METHODS

Practical solutions for capturing and processing Grey-headed Flying-foxes, *Pteropus poliocephalus*, based on a camp study at the Royal Botanic Gardens, Sydney

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ABSTRACT

Grey-headed Flying-foxes can be difficult to capture and process in sufficient numbers for population studies, and here we describe a successful method to do both and evaluate its practicality. Over the year 2006/07 (24 nights) we captured and banded with ABBBS bands 466 flying-foxes from the Royal Botanic Gardens, Sydney. Depending on weather conditions and net orientation, between 8 and 53 bats were captured per session as they returned to the roost site in the early morning. Animals were captured using a 12 m long mist-net on pulleys attached to two 13.2 m tall aluminium masts, individually assembled from 6 smaller poles. The poles were relatively light but required 4 people for safe net assembly.

Data were obtained from 259 processed individuals, except juveniles, heavily pregnant females, and females with attached young, which were banded and released immediately. We anaesthetised each individual and recorded standard morphometric measurements. Pollen, faecal, and tissue samples (blood, membrane puncture and a tooth) were collected, and 6 animals were fitted with radio collars. The processing lasted within 10 minutes/animal and bats generally recovered from the anaesthetic within an hour. When fully alert, each bat was released back into the camp by flying it across a lawn to the roost trees. No casualties resulted from capturing or processing the flying-foxes, and no processed animal was subsequently found ill or dead as a result of this study.

Key words: *Pteropus poliocephalus*, flying-fox, capturing techniques, mist-netting, intravenous anaesthesia, tissue sampling, tooth extraction.

Introduction

The limited ecological knowledge of flying-foxes in the wild is partly due to the animals being difficult to capture. Australian flying-foxes aggregate and roost in large numbers at camp sites, but live high in the canopy of vegetation such as rainforest (with a dense canopy cover) and seldom fly close to the ground. Therefore, the logistics of finding an efficient and safe method for trapping the desired sample of individuals have been challenging. The site characteristics (e.g. vegetation structure, height of roosting, road access, slope and general accessibility of the site) differ between camps, and an additional problem is that the methods applicable to a site might be difficult to implement at another.

Past techniques involved shooting the desired number of individuals in the wild (Towers and Martin 1985), a technique used as recently as 1996 (e.g. Crowley and Hall 1994; Sinclair et al. 1996). Additionally, earlier attempts included sedating the bats using a dart gun (P. Eby, pers. comm. 2007), but problems arose with respect to a suitable needle size, the amount of anaesthetic administered, and the location of the bat. The latter was an issue due to the bats' reflex action of clinging onto the branches, rather than falling down once sedated. Since then, several less harmful approaches have been implemented. One of the earliest, somewhat successful methods, involved shaking the animals down from their roosting trees (Spencer et al. 1991). The disturbed bats would fly off, and generally settle lower into nearby trees. The procedure was repeated until the bat was on a branch within reach (K. Parry-Jones, pers. comm. 2007). This technique was highly laborious and yielded comparatively little success (1–9 bats were captured in combination with a netting technique; Spencer et al. 1991).

More recently, the invention of the large harp trap (Tidemann and Loughland 1993) has changed the ecological studies of flying-foxes. The trap has been highly successful with the ability to capture over 200 animals in a session (Tidemann and Loughland 1993). Studies that used this harp trap often omit to report the trapping success per session (e.g. Webb and Tidemann 1996; Vardon and Tidemann 2000), but based on their total numbers of animals captured and the number of reported

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sessions, the average appears to be approximately 20 bats/night (e.g. Sinclair et al. 1996), an effective return on effort for megachiropteran studies. However, the installation effort and the likely cost of the equipment are high – even a standard harp trap for microbats costs generally between AU$1,200–1,500 without carrying bags and spare kits (e.g. Faunatech Austbat 2006). Additionally, the harp trap is a large (16 × 14 m) permanent structure and it requires a number of experienced personnel to operate.

Two alternative methods allowing one person to trap the animals were recently described and implemented by Welbergen (2005). The first method incorporated a noose device – a series of aluminium poles adding up to 15 m in height, with a hollow Y-shaped cord-operated fork at the end. The researcher stands underneath the bat, extends the pole, and traps the animal's neck in the fork. The pole is then gently moved until the bat lets go of the tree branch and is then brought down vertically. Although the technique permits the deliberate selection of bats captured in a study, and perhaps even a random sample of those present (with a great deal of planning), the noose-device will still be limited to the animals within reach, as well as the logistics of balancing and controlling a 15 m long pole within a dense canopy. The 2nd method Welbergen (2005) used was a large 15 × 4 m mist-net positioned in the 30 m high tree canopy. A nylon line allows the person to pull the net up or down the length of the steel cable from which the net is suspended (Welbergen 2005). This technique would be beneficial at camp sites where the trap does not have to be dismantled on a daily basis, and it would be dependent on the tree canopy to achieve the desired height and position. Despite the potential limitations, using the 2 methods, a total of 257 individuals were captured over 3 December–July seasons in the north-eastern corner of NSW (Welbergen 2005), but the number per capturing session, or hours of effort, were not reported.

Other mist-netting techniques involve a mist-net being strung between ropes (suspended from trees or light, usually bamboo, poles) on pulleys at variable height (typically under 11 m). A set of 2 or 3 nets at different locations can be operated at a time, with each net being checked 2–3 times per night (e.g. Fisher and Tasker 1997). The limitation of such systems in the past was that animals were trapped from feeding locations (i.e. a small number at a time), and the nets were not controlled at all times (with a range of species being trapped). Large number of net hours yielded relatively small number of desired trapped animals (e.g. 11,128 m^2 mist-net hours and 500 bats of which only 30 were the study species; Fisher and Tasker 1997). Similar results were achieved when using continuous mist-net walls created by stacking up to 9 mist-nets together from the ground levels up into the canopy (Hodgkison et al. 2004). Although this method can be useful for testing possible vertical stratification of flying-foxes, the capture rates remain relatively low (e.g. 352 bats of 8 species were captured during 72,306 m^2 mist-net hours; Hodgkison et al. 2004).

The location of the present study was a camp within the Royal Botanic Gardens, Sydney (33°50 'S, 151°13' E), which required equipment that was temporary, easily assembled and transported, and required the minimal number of personnel. No modifications to the site (i.e. clearance of the vegetation), nor the use of trees to suspend the net from were permitted. At first, we used a set of 11 m tall, round aluminium masts, designed by C. Smith. This set-up was previously very successful in other camp sites and for trapping other Australian flying-fox species (e.g. up to 35 Little Red Flying-foxes were trapped per session in a Northern Territory study). However, the height of these poles was inadequate in the current study and our initial trapping success rate was low (on average 7 bats were captured per session during 11 trapping nights, and a maximum number of bats captured per session was 13). Extension of the poles required a number of modifications, particularly since 11 m appears to be the height limit of the round light-weight aluminium (due to bending of the poles during set-up). Our main aim was to modify the existing mist-net design, keep it relatively inexpensive and portable, yet successful and widely applicable for flying-fox trapping. Additionally, we report our study with the aim of pointing out the benefits and encouraging collaborative work for the collection of samples from these relatively difficult to capture animals.

Methods

Mist-net assembly technique

Masts are made of 6 interlocking 2.2 m long, 32 mm square aluminium (3 mm wall thickness) hollow poles, giving them a total length of 13.2 m. The combined weight of the masts is <14 kg, making the design easily transportable by hand into a camp site. The sections are joined together by aluminium inserts (300 mm; 150 mm for the base pole; Figure 1) secured into the lower part of each section with locking pins. During the assembly, the inserts are wiped and coated with silicon-based lubricant (or dry, Teflon-based lubricant in sandy environments) to prevent scratches and jamming of the sections. Two screws are placed into each joint at right angles to each other to secure the poles together. The sections of both masts are interchangeable, providing extra flexibility to the design, and the ability to make the masts shorter if required. The base of each mast is hinged with a narrow base plate that is anchored to the ground with 4 steel pegs. Masts are assembled on the ground (Figure 2), requiring a narrow clearing of approximately 14 m (i.e. the length of the masts). Three guy-ropes (4 mm diameter, strong double-braided yachting rope) are pulled through the hooks placed on top parts of sections 2, 4, and 6 each, making a total of 9 guy-ropes per mast. Additional rope is run down the length of each mast and suspended on 2 pullies for later control of the net. The ropes on the pulley system should not exceed a 6 mm diameter (double-braided yachting rope), otherwise their weight makes it more difficult to operate the net.

Masts are raised one at a time with one person pulling the upper and middle guy-ropes at the back, while the other person walks the pole into the upright position. Two additional people are needed to stabilise the pole by pulling the upper and middle guy-ropes on the sides once the mast is beyond the 45° angle from the ground. Once erected, the mast is stabilised using 9 guy-ropes (from one another) and secured to the ground with steel pegs. The 2nd mast is erected in the position determined by stretching the net from the 1st standing mast. Any commercially made Australian Bird and Bat Banding Scheme (ABBBS) approved mist-net (we have used a 12 × 2.7 m mist-net with 31 mm mesh) can be placed between the masts, provided there is enough clear space at the trapping site. Disassembling the set-up is managed by reversing the described process.

For safety reasons, the masts should not be erected in strong winds (averaging >26 knots) and/or severe thunderstorms. However, once it is set up, the design appears to be very stable (Figure 3). During our study there was an accident with a four-wheel-drive vehicle running into the back guy-ropes at, at least, 40 km/h. The mast was bent due to the impact force, but still standing, supported by the side guy-ropes. The bent sections were straightened, but the nature of the material is such that the sections can be straightened a limited number of times before they become permanently distorted.

Operating the mist-net

Operating the net requires at least 2 experienced personnel, but having 4 people improves the trapping efficiency. The net is operated by 1 person standing at each mast and concurrently raising (and lowering) it by sliding the ropes attached to the pulley system. When a bat flies into the net, its forward movement is gently eased by the stretch in the net fabric. It is important that there is an element of looseness in the fixed net and that the operators react quickly, otherwise the bats will bounce out of the net without being entangled. The net is then lowered until the flying-fox is approximately 1 m

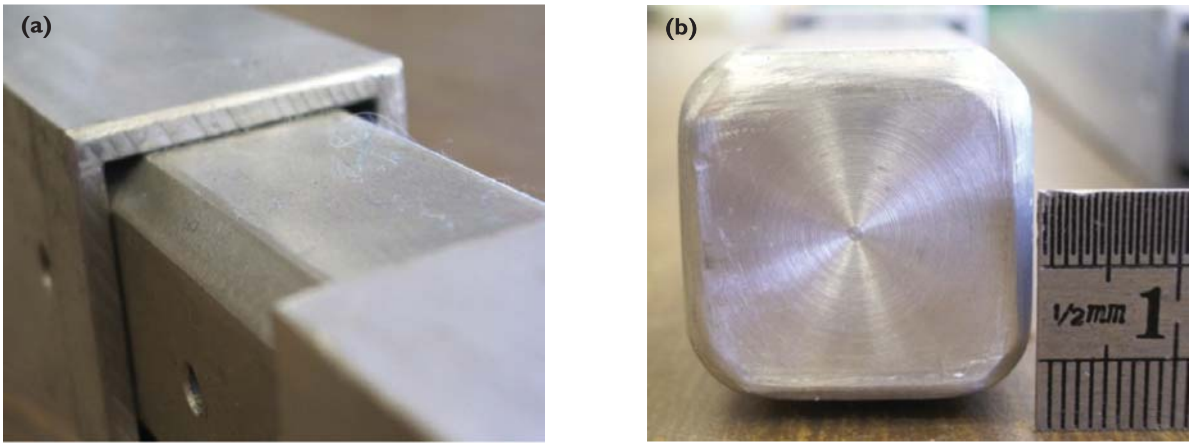

Figure 1 Aluminium inserts used to join the mast sections: a) the inserts have been machined from a round pole and have a very close tolerance fit ('snug fit') with the hollow poles to prevent any movement when the masts are erect. The corners are rounded to prevent binding between 2 mast sections. The screw holes have a narrow end, which provides access to the screw in case of it breaking inside. b) Frontal view of the insert.

Figure 2 Mast assembly on a narrow horizontal ~14 m clearing. The sections of both masts are joined together on the ground at the same time, and all ropes are pulled through the appropriate sections. The overall procedure, including the erection of the masts takes approximately 1.5 h and should be done under well lit conditions.

Figure 3 Mist-net strung between two 13.2 m aluminium masts, at the height of the tree canopy.

above the ground (Figure 4). The net operators should move in towards the animal on the same side of the net as the bat flew in from, while stretching out the net to keep the tension on it. This tension allows the bat to orient itself and start moving up the net. At the same time, a 3rd person, wearing welding gloves, runs to the animal and physically restrains it by holding the base of the head and placing the wings against the body. Flying-foxes are capable of inflicting severe bites to handlers, and wearing thick gloves and firmly restraining the head are imperative. Once the animal is under control, the handler can generally pull it out of the net without assistance. However, if the animal is entangled in the net, one of the net operators disentangles it (usually in <1 min), while the other prepares the calico bag/pillowcase for the bat to be placed in. Disentangling the bat is often aided if the net is held very loosely, rather than the bat being pulled away from it, thereby allowing it to grip the net tightly with its toes. Flying-foxes feel secure if they have something to hold onto, and the loose net will make them open up their toes in search of something secure to hold onto. When 2 flying-foxes are on occasion captured at the same time, the less entangled animal is removed first, before moving to the 2nd bat. In such instances the 3rd person restrains the other flying-fox to minimise further entanglement. After quickly recording the capture time and sex, each individual is also examined for any signs of capture-related injuries (not seen to date), or degree of pregnancy in gravid females. Individuals not needed for the study (e.g. other flying-fox species in a mixed camp, or birds trapped after sunrise) or juveniles, heavily pregnant females and those with young attached, can be banded if needed and released immediately to reduce their stress. Other captured flying-foxes are placed individually in a pillowcase and tied to a suspended pole in the order of capture. At the same time, the net is raised to the top by the 2 operators and the process is repeated for succeeding bats. The optimum number of animals that can be processed by a team at the conclusion of the capturing session is approximately 25–30.

Figure 4 When a flying-fox hits the net, it is lowered to approximately 1 m off the ground. The animal is immediately disentangled and placed in a calico bag/pillowcase.

Handling and processing techniques – an example of comprehensive data collection

In the morning, the flying-foxes were processed in the order of capture (with the exception of females in their early pregnancy or late lactation, which were processed first) within approximately 3 h following the mist-netting. Each animal was weighed still in a pillowcase to the nearest 5 g using a 1.5 kg Pesola spring scale. The right wing of a flying-fox was then exposed from the bag, and the anaesthetic Alfaxan-CD RTU (0.20–0.50 ml/kg; we used between 0.20–0.34 ml) was injected intravenously at a slow rate (10–20 seconds), to reduce a chance of apnoea (Figure 5). Animals were expected to fully recover within an hour past the induction.

Figure 5 The bat is anaesthetised by intravenous injection of Alfaxan-CD RTU. The vein running along the leading edge of the wing is easily accessible, however, vein constriction was observed under cold weather conditions.

Once anaesthetised (typically within a minute), flying-foxes were described in terms of species, sex, reproductive status and approximate age. Any additional comments about the animal (e.g. injury, description of female nipple size and condition) were also recorded and photographed at times. A numbered metal size 7 band (recommended size for *P. poliocephalus* from the ABBBS) was placed on the left thumb of each processed bat. Further measurements, including the right forearm length, thickness of the right maxillary canine, the maxillary intra-canine distance, and the length and width of right testis in males were also taken. Sample collection included tooth, blood, tissue, pollen and faecal material. The 1st premolar in the left lower jaw was extracted from each individual for ageing (Divljan *et al.* 2006; Figure 6). This is a small, at times missing, shallow-rooted tooth that lacks its complement in the upper jaw, suggesting that there should not be any major complications to the bats once the tooth is removed. The size of the root of this tooth (~1.5 mm) ensured an easy extraction using a dental elevator and pliers, usually in <1 minute. The small hole left by the tooth removal was filled with 0.05 ml of Lignocaine gel (local anaesthetic and pain relief) and the bat was subcutaneously injected with 0.1 ml of long-acting antibiotic (Amoxycillin) to reduce the chances of possible infection.

Blood (maximum 2 ml) was collected from the left wing vein using a fine needle (25 gauge). In addition, tissue samples (wing membrane) were taken with a biopsy punch by puncturing two 3 mm diameter holes in the right wing. The wing was extended and placed over a hard, flat surface (a plastic board) and the wing area used was a small, non-vascularised surface, resulting in no bleeding. To ensure fast healing of the membrane, macadamia oil was rubbed into the edges of the puncture – a procedure used by wildlife carers to aid the recovery of wing injuries in rescued flying-foxes (K. Parry-Jones, pers. comm. 2007). The actual punctures had no effect on the flying ability of the bats, and we have often seen puncture holes (from biting and other injuries) at various stages of healing. Both the blood samples and 100% ethanol-preserved membrane samples were stored in freezers until further use.

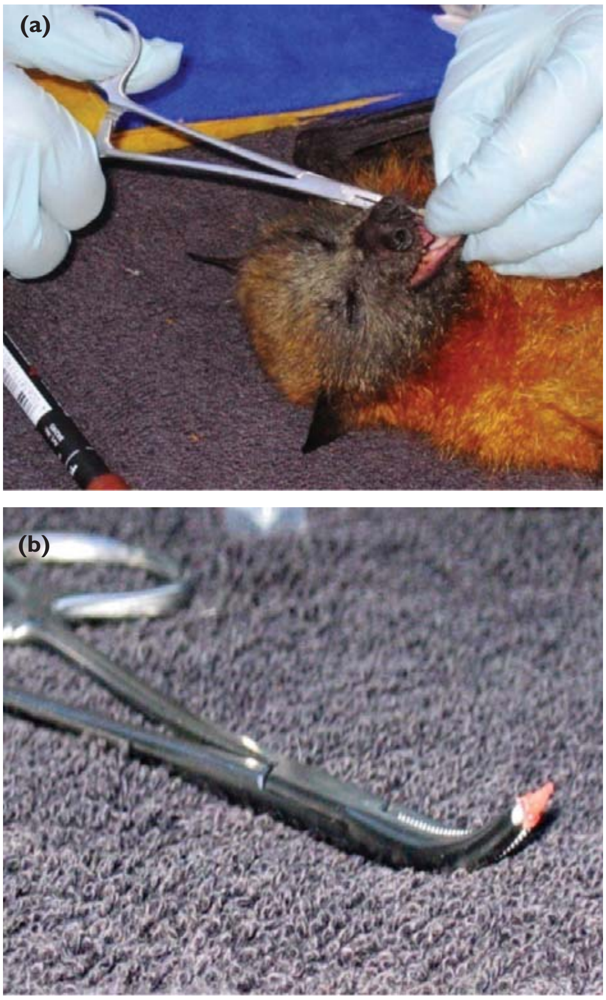

Figure 6 Tooth extraction; **a)** Left 1st premolar being removed from a live anaesthetised animal. **b)** Removed tooth (~2 mm) used for ageing.

For purposes of dietary studies (Burton 2006), pollen samples were collected from each bat's snout and head area using a sticky tape, which was then placed on clear microscope slides. Additionally, any faecal material found in the pillowcase, or on the bat was also collected. In rare instances of finding ectoparasites (wingless flies from family Nycteribiidae), they were collected and stored for future research. Six captured animals were also fitted with radio collars and detailed descriptions of the data are presented elsewhere (Burton 2006).

Each animal was processed within 10 minutes post sedation, after which it was placed in a warm bag and observed for signs of recovery (usually within 40–60 minutes). The bats were then placed back into pillowcases and left to hang in a quiet area for up to 2 h, while we processed the remaining individuals. This holding period is advised to ensure that the anaesthetic has worn off and the animals can thermoregulate properly (particularly in winter studies).

Release procedure

Animals were returned to the capture site and released from hand within a maximum of 8 hours following their capture. We used the bat's alertness and ability to fly well as a direct indication of post-anaesthetic recovery. In the majority of cases, no immediate signs of any adverse effects of the handling and processing procedures were observed, and bats flew off to the nearby trees. However, there was a small number of animals ($n < 10$) which landed on the ground. These were animals that were processed last and as a result possibly did not have sufficient time to recover fully. In these instances they were placed back into pillowcases and released successfully after further 30 minutes.

Results and discussion

Trapping success of the mist-net technique

Capturing live flying-foxes depends on a large number of factors, which can be broadly classified into time-, location- and animal-dependent factors. These can co-vary, making it often difficult to isolate and control the specific aspects to improve trapping success. Nonetheless, we have tried to outline the optimum conditions for the mist-net technique.

As far as time is concerned, animals can be trapped either on fly-out (when they leave the roosting site in search of food), at feeding grounds, or in the early morning when they return to the camp. The main advantages of capturing bats in the morning at roost sites are that the animals have fed, and large numbers of individuals are present, at least when compared to the feeding grounds. In addition, collection of samples may depend on the trapping times – pollen and faecal samples, for instance, are most easily obtained from fed animals in the morning. In our experience, majority of flying-foxes start to return to the camp about an hour prior to sunrise, and we observed several seasonal and individual-based patterns. Some adult males were generally present at the site at the start of the trapping session (2.5 hours before sunrise), probably as a result of the proximity of their foraging grounds and possibly territory defence. However, when their energetic needs increased just prior to and at the onset of the mating season, they too returned later with the females. The trapping success of juveniles increased as they became volant in summer months (late November–February). At first, the young remain in the camp and practice flying between trees while their mothers forage. Consequently, in December–January, over 50% of trapped juveniles were captured within the first 1.5 hours of the sessions. This number decreased with time, and by April, when young were foraging independently, close to 80% of juveniles were trapped later in the sessions (Divljan, 2008).

Monthly moon phase and weather patterns further affect the ease of trapping animals. The net becomes more visible at times of the full moon or increased cloud cover, when the city lights are reflected into the atmosphere illuminating the site. In such instances, we experienced that mostly animals that flew at relatively high speeds, unable to swiftly change their direction, were trapped. Others made a loud noise and avoided the net, and often inquisitively circled several times around it. The clouds can have a positive effect on the trapping success, as well, as we observed that the bats generally flew lower when it was overcast, reducing the height of the masts as a limiting factor.

In addition, every camp is unique and the access to good trapping locations within a site is sometimes limited. It is important that the mist-net design is flexible enough to allow both an easy transport of the gear to the location and its assembly on site. In cases where the canopy and the height at which the animals roost allow it, a simple set-up in which the net on the ropes is suspended from the trees might be the optimal solution. If, however, the site conditions require the use of the masts, we believe that the current design is approaching the height limit of a flexible set-up. We have made the masts sturdy, and relatively light, but we failed to raise them (using 4 people) when we added another 2.2 m section to the existing 13.2 m. Taller design would also require larger diameter of the sections to support the structure and this would compromise the weight and the portability of the set-up.

The net needs to be set up against a dark visual background, usually tree canopy, otherwise the bats tend to avoid it. For these reasons it might not be profitable to set the net directly perpendicular to an open fly-in path. Changing the net position in the weekly- or monthly-based trapping studies is not necessary, as the recapture rates appear to be extremely low (only 2 adult animals were recaptured during this study and 3 juveniles flew into the net twice in the same night). However, during a short, more intensive trapping effort (daily sessions), we recommend the net position be rotated regularly (every 2–3 nights) within the camp site. Otherwise, flying-foxes become accustomed to the net position and increasingly avoid it. Finally, the flying speed of animals appears to be an important factor. Fast-flying individuals, heading for their roost site, do not seem to have time to react and avoid the net, and it is often these animals that get trapped. Also, it is possible that younger, inexperienced bats lack the manoeuvring skills, increasing their chances of being captured.

Field use of the described design (13.2 m masts) resulted in n = 381 animals being trapped over thirteen 3-hours-long sessions (July 2006–April 2007, averaging just over 29 animals per session). The trap may be suitable for other flying-fox species in future ecological studies. The cost of the design is affordable at approximately AU$1,300 for materials.

Handling and processing techniques

The processing techniques in our study varied and were modified with time, depending on the questions and opportunities that arose during the trapping season. The main premise was that the flying-foxes are difficult to capture and we wanted to use the opportunity to ensure adequate sampling and possible collaboration with scientists outside the scope of our immediate research (which utilised only the descriptions, measurements, and tooth samples from each individual).

Additionally, our primary aim was to minimise the stress caused to the animal, whilst obtaining full information. Alfaxan-CD RTU sedated the animals in under a minute without any complications. It was the anaesthetic of choice due to its ease of application under field conditions, the fact that it provided access to the mouth of the animal (compared to the gaseous mediums) and its safety and reduced side-effects compared to the widely used Ketamine/Domitor cocktail (Heard, et al. 1996; M. Holdsworth, pers. comm. 2006). Alfaxan-CD RTU is a safe injectable steroid anaesthetic used in small animals either as an induction agent prior to the gaseous anaesthesia, or as a sole anaesthetic agent. It can be induced either intravenously or intramuscularly, and subsequent additions of up to a total dose of 1.2 ml/kg can be administered (Jurox Pty. Ltd. 2003). The alternative highly successful anaesthetic agent for flying-foxes is Isoflurane (Laser Animal Health Pty. Ltd.), a gas administered via a face mask (Jonsson et al. 2004). Its main advantage over Alfaxan-CD RTU is that animals recover from the anaesthetic within minutes. However, when working in remote locations, injectable anaesthetic agents can be more portable and practical option (Sohayati et al. 2008), eliminating the need for oxygen cylinders and expensive set-up. The use of Alfaxan-CD RTU in this study also allowed easy manoeuvring of the animal (helpful when taking multiple measurements/samples), and the individuals recovered on their own accord, without the need for a reversal.

The animals were under anaesthesia for up to one hour and required up to 2 hours of holding in a pillowcase before being released. This long time under anaesthetic could be perceived as a disadvantage to using Alfaxan-CD RTU, however once recovered from the anaesthetic, the bats were observed quietly hanging and, at times, sleeping in their pillowcase, an indication, perhaps, of reduced stress. Although, the time under anaesthetic can be reduced to minutes with the use of Isoflurane, this does not necessarily reduce the holding period and stress to the animals. The holding period depends on several factors, including: 1) the time the animal was captured, 2) the distance of the processing station from the capture site, 3) the processing procedures and the anaesthetic used, and 4) the number of bats that need to be processed. Under remote field conditions, the size of the working team is generally minimised, and people trapping the animals are the ones to process them in the morning. Therefore, with small teams it would be difficult to reduce the holding period by processing the animals (under reduced visibility) at the same time as capture. In addition, if captured animals are transported to the processing location away from the capture site in the morning, all animals should be held until the last bat is processed and recovers before they can be returned to their site and released. In such instances the advantage of the quick recovery period using Isoflurane over Alfaxan-CD RTU is reduced, particularly if 30 animals are to be processed. Therefore, depending on the site location and general field conditions, we recommend the use of Alfaxan-CD RTU as an alternative to Isoflurane in more remote field sites, and under more restricted conditions (e.g. require tooth sampling for ageing, small team size, associated costs, etc.). In most other instances, Isoflurane should be used to reduce the handling time for the bats (from up to 8 hours to up to 4 hours, assuming that capturing session takes 3 hours and the 1st bat is processed shortly after). We also found that with a processing time of under 10 minutes per animal (regardless of the samples and measurements recorded), the optimal number of animals to be processed by a team was 25–30, and a higher number of captured bats should require 2 teams working concurrently to reduce the holding time.

We were able to process 259 individuals, and no animals died or were injured in any way during our study. Released individuals flew well to the tree canopy. Some paused momentarily, before flying off (presumably to their usual sites within the camp); others settled in and resumed their normal daily activities, showing no apparent adverse effects. We further received no information on any of the banded bats dying or being found injured as a result of our study, but unfortunately 4 individuals were casualties of electrocutions and netting.

We believe that this is one of the few projects in which a large collection of measurements and samples was obtained from *P. poliocephalus*, providing an opportunity for data analyses across the different biological disciplines. We have shown that the procedures are safe and relatively simple, and we encourage other researchers to conduct detailed data collections, particularly measurements and non-destructive samples (e.g. pollen, faecal matter) for collaborative purposes which would benefit our knowledge and understanding of the flying-foxes. However, we do emphasize the need for careful consideration for the animal welfare and ethics, and to minimise the stress to the animals by choosing the appropriate data and techniques used to collect these.

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