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Full Length Research Paper

Invertebrate fauna associated with the cultivation of *Vernonia calvoana calvoana* (Asteraceae) in Yaoundé (Center-Cameroon)

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***Vernonia calvoana* is a culture spread across the world and prized for its culinary and medicinal virtues. The data was collected in Yaoundé, Central Cameroon region through manual harvesting at two stations from 2017 to 2019. A total of 168 species or morphospecies belonging to 47 families and 12 orders from two phyla were collected. The most abundant phylum was Arthropoda which represented 99.96% of the total number of all species, while Mollusca represented the rest. Within arthropoda, insecta class was found the most dominant. In Insecta, Formicidae and Aphididae were the most frequent families. At Nkolbisson, Hymenoptera and Hemiptera are more abundant on the purple variety than on the white variety. However, both are less abundant compared to those at Nlong-mvolye. The species *Uroleucon compositae* (37.01%), *Hilda cameroonensis* (4.67%) and *Sphaerocoris annulus* (2.71%) were the most abundant species recorded in all the three variants. Depending on the preference with the host plant, some orders have a distribution according to the site, but also, the variety.**

Key words: Manual harvest, insecta, hymenoptera, hemiptera, Nkolbisson, Nlong-mvolye.

INTRODUCTION

Recent demographic studies predict that by 2050, the human population could reach a total of 9.7 billion people (Godfray et al., 2010). In addition, research suggests that such an increase in population would also be accompanied by various modifications of our planet such as the reduction of arable land characteristic of urban development, or, the increase of problems related to climate change such as food insecurity and health crises (Godfray et al., 2010; Pison, 2011).

Despite efforts to find alternative and sustainable

solutions such as the implementation of increased global production and better management related to agricultural losses, the development of these strategies is slowed down by various challenges on a global or local scale. For example, many species of invertebrates are pests and vectors of diseases that hinder the development of sustainable agriculture (Liu and Sparks, 2001; Geering and Randles, 2012). This is especially in the countries of subtropical Africa. Invertebrates constitute about 80% of animal biomass. The most cited in the literature that

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causes the most crop damage are: Hemiptera, Orthoptera, Coleoptera and Lepidoptera.

The Asteraceae is one of the four most diverse families with over 1,500 genera and 23,000 species (Ehab, 2001). They are distributed throughout the world. Within the Asteraceae, the genus *Vernonia* is the most diverse with 1000 listed species (Keeley and Jones, 1979). Species of the genera *Vernonia* are prized either for their culinary or medicinal virtues. Its consumption is based on the belief that the plant is used in the management and cure of heart diseases, blindness, diabetes, malaria, stomach ache, as an anti-helminthic agent, and to prevent constipation. Preliminary pharmacological studies carried out on experimental models have validated the hypoglycemic and hypolipidemic (Iwara et al., 2015), antioxidant (Egbung et al., 2016; Iwara et al., 2017), antimicrobial (Ati et al., 2016), cardio-protective (Egbung et al., 2017), and anti-cancer properties of *V. calvoana* (Mbemi et al., 2020). In Cameroon, *Vernonia amygdalina*, *Vernonia colorata* and *Vernonia calvoana* are united under the name 'ndolè'. According to Kahane et al. (2005), 'ndolè' is the sixth Cameroonian dish. In Cameroon *V. amygdalina* and *V. calvoana* are the most common species. Moreover, *V. amygdalina* can be substituted by *V. calvoana* (Grubben and Denton, 2004). Many studies have already focused on *V. amygdalina* while *V. calvoana* has been the subject of few studies. In order to contribute to integrated pest management, knowledge of the different groups of invertebrates associated with the culture of *V. calvoana* is necessary. This present work aims to determine the diversity of the invertebrate fauna associated with the culture of *V. calvoana* in the city of Yaoundé.

MATERIALS AND METHODS

Study site

The study was conducted from August 2017 to April 2019 in two districts of the city of Yaoundé (Department of Mfoundi) (Figure 1). The city of Yaoundé is influenced by a Guinean-type equatorial climate with four seasons: a large dry season (from mid-November to mid-March); a small rainy season (from mid-March to the end of June); a small dry season (July to August); and a large rainy season (September to mid-November). Precipitation is in the range of 1400 to 1900 mm per year and temperatures vary from 18 to 35°C (Suchel, 1988).

Plant material

The plant material consists of the white and purple varieties of *V. calvoana calvoana* quite common in the Central region.

Experimental design

At the main station located in the Nkolbisson district, on an area of 530 m², three complete randomized blocks made up of eight experimental units of square shape and 16 m² of surface each, spaced 0.8 m. Each experimental unit had five rows of 5 plants

spaced 0.80 m each. At the peripheral station in Nlong-mvolye, on a surface of 412 m², we placed plants of *V. calvoana calvoana* white variety in association with *Abelmoschus esculentus*, *V. amygdalina*, *Solanum modiflorum*, *Amaranthus hybridus*, *Phaseolus vulgaris*, and *Talinum fruticosum*.

The main station included the white (12 plots) and purple (12 plots) varieties of *V. calvoana calvoana* while the peripheral station only contains the white variety. The invertebrates were collected from August 2017 to September 2018 in Nkolbisson and from March 2018 to April 2019 in Nlong-mvolye.

Sampling method

Hand collection was the sampling technique used to collect invertebrate species to occur in each location. Two persons used either forceps or aspirator to collect species on aerial organs (leaf and stems) of plant for 5 min. Collections from *V. calvoana calvoana* plants were weekly, five plants randomly selected by plots. A total of 120 plants were sampled in Nkolbisson and 60 plants in Nlong-mvolye.

The collected invertebrates were stored in tubes containing 70% alcohol and identified using the appropriate dichotomous keys. The correct specimens were deposited in the reference collection in the Zoology Laboratory of the University of Yaoundé 1.

Data analysis

After identification, cumulative and relative abundances of invertebrates hosted by each plant species/varieties were computed. For further analysis, taxa (orders, the families and the species) with $\geq 5\%$; $5\% < \text{relative abundance} \geq 1\%$ were considered dominant and less abundant, respectively. While those with abundance $< 1\%$, were considered scarce during the study. We used the Excel software for the frequency calculation and the Past 3.12 software to generate the diversity indexes.

RESULTS

Diversity associated with *V. calvoana calvoana*

A total of 168 species of invertebrates belonging to two phyla, 12 orders and 47 families identified from 13,929 individuals were collected. The phylum of Arthropoda is more diverse (11 orders, 47 families and 167 species) and abundant (13,925 individuals or 99.96% of individuals collected) than the phylum of Mollusca (one order, one family and one species). The arthropods were grouped into 03 classes: the Arachnida (with an order, 6 families and 18 species), the Diplopoda (with an order, a family and a species), and the Insecta (with 9 orders 40 families and 149 species).

Diversity at the ordinal level

Among these invertebrates, four orders of insects are the most abundant in terms of specific richness (that is 99.25% of the total number of individuals collected). These orders include the Hymenoptera (3 families and 18 species), 6850 individuals or 49.32% of the individuals

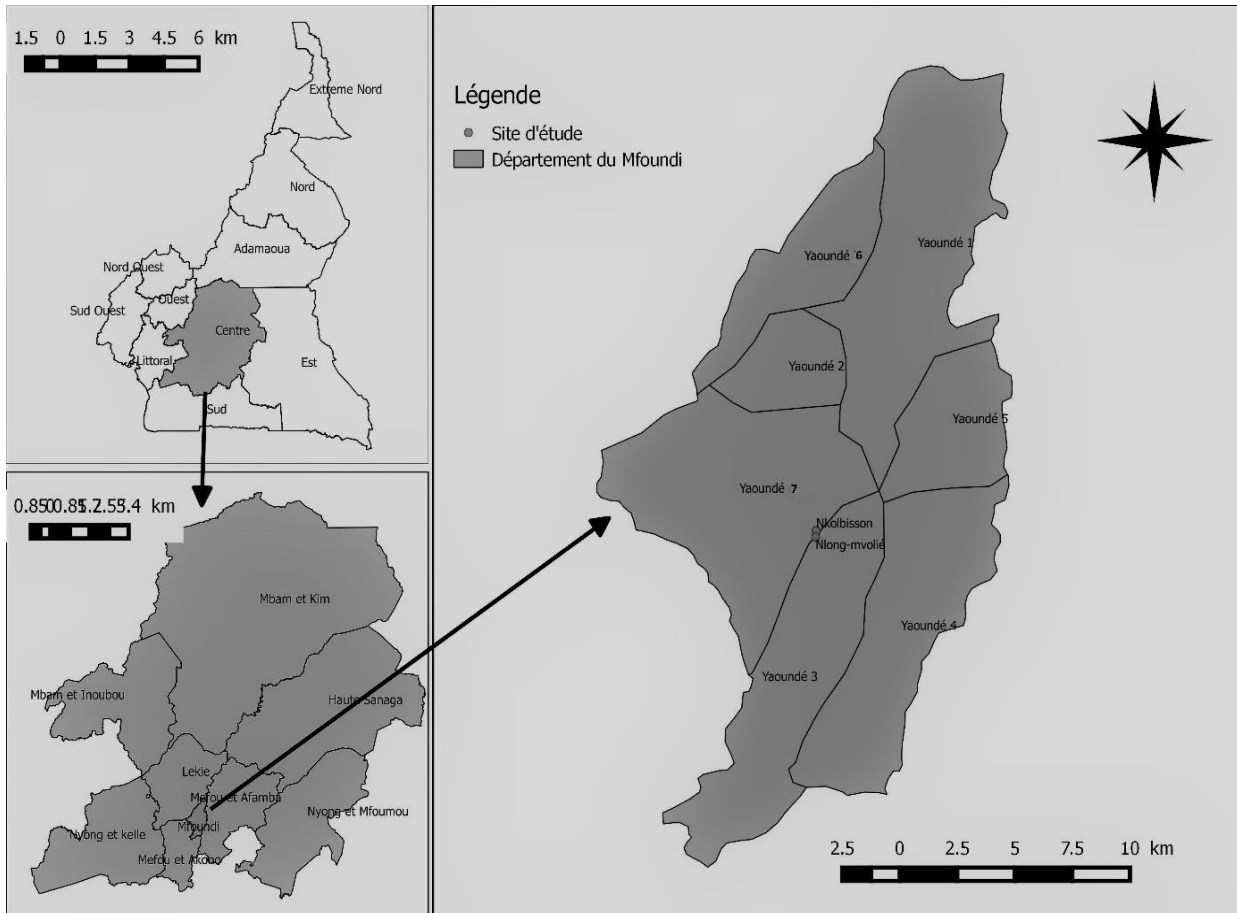


Figure 1. Locations of the surveyed sites in Nkolbisson and Nlong-mvolye in Central Region, Cameroon.

collected; Hemiptera (13 families and 41 species) 6,388 individuals, that is, 45.72% of the individuals collected; Coleoptera (7 families and 44 species) 401 individuals or 2.88% of individuals collected; and Orthoptera (8 families and 13 species) 185 individuals or 1.33% of individuals collected (Table 1).

Diversity at the family level of the most abundant orders

Without taking into account the location and the variety, 47 families were identified on *V. calvoana calvoana*, only two families belonging to Insecta have a frequency $\geq 5\%$ (Table 2). This community is dominated by the Formicidae (49.31%) and Aphididae (37.01%) families. Also, four families, namely, Tettigometridae (4.67%), Pentatomidae (2.79%), Coccinellidae (2.07%), and Pyrgomorphidae (1.03%) were numerically significant with an abundance ≥ 1 (Table 2). The purple variety was the most diverse (Table 3). The activities of the species of these families contribute to weakening the growth and the yield of the plant.

Diversity at a specific level

The Hemiptera are the most diverse with 13 families and 41 species. The most abundant species are *Uroleucon compositae* (Theobalt, 1915), *Hilda cameroonensis* (Tamesse and Dogmo, 2016) and *Sphaerocoris annulus* (Fabricius, 1775) (Table 4).

U. compositae and *H. cameroonensis* have a regular distribution regardless of site and variety, while *S. annulus* seems to have a preference for the white variety in Nkolbisson (Table 4).

The order Hymenoptera is represented by three families and 18 species. The most abundant species are *Pheidole megacephala* (Fabricius, 1793), *Diplomorium longipenne* (Mayr, 1901), *Camponotus acvapimensis* (Mayr, 1862), *Myrmicaria opaciventris* (Emery, 1893), and *Tetramorium acculeatum* (Mayr, 1866) (Table 4).

The order Coleoptera is represented by seven families and 44 species, with the most abundant species being Coccinellidae species. The latter is being better represented in Nkolbisson regardless of the variety (Table 4).

The order Orthoptera is represented by eight families

Table 1. Assemblages of orders of invertebrates collected according to different varieties.

Order	The abundance of invertebrate per varieties			
	B1	B	V	Total general
Aranea	3 (0.05)	12 (0.35)	9 (0.21)	24 (0.17)
Blattodae	0 (0)	0 (0)	1 (0.02)	1 (0.01)
Coleoptera	17 (0.28)	211 (6.08)	173 (4.01)	401 (2.88)
Dermaptera	2 (0.03)	0 (0)	5 (0.12)	7 (0.05)
Diptera	1 (0.02)	11 (0.32)	9 (0.21)	21 (0.15)
Hemiptera	3180 (51.73)	1269 (36.57)	1919 (44.5)	6368 (45.72)
Hymenoptera	2930 (47.67)	1865 (53.75)	2075 (48.12)	6870 (49.32)
Lepidoptera	6 (0.1)	12 (0.35)	20 (0.46)	38 (0.27)
Mantoptera	1 (0.02)	3 (0.09)	5 (0.12)	9 (0.06)
Orthoptera	5 (0.08)	86 (2.48)	94 (2.18)	185 (1.33)
Spirostreptida	0 (0)	0 (0)	1 (0.02)	1 (0.01)
Stylomatophora	2 (0.03)	1 (0.03)	1 (0.02)	4 (0.03)
Total general	6147 (100)	3470 (100)	4312 (100)	13929 (100)

Values in brackets represent relative abundance. B1: White variety of *V. calvoana calvoana* in Nlong-mvolye; B: White variety of *V. calvoana calvoana* from Nkolbisson; V: Purple variety of *V. calvoana calvoana* from Nkolbisson.

and 13 species, with the most abundant species being *Zonocerus variegatus* (Linnaeus, 1758) (Table 4). *Z. variegatus* is only distributed in Nkolbisson regardless of the variety (Table 4).

DISCUSSION

The present study showed that 168 species of invertebrates belonging to two phyla, 12 orders and 47 families were associated to *V. calvoana calvoana*. Our results are more diverse than the 31 families and 92 species obtained on *Carthamus tinctorius* (Asteraceae) by Saeidi et al. (2015) in Iran as well as the 30 and 36 families obtained, respectively on both *Tithonia rotundifolia* and *Tithonia diversifolia* Asteraceae by Donatti-Ricalde et al. (2018) in Brazil. This difference could be due to on one hand a much longer sampling period than theirs and to the other hand, the geographical variation of the sites. In addition, our results are less diverse than the 20 orders, 117 families and 412 species collected by Kakam et al. (2020) on 11 varieties of seven species of Curcubitaceae, in Minko'o in the South Cameroon region. This difference could be due to on one hand the number of varieties and species greater than that of our study. On the other hand, it could also be due to the fact that Curcubitaceae seems to attract more species than Asteraceae. The work also reveals the presence of Insecta, other classes like Diplopoda (Odontopygidae) and Gasteropoda.

The present results corroborate with those of Selim (1978) in Iraq and Campobasso et al. (1999) in India on *C. tinctorius* with regard to the dominance of Insecta over other invertebrate classes. This result would be because Insecta are the most abundant and diverse clade of the Invertebrate clade (Basset et al., 2012). In addition,

the present results are different from theirs when it comes to composition. Indeed, their results revealed the dominance of Coleoptera, Diptera, Hemiptera, Lepidoptera and Thysanoptera as the most abundant orders. This difference could be due to climatic variations and the number of sampling methods but also the area of the fields. Indeed, Saeidi et al. (2015) used three sampling methods: sweep netting, yellow sticky trap, and pitfall trap. Also, the size of the farms was about 1000 m² in the present work against 6000 m². The present study does not reveal the presence of Thysanoptera.

This difference would be due to the mode of organization of Formicidae and Aphididae which live in colony on one hand and their great reproductive capacity on the other hand.

U. compositae has been reported as a pest of *C. tinctorius*. Ishaq et al. (2004) specified which is at the origin of crop losses ranging from 35 to 72%. In Cameroon, this is the first time that it has been reported on *V. calvoana calvoana*. *H. cameroonensis* was listed by Tamesse and Dogmo (2016) as a pest of *V. amygdalina* (Asteraceae). It is being reported for the first time on *V. calvoana calvoana*. *Z. variegatus*, polyphagous, which can develop on *V. amygdalina* is also reported on *V. calvoana calvoana*.

Conclusion

The present study shows that the invertebrate fauna of *V. calvoana calvoana* is diverse. It is grouped into 12 orders, 47 families and 168 species. The phytophagous species are concentrated in the order Hemiptera. The most abundant species are *U. compositae*, *H. cameroonensis* and *S. annulus*. There is little variation in diversity between varieties and sites.

Table 2. List of different families of invertebrates (relative abundance above 1%) collected during the study.

Family	The abundance of invertebrate per varieties			
	B1	B	V	Total general
Achilidae	0 (0)	1 (0.03)	0 (0)	1 (0.01)
Acrididae	0 (0)	3 (0.09)	4 (0.09)	7 (0.05)
Aphididae	2859 (46.51)	872 (25.13)	1424 (33.02)	5155 (37.01)
Apidae	0 (0)	1 (0.03)	2 (0.05)	3 (0.02)
Apionidae	0 (0)	0 (0)	1 (0.02)	1 (0.01)
Araneidae	0 (0)	0 (0)	1 (0.02)	1 (0.01)
Asteiidae	0 (0)	2 (0.06)	4 (0.09)	6 (0.04)
Bibionidae	1 (0.02)	1 (0.03)	0 (0)	2 (0.01)
Blattidae	0 (0)	0 (0)	1 (0.02)	1 (0.01)
Braconidae	1 (0.02)	0 (0)	0 (0)	1 (0.01)
Cantharidae	1 (0.02)	0 (0)	0 (0)	1 (0.01)
Cercopidae	0 (0)	4 (0.12)	4 (0.09)	8 (0.06)
Chrysomelidae	6 (0.1)	56 (1.61)	39 (0.9)	101 (0.73)
Cicadellidae	1 (0.02)	5 (0.14)	4 (0.09)	10 (0.07)
Coccinellidae	8 (0.13)	153 (4.41)	127 (2.95)	288 (2.07)
Coreidae	8 (0.13)	10 (0.29)	3 (0.07)	21 (0.15)
Culicidae	0 (0)	1 (0.03)	4 (0.09)	5 (0.04)
Curculionidae	2 (0.03)	1 (0.03)	3 (0.07)	6 (0.04)
Formicidae	2929 (47.65)	1867 (53.8)	2072 (48.05)	6868 (49.31)
Gryllidae	0 (0)	1 (0.03)	5 (0.12)	6 (0.04)
Jassidae	1 (0.02)	2 (0.06)	1 (0.02)	4 (0.03)
Lauxaniidae	0 (0)	1 (0.03)	0 (0)	1 (0.01)
Lycidae	0 (0)	1 (0.03)	2 (0.05)	3 (0.02)
Lycosidae	0 (0)	1 (0.03)	2 (0.05)	3 (0.02)
Mantodae	1 (0.02)	3 (0.09)	5 (0.12)	9 (0.06)
Membracidae	5 (0.08)	2 (0.06)	5 (0.12)	12 (0.09)
Muscidae	0 (0)	2 (0.06)	0 (0)	2 (0.01)
Myodochidae	2 (0.03)	30 (0.86)	26 (0.6)	58 (0.42)
Odontopygidae	0 (0)	0 (0)	1 (0.02)	1 (0.01)
Others families	11 (0.18)	21 (0.61)	29 (0.67)	61 (0.44)
Passalidae	0 (0)	0 (0)	1 (0.02)	1 (0.01)
Pentatomidae	1 (0.02)	192 (5.53)	195 (4.52)	388 (2.79)
Pipunculidae	0 (0)	1 (0.03)	0 (0)	1 (0.01)
Plataspidae	0 (0)	2 (0.06)	6 (0.14)	8 (0.06)
Pyrgomorphidae	0 (0)	68 (1.96)	75 (1.74)	143 (1.03)
Pyrrhocoridae	10 (0.16)	19 (0.55)	13 (0.3)	42 (0.3)
Reduviidae	0 (0)	3 (0.09)	2 (0.05)	5 (0.04)
Rhaphidophoridae	2 (0.03)	4 (0.12)	2 (0.05)	8 (0.06)
Salticidae	1 (0.02)	2 (0.06)	1 (0.02)	4 (0.03)
Solenopidae	0 (0)	0 (0)	2 (0.05)	2 (0.01)
Syrphidae	0 (0)	3 (0.09)	1 (0.02)	4 (0.03)
Tetrigidae	1 (0.02)	0 (0)	1 (0.02)	2 (0.01)
Tettigometridae	293 (4.77)	122 (3.52)	236 (5.47)	651 (4.67)
Tettigoniidae	2 (0.03)	8 (0.23)	6 (0.14)	16 (0.11)
Theridiidae	1 (0.02)	1 (0.03)	0 (0)	2 (0.01)
Thomisidae	0 (0)	2 (0.06)	1 (0.02)	3 (0.02)
Trigonidiidae	0 (0)	2 (0.06)	1 (0.02)	3 (0.02)
Total general	6147 (100)	3470 (100)	4312 (100)	13929 (100)

Values in brackets represent relative abundance. B1: White variety of *V. calvoana calvoana* in Nlong-mvolye; B: White variety of *V. calvoana calvoana* from Nkolbisson; V: Purple variety of *V. calvoana calvoana* from Nkolbisson.

Table 3. Variant diversity indices at the family level.

Index	B	B1	V
Richness	39	23	35
Abundance	4450	6147	3332
Simpson_1-D	0.64	0.55	0.66
Shannon H	1.45	0.93	1.48
Equitability_J	0.40	0.30	0.42
Berger-Parker	0.54	0.48	0.47

B1: White variety of *V. calvoana calvoana* in Nlong-mvolye; B: White variety of *V. calvoana calvoana* from Nkolbisson; V: Purple variety of *V. calvoana calvoana* from Nkolbisson.

Table 4. List of the most abundant species of invertebrates (relative abundance above 1 %) collected during the study period.

Species	The abundance of invertebrate per varieties			
	B1	B	V	Total general
<i>Camponotus acvapimensis</i>	1 (0.02)	525 (15.13)	356 (8.26)	882 (6.33)
<i>Coccinellidae</i> sp.	7 (0.11)	131 (3.78)	103 (2.39)	241 (1.73)
<i>Diplomorium longipenne</i>	0 (0)	697 (20.09)	1056 (24.49)	1753 (12.59)
<i>Hilda cameroonensis</i>	293 (4.77)	121 (3.49)	236 (5.47)	650 (4.67)
<i>Myrmicaria opaciventris</i>	0 (0)	147 (4.24)	390 (9.04)	537 (3.86)
<i>Pheidole megacephala</i>	2858 (46.49)	148 (4.27)	199 (4.62)	3205 (23.01)
<i>Sphaerocoris annulus</i>	1 (0.02)	189 (5.45)	188 (4.36)	378 (2.71)
<i>Tetramorium acculeatum</i>	12 (0.2)	298 (8.59)	22 (0.51)	332 (2.38)
<i>Uroleucon compositae</i>	2859 (46.51)	872 (25.13)	1424 (33.02)	5155 (37.01)
<i>Zonocerus variegatus</i>	0 (0)	67 (1.93)	73 (1.69)	140 (1.01)
Total general	6031 (98.11)	3195 (92.07)	4047 (93.85)	13273 (95.29)

Values in brackets represent relative abundance. B1: White variety of *V. calvoana calvoana* in Nlong-mvolye; B: White variety of *V. calvoana calvoana* from Nkolbisson; V: Purple variety of *V. calvoana calvoana* from Nkolbisson.

Recommendations

For future work, the nature of the damage related to the invertebrate fauna present on *V. calvoana calvoana* will be investigated.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Studies on Shea (*Vitellaria paradoxa*) fruits storage and different preservation technologies for domestication

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In order to prevent a Shea seed from germination when stored, a preservation technology was adopted using different polythene bags which include a white transparent polythene bag of 500 mm gauge, big size perforated brown envelope, and black polythene bags of 500 mm gauge. The results revealed that the Shea seeds preserved in the white transparent polythene bags retained their viability for six months which can then be used by experienced/professional domesticators for nursery establishment. With the result obtained from this study, the viable Shea seeds would be available all year round for domesticators. Therefore, the problem of seasonality in Shea seed availability is solved. However, Shea seed domesticators are encouraged to use the successful method of preservation to ensure availability of viable seeds across the yearly seasons.

Key words: Shea seeds, preservation, technology, polythene bag, domestication.

INTRODUCTION

Shea tree is an indigenous woody plant of Savanna Parkland that is scientifically called *Vitellaria paradoxa* which belongs to the family Sapotaceae. It is found in wide belts of Africa, extending from Senegal through the Sahel savanna parkland (Elias and Carney, 2004; Schreckenber, 2004). Shea butter is processed from Shea nuts, which is also edible fruits and plays significant roles in household consumption and livelihood for rural people. Apart from providing a food supplement, Shea tree has monetary transaction importance in local economic and international trade (Lovett and Haq, 2000; Olosu, 2009).

The main constraints to domestication of karite/Shea tree are their slow growth, long juvenile phase and large yield variability. There is a high degree of variation in fruit

and nut production, nut and fruit size, pulp sweetness, oil content and quality has been documented for karite (Maraz et al., 2004). A Shea seed is a ripened ovule. At the time of separation from the parent Shea plant, the plant consists of an embryo and stored food supply, both of which are encased in a protective covering. The activation of the metabolic machinery of the embryo leading to the emergence of a new seedling plant is known as germination.

For germination to be initiated, three conditions must be fulfilled (Ching, 1972; Jann and Amen, 1977). First, the seed must be viable; that is, the embryo must be alive and capable of germination. Second, the seed must be subjected to the appropriate environmental conditions such as available water, proper temperate regimes, a

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supply of oxygen, and sometimes light. Third, any primary dormancy condition present within the seed must be overcome (Crocker, 1916). Internal processes leading to removal of primary dormancy are collectively known as after-ripened and result from the interaction of the environment with the specific primary dormancy condition.

The stage of germination involves activation of seeds, digestion and translocation of seeds and seedling growth (Bewley and Black, 1985). Seed dormancy is a form of biological adaptation that prevents germination on the plant itself (viviparia), as well as germination of seeds at an inconvenient time of year, when the seedlings would not endure adverse environmental conditions (Bewley, 1997). Seed Dormancy and Germination are very critical periods for seeds domestication. Germination is the process by which the embryo wakes up from the state of dormancy and takes to active life. The seed contains only 10 to 15% of water and this low water content is one of the factors responsible for dormancy, when certain external conditions are satisfied the dormant embryo begins to grow. Seed dormancy allows seeds to overcome periods that are unfavorable for seedling establishment and is therefore important for plant ecology and agriculture. There are other factors that affect seed germinability such as insufficient and untimely rainfall, sowing in poorly prepared land in combination with low precipitation or deep sowing etc.

Seed development comprises of two major phases, embryo development and seed maturation. Embryogenesis, which is a major photogenesis phase, starts with the formation of a single-cell-zygote and ends in the heart stage when all embryo structure has been formed (Mayer et al., 1991). Seed storage is one of the major factors of seeds domestication. Seeds that falls from a particular plant or harvested usually enters resting period. During this resting period, the seeds do not germinate until proper conditions are attained; however, they are still alive.

During dormancy, the seeds are still alive; they undergo what is referred to as cellular respiration, using up oxygen and stored sugars, while respiring and cleaning carbon dioxide. High temperature can speed respiration, adversely affecting the storage life of seeds. High humidity, especially when combined with high temperature favors the growth of fungal pathogens and subsequent rotting of seeds. Germination of seeds is a complex physiological and biochemical process controlled by many mechanisms (Gu et al., 2016).

Viability is expressed by the germination percentage, which indicates the number of seedlings produced by a given number of seeds. Additional characteristics of high-quality seed are prompt germination, vigorous seedling growth, and normal appearance (Abdul-Baki, 1980). Vigor of seed and seedling is an important attribute of 1986; McDonald, 1980). Low germination percentage, low germination rate, and low vigor are often associated. Low germination can be due to genetic properties of certain cultivars (Dickson, 1980). The objective of storage

is to keep the seeds alive in such a way that they use their food reserves slowly, remain viable for a long time and maintain energy for germination at planting time.

Domestication of Shea trees largely depends on their seeds and thereafter followed by vegetative propagation for gestation period reduction. They are constrained by some biological factors such as seasonality of production, ageing of Shea trees and local recalcitrant of shell seeds. The seeds contain high moisture content and about 45-50% oil content which shorten their dormancy and usability period in preservation for germination.

The general objective of this research is to prolong the dormancy period and viability of Shea seeds and to sustain its availability as reliable planting materials for the domesticators from a period of one week to six months as against the dormancy period and viability loss of one to two weeks as cited by Lovett and Haq (2004). The study also proffered solutions to the bottleneck of seasonality of planting of Shea seeds, short dormancy period, short period loss of viability, low effectiveness and efficiency of nursery establishment and managements at a convenient period by the domesticators or growers. The researcher carried out Shea seeds preservation and germination for domestication, using three preservation methods, namely: white transparent polythene bags, perforated brown envelope and black polythene bags for the period of one to six months as enumerated in the work.

MATERIALS AND METHODS

Description of study location

The study was conducted at Nigerian Institute for Oil Palm Research (NIFOR), Shea Tree Research Substation Nursery Site in Bida local Government Area of Niger State, Nigeria. It is located in the Guinea Savanna zone, which falls in the middle belt of Nigeria. The area lies on Latitude 08°05'78"N and Longitude 006°47.789"E. The soil type in this location is sandy loam with annual rainfall ranges between 500 to 1200 mm per annum. Minimum and maximum temperature ranges between 24 and 33°C respectively. Shea seeds/fruits were collected from already identified superior mature trees, according to farmers' criteria, which included good physical and sanitary characteristics (such as vigorous growth and absence of parasites) as well as the quality of the fruits and nuts (tasty pulp and oil-rich kernel). The collected seeds/fruits were processed for seeds by depulping the fruits manually at the substation.

Collection and preparation of Shea seeds

First, 150 Shea seeds were collected from the wild of Shea tree growing areas in Niger state. The seeds were selected and washed under a running tap, then sun dried for 5 min and stored in room temperature differently in the following storage materials with frequent observation: white transparent polythene bags of 500 mm gauge; big size perforated brown envelope; and black polythene bags of 500 mm gauge. Secondly, 150 Shea seeds were selected, washed, soaked in warm water for 2 min; sun dried and stored in room temperature differently in the same storage materials as itemized above. The first and second year experimental observation

lasted from one to five months, while the third year of the experimental observation lasted from one to six months with 180 Shea seeds for each preservation methods in 2017.

The experiment was carried out for three (3) consecutive years (2015, 2016 and 2017). For each seeds sample, six lots of 50 seeds each were used for germination tests: - Lots one to six: fresh seeds no 180 Stored after harvest (0 month of storage); Lot 1: seeds stored for 1 month after harvest; Lot 2 seeds stored for 2 months after harvest; Lot 3 seeds stored for 3 months after harvest; Lot 4 seeds stored for 4 months after harvest; Lot 5 seeds stored for 5 months after harvest; Lot 6 seeds stored for 6 months after harvest.

Experimental design, treatments and planting of Shea seeds

Treatment 1

150 Shea seeds were selected from the different preservation methods, namely: white transparent polythene bags, perforated brown envelope and black polythene bags, and 50 Shea seeds were used for each method. The seeds were washed under a running tap water, sun-dried for 5 min and stored differently in the preservation materials, planted in the appropriate soil medium of river sand, sandy loamy and cured organic manure of 1:2:1 in 8 by 12 by 500 mm guage polythene bags with adequate manual irrigation at every other day and exposed to natural precipitation. The seeds were planted on sequential months in ascending order from April to August of the year of experimental observation. This was repeated in 2015, 2016 and 2017 as indicated in tables 1-6. While in 2017, 180 Shea seeds were used for three preservation methods as indicated in Table 5 and 6 of the analysis.

Treatment 2

150 Shea seeds were selected from the different preservation methods, namely: white transparent polythene bags, perforated brown envelope and black polythene bags, and 50 Shea seeds were used for each method. The seeds were soaked in warm water for 2 min, washed with ordinary clean water, sun-dried for 5 min and stored differently in the preservation materials, planted in the appropriate soil medium of river sand, sandy loamy and cured organic manure of 1:2:1 in 8 by 12 by 500 mm guage polythene bags with adequate manual irrigation at every other day and also exposed to natural precipitation.

The seeds were planted on sequential months in ascending order from April to August of the year of experimental observation; while in 2017, 180 Shea seeds were used for three preservation methods as indicated in Tables 5 and 6 of the analysis.

Data analysis

The data for this study was collected and analyzed using frequency and percentage to show the differences in the performance of the three preservation methods with particular reference to germination.

Evaluation of seed germination

Germination is measured on two parameters, the germination percentage and the germination rate. Vigor may be indicated by measurement, but seedling growth rate and morphological appearance must also be considered. Sometime abnormal growing seedlings result from low seed quality (Heydecker, 1972). Statement of germination percentage should involve time element, indicating the number of seedlings produced within a specified

length of time. Germination rate can be measured by several methods. One determines the number of days required to produce a given germination percentage (Kotowski, 1926; Gordon, 1973; Czabator, 1962).

Germination percentage of Shea seeds was evaluated each month. Seeds are considered germinated when the plumule emerged from the testa/embryo. The percentage of germination (% G) was to:

$$\%G = \frac{NSG}{NST} \times 100$$

Where, NSG is number of seed germinated and NST is total number of seed tested.

After 6 months, the cumulative percentage of germination was determined.

RESULTS AND DISCUSSION

The results in Table 1 show that 80% of the Shea seeds preserved in the white transparent polythene bag in the first, third, and fifth months respectively were still viable and as such germinated after planting. However, 90% of the Shea seeds in the second month were still viable and germinated after planting and 70% of the Shea seeds planted in the fourth month were viable and germinated, while 0% of the Shea seeds preserved in perforated envelope and black polythene bag were recorded death.

Table 2 results indicate that in the whole five months, none of the Shea seeds preserved in white transparent polythene bag, perforated envelope and in the black polythene bag germinated after planting. This reveals that 100% of the Shea seeds were not viable after preservation. This could be attributed to warm water soaking of Shea seeds before planting in the polythene bags, which would have destroyed or damage the embryonic cells of the seeds.

From Table 3, the result reveals that 100% of the Shea seeds preserved in the white transparent polythene bag were still viable and germinated after two months of preservation. 90% of the preserved seeds were also still viable and germinated after one month and five months of preservation respectively. 80% of the seeds were also viable and germinated after four months and 60% were those that were viable and germinated at as after three months of preservation, while 0% of germination was recorded for Shea seeds that were preserved in perforated envelope and black polythene bag, this could be attributed to temperature of the growing seeds or the environmental conditions which might have influenced either positively or negatively to the seeds germination. However, Bass and Clark (1967) in his study found that the loss of viability of freshly harvested Kentucky bluegrass seed was correlated with the moisture content of the seed and length of time held at a given temperature.

The result in Table 4 shows that none of the Shea seeds preserved in the white transparent polythene bag,

Table 1. Variations in percentage germination using the different germination technologies for the year 2015.

Months of experimental observation (April to August, 2015)	White transparent polythene bags/percentage germination (%)		Perforated brown envelope /percentage germination (%)		Black polythene bag/percentage germination (%)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
First month						
Number planted	10		10		10	
Number germinated	8	80	0	-	0	-
Second month						
Number planted	10		10		10	
Number germinated	9	90	0	-	0	-
Third month						
Number planted	10		10		10	
Number germinated	8	80	0	-	0	-
Fourth month						
Number planted	10		10		10	
Number germinated	7	70	0	-	0	-
Fifth month						
Number planted	10		10		10	
Number germinated	8	80	0	-	0	-

Table 2. No viability recorded using the different preservation methods after planting for the year 2015.

Months of experimental observation (April to August, 2015)	White transparent polythene bag/percentage germination (%)		Perforated brown envelope/percentage germination (%)		Black polythene bag/percentage germination (%)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
First month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-
Second month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-
Third month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-
Fourth month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-
Fifth month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-

perforated envelope and black polythene bag germinated after one month, two months, three months, four months

and five months of preservation respectively. This result simply means that 100% of the preserved seeds in the

Table 3. Variations in percentage germination at different months after planting for the year 2016.

Months of experimental observation (April to August, 2016)	White transparent polythene bag/percentage germination (%)		Perforated brown envelope/percentage germination (%)		Black polythene bag/percentage germination (%)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
First month						
No. planted	10		10		10	
No. germinated	9	90%	0	-	0	-
Second month						
No. planted	10		10		10	
No. germinated	10	100%	0	-	0	-
Third month						
No. planted	10		10		10	
No. germinated	6	60%	0	-	0	-
Fourth month						
No. planted	10		10		10	
No. germinated	8	80%	0	-	0	-
Fifth month						
No. planted	10		10		10	
No. germinated	9	90%	0	-	0	-

Table 4. No viability recorded using the different preservation methods after planting at different months for the year 2016.

Months of Experimental Observation (April to September, 2016)	White Transparent polythene Bag/Percentage Germination (%)		Perforated Brown Envelope/Percentage Germination (%)		Black Polythene Bag/Percentage Germination (%)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
First Month						
No. Planted	10		10		10	
No Germinated	0	-	0	-	0	-
Second Month						
No. Planted	10		10		10	
No. Germinated	0	-	0	-	0	-
Third Month						
No. Planted	10		10		10	
No. Germinated	0	-	0	-	0	-
Fourth Month						
No. Planted	10		10		10	
No. Germinated	0	-	0	-	0	-
Fifth Month						
No. Planted	10		10		10	
No. Germinated	0	-	0	-	0	-

various preservation materials were not more viable after the various said months.

The result from Table 5 indicates that 90% of the Shea seeds preserved in the white transparent polythene bag

Table 5. Variations in percentage germination at different months after planting for the year 2017.

Months of experimental Observation (April to September, 2017)	White transparent polythene bag/percentage germination (%)		Perforated brown envelope/percentage germination (%)		Black polythene bag/percentage germination (%)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
First month						
No. planted	10		10		10	
No. germinated	8	80%	1	10%	0	-
Second month						
No. planted	10		10		10	
No. germinated	9	90%	0	-	0	-
Third month						
No. planted	10		10		10	
No. germinated	7	70%	0	-	0	-
Fourth month						
No. planted	10		10		10	
No. germinated	9	90%	0	-	0	-
Fifth month						
No. planted	10		10		10	
No. germinated	9	90%	0	-	0	-
Sixth month						
No. planted	10		10		10	
No. germinated	8	80%	0	-	0	-

after two months, four months, five months and six months respectively maintained their viability and as such germinated after planting. 80% of the Shea seeds were also viable and germinated after one month of preservation and 70% proved viable after three months of preservation, while only 10% of the Shea seeds preserved in perforated envelope germinated after one month of preservation and all other ones representing 90% lost their viability right from one month to six months respectively. 100% of the Shea seeds preserved in the black polythene bag lost their viability from the first month up to the last month, this might be attributed to the environmental conditions, or the chemical compositions of the seed might have been deteriorated. The results of this research is in conformity with the report of Pollock (1961) who opined that a seed with an unbalanced chemical composition, or one mechanically damaged, permitting early entry of micro-organisms, would be at a disadvantage in storage.

The result in Table 6 shows that all the Shea seeds, that is 100% of seeds preserved in transparent polythene bag, perforated envelope and black polythene bag lost their viability and as such did not germinate after one, two, three, four, five and six months of preservation respectively. The inability of the seeds to germinate at these periods might be due to environmental conditions

which may have triggered the duration of dormancy of the seeds.

Conclusion

The results of the study revealed that Shea seeds preserved in the white transparent polythene bags, washed with ordinary clean water, sun-dried for 5 min, planted in the appropriate soil medium of river sand, sandy loamy and cured organic manure of 1:2:1 in 8 by 12 by 500 mm gauge polythene bags with adequate manual irrigation of every other day and exposed to natural precipitation can retained their viability for six months and they can be used by experienced/professional domesticators for nursery establishment. Shea seeds viability and dormancy period is one of the major constraints affecting Shea tree propagation as well as its plantation establishment. With the result obtained from this study, the viable Shea seeds would be available all the year round for domesticators.

Therefore, the problem of seasonality in Shea seed availability is solved. However, Shea seed domesticators are encouraged to use the successful method of preservation to ensure availability of viable seeds across the yearly seasons.

Table 6. No viability recorded using the different preservation methods after planting at different months for the year 2017.

Months of experimental observation (April to September, 2017)	White transparent polythene bag/percentage germination (%)		Perforated brown envelope/percentage germination (%)		Black polythene bag/percentage germination (%)	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
First month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-
Second month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-
Third month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-
Fourth month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-
Fifth month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-
Sixth month						
No. planted	10		10		10	
No. germinated	0	-	0	-	0	-

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Review

Physiological ecology of ferns: Biodiversity and conservation perspectives

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Ferns have a long geological record extending over millions of years, and they are distributed in diverse environments worldwide; including swamps, coastal locations, open grasslands, mountain terrains, drylands and deserts. Consequently, their physiological ecology is rich with examples of the fern species diversity, and remarkable adaptive variability. This is a review of some major aspects of their physiological ecology (that is, water relations and desiccation tolerance, light and photosynthesis, and temperature and physiological responses), focusing on terrestrial and epiphytic ferns in diverse global geographic locales. Ferns are important economically in horticultural commerce and provide significant ecological services. Climate change and destruction of their natural habitats may lead to extensive loss of fern biodiversity; and some of the current issues related to the protection of their natural habitat and conservation of fern species are addressed.

Key words: Biological adaptation, climate change, geographic distribution, human impact, plant evolution.

INTRODUCTION

Ferns, encompassing approximately 12,000 species (PPG 1, 2016), represent only about 4% of vascular plant diversity (Mehltreter, 2010); nonetheless, they provide important ecological services (Sharpe et al., 2010), and comprise a substantial portion of commercial ornamental plant production as well as horticultural economic development (Hoshizaki and Moran, 2001; Singh and Johari, 2018). Their evolutionary and paleobiological history is extensive beginning in the Middle Devonian, approximately 390 million years ago (mya) with expanded diversity during the Cenozoic (65 mya), when angiosperms were becoming more dominant (Schneider et al., 2004; Schuettepelz and Pryer, 2009). Ferns were

dominant flora in the Carboniferous, later losing space to gymnosperms and angiosperms. Increasing forests provided suitable protective, shady environments where ferns flourished on the forest floor or as epiphytes on tree trunks and limbs (Watkins and Cardelús, 2012). Subsequently, over geological time spans, through adaptation and evolutionary radiation (Sessa, 2018), ferns proliferated to occupy a wide diversity of terrestrial environments; ranging from swamps and coastal locations to more open grasslands, mountain terrains, drylands and deserts. Consequently, their biodiversity and life histories (Figure 1) have commensurately expanded to include a rich panoply of different life forms,

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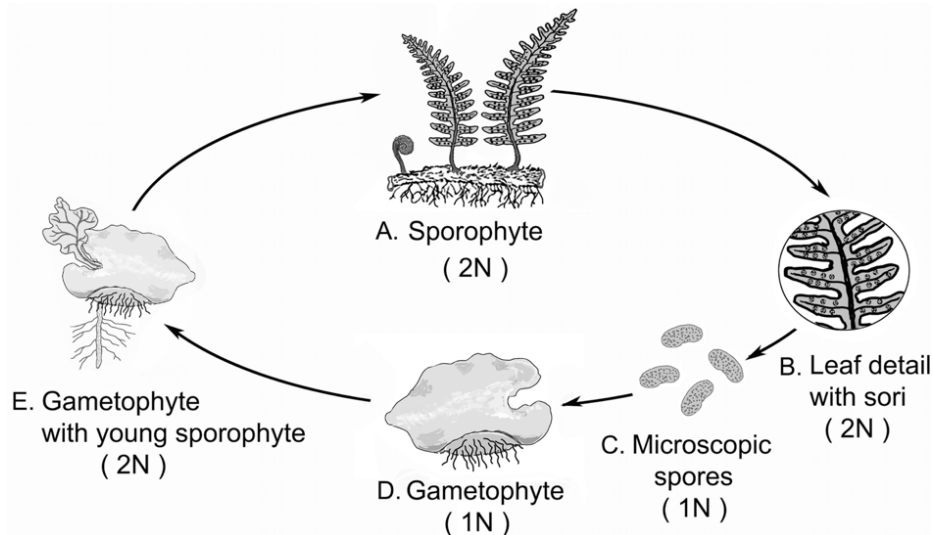


Figure 1. Fern life cycle diagram. **A.** Sporophyte, diploid phase of fern life cycle. **B.** Detail of leaf with rounded sori containing spore-producing sporangia. **C.** Spores that have been released from the sporophyte. **D.** Heart-shaped gametophyte, a haploid phase of the fern life cycle, producing sperm in antheridia and egg in archegonia; fertilized egg produces a zygote that develops into a young sporophyte. **E.** Gametophyte with first growth of a young sporophyte.

physiological adaptive strategies, and wide global habitats.

A brief comment about terminology: in prior publications, the leafy photosynthetic portion of the fern (including the stipe) was referred to as 'fronds' and this is still appropriate; but increasingly the term used is 'leaves' as applied to other vascular plants. Therefore hereafter, the term 'leaves' will be used largely, except where frond may be more appropriate.

In comparison to seed-bearing plants, spore-bearing ferns have a characteristic life cycle with alternating generations of a diploid ($2N$) spore-bearing sporophyte and a free-living, haploid ($1N$) gametophyte (prothallus) that produces egg and sperm. After fertilization of the egg producing a zygote, it gives rise to a new sporophyte generation (Figure 1). The dominant phase of the fern life cycle is the sporophyte that produces haploid spores in sporangia, typically occurring on the lower surface of fern leaves (Figure 2a, b), or in separate spikes (sporangiophores) with more specialized, usually reduced and modified fertile sporophylls. The cinnamon fern, *Osmundastrum cinnamomeum* (L.) C. Presl, observable in the natural environment and commonly grown in gardens, is an example of such a dimorphic fern. A crown of green, pinnate leaves surrounds central sporangiophores with brown, reduced fertile leaves bearing sporangia and spores.

The photosynthetic gametophyte (prothallus) that develops from the fern spore is typically thin (one-celled thick) and lacks extensive surface protective covering. Therefore, it is particularly subjected to environmental

pressures. Consequently, it can be a precarious link in the alternation of generations in the fern life cycle; because its failure would interdict development of the sporophyte stage, especially if environmental conditions are challenging. Although many species have a chordate (heart-shaped) prothallus as shown in Figure 1, it is important to recognize that in other species the prothallus is strap-shaped, ribbon-like, or even filamentous at maturity, especially varying with the habitat where the species have become adapted.

The number of spores released by the sporophyte is usually copious, and most spores are distributed in near vicinity to the mother plant (sporophyte). Consequently, a relatively dense population of gametophytes may develop in surrounding suitable locations, thus increasing the probability of successful completion of the life cycle. However, the density of gametophytes may lead to competition and only a portion of the more vigorous gametophytes survive. Moreover, there is increasing evidence that the powdery spores can be carried by wind to distant locations (including distant oceanic islands); thus, increasing dispersion of the species, assuming the new location is suitable for gametophyte survival (Sharpe et al., 2010). The evolution of very light-weight spores has promoted wide-spread dispersion of some fern species and contributed to their establishment broadly across continents and islands of the oceans in highly diverse geographic locales. Furthermore, the capacity for some gametophytes to self-fertilize also ensures that even a single gametophyte from a spore carried to distant lands may successfully give rise to a new generation at



Figure 2. Examples of fern biodiversity. **A.** *Phlebodium pseudoaureum* (Cav.) Lellinger, with large pinnatifid (lobed) leaves and rounded sori where spores are produced. **B.** *Pyrrosia lingua* (Thunb.) Farw. (Cristata cultivar), an epiphyte growing on tree bark exhibiting the abaxial surface with dense deposits of sori within a dark, purplish indumentum. **C.** *Adiantum* sp.: typically, shade adapted ferns with thin leaf laminae that have one to two celled thick mesophylls. **D.** *Davallia fejeensis* Hook. with finely divided pinnate leaves.

these distant locations. Depending on the genetic composition of the individual and potential for adaptation in the new locale, a new population may thrive and in some cases through evolution become established as endemics in that geographic locale.

The biogeography and systematics of ferns have a relatively richer, and historically longer, record of research than physiological ecology that largely expanded in quantity and sophistication during the twentieth and twenty-first centuries. There appears to be few recent reviews of fern physiological ecology addressing the particular topics treated here, although there are other substantial sources on fern ecology, more broadly (Mehltreter et al., 2010). Some representative examples of published research on fern physiological ecology are reviewed here. Ferns have diversified to occupy widely different ecological niches; including, aquatic, terrestrial, epiphytic (growing on other plants)

and epilithic (growing near to or upon rocks) species occurring in diverse geographical and climatic locales. Thus, there is a rich source of potential evidence for fern physiological ecology research. A more general treatment of fern research and natural history is provided by Fernández (2018) and Moran (2004). For information on horticultural and cultivation aspects consider Jones (1987). A comprehensive review of gametophyte developmental biology is given by Raghavan (2005). Each of the particular life forms of ferns has evolved specialized morphological and physiological adaptations to better survive in their particular habitat. With increasing concerns about the possible aversive effects of climate change for life on Earth, there is also particular interest in the likely challenges for survival of many fern species that have become adapted to particular environmental niches (Anderson, 2018). Consequently, there are enhanced efforts to better understand the physiological ecology of

ferns to more fully document and monitor the distribution and survival of biodiverse fern populations, and the biological communities where they occur.

This review of current research on fern physiological ecology focuses on terrestrial and epiphytic ferns, with particular emphasis on examples of biodiverse groups of ferns that have been published in recent decades. During the literature search, an online survey using the keywords 'Fern ecophysiology' was made for each year from present (2021) back to 1900. To delimit the categories of articles reviewed, three main topics were identified: 1) Water relations and desiccation tolerance, 2) Light and photosynthesis, 3) Temperature and physiological responses, and finally 4) Issues of conservation. These were chosen as particularly relevant aspects of physiological ecology for ferns, and consistent with the intent of a review article focusing on diverse groups of ferns in broad global environments. Additionally, articles were selected that were representative of the habitat (terrestrial or epiphytic) and addressed life stages (spores and germination, gametophyte stage, and sporophyte stage). In so far as possible, representative articles were chosen that included varied global locations (geographically and environmentally) that may be of interest to an international audience of readers.

WATER RELATIONS AND DESICCATION TOLERANCE

In addition to appropriate illumination for photosynthesis, adequate moisture is one of the most important environmental variables controlling the distribution and abundance of terrestrial and epiphytic ferns. Some species (*Acrostichum aureum* L., known as golden leather fern or swamp fern) are found in freshwater and mangrove swamps or other wet locations. By contrast 'Cheilanthoid ferns,' e.g., *Cheilanthes* spp., *Myriopteris* spp. (separated from *Cheilanthes*, sensu stricto), and *Asplenium ceterach* L., are adapted to dryland and desert locales. For example, xerophytic *Myriopteris lanosa* (Michx.) Grusz & Windham (hairy lip fern) has very small leaves, tightly curled (approximating a ball), and is covered with a dense, lanose (wooly) surface coat – particularly adapted to reduce water loss by leaf transpiration. A substantial amount of ecological and laboratory experimental research has focused on water relations and desiccation tolerance of ferns. A useful review of the topic for 'resurrection plants' in general is presented by Deeba and Pandey (2017); and more specifically for resurrection ferns by Hietz (2010), Kavitha et al. (2017), and López-Poso et al. (2018). Relevant published research topics are summarized here for terrestrial and epiphytic ferns. Subtopics within each section address environmental and physiological factors or, where relevant, cellular and biochemical adaptations. In general, review of research related to the gametophyte

is presented before addressing research with sporophytes.

Terrestrial ferns

Gametophyte environmental physiological responses

Because of the delicate and potentially fragile state of gametophytes in the fern life cycle, research attention has been given to their tolerance for desiccation stress and adaptive response to water stressful environments, including adaptive variations in tropical species (Watkins et al., 2007) and the role of competition and climate change with particular to the rare fern *Asplenium scolopendrium* var. *americanum* (Fernald) Reichst., Rasbach & Viane published by Testo and Watkins (2013). Compared to six other species, gametophytes of *A. scolopendrium* var. *americanum* had the lowest rates of germination and sporophyte production and exhibited the greatest sensitivity to interspecific competition, temperature increases, and desiccation. Given these potentially precarious characteristics, possibly threatening its survival, greater attention to conservation of this species is warranted. Particular attention has been given to gametophyte desiccation tolerance by other rare or endangered species including *Camptosorus rhizophyllus* (L.) Link, typically growing with mosses and lichens on the shaded surface of dry limestone slabs and on detached limestone slabs in open ravines and torrent beds – particularly challenging environments (Pickett, 1913).

The peculiar American species *Vittaria appalachiana* Farrar and Mickel, growing densely on shaded rock surfaces, exists only in the gametophyte form. It is sometimes known as a 'gameto-only' fern, lacking a sporophyte stage in the life cycle and reproducing asexually by photosynthetic propagules (gemmae) produced by the gametophyte. Chambers et al. (2017) report that this unique species, while limited to local sites, shows considerable adaptive variability to desiccation; indicating that it may have greater survival potential than suggested by its limited geographic range. Sato (1992) studied the effects of seasonal variables, especially spring desiccation, on survival and size of gametophytes of *Athyrium brevifrons* Nakai ex Kitag. in a transplant garden of Sapporo Japan. Gametophytes of all sizes decreased with decreasing soil moisture ($r = 0.878$, $p < 0.01$). In a similar study in the natural environment, the carbohydrate, lipid content and biomass of the gametophyte stage of the xerophytic hairy lip fern, *Myriopteris* (*Cheilanthes*) *lanosa*, collected from a sandstone bluff in midwestern U.S.A., was reported by Crow and Mack (2011). They found that each gametophyte increased in percent of total biomass (w/w) throughout development; concomitant with ability to better manage water balance during maturation.

Moreover, young gametophytes, with high carbohydrate and low lipid content, were located on substrates with a potential for small but continuous water source.

Sporophyte environmental physiological responses.

Extensive research has been given to the sporophyte stage of the fern life cycle. Mesophytic ferns, growing in environments with ample precipitation, have been investigated to determine effects of intermittent desiccation events. Liao et al. (2008) examined the effects of variation in soil moisture (80, 60, and 40%) on adaptive characteristics of *Adiantum reniforme* var. *sinensis* Y.X. Lin, an endangered species endemic to the Three Gorges Region in China. They found that drought stress decreased leaf growth and photosynthetic capacity, and hence reduced total mass, specific leaf area (SLA) and leaf area ratio (LAR). However, there was an increase in dry matter allocation into the root fraction with decreasing soil moisture. Leaf relative water content (RWC) decreased marginally as soil water was depleted. The authors concluded that these results might be the result of a physiological balance between the demand for water by the leaves and the water uptake from soil by the roots.

During the period of 2012 to 2016, California incurred severe seasonal drought. Holmlund et al. (2016) examined tissue-water relations among eight ferns in the Santa Monica Mountains to examine differential mechanisms of drought survival. They reported that five chaparral species had a wider range of tolerance (e.g., water potentials, root depths and leaf phenological traits) than two evergreen species. The evergreen species, nonetheless, were especially diverse, exhibiting wide variations in seasonal tissue water potentials. With respect to conservation perspectives, the authors predict differential survival among fern species as future drought events in California intensify, with desiccation-tolerant resurrection ferns being the most resistant and possibly leading to a reduction in diversity if less tolerant species succumb. Further research by Holmlund et al. (2020) with two species of 'resurrection ferns' documented that sufficient moisture is essential during desiccation recovery to ensure sufficient positive root pressure to drive whole-plant desiccation recovery; that is, hydration of the dried leaves alone was insufficient. Changing climate patterns that include only sporadic and limited precipitation may not be adequate to provide sufficient amount and duration of moisture at the roots to fully activate root pressure needed to revive desiccation-tolerant ferns, thus leading to their demise and decreasing biodiversity of the relatively sparse vegetation in these dryland habitats.

A rather interesting adaptive response to seasonal desiccation was reported by Farrant et al. (2009) for the unusual fern, *Mohria caffrorum* (L.) Desv., a South

African endemic, growing on Table Mountain in Western Cape, South Africa. Samples were collected during the rainy and dry seasons to determine differential responses to the changing seasonal available precipitation. Remarkably, the physiological response varies seasonally; that is, this species is desiccation tolerant during the dry season and becomes desiccation-sensitive in the rainy season, showing a differential physiological adaptive mechanism activated by changing seasonal patterns of precipitation. Its threat status is categorized as 'Least Concern' though it is growing in this rather formidable environment, and may be attributed partially to its supple capacity to alter its physiological demands to complement the changing seasonal climate.

The desiccation tolerance of ten British fern species was studied by Proctor (2009) who examined the response of excised leaves to drying. *Asplenium rutamuraria* Michx., *A. septentrionale* (L.) Hoffm., *A. trichomanes* (L.), *A. ceterach*, *Polypodium cambricum* (L.), and *P. interjectum* Shivas withstood drying for periods of a week or more to a RWC of ca. 4-7%, suggesting that they are much more tolerant to drying than most vascular-plant tissue that is irretrievably damaged at an RWC of ca. 30%. Moreover, small *Asplenium* species and *A. ceterach* dried quickly (half-drying times a few hours), suggesting little stomatal control over drying. The much slower drying of the *Polypodium* species may indicate that their stomata close under water stress. Similarly, Kessler and Siorak (2007) conducted desiccation and rehydration experiments on detached leaves of 37 fern species and six lycophytes obtained from the Botanical Garden at University of Göttingen, Germany, including a range of adaptive life forms (that is, mesomorphic, poikilohydric, xeromorphic and drought-deciduous). They reported the studied species exhibited wide variation in all measured parameters. Desiccation resistance (percentage of water loss before lethal effect) varied from 30% in the mesophytic species *Asplenium nannii* Hook. (L) to 99% in poikilohydric *Cheilanthes myriophylla* Desv. (L) and intermediate state *Adiantum macrophyllum* Sw. (L). On the other hand, the desiccation resistance of a clearly mesophytic species, *Adiantum trapeziforme* L. (L), was 51%.

Further studies by Banupriya et al. (2020) were done on the physiology of a desiccation tolerant species (*Adiantum raddianum* C. Presl) occurring widely in the Devarayanadurga forest region of Karnataka, India. They reported that during desiccation of detached leaves from healthy plants, the RWC decreased to as low as 16% after four hours with intense inward curling. Upon rehydration, the RWC of the leaves regained 85% of the initial water content within four hours; and approximated the original morphology. Physiological activities of antioxidant enzymes and molecular constituents (superoxide dismutase, peroxidase, catalase, glutathione reductase, lipid peroxidation, and proline) increased

during desiccation; however, sucrose and starch content showed differential response. Additional evidence of desiccation-protective organic constituents was reported by de Moraes et al. (2014) who analyzed the sugar content of ferns, with comparison to a lycophyte (club moss), growing in extreme rocky outcrops on the southeastern coast of Brazil. The ferns largely had glucose, fructose, and sucrose as protective osmotic compounds, and the lycophyte had glucose and trehalose. Among the ferns, the total sugar content ranged from 81.31 to 200.92 (mg g⁻¹ DW) and the RWC ranged from 80 to 88.3%.

In addition to ferns typically found in mesophytic habitats, xerophytic ferns have been studied extensively to examine their desiccation tolerance and adaptive mechanisms. Hevly (1963) examined adaptations of cheilanthoid ferns in desert environments, largely in Arizona, and summarized the morphological and physiological properties that account for their success in extremely dry environments. These adaptive properties included leaf size in microphyllous species, surface cuticles and indument with wooly coating, osmotic properties, and heat tolerance mechanisms. Harten and Eickmeier (1987) examined the comparative desiccation tolerance of two desert ferns, *Cheilanthes tomentosa* Link and *Notholaena sinuata* var. *cochisensis* (Goodd.) Weath., growing in Big Bend National Park, Texas. The high-elevation species *C. tomentosa* was most sensitive to extended desiccation and had the slowest photosynthetic recovery and the greatest membrane damage; whereas, the mid-elevation species *N. sinuata* was consistently intermediate in response. They also compared the results of these two ferns to the low elevation lycophyte, *Selaginella lepidophylla* (Hooke. & Grev.), that was least sensitive to extended desiccation and had the fastest photosynthetic recovery with least membrane damage.

With increasing evidence that some ferns growing at low elevations in coastal regions may be subjected to hypersaline conditions due to ocean flooding, interest has turned to possible effects of salinity on cultivated and naturally occurring ferns. Salanchna and Piechocki (2021) examined the salinity tolerance of four hardy ferns in the genus *Dryopteris*; namely, *D. affinis* (Lowe) Fraser-Jenk., *D. atrata* (Wall. ex Kunze) Ching, *D. filix-mas* L., and one cultivar *D. filix-mas* cv. "Linearis-Polydactylon". All were grown under different light conditions. The species that were treated with 100 mM NaCl (ca. 5.9%) exhibited reduced height, less leaf greenness index and lower fresh weight of the above-ground part. Salinity caused leaf damage in *D. affinis* and *D. atrata*, which was not observed in the other two species. The effect of NaCl depended on light treatments and individual species. Among the investigated genotypes, *D. filix-mas* seemed the most tolerant, and *D. affinis* and *D. atrata* the least tolerant to salinity and light stress. Additional aspects of light-related adaptations in terrestrial ferns are presented

in a subsequent section on Light and Photosynthesis.

Epiphytic ferns

Epiphytes, growing on other plants (particularly tree trunks and branches) are vulnerable to desiccation stress because they are particularly exposed to atmospheric and meteorological variables. In regions with rather constant climate (e.g., tropical and some subtropical locales) with sufficient humidity, moderate temperatures and more predictable precipitation, epiphytic species may thrive without specialized adaptations to avoid aversive environmental conditions. In environments with varying climate, especially unpredictable precipitation patterns, epiphytic ferns typically have adaptations to endure periods of desiccation. Consequently, a broad range of adaptive mechanisms have evolved within many taxa of epiphytic ferns to enhance desiccation tolerance. In some cases they are deciduous, shedding leaves, to avoid transpiration and water loss; or in other cases they are desiccation tolerant and endure the dry period in a dormant state ('resurrection ferns').

A comment about taxonomic nomenclature to be used in the next section: presently the accepted taxonomic name for the common polypody fern is *Pleopeltis polypodioides* (L.) E.G. Andrews and Windham (previously, *Polypodium polypodioides* (L.) Watt.). Therefore, throughout this treatment, the currently accepted name will be used and abbreviated as *P. polypodioides*.

Gametophyte environmental and physiological responses

In general, studies of gametophyte physiological ecology are fewer than those of the sporophytes; and for epiphyte species, there appears to be proportionately fewer than for studies of terrestrial species' gametophytes.

However, Watkins et al. (2007) examined the photosynthetic response of laboratory cultured gametophytes of two epiphytic species, *Phlebodium pseudoaureum* and *Microgramma reptans* (Cav.) A.R. Sm., in comparison to 10 terrestrial species during desiccation. These tropical species were collected from La Selva Biological Station in the Atlantic lowlands of north-eastern Costa Rica (37-100 m above sea level). The rate of drying varied significantly among species, with the fastest dry down in the terrestrial *Thelypteris curta* (Christ) C.F. Reed, and slowest rates in two species – the terrestrial *Cyclopeltis semicordata* (Sw.) J. Sm. and the epiphyte *Microgramma reptans* (Cav.) A.R. Sm. Depression in photochemical efficiency (Fv/Fm), as measured by photosynthetic fluorescence, in desiccated gametophytes was nonlinear and varied among species. During recovery from desiccation, *Phlebodium pseudoaureum*, compared to the other species, exhibited

relatively less depression in Fv/Fm; indicating greater resistance to photoinhibition. Moreover, *Microgramma reptans* exhibited remarkable Fv/Fm stability – no significant Fv/Fm depression occurred at any of the desiccation intensities.

Compared to terrestrial fern species, typically found on the forest floor, the desiccation stress incurred among epiphytes in the canopy may have led to evolutionary adaptations for greater desiccation tolerance. This is further exemplified in a study by Ong and Ng (1998) who found that gametophytes of the epiphytic fern, *Pyrrosia piloselloides* (L.) M.G. Price, typically found on trunks and branches of wayside trees in Singapore, were able to tolerate 50 days of drought (though with some cell death), and after rehydration were capable of partial recovery forming new offspring gametophytes. However, gametophytes desiccated for only 1–21 days recovered completely upon rehydration, without the death of any cells, and continued growth and maturation.

Further studies on the influence of simulated drought on the functioning of the photosynthetic apparatus in gametophytes of *Platyserium bifurcatum* (Cav.) C. Chr. were published by Rut et al. (2003) who reported the presence of crassulacean acid metabolism (CAM) (that is, non-photosynthetic night fixation of CO₂) in this epiphytic fern. CAM was found in the sterile cover leaves, but not in the fertile spore-producing leaves.

The photosynthetic efficiency of the gametophyte phase in the fern life cycle may be critical for subsequent sporophyte development. There is evidence that the gametophyte provides photosynthetic nourishment of the young sporophyte during its early stages of development before the one-leaf stage (Sakamaki and Ino, 1999). Consequently, desiccation stress at the gametophyte stage may incur decreases in its photosynthetic efficiency and indirectly lead to less successful development of the young sporophyte.

Sporophyte environmental and physiological responses in epiphytic ferns

Changes in water content of epiphytic ferns are one of the most significant physiological indicators of the desiccation status of poikilohydric (resurrection) species. Potts and Penfound (1948) measured the water content of *P. polypodioides* and that of the tree bark where they were growing, and found that there is close correspondence. In the active state, the ferns had mean water content (percentage of oven-dry weight) of 207 ± 32 (bark = 52 ± 16), and when desiccated and dormant the fern water content was 86 ± 13 (bark = 18 ± 4). At varying dates, they measured the water content of the ferns and the relative humidity (RH) and also found a strong correspondence varying from 8% fern water content at RH = 65%, but a much higher 23% water content at RH 94%. They also measured the change in

water content of *P. polypodioides* in laboratory experiments under controlled drying conditions and reported that over 12 hours, the amount of water lost varied from 19% during the first hour to 1% by the twelfth hour, an average change of approximately 1.6% loss per hour.

Subsequent studies by Voytena et al. (2015) reported that sporophytes of *Pleopeltis pleopeltifolia* (Raddi) Alston (commonly found in Brazil) subjected to desiccation for 0, 5, 10 and 15 days showed a sharp decline in water content when non-irrigated, reaching a final value of 9.6% after 15 days. As is typical, the ferns exhibited considerable wilting and frond rolling. A substantial increase was noted in sugar content of the fronds during desiccation – a possible osmotic adjustment and vitrification to protect tissues. During the five initial days of desiccation, the chlorophyll and carotenoid contents decreased abruptly. However, after 1 day of rehydration they partly recovered including resumption of photosynthesis.

The pliability of leaf cell walls during desiccation and folding of leaves was documented in *P. polypodioides* by Layton et al. (2010) who reported that a rise in the content of a putative 31-kDa dehydrin protein, present only during dehydration, may promote the flexibility and folding of the dehydrated leaves. They concluded that the ability to avoid cell wall damage in some desiccation-tolerant species may be partially attributed to cell wall localization of dehydrins enabling a pliable, reversible, large cell-wall deformation. This was further developed by Helseth and Fischer (2005) who derived a mathematical model to explain the physical mechanisms of changes in the pliable structure of the plant tissue during rehydration.

Further elucidation of the cellular mechanisms of dehydration and rehydration in *P. polypodioides* were reported by John (2017) using transcriptomic analysis of gene-activated protein synthesis. She found that *Pleopeltis* is prepared for desiccation at an early stage of dehydration by: a) accumulating various metabolites, b) reducing energy consuming metabolic activities, and c) subsequently catabolizing some metabolites after resumption of hydration. Additionally, many of the desiccation-induced gene transcripts are constitutively (continuously) expressed under hydrated conditions, which creates a constant supply of some essential molecules that allows the plant to adjust rapidly to desiccation.

Relatively less attention has been given to temperate epiphytic ferns. However, Klinghardt and Zotz (2021) analyzed the abundance and seasonal growth of polypod epiphytic ferns at three sites along a rainfall gradient in Western Europe; and reported, surprisingly, that seasonal frond productivity appeared to be unaffected by the amount of annual rainfall and average temperatures. Although it seems likely that these factors do play a role during gametophyte establishment. However, they reported that the abundance of epiphytic polypod ferns

strongly decreased from the wetter end of the rainfall gradient in Ireland towards the drier end in Germany; although, frond turnover was equally high at all study sites during summer months, and equally low during winter.

Considerable attention has been given to the so-called 'filmy ferns' (Hymenophyllaceae), sometimes with leaves only one-cell thick. Although they are constitutively delicate and typically grow in humid environments, some species of filmy ferns are desiccation tolerant. Other species of ferns growing in moist, shady terrestrial environments also have leaf lamina one to two cells thick; e.g., *Adiantum capillus-veneris* (L.) Hook. However, many of these are less desiccation tolerant. Ostría-Gallardo et al. (2020) applied molecular genetic analysis of gene networks to analyze desiccation tolerance mechanisms for two *Hymenophyllum* species (*H. caudiculatum* Mart. and *H. dentatum* Cav.) differing in their location within a forest canopy and experimentally studied at three different degrees of dehydration. While there were only a few distinctive genes activated comparatively in the two species, *H. caudiculatum* had ca. twice the number of activated genes than *H. dentatum*; and a higher proportion of increased-and-decreased abundance of genes occurred during dehydration. In contrast, the abundance of genes in *H. dentatum* decreased significantly when transitioning from dehydration to rehydration. Moreover, *H. caudiculatum* enhanced osmotic responses and phenylpropanoid related pathways; whereas, *H. dentatum* enhanced its defense system responses and protection against high light stress. Overall, these results provide evidence of the relationship between the species-specific response and the microhabitats that these ferns occupy in nature.

The correlation between water relations and within canopy distribution of epiphytic ferns in a Mexican cloud forest was examined by Hietz and Briones (1998). They reported that the filmy fern *Trichomanes bucinatum* Mickel & Beitel became desiccated completely within hours in moderately dry air and was confined to the stem bases; while *Asplenium cuspidatum* Lam., with no evident adaptations to cope with drought, grew in the second most shaded zone within the tree crowns. Likewise, Nitta et al. (2020) found a similar functional and habitat-specific diversity of a broad group of epiphytic ferns (including filmy ferns) in community analyses along mountain elevational gradients on the island of Moorea, French Polynesia.

Further findings include a variety of experimental and natural environmental studies as follows: a) Bravo et al. (2016) documented reversible cellular changes during desiccation and recovery of *Hymenophyllum* collected in the southern temperate rain forest of Chile; b) Flores-Bavestrello et al. (2016) made a comparative analysis of photosynthetic apparatus and responses to dehydration by *Hymenophyllum dentatum* and *Hymenoglossum cruentum* (Cav.) C. Presl isolated from different vertical

locations on a host tree in Chile; and c) Proctor (2012) published evidence of coordinated mechanisms of dehydration and photosynthetic light responses in filmy fern samples collected in New Zealand compared to those collected in Trinidad and Venezuela.

LIGHT AND PHOTOSYNTHESIS

Although available water is a major factor in fern physiological ecology, the quality and intensity of illumination is particularly important in determining fern distribution and productivity. As in previous sections, a review of research on terrestrial taxa is presented first followed by epiphytes. There is a substantial volume of published research on the topic of light and physiological adaptations of ferns. Examples of research were chosen that particularly highlight diverse geographic locales and climatic regions, globally.

Terrestrial ferns

Gametophyte environmental physiological responses

Spore germination initiates the gametophyte phase of the fern life cycle and substantial research on the effects of light on spore germination has been published, including: a) light induced spore germination (Life, 1907; Reynolds and Raghavan, 1982); b) spectral quality of light and the role of the light signaling molecule, phytochrome (Raghavan, 1971; Furuya et al., 1982; Zilberstein et al., 1984); and c) the interactions of light and temperature on spore germination (Pareek et al., 2005; Pérez-García et al., 2007). Moreover, Sugai et al. (1987) found that spores of species that normally require light to germinate can be activated by application of gibberellic acid or its methyl esters. Furthermore, complex effects are produced by adding abscisic acid (ABA) in interaction with auxin (indoleacetic acid, IAA) and kinetin (Chia and Raghavan, 1982). ABA can be inhibitory of full germination, leading to incomplete growth of the initial protonema. However, both IAA and kinetin, which alone do not promote full germination, reverse to some extent the inhibitory effect of ABA.

The importance of red light induced growth of spores is significant from a physiological ecology perspective, because overlying, shading plants absorb red light and thus may suppress underlying fern spore germination until there is sufficient full illumination to support the photosynthetic requirements of the gametophyte prothallus. It is worthy to note that ABA can take many roles, physiologically, but particularly as reported by Chia and Raghavan (1982) in the prior case, it may accumulate in spores under adverse conditions for germination, and thus delay premature germination in less than desirable environmental circumstances.

However, it is important that ABA is under regulatory control by other phytohormones (e.g., IAA and kinetins) to ensure that inhibition is released when environmental conditions are favorable for spore germination and survival of the gametophyte.

Sporophyte environmental physiological responses to light

There is a substantial literature base on this topic, and only some representative research is reviewed beginning with broad environmental factors and progressing to more detailed physiological response patterns. A rather comprehensive review of the effects of light on fern morphogenesis is presented by Kanegae and Wada (2006).

The effects of light intensity, temperature and nutrients on vernal frond emergence and biomass production in the temperate fern *Matteuccia struthiopteris* (L.) Tod. was reported by Prange (1985) who found that photosynthetic intensity, water availability, and nutrients affect frond productivity, but water availability appeared to be most critical. Maximum net photosynthesis rates of approximately $220 \mu\text{g CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ were reached at low light intensities of 300 to 600 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Jordan and Kuehnert (1975), studying *Osmundastrum cinnamomea* reported that abrupt increases in light (e.g., through local deforestation) increased leaf primordial initiation to some degree, but there was greater primordial development in leaf bud primordia that were set in prior years, suggesting a carry-over effect from one year to the next.

In a further study of temperate ferns during the spring season, Tessier (2001) assessed the adaptive value of maintaining photosynthetic (wintergreen) fronds during winter in *Dryopteris intermedia* (Muhl.) A. Gray that were found growing in the Catskill Mountains of New York. He studied the photosynthetic productivity during April to May, particularly to explore the potential photosynthetic and retranslocational benefits of wintergreen fronds in sustaining the life of the fern. Net photosynthesis occurred throughout the study indicating a potential for movement of fixed carbon from winter-green fronds to other parts of the plant, though in this study the net photosynthesis was higher in April, immediately after snow melt, compared to May.

Saldana et al. (2007) examined the effects of varying light environments on the ecophysiological traits of *Blechnum chilense* (Kaulf.) Mett. occurring widely in Chilean temperate rainforests. They particularly studied contrasts of plants growing in gaps versus those in forest understories. In gaps with higher light intensity, the survival of *B. chilense* was positively correlated with water use efficiency (WUE) and negatively correlated with leaf size. In contrast, survival in shaded understories was positively correlated with leaf size. In understories, ferns of lower respiration rate and greater leaf size showed greater fecundity. Thus, whereas control of water

loss was optimized in gaps, light capture and net carbon balance were optimized in shaded understories.

Similarly, Zhu et al. (2016) examined the response of 16 fern species distributed between open gaps and shaded understories in sub-tropical forests of China. They found that a leaf cost-benefit analysis contributes to understanding the distribution pattern of ferns in contrasting light habitats. Ferns in the open habitat, employing a quick-return strategy, can pre-empt resources and rapidly grow in the high-resource environment of the open habitats; while a slow-return strategy of ferns in understory locations allows their persistence in the shaded understory of well-established canopies of old-growth forests.

Sun flecks, caused by flutter of canopy leaves and swaying branches, produce intermittent and transient peaks in light intensity in the understory habitats. Ferns growing on the forest floor produce concomitant fluctuations in productivity, that can be very rapid, as exemplified by the response of *Polypodium virginianum* L. ferns growing on cliff edges of the Niagra Escarpment, Canada (Gildner and Larson, 1992a). Further seasonal studies of *P. virginianum* at the Niagra site (Gildner and Larson, 1992b) showed that carbon gain in the spring greatly exceeded that of any other season. However, there was little change in the photosynthetic response to light on a seasonal basis, even though plants were exposed to highly variable and highly limited light most of the time.

Nishida and Hanba (2017) examined the photosynthetic response to drought stress among four temperate fern species from different habitats by withholding irrigation in laboratory experiments. Among other outcomes, they found that *Lepisorus thunbergianus* (Kaulf.) Ching (an epiphyte) had low stomatal density and showed high water-use efficiency (WUE) retaining photosynthetic activity with low relative frond water content under drought stress. This indicated they were highly adapted to drought. In contrast, low WUE with low light-saturated photosynthetic rate in *Adiantum pedatum* L. (growing in terrestrial, shady environments) was associated with much lower photosynthesis than in the other species under drought stress, suggesting lower adaptation to drought-prone habitats.

Epiphytic ferns

This subsection focuses largely on the sporophyte phase of the life cycle, where a substantial amount of research has been done. Photosynthesis of epiphytic ferns and their within-canopy distribution in a Mexican cloud forest revealed that there was a correlation between distribution of habitats within the canopy, and physiological traits (Hietz and Briones, 2001). Maximum rates of CO_2 uptake (Amax) and photon flux densities at light compensation points (LCP) were in the range of shade plants (Amax

$=0.6 \pm 5.2 \text{ mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; $\text{LCP} = 4 \pm 6.5 \text{ mmol m}^{-2} \text{ s}^{-1}$), but their saturation light photon intensities ($270 \pm 550 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) were more typical for sun plants. Amax and nitrogen content per unit dry weight were correlated with the distribution of the species within the canopy; but LCP, apparent quantum yield and dark respiration were not.

Quinnell et al. (2017) examined the photosynthetic rate of *Davallia angustata* (Wall. ex Hook. & Grev.), an epiphyte on tree trunks and palm trees in south-east Asia. In this case it is not a crassulacean acid metabolic (CAM) plant as are some epiphyte fern species. Under well-watered conditions, *D. angustata* had a diurnal cycle of photosynthesis with maxima in mid-morning ~ 0900 hours (solar time). Under optimum irradiance $\sim 45\%$ of full sunlight (qualifying it as a 'sun plant'), the maximum photosynthetic electron transport rate (ETR_{max}) was $77.77 \pm 3.42 \text{ } \mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$; or, expressed on a Chl *a* basis = $350 \pm 36.0 \text{ } \mu\text{mol g}^{-1} \text{ (Chl } a) \text{ s}^{-1}$.

Given the recurrence of desiccation stress in many epiphytic ferns, some have adapted stress tolerance by evolving CAM photosynthetic capacity, but overall for fewer species compared to many other vascular epiphytes. Winter et al. (1986) studied CAM in a tropical epiphytic fern, *Pyrrosia longifolia* (Burm. f.), in a fully sun-exposed and in a very shaded site in Northern Queensland, Australia. Maximum rates of net CO_2 uptake and the nocturnal increase in titratable acidity (attributable to dark fixation of the CO_2 into organic acids) were lower in shade than in sun leaves. Based on carbon stable isotopic data, $\delta^{13}\text{C}$ values of sun and shade leaves were not significantly different, and ranged between -14 and -15‰ (within the range of CAM plants) suggesting that, in the long term, carbon gain was mainly via CO_2 dark fixation by crassulacean acid metabolism. Sun leaves had a higher light compensation point of photosynthesis than shade leaves, but the same quantum yield.

Additional research on CAM in *P. longifolia* and *Drymoglossum piloselloides* (L.) Presl collected in Singapore (Ong et al., 1986), under controlled environmental conditions in laboratory culture chambers, showed that CO_2 exchange under water stress in *Pyrrosia* was less than in *Drymoglossum*; showing that *Pyrrosia* was more susceptible to water stress. During water stress, there was a continuous decrease of CO_2 uptake, both in light and darkness. Moreover, notably, in both plant species a residual diurnal acid rhythm remained, even if the drought stress caused stomatal closure and the nocturnal CO_2 uptake from the environment dropped to zero. This indicated 'CAM idling,' where CAM depended entirely on recycling of respiratory CO_2 ; that is, from respiration of existing carbon already fixed into organic acids during the night.

TEMPERATURE AND PHYSIOLOGICAL RESPONSES

Based on a global perspective, a published meta-analysis

of the relative effects of temperature versus precipitation on plant traits for a broad range of plant species worldwide has shown that temperature is the better predictor of plant traits than precipitation (Moles et al., 2014). However, as reported in the review of research above; at the local level within a reasonably well-defined climatic regime, the amount and pattern of precipitation is very significant for many fern species, particularly for epiphytes (Zotz and Hietz, 2001). Nonetheless, recognizing that temperature is a major environmental variable, it is remarkable that relatively little research has been done on the role of temperature in fern physiological ecology (Hietz, 2010; Anderson, 2018). Some illustrative studies are reviewed to provide information on the range of topics that have been published. Given the relatively limited coverage of temperature in the literature, this subsection is not subdivided by subtopics such as 'Terrestrial ferns' and 'Epiphytic ferns' as was, the case in preceding sections of this paper.

Epiphytic ferns have become adapted to dwelling on a relatively wide variety of tree species in subtropical and temperate mountainous regions, adapting to varied temperature and climate regimes, including in the Himalayas (Bhakuni et al., 2021; Joshi et al., 2020). In general, montane regions are likely hotspots for fern diversity (Suissa et al., 2021). Warne and Lloyd (1980) studied the role of temperature in the germination of spores and gametophyte development in some temperate and tropical ferns with the aim of understanding the correlation of temperature responses to the habitat of the ferns. They concluded, based on laboratory-based temperature controlled experiments, that characteristics of spore germination and gametophytic responses to temperature correlate with the natural distributions and life cycles of the studied species. The tropical taxa (*Ceratopteris* spp.) had optimum development at higher temperatures than observed for the temperate taxa. For example, the pattern of growth response of gametophytes of the temperate fern *Matteuccia struthiopteris* correlates with the winter and early spring discharge of spores in this species. Moreover, Warne and Lloyd's data suggest that a limiting factor controlling the southern distribution of this species may be the inability of its gametophytes to complete normal development in warmer climates.

Gildner and Larson (1992b) reported that temperature was a significant seasonal covariate in explaining the photosynthetic response of *Polypodium virginianum* to light intensity when growing in a forest floor location in Canada. Moreover, experiments conducted in the laboratory showed that the response of photosynthesis to temperature was broad. Ong et al. (1986), studying two obligate CAM metabolizing species (*Pyrrosia longifolia* and *Drymoglossum piloselloides*), reported that the effects of temperature on CO_2 exchange were inverse compared to other CAM plants. That is, in both ferns, dark CO_2 fixation increased when the night temperature was increased, and decreased with lower night

temperatures. Increase in day temperature reduced CO₂ uptake during phase IV (CO₂ uptake during the last part of the light period); and during the following night.

In Prange's (1985) studies of the ostrich fern (*Matteuccia struthiopteris*) a minimum amount of cold exposure was required to break winter dormancy – that is, a base temperature above 5.8°C is required and may be as high as 20°C. After the ostrich fern received its chilling requirement, vegetative emergence did not occur until temperatures were at or above ca. 9.3°C. Stamps et al. (1994), using experimental temperature regimes, examined the effects of temperature on growth of *Rumohra adiantiformis* (G. Forst.) Ching, also known as the leather leaf fern. When leaves of the leather leaf fern were produced under a high temperature regime (30°C day/ 25°C night), they grew faster and produced more sori earlier than those in a low-temperature regime (20°C day/ 15°C night). Transpiration and water-use efficiency (mass basis) at light saturation were similar for leaves from both temperature regimes.

Seasonal variations in temperature have a marked influence on fern phenology—the timing of leaf emergence and bioactivity. Lee et al. (2018) analyzed a phenological dataset of 225 fern species from around the world to illustrate the distribution of studies during the past half century into leaf and spore production seasonality and the correlation with climate factors. Seasonal patterns were found in most of the phenological phases, especially in temperate regions with cold winters. In tropical to subtropical regions, seasonal patterns vary, and the seasonality of growth and reproduction in ferns may correlate with temperature or precipitation (or both), depending on the habitat locations.

Temperate ferns are particularly prone to temperature stress during winter months. Fernández-Marín et al. (2021) assessed frond freezing tolerance and xylem anatomical traits in five wintergreen fern species. They report that only desiccation tolerant species that possessed a greater fraction of narrow tracheids (< 18 µm), compared to sensitive species, tolerated freezing. They concluded that adaptation for freezing tolerance is likely associated with desiccation tolerance through complementary xylem properties (which may prevent risk of irreversible cavitation) and effective photoprotection mechanisms.

Rapp and Silman (2014), working in a Peruvian Andean cloud forest, examined the response of *Elaphoglossum* and other vascular epiphytes growing on trees to changes in temperature after being collected at varying elevations (800 m to over 4000 m), and then after they were transplanted to locations at lower elevations along the eastern slope of the Andes. When vascular epiphytes, with ramet-producing rhizomes, were transplanted down slope from the highest elevation within the cloud forest, they had lower ramet recruitment.

Furthermore, the number of ramets declined when transplanted to the lowest elevation, suggesting that

warmer temperatures, and lower cloud immersion, could cause community-level changes for species currently above the cloud base.

CONSERVATION PERSPECTIVES

A general review of current perspectives on fern conservation is given by Mehlreter (2010), including aspects of Risk assessment, Ecological data required for risk assessment and Management strategies for fern conservation. More specific issues of fern conservation related to changing climates are considered by Anderson (2018) and Sharpe (2019). The broad biogeographic distribution and wide habitat diversity of ferns, accompanied by highly diverse taxa adapted to environments ranging from aquatic to dry lands and deserts, exemplifies the remarkable evolutionary radiation of species in this group of vascular plants (Kessler, 2010). Diverse taxa such as those adapted to aquatic, xerophytic, rocky, and dryland environments further exemplify fern exploitation of widely different habitats. The presence of a broad range of epiphytic species living on the branches and trunks of trees, spanning tropical to temperate locales, provides additional witness to their adaptive capacity. Nonetheless, as with other highly evolved vascular plants, many fern species have become specialized to thrive only within particular optimal conditions. Others are endemic to limited geographic regions and in some cases have environmental requirements peculiar to their geographic locale. While all of this diversification and specialized adaptation make ferns a very attractive group of vascular plants for horticultural uses and scientific research, escalating threats by human exploitation, increasing climate change due to anthropogenic sources, and massive destruction of natural habitats (such as burning, agricultural cultivation, logging and commercial development in otherwise pristine natural environments) threaten the survival of many fern species (Arcand and Ranker, 2013; Nowicki and Kowalska, 2018).

Desert and dryland species may be considered less threatened due to their robust adaptive response to a hostile environment. However, increasing encroachment of construction for commercial purposes, and land development for human dwelling, puts ferns under additional survival pressure. Furthermore, possible increasing temperatures, and changing precipitation patterns due to climate change, may exceed the resilience of some species. Logging and massive destruction of forested regions pose particular threats to ferns in the understory of trees, where they are adapted to the usually shady, sometimes humid, and typically more constant soil moisture of the forest floor. Epiphytic ferns are particularly threatened by logging that destroys vast swaths of the forests where the epiphytes inhabit the trunks and limbs of trees. In other cases, tree thinning

increases light intensity in remaining trees. This favors invasion of open niches by high-light adapted species that proliferate to the exclusion of other species, and contribute to reduction of epiphyte biodiversity. Moreover, potential major alterations in precipitation patterns due to global climate change may produce protracted dry periods that exceed the epiphytic ferns' desiccation tolerances.

All of these potentially challenging scenarios, and many more documented in the literature, call to our attention how relatively little we know about the physiological ecology of ferns in broad and diverse habitats essential to estimating their risk status. Clearly, a more systematic research agenda is needed to categorize fern taxa into major groups that have particular environmental requirements; and better document their habitats, survival capacity and stress limitations (Anderson, 2018). For example, tree ferns (among other exotic fern species) are impressive plants and important members of plant communities, especially in tropical and sub-tropical environments; but increasing evidence suggests that their survival in some global regions is under threat and additional research and conservation measures are needed (Ramírez-Barahona et al., 2011).

It is difficult to judge how effectively *ex situ* cultivation and preservation in botanical gardens and designated natural preserves can contribute to conservation of threatened or vanishing species. Nonetheless, horticultural and research institutions committed to cultivation and preservation of fern diversity may be one of our best solutions to saving representatives of some threatened fern species, but further efforts to enhance future conservation and biodiversity of ferns within the unique circumstances of botanical garden conservatories are recommended (Mounce et al., 2017). Interestingly, innovative ideas for use of homegardens (especially, in less-developed countries and regions of the world) may be one way to preserve some indigenous fern taxa (Amberber et al., 2014).

A variety of *ex situ* protocols and methods for preservation of fern spores, gametophytes and plants have been proposed (Ballesteros, 2011; Pence, 2013; Ballesteros and Pence, 2018), or through a combination of *ex situ* and *in situ* methods (Ibars and Estrelles, 2012). Moreover, some *in vitro* conservation methods for rare and threatened fern species have been recommended (Barnicoat et al., 2011). Overall, a concentrated and coordinated program of applied research and policy analysis by major plant societies and botanical institutions may be needed to ensure that globally, the remarkable diversity and aesthetic quality of ferns will be preserved for current and subsequent generations.

CONCLUSIONS AND POSSIBLE DIRECTIONS OF FUTURE RESEARCH

The broad diversity of fern taxa, and the remarkably

varied geographic and climatic regions where they have become adapted, provides increasing opportunities to use fern species as model organisms to study ecophysiological research on the dynamics of plant and environmental interactions. With changing climatic regimes, some that threaten the survival of uniquely adapted fern species, further research is warranted on understanding the fundamental morphological and physiological processes that account for the adaptive qualities of different groups of ferns. This is especially true, if we are to better conserve environmentally threatened species.

Recent innovative approaches that combine trait analyses, functional types (that is, particular ecologically adapted taxa – some above species level) and modern molecular genetic techniques (such as transcriptomics, proteomics, and molecular phylogenetics) are particularly promising. Given the substantial diversity of ferns at the species level, and the challenges of studying species autecology in greater detail, a research strategy that focuses on broader functional groups and their ecophysiological characteristics may be a more productive approach than species-specific research. With modern experimental, controlled-environment facilities coupled with portable field-based physiological instrumentation, opportunities are open for more sophisticated combined laboratory and field-based research on fern physiological ecology. An endeavor to understand the adaptive mechanisms of ferns, that represent morphotypic and functional groups in varied environments, can be a more efficient and comprehensive approach to understanding fern physiological ecology than focusing on lower taxonomic classes of ferns, *per se*. Moreover, such comprehensive sources of evidence on the most significant ways ferns have adapted to their environmental niches may provide a more sound basis for conservation policies and practices.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

Breeding biology and diet of the Madagascar Buzzard *Buteo brachypterus* in the Bemanevika Protected Area, Madagascar

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The breeding biology and diet of the endemic Madagascar Buzzard *Buteo brachypterus*, in Bemanevika Protected Area, northern Madagascar, was studied in 2017 and 2018. Nest building occurred from early August to early September, egg laying from late August to early October, hatching between September and October and fledging between November and December. Laying peaked in September (n = 24 pairs). Twenty-four breeding pairs were observed of which nine pairs had one-egg clutch and fifteen pairs laid two-egg clutches. Average clutch size was 1.6 ± 0.5 eggs (n = 24 nests). The incubation period averaged 36.2 ± 1.1 days (range 35-38 days, n = 16 nests). Nestling periods averaged 48 ± 3.9 days of age (range 43-56 days, n = 16 nests). Of 39 eggs laid in the 24 breeding attempts, 34 (87.2%) hatched and 22 (64.7%) of those hatchling fledged. This study documented siblicide as cause of breeding failures. Of the 24 fully-documented breeding attempts, 0.91 young fledged per breeding attempt and overall nest success was 79.2%. Based on 515 identified prey items, the Madagascar Buzzards diet was composed of reptiles (37.3%), birds (35%), small mammals (19%), invertebrates (8.3%) and amphibians (0.4%).

Key words: Breeding biology, diet, endemic, Madagascar Buzzard, Bemanevika, Protected Area.

INTRODUCTION

The Madagascar Buzzard *Buteo brachypterus* is an endemic species and fairly common from sea level to 2,300 m, except on the High Plateau (Langrand, 1990). Furthermore, monitoring of common species is important for biodiversity conservation because they are good

indicators of environmental and ecological conditions and are an essential function in the ecosystems (Newton, 1998). Thirty years ago information on the breeding biology and food habits of the Madagascar Buzzard was recorded from a lowland forest on the Masoala Peninsula

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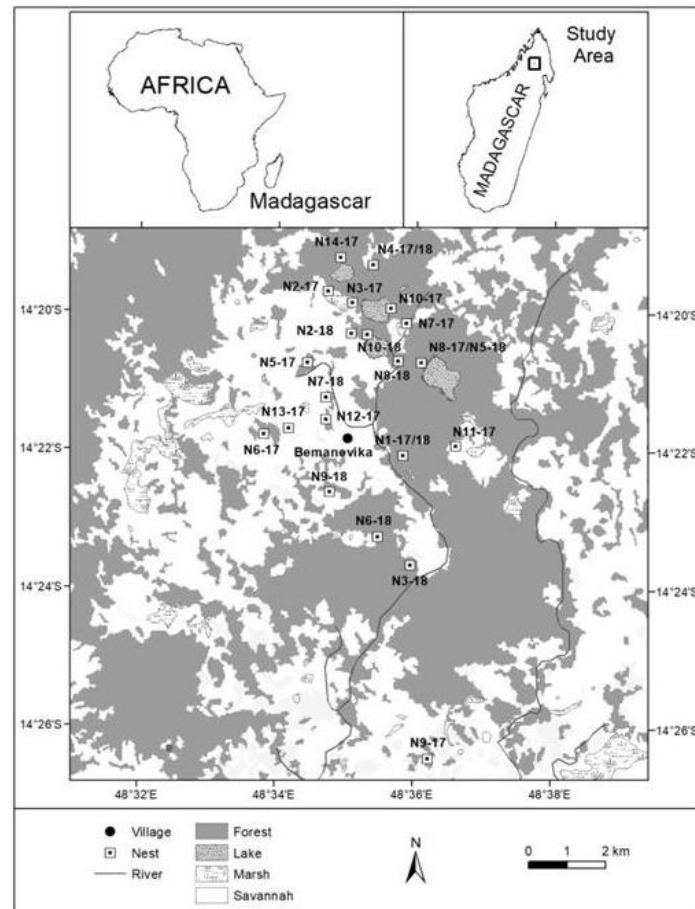


Figure 1. Distribution of Madagascar Buzzard *Buteo brachypterus* nests at Bemanevika PA, northern Madagascar.

of northeastern Madagascar (Berkelman, 1996, 1997). To increase knowledge on the Madagascar Buzzard, we conducted a study on the breeding biology and diet of this species at Bemanevika Protected Area (PA), in the northern Madagascar, during two consecutive breeding seasons (2017 and 2018). The aim of this study was to document the breeding season, behaviors, productivity and diet. The results of this study will be used for understanding the health of the ecosystems at Bemanevika PA.

MATERIALS AND METHODS

Study area

This study was conducted in and around Bemanevika PA (14°10' - 14°35' S 48°25'-48°50' E), which is located in the District of Bealanana, Sofia Region, in northern Madagascar (Figure 1). Bemanevika PA covers 35,601 ha including a mosaic of large patches of montane rainforest (20,353 ha), four volcanic lakes (158 ha), marshes (601 ha), savannas (14,489 ha) and ranges from 700 m to 1 800 m elevation (The Peregrine Fund, 2014). In December

2006, the Critically Endangered Madagascar Pochard *Aythya innotata* was rediscovered at Bemanevika (Rene de Roland et al., 2007) and in April 2015, Bemanevika became a national Protected Area. This site was also classified as a Ramsar site in 2017 and an important wetland site for conservation of biodiversity (World Wide Fund for Nature, 2017). There are two distinct seasons in Bemanevika PA: the dry season from May to October and a rainy season from November to April. During the two study seasons, the annual average rainfall at Bemanevika Field Station was 2707.5 mm. The monthly temperatures averaged in July and November were respectively 14.7 and 20.4°C, with an annual average of 18.5°C (The Peregrine Fund, 2020). Bemanevika PA was chosen as study area to collect information at different elevation across the natural range of Madagascar Buzzard. It differs in being a high elevation rainforest compared to the coastal lowland rainforest of Masoala National Park where the last study of this hawk was conducted thirty years ago.

Data collection

Fieldwork was carried out from July to January during the 2017 and 2018 breeding seasons. During July and September, we searched for individuals and nesting pairs in forest fragments and blocks by direct observations using transect lines. At every 100 m, we

stopped for 10 to 15 min to listen and search for hawks for nesting behavior (Watson, 1992). We noted all individuals found or heard and followed them to observed their behaviour and locate a suspected nesting territory. We also systematically searched all habitat suspected to hold a nesting pair (Pruvot et al., 2017). Nesting pairs were located and monitored during the two study seasons. Breeding season parameters: including nest building, egg-laying, incubation, hatching, nestling and fledging periods and breeding behaviour were determined from daily observations. Observations were made from 30-50 m from the nest with 8-10x power binoculars. When a pair was determined to be incubating, nests were climbed to recorded egg dimensions (length and width) by vernier caliper and mass with Pesola spring balance scales. During the nestling period, we described the development of young from direct observations and using trail cameras.

To identify prey items, trail cameras were used to ensure continuous sampling during the day (Trollier et al., 2014). Three trail cameras were used at five nests with two-egg clutches per breeding season. Two cameras recorded photos and one recorded videos. Trail cameras were fixed on branches about 2 m above the nest and recorded 24 h a day. To facilitate data analysis, nests and corresponding nesting pairs were coded by order of discovery and year of study: Ni/Pi-17 or Ni/Pi- 18 with N as the nest, P as the nesting pair and i is the order of discovery of the nest and 17/18 is the year.

Data analysis

Egg dimensions were calculated as mean values and standard deviations from measurements taken. A chi-square test (Fowler and Cohen, 1985; Johnson, 1992) was used to compare the quantity of food delivered to nestlings by both sexes. This test was also used to determine if the quantity of each type of prey varied during the breeding seasons. Statistical analysis was performed with STATISTICA 6.0 (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

In 2017 and 2018, we respectively found fourteen and ten nesting pairs, of which six and three nesting pairs were with a one-egg clutch while eight and seven nesting pairs had two-egg clutches. The nesting pairs were classified as P1-17 to P14-17 and P1-18 to P10-18 with their respective nests N1-17 until N14-17 and N1-18 until N10-18. Nine nests were located along the forest edge and fifteen were inside the forests. In 2018, three pairs in 2017 (P1, P4 and P8) reused their same nests (P1, P4 and P5) and seven pairs constructed new nests (P2, P3, P6, P7, P8, P9, and P10-18). Newton (1979) stated that some large raptors do change their nests from year to year, but usually placed them near the previous years' nests. In our study area, Madagascar Buzzards reused 30% of nesting pairs (N = 10) their previous years' and were much lower than the same species (50%; N = 6) in Masoala (Berkelman, 1996). This difference is due to the distribution of good food sources and according to Gill (1990): when food sites are concentrated, a bird improves its success by staying in or near sites of high food density and by moving rapidly past sites of low food density. Newton (1979) stated "the habit of breeding in

the same territory year after year is probably advantageous, so long as it is a good territory". It would be better to collect more information about the fidelity to breeding areas because it shows relationship with other factors, notably good diet and quality of breeding areas. In more, for Yellow-billed Kites *Milvus aegyptius* at the Manambolomaty Lakes Complex PA, construction of a new nest not far from a previous nest might have a relationship with the size of the nesting territory they can defend (Andriamalala, 2005). Unfortunately, we did not observe this for Madagascar Buzzard.

In Bemanevika PA, at least 24 nesting pairs of Madagascar Buzzards were documented while Berkelman (1996) found 14 nesting pairs in Masoala Peninsula. The Madagascar Buzzard is among one of the most common and abundant raptor species in Madagascar (Thiollay and Meyburg, 1981) and this could be the factor for the high number of pairs found at these two sites. This abundance could justify why the Madagascar Buzzard has a status as Least Concern (IUCN, 2020). During this study, nests were easily found in the rainforests of Bemanevika PA, while Berkelman (1993) mentioned that it was difficult to find nests in the lowland rainforests of Masoala Peninsula due to the steep topography and precipitation. In addition, our study documented Madagascar Buzzard nests were located at the forest edges and inside the forests of Bemanevika PA. However, in Masoala, the study area was primary lowland forest with some slash-and-burn clearings (Berkelman, 1995). These variations could be explained by several factors, including that the Madagascar Buzzard is an endemic species and prefers forests more than degraded habitat (Rene de Roland, 1994), and this species occupies a varied habitat such as savanna, forest edge, forest between edge and forest blocks in Bemanevika PA according to Razafindranaivo (2015). These findings are consistent with the characteristics of the species according to Langrand and Meyburg (1984): "the Madagascar Buzzard is reported to be common in wooded habitats throughout Madagascar". This confirms that *Buteo brachypterus* frequently used the forest ecosystems and it is also a ubiquitous species. Like in Bemanevika PA, Madagascar Buzzards do not appear to be sensitive to localized habitat degradation on Masoala (Berkelman, 1996) and may be less vulnerable to the effects of forest fragmentation than species with more specialized requirements (Berkelman, 1995).

Pair formation

The observation time totaled 98.7 h during the pair formation period. In 2018, we followed pairs P1 and P2 and the pair formation period was recorded from the first week of August to the first week of September. We observed 18 copulations (n = 3 pairs), lasting from 6-10 s followed by vocalizations only emitted by females. Copulations were preceded by food provisioning and

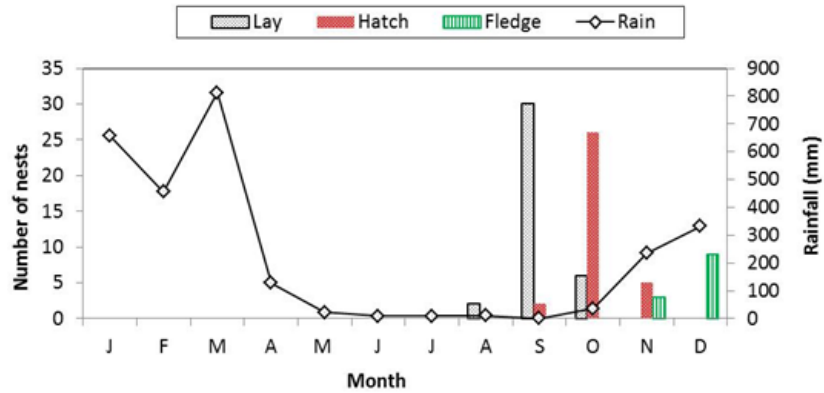


Figure 2. Breeding cycle of Madagascar Buzzard in 2017 and 2018, Bemanevika Protected Area, Madagascar.

occurred at a distance from 10 to 300 m of nest trees.

Nest construction and reconstruction

Total observation time during this stage was 372 h. Nest building occurred from early August to early September. We monitored nest construction activity at N2-18 (new nest) and nest reconstruction activity at N1-18 (or N1-17) and N5-18 (or N8-17). In 2018, of 7 nests newly constructed, the nest construction period was only recorded at pair P2-18. Nest reconstruction lasted 23 and 15 days for the pairs P1-18 and P5-18. In 2018, for P2-18, it took 33 days for nest construction to be completed which was one and six days before egg laying occurred. Nesting material (sticks and twigs) was collected from nearby trees from 10-150 m of the nest tree, but only females arranged the material in the nest. Both sexes collected nesting materials. When the male returned to the nesting territory and brought a stick or twig, the female emitted a specific call. The male answered and delivered the material into the nest. Nest construction took place between 07:30 and 12:00 in the morning ($n = 253$ items) and in the afternoon between 12:00 and 14:30 ($n = 101$ items).

Of 354 nesting materials recorded, 157 were sticks and 197 fresh cut twigs with leaves. Of 157 nesting material (sticks) delivered, males contributed 72% ($n = 113$ items) and females delivered 28% ($n = 44$ items). In 2017, the males and females delivered 71% ($n = 27$ items) and 29% ($n = 11$ items), respectively. In 2018, males delivered 72.3% ($n = 86$ items) and females delivered 27.7% ($n = 33$ items). Of 197 fresh cut twigs with leaves observed, females and males delivered 80.7% ($n = 159$ items) and 19.3% ($n = 38$ items), respectively. In 2017, males and females delivered 75% ($n = 44$ items) and 25% ($n = 11$ items), respectively. In 2018, males and females delivered 82.4% ($n = 126$ items) and 17.6% ($n =$

27 items), respectively.

The observations of sticks and fresh cut twigs with leaves making up the Madagascar Buzzard nests at Bemanevika PA is in agreement with findings of several earlier studies (Berkelman, 1993, 1995), suggesting nests are made of various kinds of plant matter, including twigs, grass, lichens, and leaves (Wimberger 1984; Collias and Collias, 1984). Moreover, these authors stated certain kinds of plants apparently help combat disease and ectoparasite infection, which can be a serious problem in fouled unsanitary nests and in reused nests such as cavities and artificial nest boxes. Gill (1990) reported green vegetation seems to be particularly useful in this regard. Similar patterns have been reported by Shutler and Campbell (2007) and imply that the green vegetation eliminate odors that may attract predators to the nest but some authors also mentioned that these green vegetation could have a repulsive action against ectoparasites, by insecticide and acaricide properties. In more, these statements could be possible according our observations which more sticks and fresh cut twigs with leaves were collected, respectively during nest building and nestling periods. Also, we think the use of sticks and fresh cut twigs with leaves are related to minimizing parasite infestation and to avoid their nestlings from falling out of the nest. Therefore, monthly monitoring would be necessary to accurately determine responses to choice of sticks and twigs in relation to specific flora taxa with its insecticide and acaricide properties in Madagascar Buzzard nests.

Egg laying

Eggs were laid during the dry season from August to October and the laying period peaked in September ($n = 24$ pairs) (Figure 2). The earliest laying date was 25 August 2017 while the latest was 04 October 2017. Eggs

were laid within an interval of one ($n = 4$ nests) to three days ($n = 11$ nests). Eggs were mainly white; but sometimes contained red spots. Mean egg dimensions were 53.3 ± 14.6 mm in length and 42.1 ± 9.5 mm in width ($n = 28$ eggs). The average egg mass was 54.8 ± 12.1 g ($n = 28$ eggs).

Brown and Amadon (1989) stated that laying period for Madagascar Buzzard was in October, and on Masoala the laying period was from late September to early October (Berkelman, 1993). During this study, the egg laying period was from late August to early October. In Masoala, from June to August, the rainy season was still present and it may be the reason the breeding season was one month later than observed at Bemanevika PA (Rene de Roland, pers._comm.). According to Brown (1976), before and during the laying period, the female needs sufficient food to form eggs. Other than climate, the availability of prey could be a factor of the timing of the laying dates (Newton, 1979). We believe these factors could explain the difference that was observed for the initiation of the laying period between Bemanevika PA and Masoala.

Regarding clutch size, this study confirmed that Madagascar Buzzards laid one to two-egg clutches, similar to that the Mountain Buzzard *B. oreophilus* (Rudebeck, 1957). Our findings were also similar to those found by Berkelman (1993) on Masoala. The size of Mountain Buzzards averaged 45 cm (Birdlife International, 2016) and the Madagascar Buzzards averaged 49.5 cm (Langrand, 1995). The minimum and maximum clutch size for raptor species related to the size of each species and the environment they inhabit. In more, clutch size can be an indirect measure habitat quality, and also an indirect indicator of the physiological condition of reproductively active females (Jacobs and Jacobs, 2002). According to our observation, both proximate factors determined eggs' number produced: Madagascar Buzzard breed in a good habitat and the female is able to produce eggs during the months when food is plentiful.

King (1973), Ricklefs (1974) and Gill (1989) confirmed the lack of food could reduce or freeze eggs productions and affect clutch size. Additionally, the maximum clutch size of two eggs for the Madagascar Buzzard is more or less small compared with other species in the same genus such as *B. platyperus* (Burns, 1911), *B. polyosoma* (Marchant, 1960) and *B. buteo* (Moore, 1957; Vanegue, 2015). The aforementioned Buteos laid three eggs and, having respectively a body size of 44, 48 and 49 cm, nearly similar to *B. brachypterus* (49.5 cm). The environment they live in is a major factor for the difference. It means Buteos in temperate climates lay larger clutches than Buteos living in the tropical environment (R. Thorstrom, pers. comm.). Also we believe that this difference is due to the body size between the species but it is a secondary factor to explain the variation. It would be better to conduct a

specific study focused on relation between clutch size and foods. It could be used to elucidate the difference of clutch size for same or different Buteos species (especially *B. brachypterus*), from one to another region.

Incubation

We totaled 114 h of nest observations during the incubation period. We only monitored incubation activity of adults at N1-18 and N5-18. The females incubated while the males provided some incubation and all the food during nest observations. At N1-18 and N5-18, the observation times were 62 and 52 h, respectively. At N1-18, the female incubated 83.8% of observation time ($n = 52$ h) while the male incubated for 3.2% ($n = 2$ h) and the nest was unattended during 13% ($n = 8$ h). At N5-18, the female incubated for 75% ($n = 39$ h), the male 2% ($n = 1$ h) and the pair was absent from the nest for 23% ($n = 12$ h) of the observation time. The incubation period was 36.2 ± 1.1 days (range 35-38 days, $n = 16$ nests). At the N1-18 and N5-18, the following behavior marked the hatching period: when the first egg hatched, the females incited the males to deliver the prey item into the nest. All eggs hatched at an interval of one ($n = 2$ nests) to three days ($n = 9$ nests). Hatching occurred between the end of the dry season and the start of the rainy season, with peak hatching in October (Figure 2).

In this study, the incubation period for the Madagascar Buzzard was from 35 to 38 days and nearly the same for Buzzards on Masoala with 34-37 days (Berkelman, 1993). These results agree with Newton (1979) who stated that in raptors, the incubation period ranges from four to eight weeks. On the other hand, the variation of incubation period of the species could be due to the difference in sizes of each species. Based on this, our result and other records confirm it. In fact, our result is more or less similar compared with other species in the same genus such as Broad-winged Hawk *B. platypterus* (28 to 31 days) (Goodrich et al., 2014.) and Common Buzzard *B. buteo* with duration of 33 to 35 days (Mebs, 1964). These two species have a body size of 44 and 49 cm that are more or less similar to *B. brachypterus* (49.5 cm). Also, the Buteos above inhabit a temperate climate whereas the Madagascar Buzzard is found in a tropical climate (Brown and Amadon, 1968).

Nestling period

The observation time during the nestling period totaled 665 hours. During the first two weeks after hatching, the female spent 45.8% ($n = 305$ h) of the time brooding and feeding young while the male provisioned the female and nestlings. Males called from a tree near the nest upon arrival with food and the female took and carried the prey



Figure 3. At N8-18, female feeding nestlings during the nestling period (© Raveloson).

item to the nest. Starting at 15 days of age, adult females started spending less time brooding and feeding their nestlings. At 20 days of age, females fed and assisted young for 37.4% ($n = 249$ h) or perched near the nest waiting for prey deliveries for 8.8% ($n = 58$ h) of total observation time. Female Madagascar Buzzards brooded and fed the young while males never brooded or fed the young (Figure 3). When two nestlings were present in the nest, the older first-hatched young was more aggressive and always received the most food. The young were able to feed themselves at 35 days of age. At this period, females spent 8% ($n = 53$ h) of the observation time hunting. Of 347 prey items delivered to the nest ($n = 11$ nests), males delivered 206 and females 141 prey items for the young in the nest. The quantity of food delivered by both sexes did not differ significantly during the feeding of young (chi-square test: $\chi^2_{cal} = 11.34$, $df = 12$, $p = 0.5$). Trail cameras recorded adult females brood the young during the night. Adult females came into the nest at 17:45 (when it started becoming dark) and went out at 5:05. This occurred from the first day of age to 25-30 days of age ($n = 5$ nests). After this period, the adult female rarely came into the nest or stayed near the nest during the night.

From one to two weeks after hatching (7- 14 days of age), young had black beaks, yellowish ceres and tarsus, and grey nails. During this period, young were directly fed by the female. By 12-16 days after hatching, the young actively moved around in the nest, preened and looked out of the nest. They emitted calls similar to the female, but much weaker. At 25-28 days of age, primary and secondary wing and tail feathers were emerging, and feathers around chest and abdomen were predominately

complete. From 33 days of age, down feathers disappeared slowly and head, chest, wing and tail feathers were emerging. At 35 days of age, the young began feeding themselves and emitted specific repetitive calls during prey deliveries. At 38-40 days of age, the young remained on a branch within 1-2 m of the nest. Prey was delivered by both adults into the nest and the young fed themselves. The young were silent except when prey was delivered to the nest. In nests with two youngs ($n = 3$ nests), the first hatched young left the nest before the second hatched young.

At 45 days of age, the young were completely covered with feathers and their plumage, ceres, nails and tarsus colors were nearly close to those of adults. Three to seven days before fledging, the young exercised their wing when the wind blew. Madagascar Buzzard young fledged at an average of 48 ± 3.9 days of age (range 43-56 days, $n = 17$ young, $N = 22$ young). During the nestling period, the nesting activities differed between males and females. Females spent almost half of their time in the nest protecting and feeding the nestlings while the male provisioned food to the female and nestlings. This is typical of nearly all raptors (Newton, 1979), for example the Common Buzzard (Moore, 1957) Jackal Buzzard *Buteo rufofuscus* (Steyn, 1983) and Yellow-billed Kite *Milvus aegyptius* (Andriamalala, 2005). However, we also suspect that the activities of this hawk considerably depend on the period and provisioned food. In fact, from the third week of the nestling period, Berkelman (1993) and this study documented that the adult females spent less time at their nests and started hunting for provisioning their nestlings. According to Simmons (1983), for raptors, the time when adult females

start to provision food for the nestlings depends on prey delivered by adult males. In spite of this, in our study area, the nestling period ranged from 43-56 days and was similar to those in Masoala (39-51 days) (Berkelman 1996).

During this study, the nestling period ended between mid-November and mid-January while Berkelman (1993) found the nestling period ended between early December and mid-January. The breeding season for Madagascar Buzzards coincides with the driest period of the year (Donque, 1972), and young fledge from nests at the start of the summer rains. Most species in eastern Madagascar breed between September and January (Langrand, 1990). We think the length of the nestling period may be determined by the variation of climate at each site, food availability and the number of young at the nest.

Fledging period and dispersal

From 49 -56 days of age, the young always perched in a tree from 10-30 m of the nest tree and waited for food delivered by adults. Young were fed in or near the nest until 60 days of age ($n = 7$ young, $n = 5$ nests). The young stayed within 150 m of the natal territory with their parents until 67 to 76 days old during fledging period. Prey transfers took place on the perch of female and female incited young to move between trees, at 57-65 days of age. For example, at 60 days of age, young began to fly farther from their nest sites, from 50 to 300 m.

The observation time during the post-fledging period was 145 h. During the post-fledging period, young became independent of food provided by their parents at 68 days of age. Young started catching prey from 69.7 ± 2.5 days ($n = 4$ young), and consequently the amount of prey delivered by adults decreased (one prey per day compared to one to three prey items per day during the first and second weeks after hatching period). Young were observed catching insects like caterpillars from leaves and branches in perching trees within their natal territory. We observed young from pairs P1-17 and P5-18 capturing insects, chameleons and a small bird. At 70 days of age, the young flew far, and adults rarely visited their nests. The restriction of prey may have stimulated the dispersal of young from natal area. For pairs that successfully fledged two young (P3-17, P5-18 and P8-18), the second hatched young became independent later than the first young. For instance, for P3-17, first young was independent at 68 days of age and second was at 77 days. Young became completely independent at an average age of 73.3 ± 3.8 days (range 68-78 days, $n = 14$ young).

During the post-fledging period, there was a decrease in prey deliveries by the adults, possibly forcing the

young to disperse from their natal areas (Moreno, 1984; Edwards, 1985). Young Madagascar Buzzards dispersed at 73 days of age, almost similar to Jackal Buzzards (70 days of age) (Steyn, 1983).

We believe that age and morphology related to faculty of young to hunt their own prey could determine the variation of dispersion period, from one to another site. Moreover, at this age, young are able to disperse from their natal areas because they were able to capture their own prey. The ability of fledged young to catch their own prey is an indication that they are close to dispersing from their natal area as also reported for *M. aegyptius* (Andriamalala, 2005). The dispersion of young might have a relationship with most raptors do not feed their young once they have left the breeding area (Newton, 1979). This author also mentioned, food is not only the factor influencing dispersion but the nesting places are also involved. It means where suitable places are widespread, many species nest solitarily in contiguous or overlapping home ranges, as described; but where suitable nesting places are concentrated, pairs of the same species may have no choice but to nest close together, and range over surrounding land to feed. Newton (1979) mentioned that the young continue to be fed by their parents until they become self-sufficient. It was concluded that Madagascar Buzzard young were able to disperse in Bemanevika and with a complete development at 73 days of age.

Rand (1936) thought that the breeding cycle extended from at least July to November for this species. In the Bemanevika PA, the breeding cycle of the Madagascar Buzzard was from the last week of July to mid-January whereas in Masoala, the breeding cycle was from August to mid-January (Berkelman, 1993). The availability of prey is the most important factor for determining the breeding cycle of raptors (Newton, 1979). Furthermore, the variation of the beginning and the end of breeding cycles are determined by the locality and season (Ferguson-Lees and Christie, 2001). For another endemic Malagasy raptor, the Madagascar Harrier-hawk *Polyboroides radiatus*, breeding activities started August and ended January (Thorstrom and La Marca, 2000). Yet, nestling hatched when conditions were at the driest (November) and most passerines were breeding and fledging occurred when the rainy season had begun (January). Consequently, for the Madagascar Buzzard, we suspect as in all such areas, increased food availability, facilitating improved body condition and egg production are the most likely proximate factors controlling the timing of breeding (Newton, 1979).

Reproductive success

In 2017 and 2018, 39 eggs were laid in 24 nests composed of 15 nests with two-egg clutches and nine

Table 1. Breeding parameters of the Madagascar Buzzard *Buteo brachypterus* during two breeding seasons, 2017 and 2018, at Bemanevika Protected Area, Madagascar.

Year	Number breeding attempts	Number of eggs	Number of eggs hatched (%)	Number of young fledged (%)	Nest success (%)	Breeding productivity
2017	14	22	20 (90.9%)	11 (55 %)	71.4% (10/14)	0.78 (11/14)
2018	10	17	14 (82.3%)	11 (78.5 %)	90% (9/10)	1.1 (9/10)
Total	24	39	34 (87.1%)	22 (64.7 %)	79.2% (19/24)	0.91 (22/24)

nest with a one egg clutch (Table 1). In the 24 nests, 87.2% hatched ($n = 34$) and 64.7% ($n = 22$) of the nestlings fledged. At N3-17, N5-18 and N8-18, both hatchlings fledged and all were completely independent. Overall productivity was 0.91 young fledged per breeding attempt. Overall nest success for 24 fully-documented was 79.2% (19 successful nesting attempts) in the two study years.

During this study, the average productivity for the Madagascar Buzzard was 0.91 young per pair (range: 0.78-1.1) for the two years combined. This result was different to that recorded from Masoala of 0.7 young per pair (Berkelman, 1996). Furthermore, we found three pairs which had two-egg clutches, hatched and fledged both young: one pair in 2017 and two pairs in 2018. However, Berkelman (1996) noted only one young survived to fledge in each successful nest although two eggs were laid in at least four nests during his study. This could explain the difference of productivity between two sites. In fact, this study showed that the reproductive success of Madagascar Buzzard could hatch and fledged two young from the same nest. In any case, the average productivity of this species varied between 0.7 and 0.91 young per pair. This study highlighted that the nest success of Madagascar Buzzard is normal in a species that the causes of breeding failures do not affect its population productivity. Consequently, the population is suspected to be stable in the absence of any declines or substantial threats (Birdlife International, 2016).

Cause of breeding failures

Of the 15 nests with two-egg clutches, in four nests (N10-17, N2-18, N4-18 and N6-18) the second egg did not hatch (possibly addle eggs). One of nine nests with a one-egg clutch failed because of predation (N6-17). Unfortunately, the predator was not identified but the corpse of female devoured was observed on the ground near the nest tree. At N13-17, both nestlings were found dead in the nest because adult female was killed by local residents for food when the nestlings were 12 days of age. At N10-17 and N7-18 respectively, both the first and second hatched nestlings were found death probably due to the insufficiency of food causing starvation during the

first week after hatching.

Siblicide was also recorded as cause of death to several second-hatched nestlings. During this study, the trail camera recorded 31,165 photos in 2017 and 96,303 photos and 707 video sequences in 2018. The photos and videos were analyzed for the presence of siblicide. Siblicide was documented at six nests (N1-17, N2-17, N4-17, N8-17, N14-17 and N1-18) with two-egg clutches. The second sibling died between 7-15 days of age, from 21 October to 23 November, and at these six nests all first hatched young survived and fledged successfully. The interval of hatching of two-egg clutches was one day for nests without siblicide (NNS, $n = 6$ nests) and three days for nests with siblicide (NS, $n = 5$ nests). Prey delivery was lower at NS than at NNS, respectively 19 and 36 prey items. The siblicide recorded for this species was facultative may or may not occur, based on environmental conditions. A case of both siblicide and cannibalism was recorded by the trail cameras at N1-18 between 24 and 25 October 2018. The adult female also increased the second nestling's death because of her pecking attacks on it. The aggression attacks were intensified on 25 October 2018 until the young died and then the adult female fed the dead young to her first-hatched nestling (Figure 4).

We recorded siblicide as a cause of breeding failures for Madagascar Buzzard in our study area. Moreover, photos and videos recorded during this study confirmed the presence of facultative siblicide in the Madagascar Buzzard. Despite the second-hatched nestling not surviving for three nests with two-egg clutches, Berkelman (1993) didn't suspected that siblicide occurred in the Madagascar Buzzard in Masoala National Park. However, Rand (1936); Milon et al. (1973); Langrand and Meyburg (1984), Brown and Amadon (1989); stated that Madagascar Buzzards exhibits caïnism and R. Thorstrom suspected it for Berkelman's study at Masoala. This observation of siblicide as cause of breeding failures for this species concurs with the result of Watson et al (1999), in the Madagascar Fish Eagle (MFE) (an Accipitridae in the same family as the Madagascar Buzzard). Additionally, siblicide is common in eagles as it occurs in at least 27 out of 59 (45.7%) eagle species worldwide (Meyburg, 1978; Brown and Amadon, 1989). Ingram (1959) also stated the existence of siblicide in five



Figure 4. At N1-18, the second-hatched nestling died from siblicide (left) (© Raveloson), and the female feeding its first nestling with the corpse of the dead nestling : cannibalism (right) (© Raveloson).

species of the genus *Buteo* such as Common Buzzard *Buteo buteo*, Rough-legged Buzzard *B. lagopus*, Swainson's Hawk *B. swainsoni*, Red-tailed Hawk *B. jamaicensis* and Red-shouldered Hawk *B. lineatus*.

Our study suggests that siblicide doesn't only occur in Madagascar Fish Eagles and Madagascar Harrier Hawks (*Polyboroides radiatus*) (Thorstrom and La Marca, 2000) in Madagascar raptors. Henceforth, our result and the statement of Ingram (1959) confirm that siblicide is found in six out of 25 (24%) species of Buteos in the world. In fact, the observation of siblicide by trail cameras suggests research has an important relationship with technology, especially research on difficult to study species like raptors. For the Madagascar Buzzard, it's interesting to check if siblicide is normal or happens occasionally throughout Madagascar, like what we recorded with trail cameras in Bemanevika PA.

Siblicide is either obligatory (a nestling is always killed by its older sibling) or facultative (apparent mortality or not of the second hatched young) (Edwards and Collopy, 1983; Mock, 1984). Our observations were of facultative siblicide with a maximum of two-egg clutches for the Madagascar Buzzard at Bemanevika PA. Watson et al. (1999) reported the Madagascar Fish Eagle was an obligatory fratricide relative to the maximum clutch size of two eggs. Therefore, we believe the maximum of clutch doesn't determine the categorization of siblicide. In addition, raptors with facultative siblicide generally have more than two eggs per clutch (Simmons, 1988). However, the Madagascar Harrier *Circus macroscleus* has a maximum clutch size of three eggs, but siblicide is obligate (the third-hatched nestling always dies on 10th day of age) (Rene de Roland et al., 2004).

In Madagascar, obligatory siblicide is well documented in the Madagascar Fish Eagle (Watson et al., 1996). The

second-hatched nestling always dies several days after hatching (Razafindramanana, 1995). In facultative siblicide species, the second hatched young sometimes dies (Edwards and Collopy, 1983). For the Madagascar Buzzard, we found siblicide in six of 11 nests with two-egg clutches and with both hatching. Edwards and Collopy (1983) mentioned that siblicide is related to asynchronous hatching and a high-percentage of volume difference within two-egg clutches, for two types of siblicide (obligate and facultative). In facultative siblicide species, siblicide usually occurred in relation to food restriction and egg volume difference is < 10%. Our study reported two reasons of siblicide occurrence: (1) asynchronous hatching of siblings, and (2) reduction food resources. Asynchronous hatching can be regarded as an adaptation to an unpredictable food supply, enabling all young to survive in times of plenty, but ensuring rapid reduction of the brood to an appropriate level in times of scarcity (Newton, 1979). Among medium-size raptor species of *Buteo* and *Accipiter*, attacks by older nestlings on smaller siblings occur only at times of great hunger (Balfour, 1957 and Newton, 1976). We believe that facultative siblicide in the Madagascar Buzzard does not affect its population at the moment. This species has a large distribution range and the bird prefers various habitat types, degraded to primary forests (Birdlife International, 2016).

Circumstantial evidence indicates that fratricide, in all probability invariably followed by cannibalism, occurs, far more frequently among birds of prey than is commonly reported and, indeed, in a few species is perhaps a normal, rather than an exception (Ingram, 1959). During this study, we discovered the presence of both siblicide and cannibalism, like in the Common Buzzard (Salter, 1904; Gilbert and Brook, 1924). A study on nesting



Figure 5. A prey *Microcebus* sp. collected by adult female and brought to N1-18 (© Raveloson).

biology and behavior of Madagascar Harrier Hawk *Polyboroides radiatus* reported the same cannibalism for this species (Thorstrom and La Marca, 2000). Hence, siblicide followed by cannibalism was not found in Madagascar Fish Eagle but only siblicide (Milon et al., 1973). Since, the Madagascar Buzzard and the Madagascar Harrier hawk are a dietary generalist while the Madagascar Fish Eagle is a dietary specialist suggesting that siblicide exists as an evolutionary breeding strategy that developed in Madagascar Buzzard. Therefore, this study reported the first case of siblicide followed by cannibalism in the community of raptors in Bemanevika PA. Based on the community of raptors, the occurrence of siblicide in the Madagascar Buzzard adds to the number of species with this behaviour in Madagascar. At present, siblicide occurs in Madagascar Fish Eagles, Madagascar Harriers, Madagascar Harrier Hawks and Madagascar Buzzards.

Food habits and hunting behavior

During this study, we recorded 541 prey items of which 312 (57.7%) were documented by trail cameras and 229 (42.3%) by direct observation for a total of 515 identified and 26 not identified. Of the 541 prey items, 28 and 513 were recorded during the incubation and nestling periods, respectively. Based on the 515 identified prey items, the diet of the Madagascar Buzzard was composed of 37.3% (192) reptiles, 35% (180) birds, 19% (98) mammals (Figure 5), 8.3% (43) invertebrates and 0.4% (2)

amphibians. Reptiles were composed of 98% (188) chameleons and 2% (4) other lizards. Of the 98 mammals identified, rats made up 98% ($n = 96$) and lemurs 2% ($n = 2$) (Figure 6). The quantity of each prey type differed significantly between the two study seasons (chi-square test: $\chi^2 = 241.7$, $df = 8$, $p = 0.0001$). Madagascar Buzzards hunted alone and from a raised perch in the forest or on the ground in the savannas. In the forest, when prey was observed they flew directly at the prey. In the savanna, after a long stationary flight and when prey was spotted, they descended slowly to the ground and then dropping onto the prey grasping it with their beak or feet. Males delivered at least two preys items per day to the nest from the hatching to fledging periods. At N8-18, the female was observed delivering two small birds at the same time: one carried in a foot and the second one in the beak. The consumption of prey items varied from two seconds to five minutes ($n = 904$ beakfuls).

During the two breeding seasons, we recorded a variety of prey items in the Madagascar Buzzard's diet such as reptiles, birds, amphibians, invertebrates and micro-mammals. In Masoala, micro-mammals were not reported as a prey item by this species (Berkelman, 1997). Rand (1936); Milon et al. (1973); Brown and Amadon (1989) and Langrand (1990) reported the same, as well as the existence of carrion in the diet of the Madagascar Buzzards. These previous records and our study showed that two prey categories are the most taken from several localities by Madagascar Buzzards, reptiles and birds, then followed by others prey types.

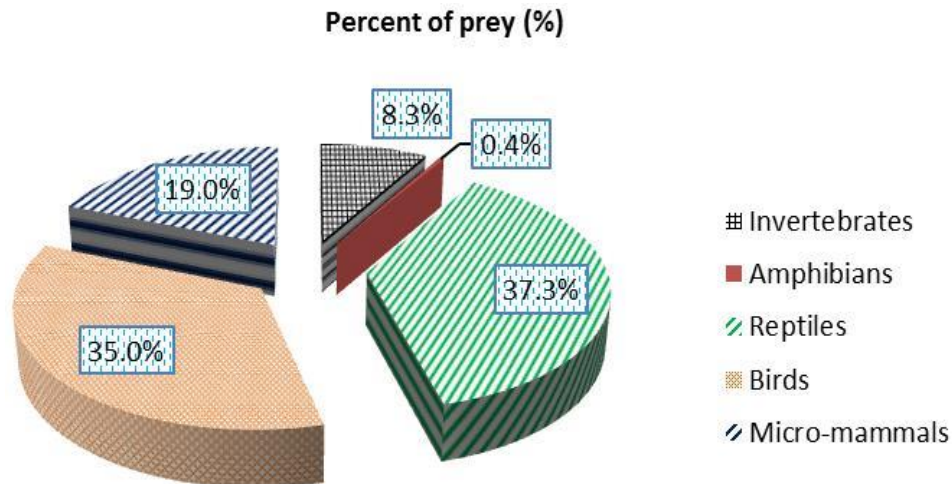


Figure 6. Percent of prey items recorded at nests of *Buteo brachypterus* during two breeding seasons, Bemanevika PA, Madagascar.

Thus, our result confirms that *Buteo brachypterus* is a dietary generalist species. In addition, the specific distribution of each prey species from one to another locality in Madagascar may explain this diversity of prey types for *B. brachypterus*. During this study, reptiles (37.3%) were predominantly chameleons (98%), and birds (35%) made up the greatest portion of the diet of Madagascar Buzzards at Bemanevika PA. Berkelman (1993) found that birds (33.2%) were taken more than chameleons (29.1%). This difference appears to be explained by prey availability and forest types at these two sites and the breeding period of chameleons seems to coincide to those of Madagascar Buzzard in Bemanevika PA (Angelinah Rene de Roland, comm.pers). In October and November, chameleons are known to lay their eggs on the ground, making them susceptible to predators (J Rabearivony, pers. obs.) like Madagascar Buzzards. Like chameleons, small fledgling birds were among the most consumed prey as they are easier to capture because of their lack of experience and flying skills. This was similar to what was observed in the Frances's Sparrowhawk *Accipiter francesiae*, especially taking small fledgling passerines on Masoala (Rene de Roland, 2000b). We suggest the agility of each individual's hunting skills and methods used could explain these differences in prey types taken by Madagascar Buzzards.

Conclusion

The study was focused on the breeding biology and diet of *Buteo brachypterus*, an endemic raptor of Madagascar, during two breeding seasons 2017 and 2018, in the montane rainforest of Bemanevika Protected Area. This

study highlighted the trail camera as an efficient method for research in biology of raptors. We documented new information on several aspects of biology and ecology of this raptor species, especially regarding its diets and siblicide behaviour. We recorded 24 nesting attempts including nine nests located at the forest edge and fifteen in the forest interior. The breeding period lasted at least six months, from the last week of July to mid-January. The clutch size was from one to two eggs. Of 24 nests attempts, nine were with a one egg- clutch and 15 were with two egg-clutches. Breeding productivity of *B. brachypterus* ranged between 0.78 and 1.1 young fledged for the two breeding seasons. Three factors were determined for breeding failures: predation, siblicide and female persecuted for food by local villagers. We reported an evolutionary breeding system called facultative siblicide occurs in the Madagascar Buzzard. This study was the first case of this occurrence of siblicide followed by cannibalism in the Madagascar Buzzard. Food habits identified chameleons, birds, lemurs, rats, snakes and amphibians as the source of food for this raptor. Our study is consistent with other results that *B. brachypterus* is a dietary generalist with chameleons and birds the most frequent prey taken. This was the first study of the Madagascar Buzzards at Bemanevika PA, even though the data reported the buzzards were successfully in fledging 0.9 young per nesting attempt, we recommend further studies to understand more about siblicide in this species, in other raptors in the study area and in other regions of Madagascar. It is important to elucidate more on this species' reproductive strategy and the mechanisms that regulates its population, it is important to assess their population size and ranging behavior during the non-breeding and breeding periods, and to determine their

distribution and global status.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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