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The Flying Fox *Pteropus seychellensis* of Mayotte (Comoros): Method of Capture and Blood Sampling

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ABSTRACT: *Pteropus seychellensis comorensis* is the only Pteropodidae bat species on the island of Mayotte (Comoros Archipelago), and most aspects of its biology are unknown. In order to catch this large bat, we used a simple and low-cost method, consisting of raised mist nets that were set close to foraging sites. Major factors driving catch success were high food availability, good positioning of mist nets, and careful observation of movement patterns to identify foraging sites where the chances of capture are high. Blood was collected from the alar and humeral veins, which appear to be more practical for this purpose than other parts of the venous system.

Key words: Bat, blood sample, capture, Comoros, Mayotte, Pteropus seychellensis.

Mayotte (376 km²; highest point 660 m above sea level [a.s.l.], $12^{\circ}50S$, $45^{\circ}10E$), the eastern and oldest island of the volcanic Comoros Archipelago (Indian Ocean), lies 400 km to the northwest of Madagascar. All of the terrestrial mammals occurring on Mayotte have been introduced with the exception of bats: two Yangochiroptera species, Taphozous mauritianus and Chaerephon pumilus, and one Pteropodidae, the Mayotte flying fox, Pteropus seychellensis (Cheke and Dahl, 1981; Bouttemy et al., 2004). This latter species belongs to a genus that has a broad distribution from offshore east African islands to Australia including southern Asia and Pacific islands (Simmons, 2005). Pteropus seychellensis is thought to be divided into two subspecies, Pteropus seychellensis seychellensis and Pteropus seychellensis comorensis. The latter is considered endemic to the Comoros Archipelago and the island of Mafia, Tanzania (Simmons, 2005). However, recent studies have shown that both subspecies are not sister taxa, which renders the *P. seychellensis* species paraphyletic and may lead to a new classification (O'Brien, 2005).

Pteropus s. comorensis of the Comoros Archipelago has been the subject of a limited number of studies (Cheke and Dahl, 1981; Moutou, 1988; Trewhella et al., 1995; Clark et al., 1997; Lindhe Norberg et al., 2000), but none have been specifically devoted to the Mayotte population, despite its high abundance (Cheke and Dahl, 1981). The 2005-06 Indian Ocean Chikungunya virus outbreak affected 35% of La Réunion's human inhabitants (Renault et al., 2007) and the same proportion of inhabitants on Mayotte. This triggered a considerable research effort toward this disease, including an assessment of potential involvement of the local vertebrate fauna in the epidemiology of the disease. Pteropus s. comorensis was one of the target species from which blood was collected to test for Chikungunya virus and other antibodies. The aim of this paper is to present the simple method of capture and blood sampling that was used in these epidemiologic studies of *P. s.* comorensis on Mayotte.

Flying foxes were captured between 29 March and 13 May 2007. Captures were performed at two sites. The first, near the village of Coconi (45°8'15.64"E, 12°49'52.878"S; 95 m a.s.l.), was a small

Date	Site, time range	No. captured bats per sex ^a	No. mist nets, total length of nests	Sampling effort (hr/m ² of net)
2 April 2007	Coconi, 17–19 hr	10*	1, 12 m	58
5 April 2007	Coconi, 16–19 hr	29, 1°	2, 24 m	173
6 April 2007	Coconi, 16–19 hr	49, 10'	2, 24 m	173
8 April 2007	Coconi, 16–19 hr	7 <u>9</u> , 10°	3, 30 m	216
9 April 2007	Coconi, 16–19 hr	59, 4°	3, 30 m	216
10 April 2007	Coconi, 16–19 hr	29, 20	3, 30 m	216
17 April 2007	Saziley, 10–13 hr	3 <u>9</u> , 10'	2, 24 m	202
21 April 2007	Coconi, 16–19 hr	49, 30°	3, 30 m	216
26 April 2007	Coconi, 16–19 hr	29, 30°	2, 18 m	130
30 April 2007	Coconi, 16–19 hr	0	2, 18 m	130
6 May 2007	Coconi, 16–19 hr	lç	2, 18 m	130
7 May 2007	Coconi, 16–19 hr	10	2, 18 m	130
8 May 2007	Coconi, 16–19 hr	10'	2, 18 m	130

TABLE 1. Description of capture sessions for P. seychellensis.

^a φ = female, \circ = male.

plantation of introduced guava (Psidium guajava) and coconut trees (Coco nucifera) surrounded by a secondary forest dominated by *Erythrina* sp., where numerous Pteropus were sighted foraging on guava fruits. The second site was located on the seashore of the Saziley Nature Reserve (45°10′49.505″E, 12°59′4.833″S; 1 m a.s.l.), where Pteropus were found foraging on an umbrella tree (Terminalia *catappa*) and a baobab (Adansonia sp). Flight patterns and periods of activity of *P*. s. comorensis are known to be very regular (Verschuren, 1985). In addition, although this species has a nocturnal pattern of activity, it can often be seen foraging during the daytime and more specifically 1–2 hr before sunset after having left the larger trees that are used as day roosting sites (Cheke and Dahl, 1981; Amélie Desvars, pers. obs.). Capture sessions at Coconi were performed from late afternoon until sunset. However, at Saziley captures took place in the early afternoon because bats were observed foraging (Table 1).

Two styles of mist net were used: 1) two black nylon Japanese mist nets (110 D, mesh of 16 or 19 mm, 12 m long, 2.4 m high, five pockets) and 2) one black nylon mist net (110 D, mesh of 45 mm, 12 m long, 3.2 m high, four pockets). To opti-

mize capture, two or three mist nets were set simultaneously (Trewhella et al., 1995), on 7 m high carbon fiber fishing poles, each inserted on a stick and guided with strings attached to surrounding vegetation. The fishing pole system allows the nets to be rapidly raised or lowered. Because of the elasticity of the mist net and the poles, bats are not immediately stopped when bumping into the net, and the net can move more than 1 m following the strike. Therefore, vegetation must be cleared 2 m on each side of the mist net. Sampling effort were summarized in mist-net hours (1 net-effort unit = 1 hr per square meter)of net; O'Malley et al., 2006).

Once removed from the nets, bats were placed in a handmade wicker basket (80 cm diameter, 60 cm high) where they could hang upside down. Up to nine bats were put in the same basket and were sprayed with water approximately every 30 min to avoid dehydration. For each bat, we recorded sex, reproductive condition for females (i.e., pregnant or lactating), radius length, and body weight (data not shown). A blood sample of 1 ml, representing less than 1% of the total body mass, was collected with a 1 ml syringe and 25 G, 16 mm needle. Two puncture sites were compared: the alar vein and humoral vein. Hemostasis was performed

by direct pressure. Bats were subsequently fed fruit juice, and hair from the cervical area was partially removed for identification in case of subsequent recapture. Animals were released within 4 hr in proximity to the capture site.

During the 13 capture sessions, 49 P. s. comorensis (31 females, 18 males) were netted (Table 1). The mean number of captured bats per session $(\pm SD)$ was 3.8 ± 2.9 , range [0; 9]. Following 30 April 2007 (last four sessions), when guava harvest was started, a significant drop in the number of captures was observed. Four bats entered the net at high speed and bounced out of it; these were consequently excluded from the table and our calculations, as were two bats that were recaptured and immediately released (on 10 April 2007). We sometimes observed individuals with light bleeding of the gums and palate where the mist net had become entangled in the teeth.

In several previous studies, nets to catch Pteropus spp. were raised above the canopy level (i.e., about 20 m in height; Trewhella et al., 1995; Clark et al., 1997) and were sometimes operated on a pulley system (de Jong et al., 2005; O'Malley et al., 2006). In the Philippines O'Malley et al. successfully captured Pteropus spp using two sizes of mist net $(10 \text{ m} \times 2.6 \text{ m})$ and 6 m \times 2 m, 38 mm mesh) that were set 1–10 m above the ground (O'Malley et al., 2006). The capture method we used with nets raised between 5 and 7 m off the ground was more practical, lower in cost, and reduced risks for captured animals. Two main justifications can be given for capture at feeding sites rather than at roosting ones (Trewhella et al., 1995; Clark et al., 1997). First, roosts are hard to locate and are primarily found in places where mist net placement may be difficult. This results from factors such as the presence of trees and the steepness of the slope or because the site is protected or being used for human activities. Second, the concern with roost sampling

relates to roost disturbance and subsequent abandonment of the roosting site. Local food abundance has a major impact on capture success, and the high number of captures we obtained at Coconi was associated with the abundance of ripe guavas.

Bat anesthesia is sometimes recommended to ease handling and to reduce the risk of being bitten (Wimsatt et al., 2005). We demonstrated that working safely without anesthesia is possible and allowed bats to be released immediately after blood sampling. In our experience we found that the humeral vessel was preferred for blood collection because the alar vein rapidly collapses, the humeral vessel can easily be located with puncture achieved without vein compression, and a stronger blood flow in the humeral vein allows more rapid blood collection.

The epidemiologic role that *P. comor*ensis plays in human diseases is of considerable importance as these bats have been shown to be reservoir for numerous viruses and bacteria, including zoonotic pathogens (Calisher et al., 2006). For example, *Pteropus* spp. have been shown to carry *Leptospira* spp. (Cox et al., 2005) and can be infected with a diverse group of viruses (Moutou, 2000; Calisher et al., 2006; Pritchard et al., 2006; Reynes, 2006; Lehlé et al., 2007).

Our technical results provide the means to capture *Pteropus* for a variety of studies, including ecologic monitoring, studies on the dynamic of populations by the way of the capture-mark-recapture technique, and epidemiologic research.

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