Bonaccorso, 1997). In south-central Mexico, fruits of cacti are eaten by some phyllostomid bats (*Leptonycteris curasoe*, *Choeronycteris mexicana* and *Artibeus jamaicensis*). Only one species of cactus (*Pachycereus hollianus*) out of 7 studied increased its germination after the seeds were soaked in acid solution, meant to mimic passage through a bat's gut. In the other 6 species no increase in germination success was recorded (Godinez-Alvarez and Veliente-Banuet, 1998). Recent study on *A. jamaicensis* (Heer et al., 2010) on Barro Colorado Island, Panama, reviled that bats have positive effect on germination of six native fig species (free-standing figs: *F. insipida*, *F. maxima* and *F. yoponensis,* and strangler figs: *F. nymphiifolia*, *F. obtusifolia* and *F. popenoei*). Although there was not much difference in germination success of seeds removed from fruits manually and those extracted from bat faeces, the removal of seeds from the fruit pulp was a crucial step to facilitate germination. The control seeds which were left in the fruit palp showed no germination and got infected by fungi and other microorganisms.

Germination results for *Polyscias*, *Psidium* and *Ficus* species after ingestion by *Eidolon dupreanum* in Madagascar revealed that there was significantly different germination success between seeds extracted from faecal samples and those taken from ripe fruits. *Ficus brachyclada* and *F. pyrifolia* germinated only after passage through digestive system of bats, whereas more seeds of *Polyscias ornifolia* germinated from faecal samples compared with ripe fruits, and none germinated from ejecta. However, sample sizes were too small to allow statistical analyses (Picot et al., 2007).

Nevertheless, most of the studies were conducted in laboratory conditions and focused on a limited range of seed species (e.g. Tang et al., 2007; Iudica and Bonaccorso, 1997; Izhaki et al., 1995). The study on *Eidolon dupreanum* in Madagascar showed that the transit of some fig seeds through bat guts was necessary for successful germination (Picot et al., 2007), although whether this is a generic coevolutionary response by *Ficus* species on the island to all bat species remains untested. As mentioned before, any influence of digestion on seed germination may depend on seed structure rather than on digestive processes. Thus, it is essential to test the rate of germination of a wide range of bat-digested seeds found in natural conditions to obtain a more reliable perspective on whether seed germination is affected by gut passage. All over the world, fruit bat populations are suffering from habitat fragmentation, persecution, and the loss of suitable roost sites and these factors influence their foraging behaviour and diet selection (Jenkins et al., 2007; Long and Racey, 2007; Kofoky et al., 2007; Gumal, 2004; Webb and Tidemann, 1996). Old World fruit bats are of major conservation concern. Of the 200 or so species described (Simmons, 2007), seven are extinct, and nearly half face significant threat to population viability (Mickleburgh et al., 1992).

Aims of the project

This study aimed to determine the efficiency of Malagasy bats in promoting forest regeneration by comparing the germination success of seeds from faecal and ejecta pellets with those of ripe fruits. I have continued to expose bat-processed seeds to several different, progressively more natural conditions. Beginning from basic laboratory treatment, when sterilized and unsterilized seeds are places on the filter paper in Petri dishes, through soil with no invertebrates, untreated soil and finally on the ground with no additional protections from environmental factors like rain or wind and predators like ants.

I also aimed to collect information about movement pattern of *Pteropus rufus*, distances it travels over the night, habitat selection and possible places it deposits the seeds.

Additionally, I investigated gut retention time of *P.rufus* to estimate the time from feeding to defecation and how long seeds can be retained in the bats' gut.

The main hypothesis of my study are summarised in Table 1.

Table 1. – Summary of the main hypothesis of the study with methods and type of collected data.

Hypothesis	Data collection methods	Type of collected data
Germination rate is affected by passage of seeds through bats	Seed germination from faecal pellets under different, progressively more natural conditions	Influence of bat- ingestion and environmental factors on seed germination
Bat movements promote dispersal of seeds into cleared areas and between forest patches	GPS tagging of bats	Maps of bat movements across agricultural landscapes and scattered forest fragments; habitat preferences of bats
Food transit time in bats is rapid, although bats can retain seeds in the guts for relatively long time	Monitoring of captured bats	Time between feeding and defecation of seeds

Methods and results

1. ***Germination rate is affected by passage of seeds through bats' gut.***

To test this hypothesis, seed germination experiments were conducted between 18th August 2012 and 25th September 2012. All the seeds extracted from the bat faeces in Berenty were identified as *Ficus grevei* (Baillon 1985), a semi – epiphytic fig which is endemic to Madagascar (Dalecky et al., 2003.)

1.1 Methods

The seeds (faecal and unprocessed) were sown 1) on filter paper (100mm) in Petri dishes (91mm) 2) on 15g of ferralitic soil treated in a Tullgren funnel for 48h to remove invertebrates and 3) on untreated soil. Each Petri dish contained 20 seeds and was replicated 15 times, and was moisturised with 3ml of water. Additionally, seeds sown on filter paper were divided into two groups: sterilized (seed washed in 10% ethanol to prevent any possible fungal or bacterial infection) and unsterilized. In total 120 Petri dishes with 2400 seeds (20 seeds/dish) were randomly distributed on an outside table (W 100cm, H 100cm, L 300cm) in the Berenty Reserve Naturaliste Camp in semi-shaded conditions under tree branches to prevent overheating and to represent natural conditions.

In a second experiment, seeds (bat-processed and unprocessed) were sown on the ground, next to the table in the same manner (15 replicates of 20 seeds). The seeds were fully exposed to the outside environment. Because the experiment took place in the dry season, where rain is very occasional, the seeds were watered twice a day, in the morning and in the evening.

All the seeds were randomly chosen from the collected pool of seeds. Seeds were rejected only when they were obviously damaged. The seeds were checked first after 5 days and then they were checked every second day and moisture was provided when necessary with all dishes treated consistently. The experiment ended once there was no sign of seed germination for seven continuous days.

1.2 Analysis and results

I used two-way ANOVA in the PASW Statistics 20 package to investigate the effect of seed type (processed and unprocessed) and sterilization (sterilized and unsterilized seeds) on germination success. Also, I have tested whether different soil substrate had an influence on the germination of both processed and unprocessed seeds.

1.2.1 Seeds sown on filter paper

In this experiment unsterilized bat-processed seeds (F1) showed the highest germination success with a mean of 11.73 (± 0.58 SE) seeds germinating out of the 20 placed in the petri-dish (Fig. 1). Unsterilized seeds extracted from ripe fruits (N1) achieved a germination rate of only 3.33 (± 0.49 SE).

The ANOVA was significant ($F_{3,56}$ = 38. 434, $P < 0.001$) and Levene's test showed that the error variances were homogenous ($F_{3,56}$ = 0.856; $P = 0.469$)

The results showed that the difference in germination success between processed and unprocessed seeds is highly significant ($F_{1,56}$ = 66.27; $P < 0.001$). There was significant difference in seed germination according to whether the seeds were sterilized or unsterilized ($F_{1,56} = 10.529$; $P = 0.002$). Bat-processed seeds (Fs1) slightly decreased their germination success when sterilised but a substantial increase in germination occurred in unprocessed seeds following sterilisation (Ns1). The interaction between seed type and sterilization was highly significant ($F_{1,56} = 38.503$; $P < 0.001$) showing that processed and unprocessed seeds respond in different ways to sterilization.

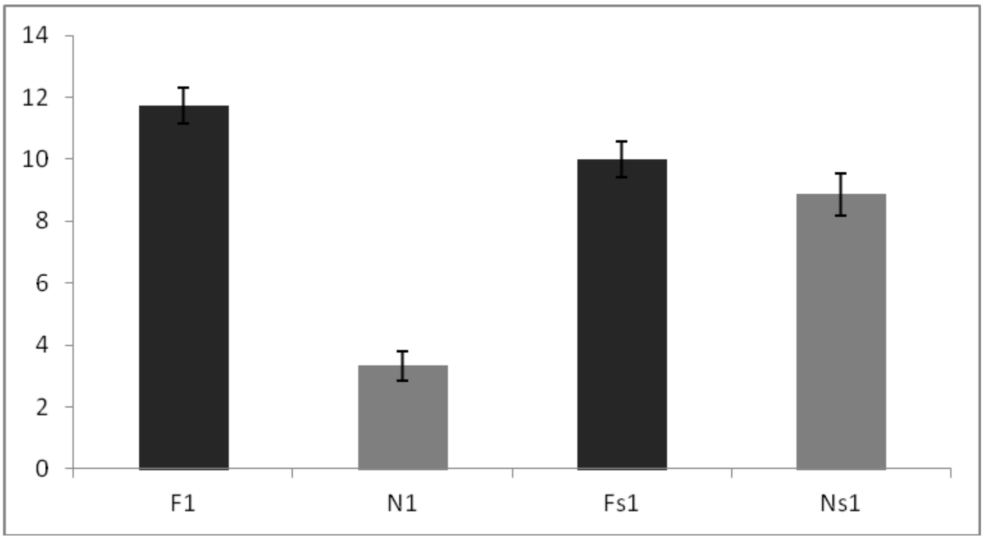

Figure 1. – Mean germination success of processed and unprocessed seeds sown on filter paper. There are 15 replicates of 20 seeds/ treatment. Bars represent means and standard errors. Codes: F1 – faecal seeds sown on filter paper; Fs1 – faecal sterilized seeds on filter paper; N1 – seeds from ripe fruits on filter paper; Ns1 – sterilized seeds from ripe fruits on filter paper.

1.2.2 Seeds sown on different substrates

The results showed homogenous error variances (Levene's test, $F_{3,56} = 1.246$; $P = 0.302$) but ANOVA was not significant ($F_{3,56} = 1.195$; $P = 0.32$). Therefore, there was no significant difference in germination success of seeds sown on differently treated soil ($F_{1,56} = 0.159$; $P = 0.691$) (Fig. 2) and no difference was found between germination success of bat- processed and unprocessed seeds ($F_{1,56} = 2.372$; $P = 0.129$). Overall, germination success was considerably lower than on filter paper with between 3.8 and 6.8 of the 20 seeds germinating in the 4 treatments.

The seeds which were sown on the ground with no protection showed no success in germination. The majority of them were seen to be removed by ants.

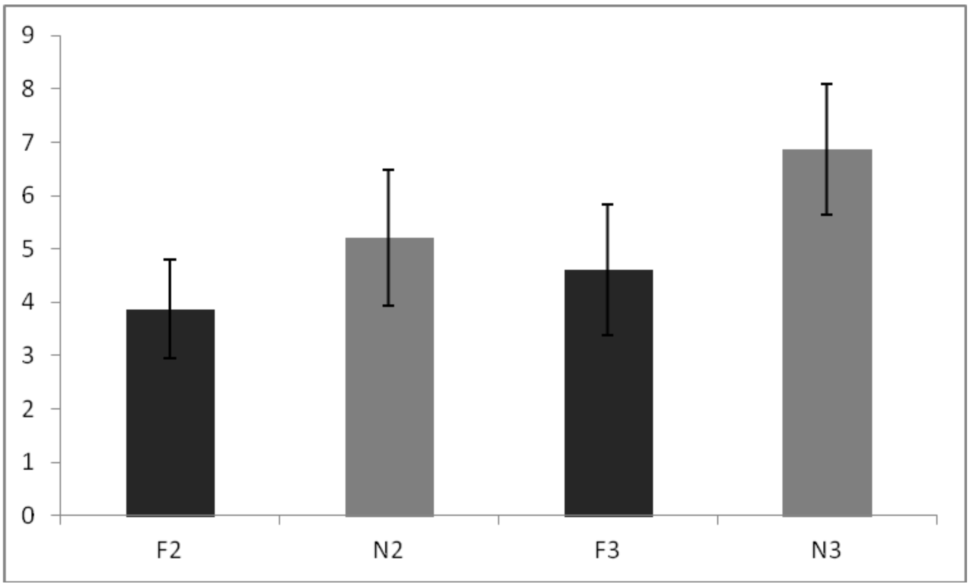

Figure 2. – Mean germination success of processed and unprocessed seeds on different soil substrate. There are 15 replicates of 20 seeds/ treatment. Bars represent means and standard errors. Codes: F2 – faecal seeds on untreated soil; N2 – ripe fruit seeds on untreated soil; F3 – faecal seeds on soil with no invertebrates; N3 – ripe fruit seeds on soil with no invertebrates.

1.2.3 Germination rate

The germination of seeds began on day seven and faecal seeds on filter paper were the first to germinate. On day nine, seeds from six out of 12 treatments showed germination and on day 11 germination was apparent in all treatments in Petri dishes and none on the ground. The faecal seeds on filter paper showed also the highest cumulative germination rate (sum of all the numbers of germinated seeds from all the replicates) among all the treatments with 9.33%, 27.33% and 38% (day nine, 11 and 13 respectively).

2. ***Bat movements promote dispersal of seeds***

To test this hypothesis, between 23rd July 2012 and 3rd September 2012, 16 bats were tagged with GPS devices (GPS – ACC – Logger – Tag, E-obs GmbH digital telemetry, Germany, ca. 26g) and two with Alana Ecology GPS tags, UK (MicroTraX TSTX GPS Tag, Code: 070602, ca. 30g) to monitor their movements at night.

2.1 Methods

The bats were captured using a handmade nylon net (5m x 2m; 10cm mesh) placed on the top of sisal flowers during the bats' feeding time between 18.00h and 01.00h in sisal (*Agave sisalana*) plantations adjacent to the Berenty Reserve (ca. 3km distance) and on the *F. grevei* trees (placed in the canopy) in the gallery forest, near the roosting site of the bats (ca. 1.5km distant). After capture, each bat was weighed and measured. In order to attach a tag, a strip of fur (6cm x 2cm) was shaved from between the shoulders and skin bounding latex glue (Ostomy Adhesive Solution, Salts Healthcare, UK, Product code: 833005) was applied to both the skin and the tag. Only bats weighing 650g and were tagged to ensure the tag did not exceed 5% of body mass. After the glue started to dry, the tag was applied and held down for around 5min. Once the glue set, the bat was released. The data were downloaded remotely every day between 13.00h and 14.40h as only within this time window was the built-in pinger on the tags programmed to emit a radio frequency allowing me to locate the tag.

At the end of the study, three working tags were used to establish accuracy of the fixes. The tags where left firmly attached to a leafless tree for three nights to acquire constant fixes. The distances from the attachment point and recorded fixes were then measured in Google Earth and the mean distance calculated.

2.2 Analysis

The tracking points and paths of each bat were mapped in ArcGIS 10 using a built-in base map (Bing Map Aerial). Using Ranges 7 (Antrack Ltd, Wareham, UK), 100% minimum complex polygons (MCPs) were constructed to determine home ranges for each individual bat and for all bats tracked from the colony. The habitat within the whole range was then classified into 13 categories (Table 2) which were ground-truthed during the field work. To define core foraging areas, cluster polygons were used. Analysis of utilisation distribution discontinuities showed that on average 80% (± 4.22 SD) cluster cores were used by bats as foraging areas. The remaining 20% caused disproportional increase in areas utilised by the bats, as it included the paths bats used to travel to and from the feeding sites.

Table 2. – Habitat types, area and its percentage defined within the whole colony's home range.

Habitat code	Habitat type	Area (ha)	Percentage (%)
1	gallery forest	331.92	0.57
2	degraded gallery forest	740.37	1.27
3	sisal plantation	13345.38	22.82
4	mixed forest	974.79	1.67
5	spiny forest	23376.65	39.97
6	agricultural land (maize, manioc, sweet potatoes)	10178.52	17.40
7	rice paddies	716.84	1.23
8	agriculture trees	391.74	0.67
9	overgrown sisal (with trees and shrubs)	97.11	0.17
10	water bodies	2028.24	3.47
11	human settlement	410.20	0.70
12	open areas	262.67	0.45
13	degraded spiny forest	5628.04	9.62
	Total	**58482.48**	**100**

Habitat preferences of the bats were defined by comparing composition of the habitat in each bat's home range (100% MCP) to the habitat used during foraging (80% cluster cores of all the foraging records). The used versus available habitat was compared using compositional analysis (Compositional Analysis Plus Microsoft Excel tool 6.2, Smith Ecology Ltd, UK). This analysis highlights whether the available habitat was used at random (i.e. in proportion to availability) or whether specific habitats were selected by bats. It also ranks the habitat types according to bats' preferences and weights it in relation to the number of fixes collected from each bat.

For all recorded fixes, a 15m buffer was applied when calculating MCPs and cluster areas which accounted for the accuracy of GPS tags obtained during the fieldwork (mean 14.36m ± 4.39 SD; n = 370 fixes).

2.3 Results

In total 101 nights of data were collected from 15 bats (nine males and six females of which two were pregnant) using e-obs GPS tags (Table 3). Three tagged bats either lost the tag just after the attachment or could not be located the following day. To minimises biases that may result from bats being held for tag attachment, the first night after tag attachment was excluded from analysis, giving in total 86 nights of viable data. On average, for each tagged bat almost a week of data (6.73 days ± 3.58 SD) and 348.93 location fixes (± 196.06 SD) were recorded.

Table 3. – Description of all captured and tagged *P. rufus* bats in Berenty Reserve. Female (p) represents pregnant females .

Date from	Tag ID	Date to	Nights of data	Sex	Age	Body mass (g)	Forearm length (cm)
23/07/2012	2407	08/08/2012	16	male	adult	1100	18
01/08/2012	2412	06/08/2012	5	female	adult	700	16
06/08/2012	24091	14/08/2012	8	male	juvenile	760	16.5
06/08/2012	2406	10/08/2012	4	male	adult	750	16
06/08/2012	2414	11/08/2012	5	female	adult	650	16
09/08/2012	2408	15/08/2012	6	male	juvenile	650	15
09/08/2012	24131	18/08/2012	9	male	juvenile	650	16
12/08/2012	2410	24/08/2012	12	female(p)	adult	810	18
12/08/2012	24111	15/08/2012	3	male	adult	800	17
14/08/2012	24051	23/08/2012	8	female(p)	adult	710	17
23/08/2012	24112	27/08/2012	4	male	juvenile	650	15
23/08/2012	24052	26/08/2012	3	male	juvenile	600	16
30/08/2012	2408	06/09/2012	7	male	adult	800	17
30/08/2012	24132	03/09/2012	4	female	juvenile	750	16
30/08/2012	24053	lost	0	male	adult	900	19
30/08/2012	Alana1	lost	0	male	adult	900	18
03/09/2012	24092	10/09/2012	7	female	adult	700	16
03/09/2012	Alana2	lost	0	male	juvenile	600	15
Total nights			**101**				
Total excluding 1st night			**86**				
Mean			**6.73**			**748.89**	**16.53**
SD			**3.58**			**125.51**	**1.14**

2.3.1 Habitat availability

The area of MCP for the whole tracked population (Table 3) was 58482 ha with its habitat mainly composed of spiny forest (39.97%), sisal plantations (22.82%), agricultural land (17.40%) and degraded spiny forest (9.62%).

Table 3. – Habitat types, area and its percentage defined within the whole colony's home range.

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	Total	**58482.48**	**100**

Individual home range areas (100% MCP) (Tab.4.) varied greatly among bats (from 15777.9ha to 24.88ha) with a mean of 5190.53ha (± 5667.09 SD). The total area of each bat's 80% cluster cores (Table 4) was considerably smaller than the home ranges of the bats with a mean area of 86.97ha (± 124.89 SD) and great size variation (414.04ha to 1.7ha). On average, the most widely available habitat within each bat's home range was sisal (45.44% ± 24.21 SD), spiny forest (13.49% ± 11.98 SD), agricultural land (13.03% ± 10.81 SD) and gallery forest (8.97% ± 14.93 SD).

Table 4. – 100% MCP and 80% cluster core home range areas (i.e. total area of all 80% cluster cores over the tagging period) for 15 GPS tagged *P. rufus* bats in Berenty Reserve.

Bat ID	**100% MCP area (ha)**	**80% cluster core area (ha)**
24051	14687.70	314.24
24052	422.14	7.10
2406	9162.59	414.04
2407	645.98	6.42
24081	786.75	12.64
24082	13949.20	20.39
24091	44.74	2.38
24092	3928.67	171.84
2410	6968.38	67.13
24111	24.88	1.70
24112	3553.77	32.26
24113	5089.41	27.80
2412	15777.90	125.67
2413	217.00	4.51
2414	2598.79	96.49
mean	**5190.53**	**86.97**
SD	**5667.09**	**124.89**

2.3.2 Habitat preferences

The compositional analysis showed that habitats used by bats was significantly different from what was expected in relation to their availability (weighted mean Wilk's $\Lambda = 0.0002$, $X^2 = 128.4587$, d.f = 12, $P < 0.0001$, randomisation $p = 0.018$). The mean percentage of used habitat vs. available is summarised in Figure 3.

The ranking matrix of the habitats (Table 5) ordered the preferences of the habitat from the most to least selected by bats as follow: overgrown sisal > gallery forest > sisal plantations >>> degraded gallery forest > agriculture trees= water bodies > mixed forest > rice paddies = agricultural land > open areas > human settlement > degraded spiny forest > spiny forest, where '>' means positively selected over the following habitat type, '>>>' significantly selected over the following habitat type and '=' equally selected to the following habitat type. When comparing all of the habitats, sisal plantation and gallery forest were significantly selected over most of the other available habitats.

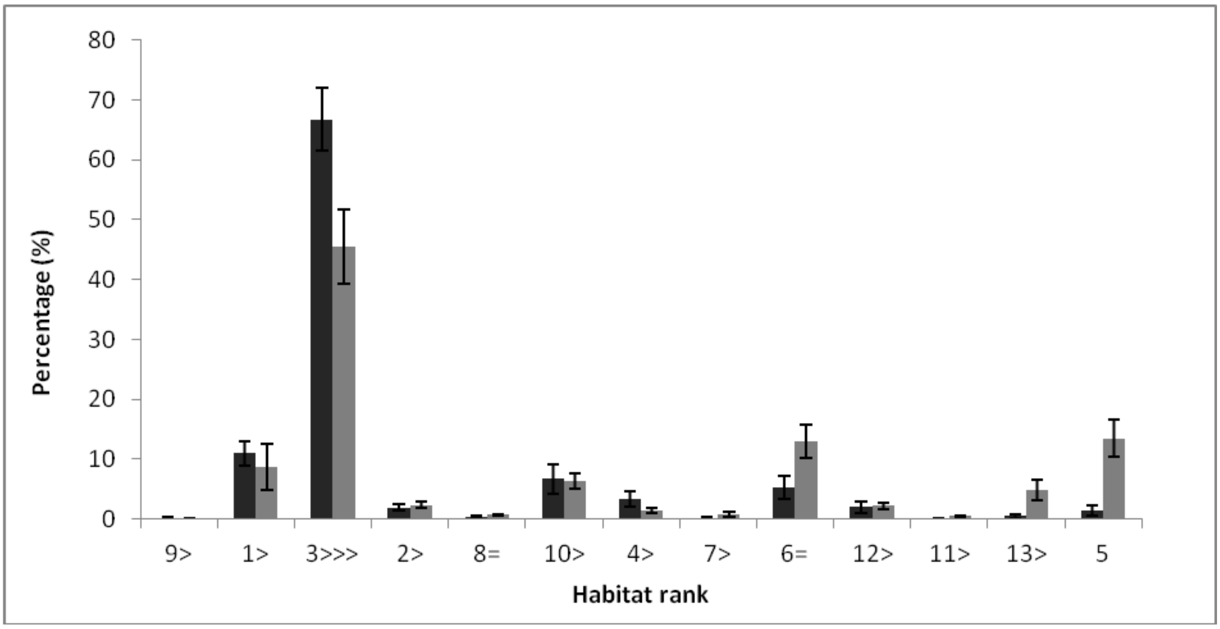


Figure 3. – Available habitat (100% MCP, grey bars) vs. used habitat (80% cluster, black bars) based on mean percentage area for all 15 tagged bats (bars represents SE). Habitat ranked from the most selected (left) where '>' means selected over the adjacent habitat to the right with '>>>' indicating a significant difference compared with the following habitat in the ranking. Habitat codes: 1 – gallery forest; 2 – degraded gallery forest; 3 – sisal plantations; 4 – mixed forest; 5 – spiny forest; 6 – agricultural land; 7 – rice paddies; 8 – agriculture trees; 9 - overgrown sisal; 10 – water bodies; 11 – human settlement; 12 – open areas; 13 – degraded spiny forest.

Table 5. – Ranking matrix for habitat preferences (for each category on every row compared to corresponding habitat in each column) of all tracked *P. rufus* bats. The matrix compares proportion of habitat within 80% clusters (used habitat) and 100% MCP (available habitat). A significant difference between habitat types is shown by +++ (positively selected) and --- (negatively selected) with + or – showing no significance. The rank list of the habitats ranges from 0 (the least selected) to 12 (the most selected). Habitat codes: 1 – gallery forest; 2 – degraded gallery forest; 3 – sisal plantations; 4 – mixed forest; 5 – spiny forest; 6 – agricultural land; 7 – rice paddies; 8 – agriculture trees; 9 - overgrown sisal; 10 – water bodies; 11 – human settlement; 12 – open areas; 13 – degraded spiny forest.

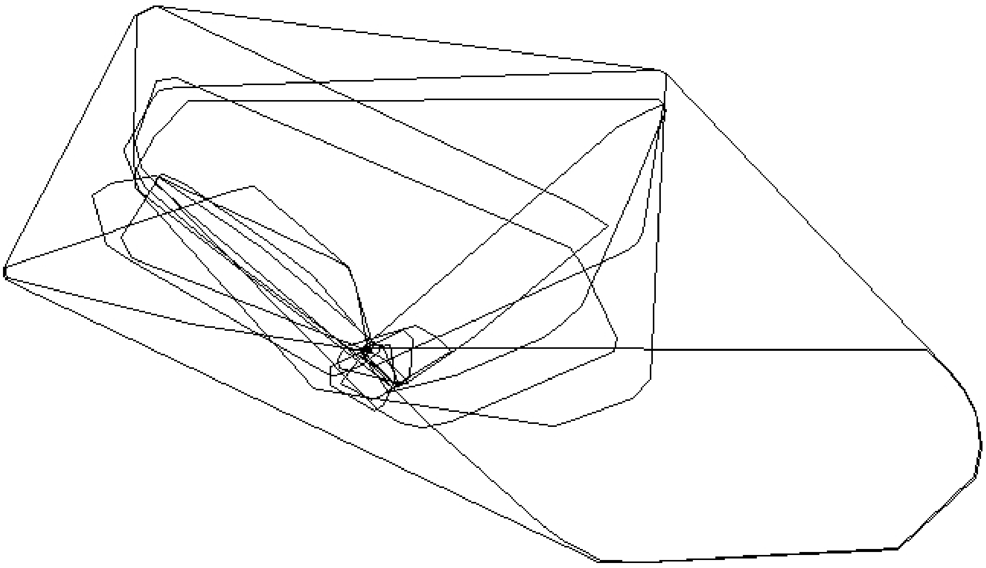
	1	2	3	4	5	6	7	8	9	10	11	12	13	Rank
1		+++	+	+++	+++	+++	+	+++	-	+++	+++	+++	+++	11
2	---		---	+	+++	+	+	+	-	+	+++	+	+	9
3	-	+++		+	+++	+++	+	+++	-	+++	+++	+++	+++	10
4	---	-	-		+++	+	-	-	-	+	+	+	+++	6
5	---	---	---	---		---	-	---	-	---	---	-	-	0
6	---	-	---	-	+++		+	-	-	-	+	+	+	5
7	-	-	-	+	+	-		-	-	-	+	+	+	5
8	---	-	---	+	+++	+	+		-	-	+	+	+++	7
9	+	+	+	+	+	+	+	+		+	+	+	+	12
10	---	-	---	-	+++	+	+	+	-		+	+	+	7
11	---	---	---	-	+++	-	-	-	-	-		-	+	2
12	---	-	---	-	+	-	-	-	-	-	+		+	3
13	---	-	---	---	+	-	-	---	-	-	-	-		1

2.3.3 Foraging behaviour

The mean time at which bats emerged from the roost was 19:23h (± 32 min SD, n = 15) and they returned at 04:55h (± 51min SD, n = 15). The study took place during the Austral winter, when the day was getting progressively longer with sunrise at 06:21h and sunset at 17:32h on the 23rd of July while at the end of the GPS tracking study (10th September) sunrise was at 05:50h and sunset at 17:44h.

Spatial distribution of the individual home ranges (Fig.3.) showed small overlap between each MCP with a mean of overlap of 32.90% (± 17.49 SD). Three bats with the smallest ranges (2406, 2410 and 24051) overlapped with almost every other bat to a greater extent, with a mean of 85.1% (± 23.39 SD), 70.03% (± 27.89 SD) and 64.17% (± 28.63 SD) respectively. Their home ranges were narrowed to the gallery forest within which they roost and nearby sisal plantation, usually included within the large home ranges of other bats. The core foraging sites vary in size among the bats, however they show that bats are faithful to their feeding sites with little overlap between each bat’s foraging area (mean overlap was only 6.11% ± 8.36 SD).

a)



b)

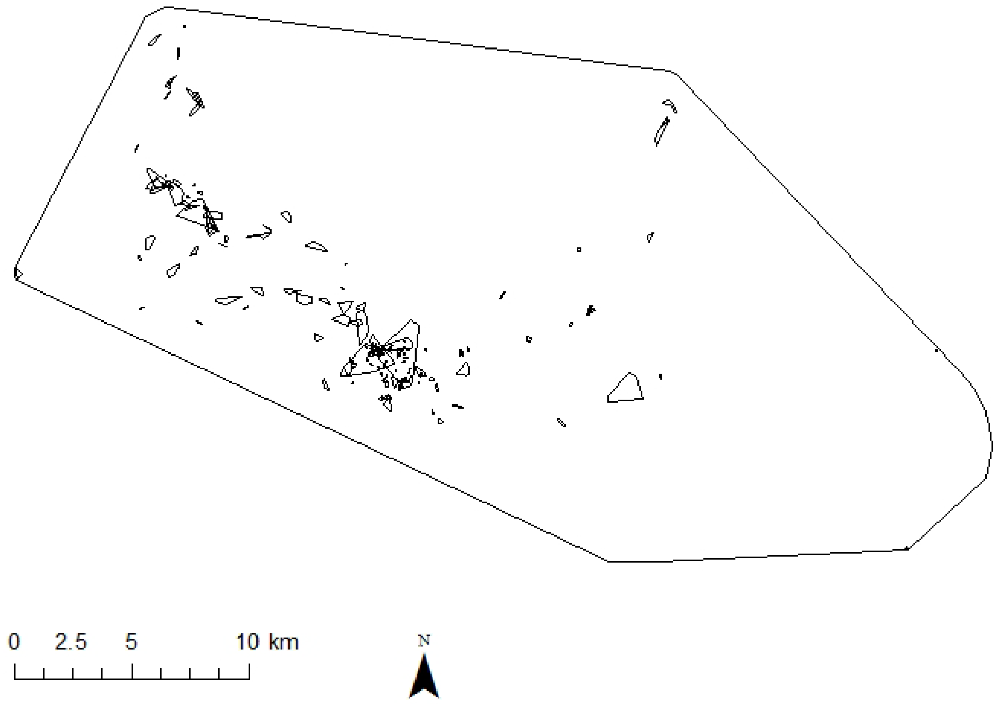


Figure 3. – Home range area for 15 tagged bats with individual 100% MCP home range areas (a) and 80% cluster core foraging areas (b).

3. *Gut retention time*

3.1 Methods

Nine bats were kept for observations in cages (1m x 1m x 1m) for around three nights. The bats were captured on different nights using handmade nets (refer to 2.1) and transported to the Berenty Naturaliste Camp in cotton bags. Each bat was kept separately and had water provided during the whole period of the study. During the first night bat usually refused to eat, therefore the food was provided on second night at 18.00h. Each bat was given 200g of sliced banana. Each slice was either with seeds extracted from ripe fig fruits (one fig per slice) or plain as a control. The slices given to each bat were equal in number and size. The bat was then monitored every 30min and number of eaten slices and produced droppings counted. Additionally, the droppings were removed from the cages and number of seeds inside was counted. If there were more than 25 seeds in the faeces, the counting stopped and a score of 25 and over was given. Any smaller amount of seeds was counted individually. In the morning (around 06.00h) the food was removed from the bat, and the caged cleaned during the day. On the fourth night around 20.00h bats were released.

3.2 Results

The results showed that on average bats produced 2.39 (± 0.63 SD, n = 9) droppings every 30min when fed *ad libitum.* Even when bats stopped feeding droppings were produced by the first inspection (30min after feeding) and continued to be produced. When looking at bats' ability to disperse the seed (Fig.5.), it can be seen that in four cases the maximum amount of seeds was dispersed during the first hour since the last feeding. However, one bat continue to produce fully seeded dropping up to three hours after the seed ingestion and one managed to retain 23 seeds for over two hours. Therefore, I assumed that on average bat will produce one dropping every 12min since the start of feeding and will continue to produce seeded droppings up to three hours after feeding. This frequency of dropping production was used in further analysis (i.e. for calculations of seed dispersal distances and seed shadows). In general, the seeds can be retained in the bat's gut for over 20h (n = 5). On average, a fully seeded dropping (with a score of 25 seeds and over) can stay in the gut for 6.6h (± 8.04 SD, n = 5) and smaller numbers of seeds can be retained for over 15h (4 seeds ± 6.3 SD; 15.21h ± 6.28; n= 5).

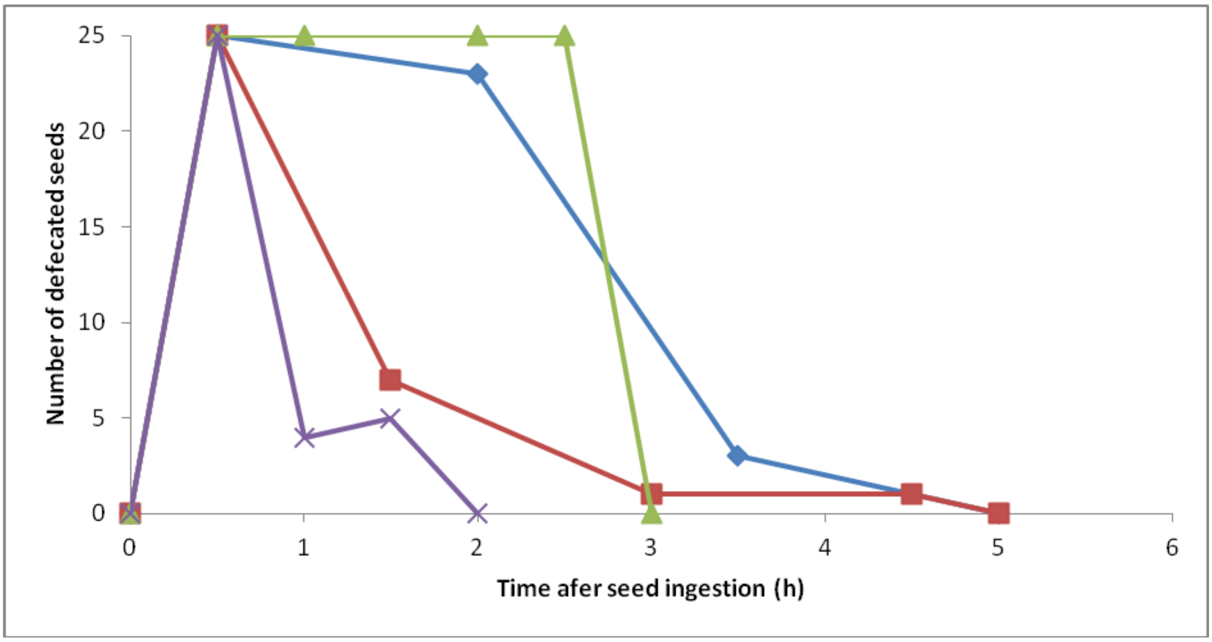


Figure 5.- Seed retention time in four individual bats. The Y axis represents number of fig seeds present in the faeces, the maximum of 25 refers to a score of '25 and over'. The X axis represents time since the last feeding of a bat (seed ingestion).

The foraging behaviour of bats influences their ability to disperse seeds. A case study (Fig. 6) reveals that a bat can disperse seeds from three individual fig trees in different directions. The majority of seeds would be dispersed under the feeding tree or at the subsequent feeding site. However, some seeds will also be dispersed during flights to and from a feeding site. A bat can travel as far as 10km from the food source within only 12min (Fig.7.). The maximal achieved distance was 19km and this distance can be covered within 84min. On average a bat will deposit the seeds within three to five km from the seed source.

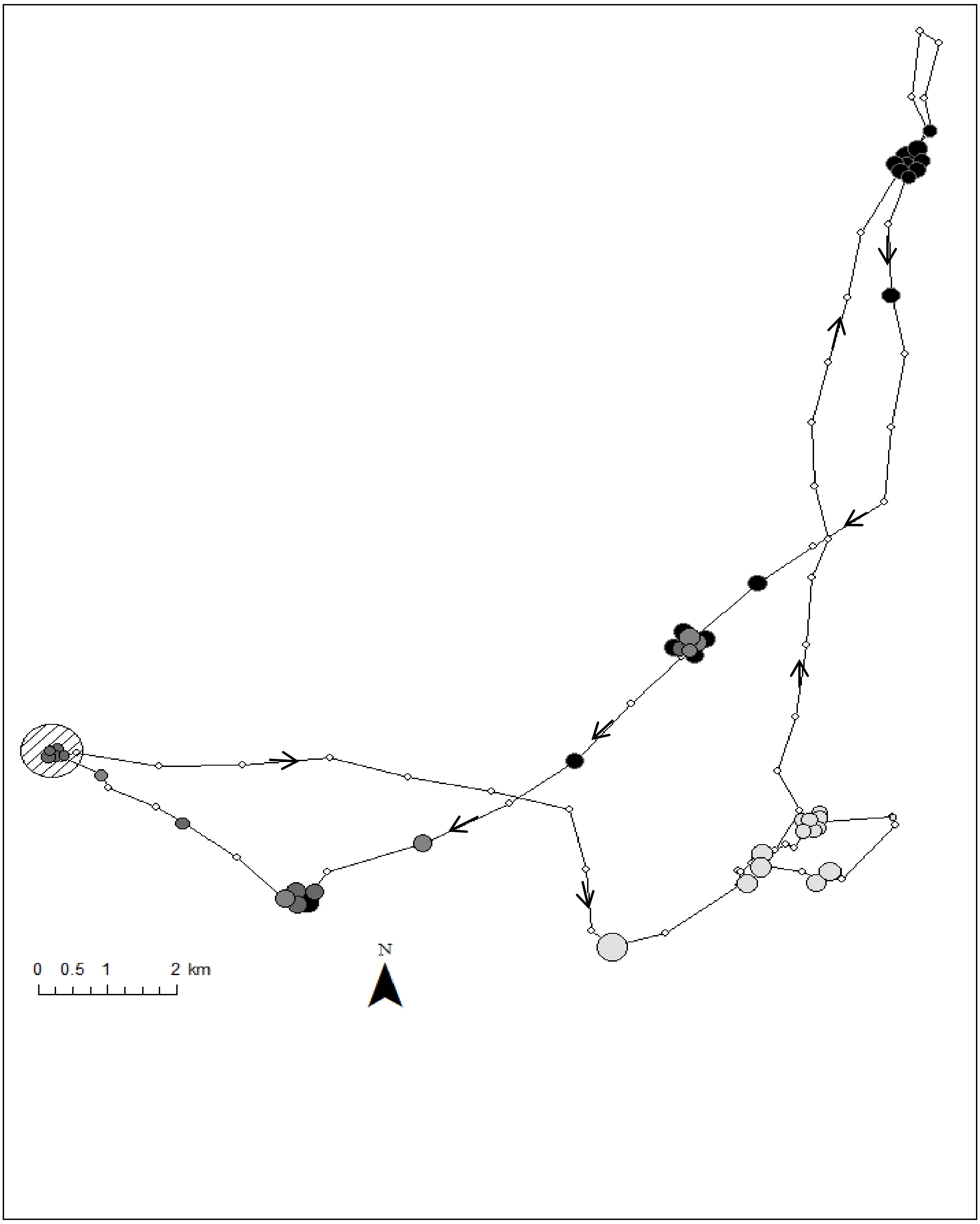


Fig.6. – Seed dispersal of bat 2406 during one night of foraging. The differently shaded circles represent seeds shadows coming from individual fig trees (the size of the circles differs in order to show their number). Small open circles denote fixes. The shadows where drawn every 12 min since the start of feeding and continued to up to 3h since the bat stoped feeding and left the feeding site (refer to Fig. 5 about seed retention time). The patterned circle represents roost site and arrows denote the flight direction.

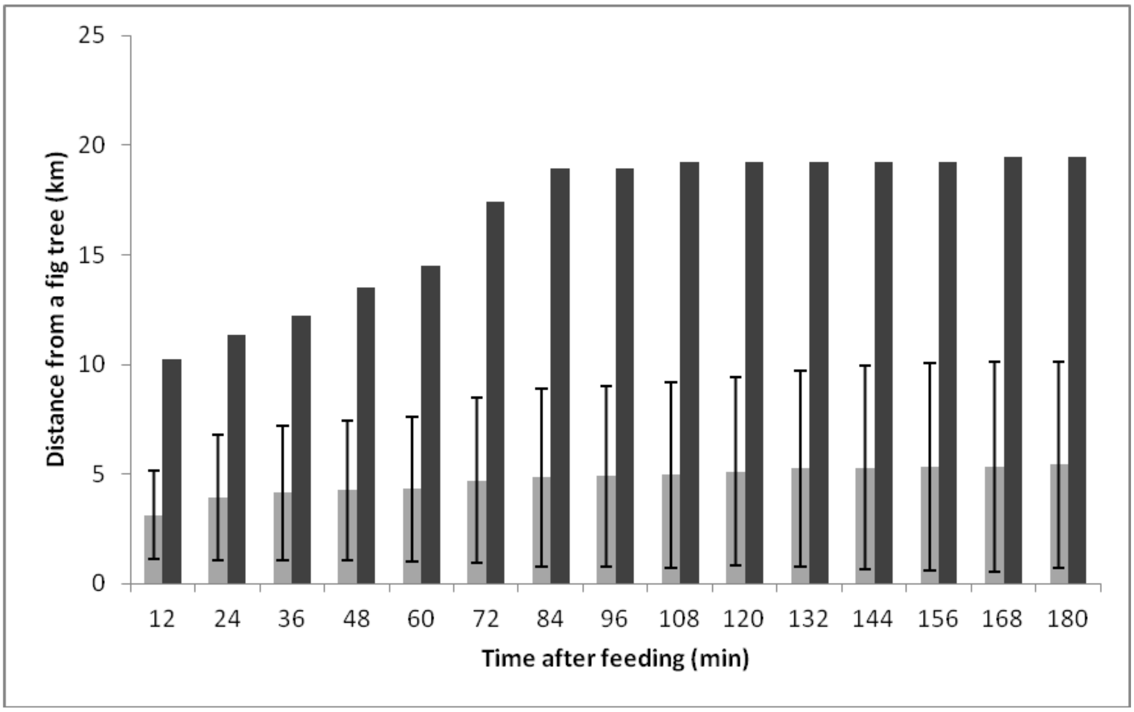


Figure 7. – Distance travelled by *P. rufus* from a fig tree for up to 3h after feeding (measured every 12min). The dark colour represents maximum recorded distance and light colour represents the mean with the standard deviation (n = 71). The data come from the whole tracked colony.

Discussion

Seed germination

The results of this experiment showed that bat-processed seeds germinate significantly better than those extracted from ripe fruits only when sown on filter paper. This may be due to the fact that small amounts of material potentially remaining from the bat faeces on the seeds may act as fertilizer and promote germination (Heer at al., 2012). Control seeds in this case benefit only from the moist environment which has not much to offer in terms of nutrients. Taking into account that bats would defecate their seeds while in flight and deposit them not only on the ground but also on vegetation, the manure around seeds may increase the chances for germination and seedling establishment. The sterilization of these seeds slightly decreases their success which may be caused by changes in the microflora. Microorganisms may affect germination of seeds as well as their mortality (Robertson et al., 2006). Therefore alcohol-washed seeds may lack beneficial organisms which otherwise would promote germination. The procedure may also wash faecal material off the seed. In contrast, the sterilization of seeds extracted from the ripe fruits increased their germination success. This could be because harmful pathogens benefit by growing on fragments of fruit material that remain attached to the seeds. A similar pattern was observed in my previous study (2011, with *Ficus polita*), indicating that sterilization of seed may increase germination in seeds from fruits and decrease the germination success of bat-processed seeds.

Increased germination in sterilized control seeds may be caused by alcohol which can soften the coat around the seed, break the dormancy and promote germination. On the other hand, the coat may protect the seed from osmotic changes which could be caused by the alcohol wash, and from direct contact of the seed shell with the solution. Both cases may explain why the two groups of the same seed species react differently to that treatment. Nonetheless the seeds which are dispersed by bats over long distances benefit from the pulp extraction and fertile manure which also keep the moist in. Furthermore, *F. grevei* is a strangler tree which will initiate its germination on bark of another trees and then over time send its roots to the ground (Dalecky et al., 2003). Dispersal by bats increases opportunities for the seeds to be deposited on other trees and manure provide a good start for germination. Therefore, the soil substrate experiment may not be relevant to the ecology of this plant.

In the treatments with soil in, there was no significant difference in the number of germinated seeds regardless of whether they were bat-processed, sterilised, or whether invertebrates were removed from the soil. Both seed types (processed and control) had much lower germination success when compared to the filter paper treatment.

The seeds which were sown on the ground showed no success in germinating. This was because all of them got eaten or carried away by invertebrates, mostly ants which were seen to carry the seeds away. The ants may bring several advantages to the seeds. They help disperse accumulated seed shadows from bat droppings (Kaufmann et al., 1991), arboreal ants may carry the seeds to their nest which provide anchorage, and a moist and nutrient rich environment (Kaufmann et al., 1991). Therefore, epiphytic seeds dropped on the ground may still have chance to be transported onto a tree with desirable growing environment.

Habitat preferences

The home range for the whole colony is composed mainly of spiny forest (intact or degraded) which does not provide any food resources for the bats. Thus, the bats focused their foraging on sisal plantations and in small patches of native forest scattered along the river.

Habitat ranking showed that *P. rufus* at Berenty prefers overgrown sisal plantations. Although this type of habitat is relatively limited in the area, it brings several advantages to the bat. The habitat is a mixture of shrubs, trees and sisal plants. Native plants in unmanaged sisal plantation probably arrived as seeds dispersed by birds and bats, some plants were invaders from adjacent spiny forest. Strips of vegetation were also left in the plantation to protect the sisal from strong winds. In all cases, the presence of other vegetation, especially trees provides resting places and protection for bats. Additionally, fruiting tamarind trees (*Tamarindus indica*) are present in overgrown sisal plantations, and sometimes as single standing trees in the managed plantations, and these provide food for the bats.

Gallery forest was the second most highly ranked habitat selected by the bats. This is most natural and productive habitat available in the area and a roosting place for bats. An abundance of tamarind trees and at least four large fig (*F.* grevei) trees provides food resources during the dry season. Bats also visited several remnant forest patches in the area hence promoting seed exchange to and from these patches.

In their review, Shanahan et al., (2001) indicate that among mammals, some bat species in the family Pteropodidae are among the major consumers of figs. They largely contribute to long-distance dispersal of fig seeds and the recruitment of new trees in the forest as well as in isolated areas. Observations from this study support their statement. In their research in Madagascar, Martin et al.,

(2009) found that the activity and species richness of birds was significantly higher in open areas where isolated trees were found, especially *Ficus* spp. compared with areas with no trees. Those birds were mainly frugivores capable of dispersing seeds. My results raise the possibility that bats may act as 'stepping stones' for regeneration of forest. The GPS data indicate that tagged bats frequently crossed cleared and agricultural fields. Taking into account their ability to disperse seeds while flying, bats may be key species for recruiting new trees to open areas and small forest patches that are unattractive to any other frugivores. According to Martin et al., (2009), such isolated trees may attract many potential frugivorous birds which will then in turn enrich the soil bank by bringing in seeds from even more plant species. My results support the hypothesis that bats promote the dispersal of seeds in cleared areas and additionally, that they may provide new feeding grounds for other frugivores and thus ultimately promote regeneration of forests.

Sisal plantation was significantly selected in relation to its availability and thus was important for bats, especially as it is the dominant habitat in the area. The plantation (30 000ha) brings to bats easily accessible food sources available all over the year. The Berenty plantation was established in the 1930s and the sisal plants are exotic to Madagascar, originating from the New World. Foraging bats would spend significant amounts of time feeding on sisal nectar and pollen. Sisal flowers produce their pollen and nectar at night and have a strong musty smell which encourages bats to visit (Long and Racey, 2007).

Agricultural trees were also used by bats. They were usually in form of hedges which separated individual land patches and water bodies. This habitat was equally selected with water bodies, perhaps because both habitats are usually adjacent to each other and bats may fly over water to reach a tree. The trees include cultivated species such as mango and avocado, but most importantly fruiting tamarind trees and figs. Agricultural land ranked equally with open areas was avoided by bats. The use of these habitats was lower than expected from their availability. Both habitats are largely treeless, though may have included single trees which were too small or hidden to be detected in my habitat mapping, but which were still visited by bats on some occasions. The spiny forest was the habitat least selected by bats as it does not offer any food sources.

Dietary analysis (Long and Racey, 2007) showed that *P. rufus* in Berenty mainly feeds on sisal pollen and nectar (56%) and tamarind fruits (39%). Results of my study confirm that *P. rufus* bats at Berenty prefer to forage in habitats in which both these food sources are most abundant.

Home range and foraging behaviour

The home range of tracked bats varied greatly. Some bats preferred to feed in nearby sisal only and showed strong fidelity to their feeding sites. This used small home ranges presumably because they had no need to explore other resources. Bats with the largest home ranges were foraging for other food sources, most likely figs as no other fruits were available to them during the study (apart from tamarind abundant close to the roost site). Nonetheless, each of the bats fed to some extent on sisal every night usually in specific places to which they returned regularly. When I was capturing bats feeding on sisal, I observed that single bats flew over the area in a 0.5h - 1h intervals. This suggests that the bats are widely dispersed over the plantation and that they do not interfere with each other, a suggestion supported by the general lack of overlap among the foraging areas of bats. The abundance of sisal flowers on the plantation and its large extent provides plenty of resources for the colony.

Gut retention time and seed dispersal

Average food transit time in *P. rufus* takes around 12min, assuming droppings are produced at equal intervals during my inspection times (every 30 min). If all droppings were produced near the end of the time between inspections, transmit times may be closer to 30 min. Either way, it is clear that the transit time can be short. As explained in the methods, a knowledge of seed transit times is necessary to conduct further analysis regarding how seeds may be dispersed in the landscape. The transit time I measured corresponds with previous findings where food transit time in fruit bats ranges between 12 and 114min (and is usually less than 30min) (Nakamaoto et al, 2009; Tang et al., 2007; Traveset, 1998; Fleming and Heithaus, 1981). However, seeds can be retained in the gut for over 24h. Long retention times were also recorded by Shilton et al. (1999) for *Cynopterus sphinx*, where seeds were retained for more than 12h and 18h. Generally, bats can disperse larger quantities of seeds over periods of <3h since eating (Fig. 5), but smaller numbers of seeds may be retained for as long as one day. Given that the bat can travel as far as 10km within 12min directly from the feeding site, it makes *P. rufus* a very efficient as a long distance seed disperser. The average distance that a bat would fly from a feeding site is 3km to 5km. However, this only considers journeys up to 3h after eating figs. Taking into account that bats can retain small amounts of seed for a day, that gives the potential for a small number of seeds to be dispersed over much greater distances and in a wide range of directions.

Conclusion

The results of this study supports the hypothesis that *P. rufus* is an important seed disperser in Madagascar. The seeds dispersed by these bats show an ability to germinate with higher success than a control group, at least in exposed conditions. Bats can travel long distances within relatively short periods of time. Therefore, even with very fast gut transit times, they can be successful dispersal agents in the tropics. Their ability to retain seeds for several hours ensures that a few of the seeds may potentially be dispersed far from the parental tree. Movement behaviour of those bats shows small overlap within feeding sites of individual bats. This also benefits seed dispersal as each bat creates individual seed shadows on its paths. The rank of the habitat used by bats shows their ability to travel within cleared areas and thus contributing potentially to the regeneration of forest fragments.

This study provides valuable insights into bats' feeding behaviour and there are still several aspects to be analysed. More analysis will be conducted to construct landscapes to identify hotspots of seed dispersal in the study area. The speed of flight and travel distances are being measured. More attention needs to be paid toward fragments of gallery forest in bats' home range and frequency at which bats visits these sites. Nonetheless, the presented results indicate that bats can contribute to forest regeneration and maintenance in Madagascar. In addition to the studies on seed germination and GPS tagging described, I have completed an analysis predicting the distribution of *P. rufus* in Madagascar by using presence-only modelling, and projected how distributions might change under climate change scenarios. This will form a chapter of my PhD thesis.

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