**Title**: Seasonal morphological shifts track resource availability for endemic Madagascar fruit bats

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**Abstract**

**Introduction.**

The bat family Pteropodidae, known as the so-called Old World Fruit Bats and flying foxes, makes up one of the most endangered groups of mammals on Earth, with some 35% of species currently extinct or threatened, a proportion almost three times higher than that reported for all other bat families combined (*1*). Fruit bats experience disproportionate rates of persecution, likely as a result of their propensity for small island endemism (*2*) and their large sizes, which make them targets for the bushmeat trade (*3*–*9*): wingspans can reach up to two meters in the case of *Pteropus vampyrus,* the world’s largest bat (*10*). The diverse ecosystem services offered by pteropodids have been reviewed before: these bats play important roles in the pollination and seed dispersal of numerous plant species across the Old World, particularly in island ecosystems often depauperate in other frugivores (*11*, *12*).

Madagascar is one such island ecosystem recognized for its unusually depauperate frugivorous fauna (*13*). Primates (lemurs), rather than birds, are considered the primary seed dispersers on the island (*14*, *15*), in contrast to otherwise comparable tropical ecosystems in the New World (*16*, *17*). In addition to lemurs, Madagascar is home to three endemic species of frugivorous bats from the family Pteropodidae, *Pteropus rufus, Eidolon dupreanum,* and *Rousettus madagascariensis,* all of which have been shown to pollinate flowers and disperse seeds from both native Malagasy and exotic plants (*18*–*24*). To date, the relative contributions of fruit bats vs. frugivorous lemurs to community assembly have yet to be rigorously quantified in a specific Malagasy ecosystem. Importantly, *E. dupreanum* may be the only extant pollinator of the endangered, endemic Malagasy baobab, *Adansonia suarezensis* (*24*).

Despite their value, Madagascar’s fruit bats are heavily persecuted. All three species are consumed across the island as a source of human food (*4*, *8*, *25*–*29*), and *P. rufus,* the largest and most heavily hunted, is sometimes targeted in response to its largely inaccurate characterization as a predator of human fruit crops (*30*). Respectively, *P. rufus, E. dupreanum,* and *R. madagascariensis* are currently IUCN Red-listed as ‘Vulnerable,’ ‘Vulnerable,’ and ‘Near-Threatened’ species (*1*), though recent population viability analyses suggest that *P. rufus*, in particular, may be experiencing more severe population declines than have been previously reported (*28*). Globally, anti-bat sentiments have been on the rise as a result of the COVID-19 pandemic (*31*); though no specific instances of COVID-related persecution have yet been documented for the Malagasy fruit bats, all three species are known to host potentially zoonotic pathogens (*32*–*39*), posing risks that negative public reactions may arise in the future.

All three Malagasy fruit bats are known to reproduce seasonally in largely synchronous birth pulses (*28*, *40*), and previous work suggests that roost population sizes and survival rates vary across the year for these three species, as well (*28*, *41*). Temporal fluctuations in nutritional status may alter bat immune responses, thus influencing pathogen dynamics (*33*), as well as modulate bats’ vulnerability to seasonally variable hunting pressures (*28*). We sought to expand existing knowledge of seasonal variation in the reproductive calendar and nutritional status of all three Malagasy fruit bat species, in order to facilitate future conservation assessments. In particular, we aimed to (a) quantify life history traits needed for population modeling for these three species, (b) document seasonal variation in their morphometrics and body conditions, and (c) calculate juvenile growth rates throughout the post-reproductive period. Our work emphasizes the importance of longitudinal field studies in accurately describing the ecology of frugivorous bats.

**Materials and Methods.**

**Study periods and sites**

Field studies were carried out between 2013 and 2020 in part with previously published work examining population viability (*28*) and the dynamics of potentially zoonotic infections circulating in Malagasy fruit bats (*32*–*34*). Bats were captured periodically throughout each year, with sampling spanning all months and all seasons (dry, wet, shoulder), interspersed with some gaps in temporal continuity. Captures took place in several regions of Madagascar (Figure 1): (1) Ankarana National Park in the northwest, (2) Makira Natural Park in the northeast, (3) Mahabo forest in the center-west, and (4) several sub-localities of the Moramanga District in the center-east, including: the fragmented forests of Ambakoana, Mangarivotra, Marotsipohy, Marovitsika, Lakato, and Mahialambo, the special reserves of Angavokely and Angavobe, and the new protected area of Maromizaha.

**Netting**

Mist nets were deployed from 6:00 p.m. to midnight and from 3:00 a.m. to 8:00 a.m. around roosting or feeding sites of *P. rufus*, *E. dupreanum* and *R. madagascariensis* and monitored continuously. Captured bats were placed in individual clean cloth bags while awaiting processing for disease studies, as has been previously described (*32*–*34*). For each sampling session, we conducted between 1 and 10 nights of netting, ending sessions early when 30 individuals of each species present at the site were captured. Upon capture, all bats were weighed (in g) with a Pesola scale attached to the cloth bag and forearm, tibia, and ear were measured with a caliper or tape measure (in mm). All raw data used in this study are accessible in our open-access github repository at: github.com/brooklabteam/Mada-Bat-Morphology.

**Literature review**

To place our Malagasy bats in a broader context, we compiled information from the literature concerning the morphology of other bats in the family Pteropodidae. From the ‘Bat Species of the World’ database (*42*), we compiled a list of some XXX previously described pteropodid species, then searched google scholar and Web of Science for any records documenting the mass, forearm, tibia, and ear length of each species. We only collected records that were sex-specific, and where possible, we documented the sample size from which those records were derived, if reported as an average. In cases where no sample size was reported, we assumed that sample size to be one individual. In total, we were able to compile mass records from XXX pteropodid species for females and XXX species for males; forearm records from XXX species for females and XXX species for males; tibia records from XXX species for females and XXX species for males; and ear length records from XXX species for females and XXX species for males. All raw data and references are accessible in our open-access github repository at: github.com/brooklabteam/Mada-Bat-Morphology.

from the literature We additionally reviewed the literature to document also collected information about morphology from the literature to see how these bats fit in the broader field biological literature. Research shows that populations of *Balionycteris maculata* are smaller in a number of traits and that some differences in shape occur between populations (Maryanto, 2003). Studies by Campbell et al. (2007) about *Cynopterus brachyotis*, *C. horsfieldii* and *C. brachyotis sunda* species show that sex had a significant effect on forearm length and tibia length. The species *Dobsonia minor* and *D. beauforti* have shown sexual dimorphism and that *D. emersa* is separated from these two species by its body and cranial characteristics (Yohanita et al. 2010). Reviews by Helgen et al. (2007) suggest that *Dyacopterus rickarti* differs from Sundaic taxa by its much larger size. The study on *Pteropus personatus* specimens shows that there is variation in skull size, dental measurements and other external measurements between populations (Wiantoro and Maryanto, 2016).

**Statistical Analysis**

Data analysis was performed using R 3.6.1 software (R Core Team, 2019). We distributed the number of individuals captured for each species for each site during our study and defined the period and duration of gestation and lactation for each species. A type 2 linear regression model with the R package “lmodel2” (Legendre, 2018) was fitted using the standard major axis method (SMA) to observe the relationship between body mass and forearm. Then, we determined the length of the tibia, ear and forearm for both sexes and in Malagasy and non-Malagasy species. Seasonal variation was determined by fitting a generalized additive model (GAM) (Wood, 2011) to the residual mass of the forearm using the “mgcv” R package (Wood et al 2016). The duration of Malagasy bats encompasses the whole range of non-Malagasy bats because they are studied through all seasons. We estimate the growth rate of juveniles (length of the tibia, forearm and ear) from the first day we observe a newborn or a juvenile bat for the three Malagasy species using a linear model for the first 60 days. Then we adjusted a GAM to the length of the forearm, the length of the tibia and the ear and observed the growth rate.

**Results.**

*Capture Demographics*

In total, 2396 fruit bats were captured, processed, and marked across all four field sites between 2012 and 2019; and among those, 250 (*Eidolon* n=151, *Rousettus*: n=99) were captured more than once (Fig. 1b). The Moramanga region boasted the most captures of all three species with just over 1700 bats (*Eidolon* n=732, *Rousettus* n=653, *Pteropus* n=317). Of the 380 bats captured in Ankarana National park, 172 were *Eidolon* and 208 were *Rousettus*. Fourteen *Eidolon* and nineteen *Pteropus* bats were captured in the Mahabo Forest. Lastly, a total of 40 (*Rousettus* n=32, *Pteropus* n=8) bats were captured at Makira National Park.

Monthly capture data has allowed us to track the onset of the birth pulse for each species. The length of the observed gestation period was calculated by counting the days between the first sighting of a pregnant female (confirmed by gentle palpation of the abdomen) to the first sighting of a juvenile. Likewise, the observed lactation period was calculated as the number of days since the first sighting of a juvenile to the last capture of a lactating female. As shown in Figure 1a, *P. rufus* has the shortest observed gestation period of approximately ~80 days starting in July and ending in September. Although, it is likely that gestation began sometime in April and is a total length of ~180 days as is seen in its Australian sister species, P. alecto, P. poliocephalus and P. scapulatus (McIlwee & Martin, 2002). The *P. rufus* had the longest observed lactation period of ~115 days - from September to January.

Based on their African sister species *Rousettus aegyptiacus* (Korine et al., 1994) and *Eidolon helvum* (Odukoya et al., 2008), the Malagasy *Rousettus* and *Eidolon* bats likely have a total gestation period of 120 days that begins in either August or July. However, we observed a ~90 and ~105 day period that began in August and September for the two species respectively. The observed lactation period was ~60 days for *Rousettus* that began in December and ended in February, and the *Eidolon* bats had an ~80 day lactation period that began in November and ended in February. The gestation and lactation data collected closely matches the birth pulse of other Malagasy frugivorous mammals which coincides with emergence of fruit in the wet season (Wright et al., 2005).

In Malagasy fruit bats, the body morphometrics differed largely among but did not have a pronounced difference between sex. For *R. madagascariensis* (the smallest species), the median of male forearm measurement is 71.41mm (Q1= 64.58mm, Q3= 76.5mm) and69.8mm (Q1= 62.16mm, Q3= 74.44mm) for female. Concerning the medium-sized bat *E. dupreanum*, the median of male is 129.4mm (Q1= 120.09mm, Q3=136.53mm) and 129.6mm (Q1= 120mm,Q3=138mm). And about the largest Malagasy bat, *Pteropus rufus*, the length of male forearm ranged from 145.91mm to 185.65mm (median=169mm) and between 144.04mm and 175mm (median=163.5mm) for female The ear lengths of these three species varies extensively with a median 12.9mm (Q1=9.16mm, Q3=17.73mm) for male *R. madagascariensis* and 13.5mm (Q1=10mm, Q3=17.7mm)for female. The median value is the same (median=28mm) for both sexes of *E*.*dupreanum* and 28.7mm for male *P. rufus* (Q1= 20.54mm, Q3=37.72mm) and 29mm for the female (Q1=21.84mm, Q3=37.94mm. The tibia length diverges among the three species. The median measurement *R. madagascariensis* if is 33.9mm for male (Q1=28.83, Q3=37.77) and 32.3mm for female (Q1=26.2, Q3=36.84). For the medium size bat, the median tibia is 62.3mm for male (Q1=55mm, Q3=68.45mm) and 61.8mm for female (Q1=52.13mm, Q3=68.13. And for the largest one the tibia median is 81.87mm for male (Q1= 67.27mm, Q3= 92.06mm) and 79.27mm for female (Q1=69.15mm, Q3= 83.72). The measures of Malagasy bats are encompassed to the whole range of the non-Malagasy bats because they are studied and sampled across all seasons. The median of forearm length of non-Malagasy fruit bats is 118.8mm (Q1=53.20mm, Q3=181.03mm) for male and 114.45mm (Q1=45.56mm, Q3=148.63mm) for female.

*Mass:Forearm Relationships*

*(Fig. 2,3)*

When we fitted a linear model to observed a relation between body mass and forearm length (Figure 2), a high correlation is observed in the three species of Malagasy fruits bats for both male (r-square: 0.959) and female (r-square: 0.958). And it shows the same result for the non-Malagasy fruits bats for both male (r-square: 0.981) and female (r-square: 0.956). More the body mass is big, more the length of forearms is long in the three species.

Then we fitted a GAM to the residual values and saw that for both sex of *E.dupreanum*, the season has an effect on body mass (F=4.576, P<0.0001 for female and F=2.578, P=0.001 for male). For *P.rufus*, the body mass change over the season is strong for male only (F=6.816, P<0.0001) And we observed also a slight change for *R.madagascariensis* male (F=0.773, P=0.0255).This result suggest that bats' nutritional status varies with seasonal food changes in food availability.

*Juvenile Growth Rates (Santino + Angelo)*

*(Fig. 4)*

**Discussion. Add in frugivore timing of weaning**

The reproduction activities of the three species are well spread across the year (from April to February). For *P. rufus*, the gestation begins at the end of the wet season and the parturition begins on late dry season.  For both *E. dupreanum* and *R. madagascariensis*, gestation period begins at the mid of dry season and the parturition occurs at the onset of wet season. The weaning time is at mid wet season, that should coincide to the peak of fruit abundance.

The morphometrics analyses shown that there is no strong dimorphism found among both sex of Malagasy fruit bats. But we found that the forearm length and the body mass are highly correlated for both male (r-square: 0.959) and female (r-square: 0.958). This trait is also found for non-Malagasy bats.

Season affected bats in different way when we plotted the residual value of body mass by forearm. For both sex of *E. dupreanum*, the we noticed that their body mass are lower than expected. For male, the declining was really pronounced during the wet season (F=4.576, p<0.0001). For the female the lower body mass was noted at the end of wet season (F=2.578, p=0.001). Concerning *P. rufus* species, all body mass is greater than expected. For male there was a strong variation of body mass over the year and the lowest is found during the dry season (F=6.816, p<0.0001) and no significant change found for female. All body mass also was greater than expected for *R. madagascariensis* and no significant change found for both sex.

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**Literature Cited.**

**Tables.**

**Figures.**