

Final Kate Barlow Award Project Report

Title: Assessing the Nutritional Content and Stable Isotope Ratios of Native and Non-native Food Sources Used by the Endangered Christmas Island Flying-Fox (*Pteropus melanotus natalis*)

Project Leader:

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Project Summary:

Project Aims and Objectives: The Christmas Island Flying-fox (CIFF) is the last remaining mammal on Christmas Island and is critically endangered. Nutritional imbalances stemming from preferential consumption of introduced plants may be a contributing to its decline. This study first aimed to determine how the CIFF used native plants to meet its nutritional requirements historically and if the nutritional content of introduced plants differed from native plants. Then to assess if stable isotope analysis could be used to determine the percentage of the CIFF diet composed of native and introduced plants.

Methods: Crude protein, non-fiber carbohydrates (NFC) and mineral content were determined from 27 fruit species, 6 flower species, and 5 species of leaves, petioles or stems commonly eaten by the CIFF. Sugar content of nectar was determined by refractometry. Stable isotope ratios (¹³C:¹²C and ¹⁵N:¹⁴N) were determined from a subset of dried plant samples.

Results: In comparison to native fruit, introduced fruits contained higher concentrations of NFC and zinc but lower concentrations of crude protein, calcium, magnesium, sodium, copper, iron and manganese. Two exceptions were introduced Japanese cherry and Breadfruit, which contained similar calcium and protein concentrations to native figs. Leaves and petioles contained higher concentrations of crude protein, calcium, magnesium, sodium, iron, and manganese but lower levels of NFC and zinc than fruit. Sugar content (11.5 and 16%) of native flower species was similar to non-native flower species (12 and 17.5%), apart from passionfruit (47%). Stable isotope ratios did not differ between native and introduced fruits.

Impact: This study shows that historically, CIFFs obtained the bulk of their energy from fruit and nectar. These food sources, however, have low percentages of protein and calcium. Therefore CIFFs, particularly lactating females, must have selectively fed on the leaves, stems and petioles of specific native plants to obtain these nutrients. We also speculate that pollen played an important role in supplying protein. Thus the CIFF, and likely other flying-foxes, require an ecosystem containing a diverse array of plants to meet their nutritional needs. In general, introduced plant species contain less protein and essential minerals. If the CIFFs preferentially feed on them, they may have to consume more of them to meet their protein requirements which could lead to obesity. Introduced plants including Japanese cherry and breadfruit, however, had nutrient content similar to the native fig and may be an important contemporaneous food source for the CIFF.

Introduction:

The Christmas Island flying-fox (*Pteropus melanotus natalis*, CIFF) is the last endemic mammal on Christmas Island (CI) and is critically endangered and declining. In the last 30 years, the CIFF population has declined from 6,000 individuals to fewer than 2,500 [1]. As a pollinator and seed disperser, the loss of this keystone species would prove catastrophic to the CI ecosystem. While factors leading to its decline are not well understood, a working group sponsored by the National Environmental Research Program (NERP) identified multiple key threatening processes which could be contributing to the CIFF decline [1]. Potential threats include intoxication with heavy metals released by phosphate mining, nutritional imbalances from preferential feeding on introduced plants, and diseases caused by infectious agents. **The purpose of this project was to determine if preferential foraging on introduced plants could result in nutritional imbalances in the CIFF.**

A significant portion of eastern CI is mined and the bulk of plants used for re-vegetation in these areas are not native. In addition, multiple species of fruit trees including Japanese cherry, mango, papaya, and banana have been introduced to CI and our work shows that CIFF feed on them alongside many native fruits. Limited studies suggest that the fruit from non-native food sources contain less energy, protein, calcium, iron and sodium than do native food sources [2]. Reliance on non-native food plants could ultimately contribute to nutritional diseases, reduced lifespan and fecundity in flying-fox populations. **The first aim of this study was to determine how CIFFs used native plants to meet their nutritional requirements historically and if the nutritional content of non-native food plants differ from native food sources.**

To further understand how a reliance on non-native food sources could contribute to nutritional disease in the CIFF, the proportions of each food plant within the CIFF diet needs to be determined. This would normally be done by following specific individuals over the course of many nights to determine what proportion of time they spend foraging on different plants. However, this is not feasible in flying-foxes because of the distances they fly in a given night. Stable isotope analysis is an alternative method that has been used to determine diet proportions in wildlife populations [3]. If isotopic signatures vary significantly among food groups, then diet proportions can be determined. **Therefore, the second aim of this project was to compare stable isotope ratios in native and non-native food sources.**

Project Results & Impact:

Methods:

Common food plants consumed by the CIFF (nectar, fruit, leaves, petioles, stems) were collected in the wet and dry season. Nectar samples were collected and read on a refractometer to determine sugar content. Plant samples were collected, sliced, and frozen until further processing. Plants were then desiccated in an oven, milled, weighed, and submitted to a commercial laboratory to determine macronutrients (dry matter, crude protein (CP), non-fibre carbohydrates (NFC)) and micronutrients (calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn)). A Wilcoxon Rank Sum Test was used to assess nutritional differences between native and non-native food plants.

A subset of fruit and leaf species was additionally submitted to a commercial laboratory to determine stable isotope ratios (^{13}C : ^{12}C and ^{15}N : ^{14}N). Differences in stable isotopes was determined with a MANOVA followed by a Tukey's Test of Honestly Significant Difference (THSD).

Results:

From May, 2018 to February, 2019, 134 plant samples were collected including 16 native fruits, 11 non-native fruits, 5 native leaves, stems, and petioles, 2 native flowers, and 4 non-native flowers.

Aim 1: One-hundred and eighteen samples were submitted for nutritional analysis. Non-native fruit on average had lower percentages/concentrations of CP, Ca, Mg, Na, Cu, Fe, and Mn but higher percentages/concentrations of moisture, NFC, and Zn (Table 1, 2, and 5). In comparison to fruit, native leaves had higher percentages/concentrations of CP, Ca, Mg, Na, Cu, and Mn but lower percentages/concentrations of NFC, Fe, and Zn (Table 3 and 5).

Sugar content (11.5 and 16%) of native flowers was similar to non-native flowers (12 and 17.5%), apart from passionfruit (47%) (Table 4).

Aim 2: Twenty plant species were submitted for stable isotope analysis. There was no significant difference between $^{15}\text{N}:^{14}\text{N}$ for non-native fruit (mean = 1.61, range = (-5.4 – 8.6)), native fruit (1.94 (-8.5 – 7.8)), and leaves (6.7 (-0.1 – 14.1)) (ANOVA $F_2 = 0.2$, $p=0.83$). There was a significant difference between $^{13}\text{C}:^{12}\text{C}$ for non-native fruit (-28.1 (-32.9 – -23.6)), native fruit (-29.2 (-31.7 – -25.3)), and leaves (-33.5, (-36.2 – -31.1)) (ANOVA $F_2 = 14.1$, $p < 0.001$). However after running a THSD the difference between means of native and non-native fruits was not significantly different (difference = -1.7, $p = 0.2$). However, there was a significant difference in $^{13}\text{C}:^{12}\text{C}$ between all fruit samples combined and native leaf samples (ANOVA $F_1 = 23.2$, $p < 0.001$).

Discussion and Significance:

Aim 1: Our data is two-fold in that it provides evidence on how CIFF used native foraging resources prior to the introduction of non-native plants and how the introduction of non-native food species could play a role in nutritional imbalances in the population. Nutritional analysis of native food sources provides evidence that historically CIFFs obtained the bulk of their energy from native fruit and nectar, however these plants are generally low in protein and calcium which are important for energy, growth, and reproduction [4]. Therefore, CIFFs supplement their diets with leaves, petioles, and stems. Pollen is also high in protein and we know that CIFFs eat pollen of native flowers. Therefore, pollen likely plays an important role in maintaining sufficient protein in the CIFF diet. This provides growing evidence that a diversity of plants is required to meet the nutritional needs of the CIFF and this needs to be replicated when considering revegetation efforts across the island.

Christmas Island National Park (CINP) staff regularly plant non-native food plants in newly rehabilitated mine sites where it naturally aids in breaking up the soil while also attracting frugivorous species to these areas to promote regeneration of native rainforest. This is concerning as non-native plant species on average contain less protein and essential minerals in comparison to native plant species. In comparison to native fruits, non-native fruits also have higher amounts of NFC. Thus, if the CIFF is preferentially foraging on non-native fruits, they may consume more of them to meet protein requirements which may lead to obesity in the population. Introduced Japanese cherries and breadfruit, however, had nutritional content similar to that of native figs. Japanese cherry in particular is commonly planted in rehabilitated areas and is a preferential food source for CIFFs and therefore may be an important non-native food source. Therefore, when choosing to plant non-native food plants we recommend choosing those plants which have similar nutritional profiles to those of native plants to maximize nutrition of the population.

Wider Significance:

Foraging studies in flying-foxes typically only focus on utilisation of food resources and not their nutritional content which limits our ability to understand the nutritional drivers or demands of flying-fox populations. This study is only one of a handful of studies which has determined nutritional content of plants consumed by flying-foxes and has implications for other flying-fox populations who consume similar plants. Our data provides evidence that multiple food sources are necessary to meet nutritional demands of flying-foxes, this is particularly true for females who have higher nutritional demands for reproduction. In addition, the introduction of non-native vegetation is not a unique problem to Christmas Island and is seen all over the world, including mainland Australia. This study supports the theory that many non-native plants are nutritionally deficient which may lead to decreased fecundity and overall health of flying-foxes if other food sources are not available.

Lessons Learned:**Which project activities and outcomes went well and why:**

Collection of food plants for aim 1 went very well. Having good collaboration with other graduate students, some of whom were working on the same species, was key for this aim. Their willingness to share knowledge of foraging ecology and behaviour allowed me to prioritize collection of common food plants. Having relationships with CINP staff also allowed me to tap into local knowledge and expertise regarding fruit and flowering times on the island and knowledge of where to find many of the plants I needed to collect. CINP staff were also key in helping me organize permits and licenses to collect and transport food plants for analysis.

With this knowledge and ability to collaborate with others I was very successful in my collection of food plants. Although it was difficult to find CIFF foraging on food plants in my first season, particularly native fruit, I was able to reach out to CINP staff and student researchers who were able to assist me in finding plants available at the time. Unfortunately, I wasn't able to collect as many plants as I had hoped for, potentially due to annual weather variation, however my second field season was much more successful. In the end having collections during both seasons was useful as it aided me in obtaining a larger variety of species and a larger sample size.

Problems encountered during the project and how these problems were addressed:

Collection of flowers for nectar and pollen analysis was particularly difficult in this study. While we were hoping to collect nectar for nutritional analysis we quickly realized during some of my preliminary trials that it was nearly impossible to collect adequate amounts of nectar (~20 g) for nutritional analysis. We therefore corresponded with a nutritional expert and came to the conclusion that it would be better to collect nectar and take readings on a hand held refractometer for sugar analysis. This is commonly done in nutritional studies and therefore we decided this was an adequate reason to deviate from our original plan.

Once I arrived in the field, finding flowers for nectar collection was even more challenging than expected. In general, it was difficult to find flowers that were blooming during the times I was present on the island and in areas where I could easily collect them. Differences in annual weather variation could have accounted for the difficulties in finding flowers during times when they should have been blooming. For example, this year had significantly lower rainfall in comparison to previous years. My ability to collect flowers was also limited by the ability to reach flowers, as many native trees can grow up to 40 meters high and therefore it was impossible to be able to collect flowers at this height. Additionally, upon arriving on the island I found that many flowers preferred by the CIFF were much smaller than I was anticipating and I was unable to extract ample amount of nectar from these flowers

for refractometry. This is a limitation that we are not able to adjust for but I have collected nectar from most preferred species of flowers on which we commonly see the CIFF feeding. I also plan to explore the literature to see if there is more information on sugar values for any flower species we are missing.

Another challenge was the ability to transport plant samples from CI to mainland Australia. As CI is an external territory there are very strict regulations on imports allowed into mainland Australia. While we were planning to determine nutritional content of pollen, after obtaining this grant we were advised that pollen could not be imported into mainland Australia without first undergoing ethanol fixation or gamma irradiation both of which would significantly impact nutritional analysis. Therefore, we elected to qualitatively assess pollen digestion in archived CIFF faecal samples and supplement this with nutritional data of pollen eaten by other frugivorous species.

Although we thought that non-native fruits may have different stable isotope signatures due to their localities in disturbed and potentially fertilized soils we did not see any significant differences to stable isotope signatures of native plants. We are instead using GPS telemetry data to determine the average time CIFF spend foraging in areas primarily comprised of non-native vegetation in comparison to those areas with primarily native vegetation.

Lessons learned:

Nutritional ecology studies of cryptic aerial wildlife on a remote tropical island is challenging. Being highly prepared and discussing methods with collaborators in advance of each field trip maximized the value of our efforts on the ground. Conducting literature reviews and testing methods in advance was key to the success of this project. Additionally, being prepared to adjust plans accordingly depending on the situation and flower/fruiting opportunities was also important.

Conclusion: (up to 400 words)

- Provide accurate and specific conclusions, avoiding general inferences
- Describe the project's overall contribution and answer questions raised in the introduction, adding any new information exposed by the project process
- Explain what efforts you will be taking to sustain this work beyond the grant period and what further work would be useful for the conservation of the target species/area

Support from Bat Conservation Trusts Kate Barlow Award has allowed us to generate nutritional data to understand nutritional requirements of the CIFF and determine if non-native species are less nutritious compared to native food plants. This study is unique in that it is one of a handful of studies which have looked at nutritional differences between native and non-native fruits, many of which are eaten by flying-fox populations worldwide. From this research it is evident that the CIFF needs access to a diverse array of plant species in order to meet nutritional demands. Nutritional data on native food plants gives us a snapshot of what CIFF historically foraged on and what nutritional requirements are necessary to sustain the population.

While most non-native fruit species were nutritionally deficient in comparison to their native counterparts, this was not necessarily the case for all non-native fruit. It is important to take this into consideration in our context as CINP staff regularly plant non-native food plants in rehabilitated mine sites. Japanese cherry is one such fruit commonly planted in rehabilitation fields and is highly preferred by the CIFF, our nutritional analysis found that nutritional levels of Japanese cherry were nearly equivalent to that of native figs. However, Japanese cherries are low growing trees possibly making CIFF more susceptible to predation by feral cats. Feral cat baiting programs are currently taking place on CI,

however the risk of predation by feral cats has not been well studied in the CIFF and should be an area of future research. These factors need to be taken into consideration when implementing revegetation plans across the island.

This project will be further sustained by looking at GPS telemetry data to determine the amount of time CIFF spend foraging in areas containing primarily non-native food plants. To our knowledge this will be the first study using GPS telemetry data coupled with nutritional data to explore nutritional demands in flying-foxes. Further work which would be useful for the conservation of CIFF includes determining the threat of heavy metals in the population which my PhD is also addressing. In addition to this, the impact of seasonal or climactic changes on food availability, and the impact of predation by feral cats, invasive centipedes, and wolf snakes needs to be further explored.

References:

1. Walshe, T., E. McDonald-Madden, and D. Southwell, *Christmas Island Flying Fox risk-based decision support: outcomes of a workshop held 28-29 November 2012*. . 2012, NERP Environmental Decisions Hub.
2. Nelson, S.L., et al., *Nutritional Consequences of a Change in Diet from Native to Agricultural Fruits for the Samoan Fruit Bat*. *Ecography*, 2000. **23**: p. 393-401.
3. Crawford, K., R.A. McDonald, and S. Bearhop, *Applications of stable isotope techniques to the ecology of mammals*. *Mammal Review*, 2008. **38**(1): p. 87-107.
4. Barboza, P.S., *Integrative wildlife nutrition*, ed. K.L. Parker and I.D. Hume. 2009, Berlin: Springer.