THE TROPHIC ECOLOGY OF THE ENDANGERED ENDEMIC BARAU'S PETREL (*PTERODROMA BARAUI*) FROM RÉUNION ISLAND, SOUTH-WESTERN INDIAN OCEAN.

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“The richness I achieve in life comes from nature, the source of my inspiration”

:- Claude Monet -:

ABSTRACT:

Réunion is the only tropical island that supports two endemic gadfly petrels. Population modelling has indicated that the current threats to the Barau's Petrel (*Pterodroma baraui*) may drive it to extinction; this fate is almost definite for the Mascarene Petrel (*Pseudobulweria aterrima*). Management interventions have therefore been implemented, but conservation potential is handicapped since virtually nothing is known about the former species' at-sea biology. Thus, following numerous recommendations, this study aimed to combine stomach content, stable isotope, and fatty acid analyses so to provide new information on the at-sea ecology of Barau's Petrel. Breeding colonies were repeatedly visited over the same season and samples gathered from adults, fledglings, and downy chicks. Stomach contents consisted mostly of accumulated cephalopod beaks whereas structures from fishes, molluscs, arthropods, and crustaceans were less frequently encountered. Fatty acid profiles of blood varied greatly among individuals and the low incidences of monounsaturated and n-3 fatty acids discounted fish as an important dietary component. $\delta^{15}N$ of blood indicated a niche between the fourth and fifth trophic levels, which proposes that these birds scavenge to a greater degree than other sympatric seabirds and suggests that adults are also reliant on their endogenous reserves during breeding. $\delta^{13}C$ values confirmed the migratory behaviour of adults since birds returning from the non-breeding grounds were enriched relative to individuals sampled through the breeding period. Significant intra-breeding season variations in $\delta^{13}C$ were also observed, which matched this species' patterns of habitat use as have recently described. These results collectively indicate an opportunistic behaviour, which implies some degree of resilience against shifts in prey availability/accessibility, and suggest that this species' reproduction is

dependent on distant foraging areas. This breeding strategy is synonymous with great vulnerability as over-investing into a single breeding episode may jeopardize future survival and fecundity. Thus, in light of environmental conditions that are becoming increasingly more susceptible to dramatic changes, the birds could rather be prioritising adult survival, over reproductive output. Further work is obviously necessary and should benefit from databases of fatty acid profiles and isotope signatures of potential prey species.

TABLE OF CONTENTS:

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LIST OF NON-STANDARD ABBREVIATIONS:

ECOMAR – Labatoire d'Ecologie Marine de l'université de La Réunion

m a.s.l. – metres above-sea-level

IUCN – International Union for the Conservation of Nature

GPS-PTTs – global positioning system platform transmitter terminals

La SEOR – La Société d'Etudes Ornithologiques de La Réunion

rpm – revolutions per minute

PCR – polymerase chain reaction

bp – base pairs

WW – wet weight

FO – frequency of occurrence

RPO – relative percentage occurrence

LRL – lower rostral length

ML – mantle length

JA – jaw angle

RT – rostrum tip

GLS – global location sensors

SIA – stable isotope analysis

SI – stable isotope

FA – fatty acid

SFA – saturated fatty acids

MUFA – monounsaturated fatty acids

PUFA – polyunsaturated fatty acids

i – iso fatty acids

ai- – anti-iso fatty acids

TFA – total fatty acid

FASA – fatty acid signature analysis

GC – gas chromatography

FAME – fatty acid methyl esters

ANOSIM – analysis of similarities

SIMER – similarity percentage

MDS – non-metric multidimensional scaling

PCA – principal component analysis

LIST OF FIGURES:

a typical burrow, usually located in cinder soils under rocks and in natural crevices, containing the single large egg (Photo credit: Daniel Keith Danckwerts).

Figure 2.1: Location of Réunion Island (colony 45 – represented as black star) relative to all other seabird breeding colonies in the western Indian Ocean as adapted from Danckwerts *et al.* (in press). Colonies are listed as follows: (1) Jasiira Ceebaad and Jasiira Sacada Din, (2) Jasiira Maydh, (3) Socotra Group, (4) Raas Xaafun and Raas Gumbax, (5) Mogadisho Islets, (6) Bajan islands, (7) Kiunga Marine Nature Reserve, (8) Whale Island, (9) Kisite Island, (10) Zanzibar Chumbe Island, (11) Latham, (12) Mafia Island, (13) Aride, (14) Bird Island, (15) Cousin, (16) Cousine, (17) Frégate, (18) Récif, (19) African Banks, (20) Boudeuse, (21) D'Arros, (22) Desnoeufs, (23) Marie-Louise, (24) Farquhar, (25) Goelettes, (26) Aldabra, (27) Cosmoledo, (28) Glorieuses, (29) Mayotte, (30) Mohéli, (31) Cape Anorontany Archipelago, (32) east coast of Ansiranana, (33) Mitsio Archipelago, (34) Nosy Bé and other islets, (35) Etoile, (36) Puga-Puga, (37) Juan de Nova, (38) Nosy Barren, (39) Europa, (40) Islets off Morombé, (41) Nosy Vé, (42) Nosy Manitra and other islets, (43) Ilôts off Toamasina, (44) Tromelin, (45) Réunion, (46) Flat Island, (47) Gabriel Island, (48) Gunner's Quoin, (49) Pigeon Rock, (50) Round Island, (51) Serpent Island, (52) Saint-Brandon, (53) Rodrigues Islets, and (54) Chagos Archipelago.

Figure 2.2: Barau's Petrel (*Pterodroma baraui*) are sexually monomorphic, implying that all adult birds needed to be sexed using molecular techniques. The restriction enzyme Hae III (pRomega) cuts the CHD1Z intron, but not CHD1W, so that males (ZZ) display two fragments at 65 and 45 base pairs (bp; seen as two separate bands) and females (ZW) show three fragments at 45, 65, and 110 bp (visualized as one dark band). Bands in the 2 % agarose TBE

LIST OF TABLES:

CHAPTER 1: GENERAL INTRODUCTION AND MOTIVATION

1.1. Seabirds as biological models

The term 'seabird' has been used to collectively describe birds from the Orders Sphenisciformes, Procellariiformes, Pelecaniformes, and Charadriiformes (Schreiber & Burger 2002a). They are among the most visible of all marine organisms, having established populations in every sea/ocean on Earth, and include representatives from almost every available ecological niche. Some auks (e.g. Little Auk, *Alle alle*), for example, are specialist planktivores that feed relatively close to their cliffside colonies (Węslawski *et al.* 1999; Kwasniewski *et al.* 2012; Boehnke *et al.* in press), whereas albatrosses (*Thalassarche*, *Phoebastria*, *Diomedea,* and *Phoebetria*) and petrels (e.g. *Pterodroma,* and *Daption*) are dietary opportunists that fly thousands of kilometres away from their isolated nesting islands (Lipinski & Jackson 1989; Rodhouse *et al.* 1990; Warham 1990; Weimerskirch *et al.* 1993; Cherel & Weimerskirch 1995; Imber *et al.* 1995; Ballance & Pitman 1999; Bester 2003; Bester *et al.* 2010; Connan *et al.* 2014). Yet, despite this apparent diversity, all seabirds share one common trait – adults of most species disperse out at sea during the inter-breeding period but all must return to a few isolated colonies to reproduce at which time the energy demands of the young limits the adults' foraging trip duration and range (Phillips *et al.* 2005; Pinaud & Weimerskirch 2005, 2007; González-Solís *et al.* 2007; Catry *et al.* 2009b; Egevang *et al.* 2010; Quillfeldt *et al.* 2010a, 2010b). Such 'central place foraging' demands unique behavioural, demographical, physiological, and morphological adaptations (Stearns 1992; Pinaud & Weimerskirch 2005, 2007), which together make seabirds extremely useful as biological models for studying ecosystem state, function, and change (Piatt *et al.* 2007; Birdlife International 2009). Indeed, most seabirds breed synchronously in vast colonies

(Danchin & Wagner 1997; Coulson 2002), implying that large numbers of birds are often extremely accessible and a variety of demographic, behavioural, and ecological parameters can easily be monitored together (Ballance & Pitman 1999; Schreiber & Burger 2002b; Weimerskirch 2002; Piatt *et al.* 2007). Seabirds also have dramatically different life-history characteristics from most other bird groups that augment their usefulness for study purposes. They show long life (20 to 60 years), delayed sexual maturity (breeding age sometimes delayed until 10 years of age), small clutch size (in many cases one egg), extended chick-rearing periods (up to a year in some species), and high philopatry (Hamer *et al.* 2002; Schreiber & Burger 2002b; Weimerskirch 2002). These traits render them unable to adapt to drastic temporal changes in marine (on foraging grounds) and terrestrial (at colonies) ecosystem state and function, however, as environmental unpredictability limits the adults' ability to control the level of investment required to ensure successful breeding (Stearns 1992; Tavecchia *et al.* 2007; Le Bohec *et al.* 2008; Green *et al.* 2009). Devoting too much into a particular breeding episode may jeopardize future individual survival and reproductive output, potentially also having effects at the population and species levels (Stearns 1992). Indeed, whilst there has been some variation in response (Furness & Camphuysen 1997; Kitaysky & Golubova 2000; Jenouvrier *et al.* 2005), numerous examples exist whereby environmental unpredictability has negatively affected marine bird populations (e.g. Wilson 1991; Montevecchi & Myers 1995, 1996; Harris & Wanless 1996; Croxall *et al.* 1999; Barbraud & Weimerskirch 2003; Frederiksen *et al.* 2004; Bertram *et al.* 2005; Grosbois & Thompson 2005).

1.2. Research, management, and conservation priorities for procellariiform seabirds

Seabird populations around the world have been reduced to a fraction of their historical numbers, largely as a consequence of human-related perturbations (Feare 1978; Schreiber & Burger 2002b; Butchart *et al.* 2004; Feare *et al.* 2007). A number of species (e.g. Jamaican Petrel, *Pterodroma caribbaea;* Imber 1991) and many breeding colonies (Feare *et al.* 2007) that once existed have now gone extinct, whilst those populations that remain are greatly reduced in size (e.g. Feare 1978; Feare *et al.* 2007). Feare (1978) recognised the importance of quantifying the remaining seabird populations and identifying the small and large-scale pressures that they are faced with. Seabirds have since been exceptionally well-studied and knowledge of their conservation statuses has become more comprehensive than for any other group of marine organisms (Vie *et al.* 2008). Nevertheless, numerous knowledge gaps still remain that severely limit conservation efforts. For this reason, and because their status typically reflects the underlying state of important coastal and oceanic marine ecosystems (Furness & Camphuysen 1997), Croxall *et al.* (2012) stressed the need to begin determining how seabirds might be faring against present and future pressures, how their statuses have changed in recent times, what actions are needed to address the main current threats, and what kind of baselines exist against which to measure future changes. Many threats, which have contributed to the significant population declines, have been investigated including direct harvesting of birds and their eggs (Feare & Lesperance 2002; Merkel & Barry 2008), incidental mortality in line-based and trawl fisheries (e.g. Brothers 1991; Montevecchi 2002; Baker *et al.* 2007; Croxall 2008; Anderson *et al.* 2009, 2011), trace metal bioaccumulation and choking hazards associated with plastic pollution (e.g. Laist 1997; Lavers *et al.* 2014; Lavers & Bond in press), chronic and catastrophic oil spills linked to increasing maritime traffic (e.g. Piatt *et al.* 1990; Evans *et al.* 1993; Ford *et al.* 1996; Flint *et*

al. 1999; Wiese & Robertson 2004; Velando *et al.* 2005a, 2005b; Votier *et al.* 2008; Munilla *et al.* 2011), predation of eggs and birds by non-native species (Courchamp *et al.* 2003; Peck *et al.* 2008; Angel *et al.* 2009; Brooke *et al.* 2010), and changes in prey availability and/or accessibility as a consequence of fisheries (e.g. Schaefer 1970; Vader *et al.* 1990a, 1990b; Furness *et al.* 1992; Garthe *et al.* 1996; Frank *et al.* 2005), and/or changing oceanographic conditions (e.g. Croxall *et al.* 1999; Oro *et al.* 2004). More recently, Lewison *et al.* (2012) identified six research themes that require additional attention and which are of pivotal importance to bettering conservation and management practices for seabird populations. These include aspects of (1) seabird population dynamics, (2) at-sea spatial ecology, (3) trophic dynamics, (4) community roles of seabirds, (5) impacts of fisheries, (6) population response to environmental variability, and (7) managing anthropogenic impacts on their populations. Understanding seabird at-sea behaviour and their functional role in marine food webs is of paramount importance to the effective conservation and management of their populations since this is often when they are most vulnerable (Furness & Camphuysen 1997). Such a task is not easy and requires sound knowledge of basic biological and ecological factors, such as dietary habits, temporal variability in foraging grounds and at-sea behaviour, and interactions with abiotic and biotic components of their environments. Generating such knowledge could also improve our ability to predict the long-term outcomes of global climate change on ecosystem structure and function (Furness & Camphuysen 1997), and alert us to impeding changes by effectively using these birds as sentinel organisms.

Albatrosses, petrels, and shearwaters (collectively included into the Procellariiformes) show extreme life-history characteristics that render them highly sensitive to slight changes in adult mortality, whilst variations in recruitment have longer-term effects on their

populations (Montevecchi 2002; Weimerskirch 2002; Finkelstein *et al.* 2010; Croxall *et al.* 2012). These birds face perturbations on land, but the current principal threats to their populations, which relate to commercial fishing practices, climate change, and pollution (Laist 1997; Montevecchi 2002; Anderson *et al.* 2009, 2011; Lavers *et al.* 2014; Lavers & Bond in press), are at sea. Indeed, they are the most oceanic/pelagic of all marine birds, spending months and sometimes even years away from land, and will often cross multiple management jurisdictions (Inchausti & Weimerskirch 2002; Weimerskirch *et al.* 2006; Gutowsky *et al.* 2014). Their unsurpassed level of sensitivity and the degree to which they have remained unprotected in their marine habitats (Game *et al.* 2009) have together rendered the Procellariiformes the most threatened bird Order of all; 19 of the 22 albatrosses and more than half of the 125 petrel species are now recognised as being threatened with extinction by the International Union for the Conservation of Nature (IUCN; Bugoni *et al.* 2008; Bull 2009; Anderson *et al.* 2011; Croxall *et al.* 2012; ACAP 2014). These birds have, however, adopted three unique strategies that allow them to overcome the constraints of central place foraging and spatio-temporal variability in prey availability and/or accessibility (Baduini & Hyrenbach 2003). Firstly, they are known to over feed their young during development to the point that the chicks may far outweigh even the adults (Hamer *et al.* 2002; Baduini & Hyrenbach 2003). Prior to fledging, the chicks accumulate large quantities of non-structural body fat, most of which is lost just before or soon after fledging (Hamer *et al.* 2002). Many hypotheses have been suggested to explain this 'nestling obesity,' although traditional explanations propose that the chicks require large fat reserves to sustain themselves over long intervals between feeds and during a critical period after fledging when they learn to forage for themselves (Perrins *et al.* 1973; Ricklefs & Schew 1994; Hamer *et al.* 2000; Baduini & Hyrenbach 2003). Another hypothesis proposes that the

fat reserves may fuel chicks during the final stages of development, thus allowing adults to commence their early post-breeding migrations (Brooke 1990). Secondly, most species have developed a mechanism through which they alter the chemical composition of the prey between foraging grounds and the nest so that chicks are fed (in part) with energy-rich stomach oil (Hamer *et al.* 2002; Baduini & Hyrenbach 2003). This oil is known to have a caloric content up to 30 times greater than that of the whole prey per unit of weight (Warham *et al.* 1976; Warham 1977; Roby *et al.* 1997), which supposedly allows adults to carry enough energy back to the chicks after prolonged periods at-sea (Ricklefs *et al.* 1985; Roby 1991; Roby *et al.* 1997; Baduini & Hyrenbach 2003). Thirdly, a number of species have adopted bimodal foraging patterns, whereby adults alternate long (usually in distant locations) and short (closer to colonies) trips to sustain themselves and their young, respectively (e.g. Weimerskirch *et al.* 1993, 1994a; Chaurand & Weimerskirch 1994; Baduini & Hyrenbach 2003; Congdon *et al.* 2005; Pinet *et al.* 2012). Dual foraging trips appear to have a high degree of plasticity within or across allopatric populations of the same species (Baduini & Hyrenbach 2003). Some species/populations are known to switch between single long and short trips whereas others undertake several short trips before each single long trip (Weimerskirch *et al.* 1994a; Baduini & Hyrenbach 2003). This appears to be dependent on oceanographic conditions as well as on the temporal variability in energy demands of the adults and their chicks (Baduini & Hyrenbach 2003). These strategies have become greatly pronounced in tropical Procellariiformes, especially the smaller (< 800 g) petrels, together allowing sustainable breeding while adults maintain balanced energy budgets under conditions of extremely sparse, patchy, and temporarily variable resources (Baduini & Hyrenbach 2003; Pinet *et al.* 2012). Tropical Procellariiformes are also mostly endemic to one or a few isolated islands (Chown *et al.* 1998; Worm *et al.* 2009), implying that they have

low resilience to conditions that are becoming increasingly more susceptible to dramatic shifts over short periods of time. These are among the least known and most threatened of all seabirds (particularly the tropical gadfly petrels of the genera *Pterodroma* and *Pseudobulweria*), and immediate conservation and/or management interventions are necessary to prevent further population declines.

Effective conservation of threatened or near threatened seabird species, such as these tropical Procellariiformes, requires some indication of their main management priorities. Salafsky *et al.* (2008; following Birdlife International) broadly classified these as follow: the need for on-going monitoring, the control/eradication of invasive species, studies to understand genetic diversity within and among colonies as well as the nature of gene flow between colonies, breeding colony discovery and increased or enhanced site/area protection, improved legislation/regulation/best-practice standards, education to promote awareness, harvest management, reintroductions to promote species recovery, and assessments of the potential impacts of global warming on the bird populations. The relative importance of these varies among species, as does the ease of implementing these generic recommendations for conservation action (Lewison *et al.* 2012). It is clear though, for most species at least, that the first step should be commencing and sustaining multi-disciplinary research initiatives from which species and colony specific management plans can then be derived. Indeed, using basic demographic parameters as a proxy, Lewison *et al.* (2012) highlighted that the percentage of species for which adequate information for conservation action exists is very low; the age of first breeding is known for 60 % of all species, but estimates of adult survival exist for only 20 % of all seabirds, 5 % of species have a known recruitment curve, and fewer than 2 % of all marine birds have estimates of immature survival. These, and other knowledge gaps, must be addressed before we can understand

seabird response to episodic environmental unpredictability and the capacity of seabird populations for recovery thereafter (Lewison *et al.* 2012).

1.3. Réunion Island's endemic Petrels

The islands of the south-western Indian Ocean host a surprising diversity of gadfly petrels (Pinet *et al.* 2009; Brown *et al.* 2011; Shirihai *et al.* 2014). Réunion Island, in particular, is the only island in the world that supports two endemic extant gadfly petrels. These birds, the Mascarene (*Pseudobulweria aterrima*) and the Barau's Petrels (*Pterodroma baraui*), both have unfavourable conservation statuses (Pinet *et al.* 2009; Shirihai *et al.* 2014). Yet, despite their vulnerability, virtually nothing was known about both species until very recently. The Mascarene Petrel remains little known since its population is drastically low (the most recent estimates suggest a breeding population of about 100 individuals; M. Le Corre. unpub. database) and because its active breeding colonies have not yet been discovered (Shirihai *et al.* 2014). In contrast, although a few knowledge gaps still exist, more information is available for the Barau's Petrel.

1.3.1. Description

The Barau's Petrel, known locally on Réunion Island as Pétrel de Barau or Taille Vent, is a small (Table 1.1) monomorphic gadfly petrel with an overall grey-black-and-white plumage with a vague bluish tinge (Sinclair & Langrand 2003; Hockey *et al.* 2005; Sinclair *et al.* 2011; Birdlife International 2013; Safford 2013; Figure 1.1). The pale underparts contrast with the grey upperparts, blackish-brown cap, and black face patch extending to just in front of and below the eye (Sinclair & Langrand 2003; Hockey *et al.* 2005; Sinclair *et al.* 2011; Birdlife International 2013; Safford 2013). The upperwing is dark grey with paler inner lesser and

median coverts, which together form a dark 'M' across the back and wings, and the underwing is mostly white but is bisected by strongly defined black carpal lines that extend towards the axillaries across the medium wing coverts (Sinclair & Langrand 2003; Hockey *et al.* 2005; Sinclair *et al.* 2011; Birdlife International 2013; Safford 2013). The bill is short and black, as are the legs, and the feet are pale pink (Sinclair & Langrand 2003; Hockey *et al.* 2005; Sinclair *et al.* 2011; Birdlife International 2013; Safford 2013). Chicks are covered in thick grey-brown down and fledglings resemble the adults although the adult plumage tends to be browner (Sinclair & Langrand 2003; Safford 2013).

Figure 1.1: Barau's Petrel (*Pterodroma baraui*) is a small monomorphic petrel with an overall grey-black-and-white plumage (photo credit: yabalex; www.oiseaux.net).

Table 1.1: Morphometric data (mean ± SD) for Barau's Petrel (*Pterodroma baraui*) adults and fledglings (March-May). Adapted from Laboratoire d'Ecologie Marine (ECOMAR), Université de La Réunion, ringing database.

Measurement	Adults (n = 553)	Fledglings (n = 5 812)
Wing chord (mm)	293.4 ± 13.4	286.0 ± 13.1
Tarsus length (mm)	38.5 ± 1.4	38.8 ± 1.3
Culmen length (mm)	33.1 ± 1.4	32.5 ± 1.2
Bill depth* (mm)	12.3 ± 0.8	11.2 ± 0.6
Maxillary unguis length (mm)	19.5 ± 1.2	18.3 ± 1.0
Body mass (g)	418.0 ± 55.9	397.8 ± 81.8

* measurement taken at maximum gonydeal expansion.

1.3.2. History and taxonomy

Written observations of Taille Vent are known from Réunion Island from early as 1804 (Jouanin & Gill 1987). These references supposedly described the Barau's Petrel and the distinctive 'arcing' flight action adopted by the *Pterodroma* petrels, as well as the sound produced by the birds wings in flight; loosely translated 'Taille Vent' refers to the 'air cutter' or 'wind cutter.' Despite the early 'knowledge' of this species, it was only formally described to science in 1963 after the collection of specimens on one of Réunion's northern beaches (Jouanin 1963). It was initially named *Bulweria baraui,* after the ornithologist Armand Barau, but was later reclassified into the genus *Pterodroma* based on morphological and plumage characteristics (Jouanin 1963, 1964). An analysis of vocal behaviour related this species to the Mottled (*P. inexpectata*), and Galapagos Petrels (*P. phaeopygia*; Bretagnolle & Attié 1991), although, using plumage characteristics, Barau's Petrel was allied with the Trinidade (*P. arminjoniana*), Juan Fernandez (*P. externa*), Kermadec (*P. neglecta*), Galapagos, Murphy's (*P. ultima*), and Black-capped Petrels (*P. hasitata;* Brooke 1978; Bretagnolle & Attié 1991). Recent genetic analyses have confirmed this species' reclassification into the genus

Pterodroma but suggested that Barau's Petrel is most closely related to the Black-winged Petrel (*P. nigripennis*) from the Pacific Ocean (Bretagnolle *et al.* 1998). Most other Indian Ocean petrels have also been linked to species with wider distributions in the Pacific Ocean (Brown *et al.* 2011).

1.3.3. At-sea distribution and habitat

The exact at-sea distribution of Barau's Petrel was virtually unknown until 2011. The, apparently misleading (Pinet *et al.* 2009, 2011b, 2012), knowledge that existed prior to that time was based entirely on ship-based observations of the birds from areas all around the Indian Ocean (Chapman & Cheshire 1987; Dunlop *et al.* 1988; van Marle & Voous 1988; Bourne 1989; Stahl & Bartle 1991; Van Den Berg *et al.* 1991; Lambert 2001; reviewed in Pinet *et al.* 2009). More recently, Pinet *et al.* (2011b, 2012) attached biologgers onto adult birds and, in doing so, identified this species' principal foraging grounds during various stages of the breeding season and over the interbreeding period.

Pinet *et al.* (2012) attached Argos global positioning system platform transmitter terminals (GPS-PTTs) onto 17 petrels (nine males, eight females) for two consecutive years and subsequently described the foraging behaviour and habitat use of Barau's Petrel during the austral summer (September-April). Their observations have built on fairly accurate pre-existing knowledge of this species' at-sea distribution during the breeding season (Stahl & Bartle 1991). The birds arrived back at the colonies on the 10 September ± 7 days (usually timed with the full moon; Pinet *et al.* 2011a), where they stayed for 11 ± 6 days. They then departed on an extended pre-laying exodus (46 ± 6 days) during which the birds travelled some 3 000 km to a large area in the south-western Indian Ocean, between 21°E and 60°E and from 19°S to 40°S, where they presumably gathered the resources required for

reproduction (Mallory *et al.* 2008; Pinet *et al.* 2011b). Interestingly, Pinet *et al.* (2012) reported almost complete segregation between the sexes at this time (2.2 % overlap in foraging distribution) – males tended to forage off the Mozambique Plateau whereas females remained in the Madagascar Basin and along the Madagascar Plateau (Pinet *et al.* 2012). Later, during the incubation period, the foraging overlap between the sexes increased to between 40 and 60 % at which time the birds consistently used the area between southern Madagascar and the southeast African shelf (Bourne 1989; Pinet *et al.* 2012; Safford 2013; Figure 1.2). The spatial overlap in core foraging distribution of males and females further increased (68 and 91 %) towards the chick-rearing period, when both sexes foraged in an area 2° east of the area used during incubation. At this time, trips by males were significantly longer than those of females and both sexes alternated long (10 ± 5 days) and short (< 3 days) foraging trips (Pinet *et al.* 2012; Figure 1.2). The adults travelled southeast towards Madagascar, flying up to 1 300km away from the colony, during long trips but remained in deep water around Réunion Island on short trips (Pinet *et al.* 2012). The main foraging areas during the early breeding season were characterised by low mean depths, high wind speeds, and high productivity (Pinet *et al.* 2012; Safford 2013). During the chick-rearing period, the birds tended to forage over waters of lower productivity closer to Réunion Island (Pinet *et al.* 2012). Together these results suggest that Barau's Petrels target two specific subtropical regions during the breeding season, including an area between the Agulhas Current and the South East Madagascar Current, as well as an area over the South Indian Ocean Counter Current (Pinet *et al.* 2012). The first is characterised by strong mesoscale activity close to the origin of the Agulhas Current (Donguy & Piton 1991; De Ruijter *et al.* 2002, 2005; Lutjeharms 2006), whilst the second is associated with a seasonal

and persistent phytoplankton bloom in the south-central Indian Ocean (De Ruijter *et al.* 2004; Srokosz *et al.* 2004; Penven *et al.* 2006; Siedler *et al.* 2006; Lutjeharms 2007).

Ship-based observations of the birds during the austral winter have suggested a collective post-breeding migration to the productive Arabian Sea (Van Den Berg *et al.* 1991), whilst other sight records exist from areas immediately offshore of South Africa, Namibia, Indonesia, Australia, Sri Lanka, and India (Dunlop *et al.* 1988; Stahl & Bartle 1991; Lambert 2001; Pinet *et al.* 2009). These contrast with the recent global location sensor (GLS) tracking study on this species, which ran over two consecutive years. Indeed, whilst Pinet *et al.* (2011b) noted some individual variation in migratory routes of 23 petrels (12 males, 11 females), all consistently used the same area in the central Indian Ocean (around 10°S and focussed on the Ninety East Ridge) and no birds were recorded flying north of 10°N (Figure 1.2). Their study was limited to adult birds, however, and the post-fledging dispersal of young birds has yet to be successfully addressed (M. Le Corre. pers. comm.). South of 10°S, the persistent southeast trade winds reach their seasonal maximum and most northerly extent during the southern winter, promoting vertical mixing of the water column and, thus, primary production (Schott *et al.* 2002, 2009; Talley *et al.* 2011). Lévy *et al.* (2007) conducted a regional analysis of phytoplankton blooms and showed a well-marked seasonal bloom occurring in the central Indian Ocean, associated with these winter wind systems. It appears that the non-breeding adult Barau's Petrels targeted these oligotrophic (Chlα < 0.1 mg/m^{-3}) and mesotrophic (Chlα > 0.1 but ≤ 0.3 mg/m^{-3}) waters with characteristic strong wind systems (Longhurst & Pauly 1987; Kahru & Mitchell 2000; Safford 2013). The region remains little known and may also be an important breeding ground for pelagic cephalopods (M. Le Corre. pers. comm.).

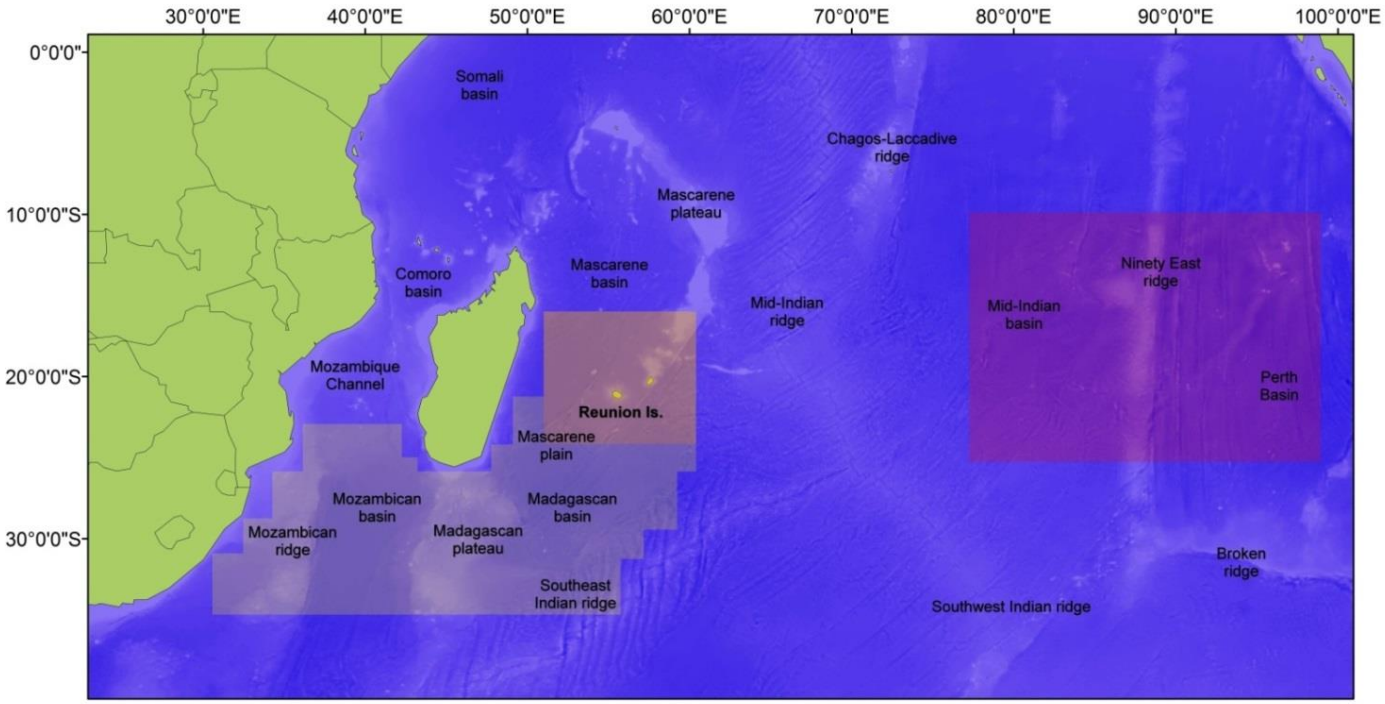

Figure 1.2: Approximate breeding (orange; September - April) and non-breeding (purple; May-October) distribution of Barau's Petrel (*Pterodroma baraui*) in relation to the main bathymetrical features of the Indian Ocean. Breeding distribution indicates approximate distribution of adults during long (pale yellow) and short (orange) trips. Species' distribution adapted from Pinet *et al.* (2011b, 2012) and bathymetry grid from National Oceanic and Atmospheric Administration (NOAA; http://www.ngdc.noaa.gov/mgg/bathymetry/relief.html).

1.3.4. Reproductive behaviour

Little is known about the reproductive behaviour of Barau's Petrel but, based on what information is currently available, this species seems to be similar to other high-altitude nesting petrels, most notably the Hawaiian Petrel (*P. sandwichensis;* Simons 1984, 1985). The Barau's Petrel is unusual in that its nesting habitats are amongst the highest (up to

2 850 m above-sea-level (m a.s.l.); Figure 1.3; pers. obs.) of all seabirds and that, unlike most other small Procellariiformes*,* they tend to arrive at colonies by day (Bretagnolle & Attié 1991; Probst 1995; Safford 2013). Brooke (1978) suggested that this day-time arrival may be related to the use of warm air thermals for flying inland.

The first Barau's Petrel nest was found on Rodrigues Island in 1974 (Cheke 1987). Birds prospecting at the Valley of Black River Nature Reserve on Mauritius have also led biologists to believe that breeding may occur there – no nests have been found, however (Stahl & Bartle 1991; Pinet *et al.* 2009). In addition, bones found on Amsterdam Island suggest that this species may have once bred there (Worthy & Jouventin 1999). Today breeding occurs only on Réunion Island (Pinet *et al.* 2009; Figure 1.3). Réunion is incredibly rugged and many parts of the island (particularly the central regions) are completely inaccessible. It is of no surprise then that the locations of the active breeding colonies were unknown until very recently. The first active colony on Réunion was discovered in 1995, at about 2 400 m a.s.l., on a ridge that joins Grand Bénare to Piton des Neiges (Probst 1995; Probst *et al.* 1995, 2000). In 2001, a second much larger colony, hosting some 900 breeding pairs, was discovered on Piton des Neiges (Pinet *et al.* 2009). To date, this colony remains the largest of all. Several smaller colonies have also been discovered in the central parts of Réunion Island, mainly on the higher slopes of Cirque de Cilaos, Cirque de Mafate, and Cirque de Salazie (Le Corre & Safford 2001). Most of these are inaccessible for study purposes, however, and it was only in 2006 that a relatively large (50 - 100 nests) and reasonably accessible colony was discovered at La Vallée des Deux Miches on the higher slopes of Grand Bénare (2 700 m a.s.l.). This has since facilitated more intensive research into the biology and ecology of the Barau's Petrel.

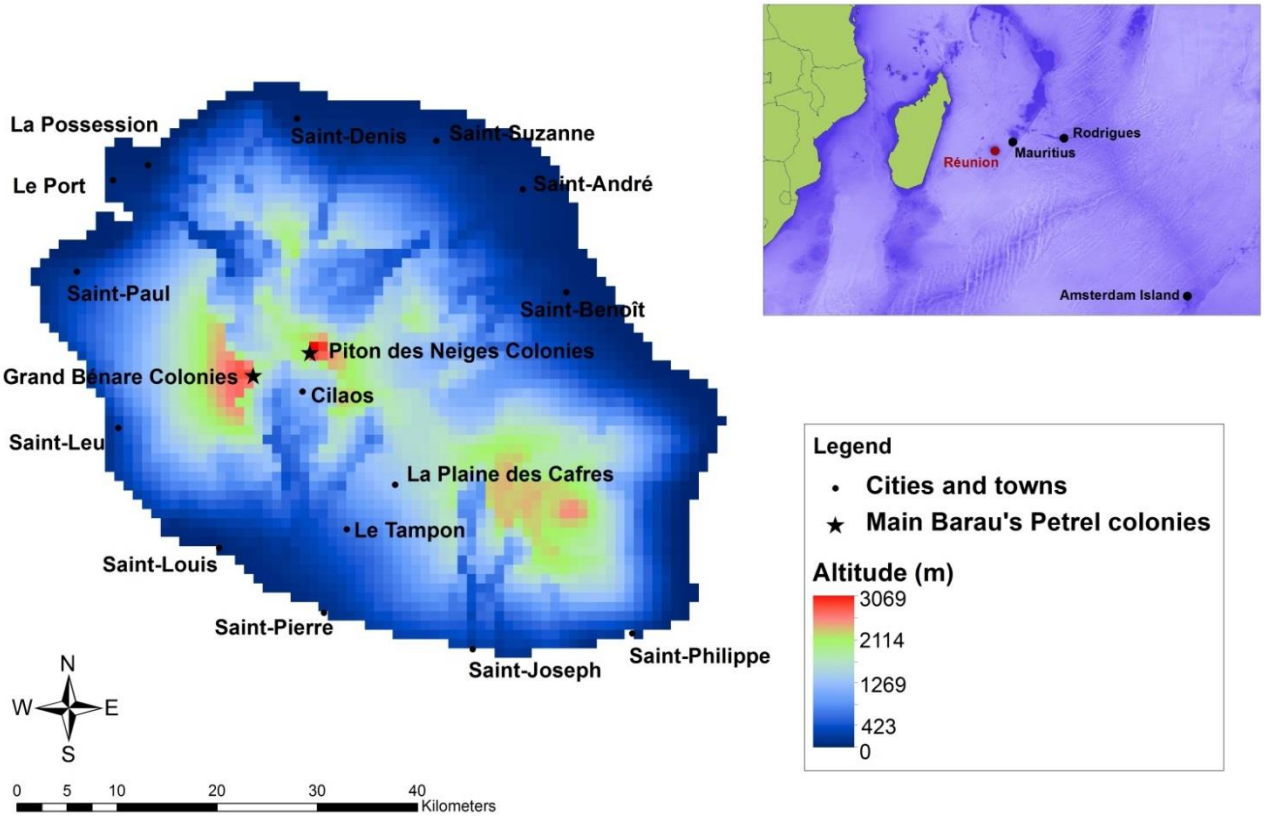

Figure 1.3: Barau's Petrel (*Pterodroma baraui*) is an endemic gadfly petrel of Réunion Island in the south-western Indian Ocean. All active breeding colonies are located in mountain forest and shrublands on the higher (suitable habitat between 2 400 - 3 069 m a.s.l.; red on map) slopes of Grand Bénare and Piton des Neiges (Strasberg *et al.* 2005). The first nest was found on Rodrigues Island, however, and breeding has been suspected for both Mauritius and Amsterdam Islands. Relief grid downloaded from Diva-GIS (http://www.diva-gis.org/gdata).

The breeding colonies of Barau's Petrel are located in mountain forest and sub-alpine shrublands where the dominant vegetation includes *Erica reunionensis, Hypericum lanceolatum, Stoebe passerinoides, Sophora denudate,* and *Phillippa montana* (Bretagnolle

& Attié 1991; Strasberg *et al.* 2005; Figure 1.4). Like most other *Pterodroma* petrels, Barau's Petrel nest in burrows in natural crevices or under rocks, favouring areas of cinder soils (Bretagnolle & Attié 1991; Safford 2013). Burrows are approximately 98 cm long, 19 cm wide, and 11 cm high and are found at a density of between 0.03 /m^2 and 0.35 /m^2 on ridges and plateaus, respectively (Probst *et al.* 1995, 2000). Factors that influence nest selection are thought to include vegetation cover, soil type, slope, and altitude (Probst *et al.* 2000).

Barau's Petrels reproduce annually, arriving back at colonies from the wintering grounds during mid-September. In November, after an extended pre-laying exodus, the female lays a single large white egg before departing to forage once more (Pinet *et al.* 2012). This egg is among one the largest and most nutrient rich of all avian eggs, relative to female body size (Warham 1990; Mallory *et al.* 2008), and it is believed that the females' second exodus allows them to restore the body condition lost during egg production as the males invariably take the first incubation shift (Pinet *et al.* 2012). This initial incubation shift is always the longest (± 16.9 days) and the higher foraging effort of male birds, during the pre-laying exodus, may be necessary to ensure sufficient body condition for this long period of fasting (Pinet *et al.* 2012). Indeed, if the males' body condition is too poor for them to endure the first shift or if the females' second exodus is too long, then the males may abandon the eggs. Upon return to colonies, male (≤ 535 g) Barau's Petrels are significantly heavier than females (≤ 480 g) providing additional support for this hypothesis (Pinet *et al.* 2012). Although the exact timing is unknown, Pinet *et al.* (2009) calculated that the incubation period is approximately 45 days long and the chick-rearing period lasts about three months with peak fledging occurring in April. This timing is in accordance with other similar sized and related *Pterodroma* petrels (refer to Appendix 2 in Schreiber & Burger

2002a), and is supported by the sightings of newly fledged birds in early- to mid- April (Stahl & Bartle 1991).

Figure 1.4: Barau's Petrel colonies are located in pockets of high altitude (suitable habitat between 2 400 - 3 069 m a.s.l.) cloud forest, dominated by *Erica reunionensis, Hypericum lanceolatum, Sophora denudate,* and *Phillippa montana* (Strasberg *et al.* 2005). Insert shows a typical burrow, usually located in cinder soils under rocks and in natural crevices, containing the single large egg (Photo credit: Daniel Keith Danckwerts).

1.3.5. Relationship with man and conservation

It is difficult to assess the population trajectory of Barau's Petrel in the absence of population estimates from prior to 1990. In 1991 the population was estimated, using ship-based surveys, at between 7 500 and 10 000 pairs (Stahl & Bartle 1991). Thereafter, annual population counts (beginning in 1996) have increased our understanding of the population dynamics and trajectory of Barau's Petrel. In 2000, the estimates suggested a population of

about 3 800 breeding pairs (Probst *et al.* 2000), but this has risen to between 4 000 and 6 000 pairs since the discovery of new colonies in 2001 (Le Corre & Safford 2001; Birdlife International 2013). These data are rather difficult to interpret, because of methodological inconsistencies, but indicate a decline in the breeding population of Barau's Petrel between 1991 and 2001, presumably as a result of an array of anthropogenic pressures. Key threats include the predation of birds and their eggs at colonies by invasive mammals (cats, rats, and mice), disturbance at and around colonies, light-induced mortality, increasing tourism activity, and direct exploitation (although illegal; Le Corre *et al.* 2002; Le Corre 2008; Falquier *et al.* 2009; Pinet *et al.* 2009; Dumont *et al.* 2010). The most recent estimates remain at between 4 000 and 6 000 breeding pairs suggesting that the population is now stable (Pinet *et al.* 2009; Birdlife International 2013). In spite of this, Barau's Petrel is considered endangered by the IUCN on the grounds of its small and fragmented (< 6 known locations) breeding range, a projected decrease in its extent of occurrence, area of occupancy, quality of habitat, number of breeding locations, and the number of mature individuals (Birdlife International 2000; Birdlife International 2013).

1.3.6. Trophic ecology

Although huge advances have been made in our knowledge of Barau's Petrel, almost nothing is known about its trophic ecology. This is one of the few aspects of its biology that has never been formally addressed and the data that are currently available are strongly contradictory (Pinet *et al.* 2009). Stahl and Bartle (1991) reported that the diet of Barau's Petrel consisted almost entirely of fish, whereas Kojadinovic *et al.* (2007) identified mainly cephalopod prey (98 %; dominated by *Stenoteuthis oualaniensis* and *Toanius* sp.) and fish were only infrequently recorded (2 %). Kojadinovic *et al.* (2008) also noted differences in ^{13}C

and ^{15}N values in the liver between fledglings and adults, which suggested ontogenetic differences in diet, but they failed to discuss this result in any great depth.

1.4. Motivation, objectives, and hypotheses

Population modelling has indicated that the current known threats may drive Barau's Petrel to extinction (Le Corre 2008; Russell & Le Corre 2009; Dumont *et al.* 2010). A conservation action plan was structured and has been implemented (as of 2008) as a means of maintaining the stability of this species' population (Salamolard 2007). One of the issues identified in this conservation plan was the uncertainty regarding the marine component of the Barau's Petrels' ecology (including the species' trophic ecology), which prevents assessment and control for the threats that the species likely faces whilst at sea. Thus, following the recommendations made in Salamolard (2007), which were reiterated by Pinet *et al.* (2009, 2012), the principal objectives of this research are to use a combination of stomach content, stable isotope, and qualitative fatty acid analyses to (1) describe the diet of adult, downy chick, and fledgling Barau's Petrel during various stages of the breeding season and (2) describe the trophic ecology of adults during the non-breeding season. In doing so, the principal resources on which this species relies will be identified and it is hoped that this information may shed light into their foraging behaviour. Furthermore, it is hoped that any additional threats that the birds' might face whilst at sea will be identified. This research closely follows the work done by Pinet *et al.* (2011a, 2011b, 2012) on the biology and foraging ecology of the Barau's Petrel and will also address the foraging habitats used by this species during the breeding and non-breeding seasons. Based on these cumulative research efforts, the conservation action plan for Barau's Petrel may then be restructured so as to facilitate population recovery. In addition, the present research will provide baseline

data such that any additional information on this species' diet may provide insight into the potential outcomes of climate change on the globally important south-western and south-central Indian Ocean.

The use of techniques that clarify the diet over different time periods (stomach content, stable isotope, and qualitative fatty acid analyses) and of tissues with different turnover times (blood and feathers) allows the testing of various predictions. Firstly, adult Barau's Petrel employ a dual foraging strategy during the final stages of the breeding season so that the diets of adults are expected to differ from those of the downy chicks and fledglings. This might not imply that the prey species assemblage differs among the ontogenetic stages, however, as the adults could feed their young on size-specific (i.e. greater proportion of juvenile animals) prey. Adults also begin foraging closer to Réunion, as the energy demands of their offspring increases, so that differences in diet are expected between downy chicks and fledglings through the course of the chick-rearing period. Spatio-temporal differences in isotope baseline and fatty acid composition of prey should also be reflected in the signatures of the birds and have the potential to confirm the complex patterns of habitat use by adult Barau's Petrel. Indeed, adult birds show different at-sea distributions at different stages of the breeding season (Pinet *et al.* 2012), and the trophic pathways leading to the birds are expected to differ between males and females when they are segregated in their foraging areas. Differences are expected during the pre-laying exodus when males forage offshore of South Africa whilst females remain in less productive waters closer to Madagascar. The same habitats are then used by both sexes during the incubation and chick-rearing periods so that no differences are expected between the sexes during these times. The similarity in habitats used by females during the pre-laying exodus and by both sexes during the incubation periods would suggest no differences in chemical

signatures among these birds. Differences are expected between the earlier breeding stages (i.e. pre-laying exodus and incubation) and the chick-rearing period, however. In this final stage, adults forage closer to Réunion than during any other stage of the breeding season and their trophic signatures should reflect this. Adult birds also occupy extremely different habitats during the non-breeding season so that variations in diet and trophic position are anticipated. Finally, ontogenetic, sexual, and seasonal physiological differences among birds should also be reflected in the stable isotope and fatty acid results, although it is difficult to predict how so. Insufficient information exists about the regions in question to determine the precise areas and/or resources that the birds are using with these results, however, and differences will be interpreted using the available tracking information.

CHAPTER 2: STUDY SITE AND GENERAL METHODOLOGY

2.1. Study site

Réunion (21° 6’ S; 57° 29’ E), together with Rodrigues (19° 43’S; 63° 25’ E), and Mauritius (20° 17’ S; 57° 32’ E), form the Mascarene Archipelago in the south-western Indian Ocean (Figure 2.1). These islands are collectively included into the ‘Madagascar and associated islands’ hotspot of marine and terrestrial biodiversity (Meyers *et al.* 2000; Safford & Hawkins 2013). However, despite their close proximity, the three islands have had fairly distinct geological, biological, and human histories (Olson 2008; Hume 2013; Safford & Hawkins 2013).

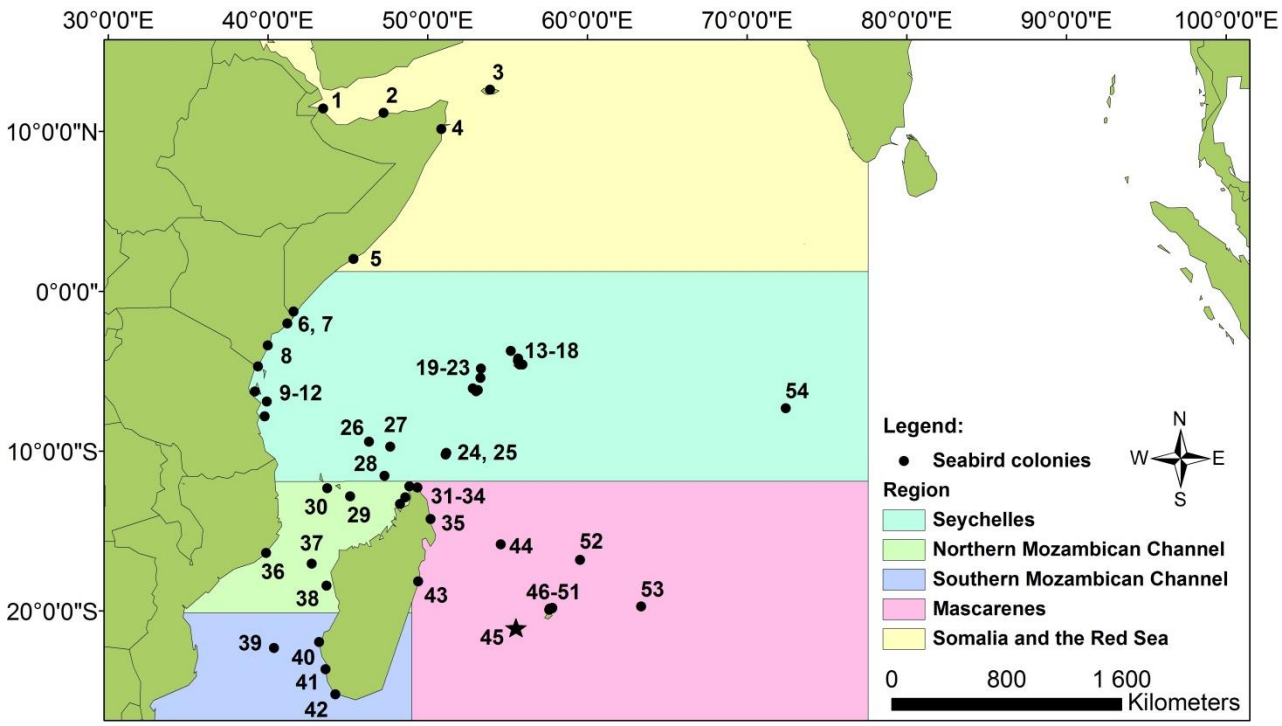

Figure 2.1: Location of Réunion Island (colony 45 – represented as black star) relative to all other seabird breeding colonies in the western Indian Ocean as adapted from Danckwerts *et al.* (in press). Colonies are listed as follows: (1) Jasiira Ceebaad and Jasiira Sacada Din, (2)

Jasiira Maydh, (3) Socotra Group, (4) Raas Xaafun and Raas Gumbax, (5) Mogadisho Islets, (6) Bajan islands, (7) Kiunga Marine Nature Reserve, (8) Whale Island, (9) Kisite Island, (10) Zanzibar Chumbe Island, (11) Latham, (12) Mafia Island, (13) Aride, (14) Bird Island, (15) Cousin, (16) Cousine, (17) Frégate, (18) Récif, (19) African Banks, (20) Boudeuse, (21) D'Arros, (22) Desnoeufs, (23) Marie-Louise, (24) Farquhar, (25) Goelettes, (26) Aldabra, (27) Cosmoledo, (28) Glorieuses, (29) Mayotte, (30) Mohéli, (31) Cape Anorontany Archipelago, (32) east coast of Ansiranana, (33) Mitsio Archipelago, (34) Nosy Bé and other islets, (35) Etoile, (36) Puga-Puga, (37) Juan de Nova, (38) Nosy Barren, (39) Europa, (40) Islets off Morombé, (41) Nosy Vé, (42) Nosy Manitra and other islets, (43) Ilôts off Toamasina, (44) Tromelin, (45) Réunion, (46) Flat Island, (47) Gabriel Island, (48) Gunner's Quoin, (49) Pigeon Rock, (50) Round Island, (51) Serpent Island, (52) Saint-Brandon, (53) Rodrigues Islets, and (54) Chagos Archipelago.

2.1.1. Geography and topography

Réunion is a young volcanic island (2.8 Ma; McDougall 1971) situated 600 km to the east of Madagascar with an area of 2 512 km^2. The central part of the island consists of a volcanic massif comprising two main shield volcanoes, one of which is still active (Duncan & Hargraves 1990). The largest volcano is inactive and is centred on Piton des Neiges, the highest peak (3 069 m a.s.l.) on Réunion and across the western Indian Ocean (Rivals & Hugot 1989; Le Corre & Safford 2001; Hume 2013; Safford & Hawkins 2013). This massif has been eroded into three main valleys or cirques (named Cilaos, Mafate, and Salazie) resembling large amphitheatres, each approximately 10 km across (Rivals & Hugot 1989). The younger and smaller volcano in the south of the Island, named Piton de la Fournaise

(2 631 m a.s.l.), remains active and has retained its dome-like shape with a central crater (Rivals & Hugot 1989).

2.1.2. Flora

Réunion is dominated by a diverse array of dry and wet scrublands and forest (Cadet 1977; Strasberg *et al.* 2005). Five hundred and forty six flowering plant species have been described from the island, of which 30 % are endemic (Le Corre & Safford 2001). An additional 592 species have been introduced and have become naturalized (Le Corre & Safford 2001). Since its colonisation in the 1600s, there has been an estimated loss of 75 % of the native vegetation and 18 % of the native flora has now been listed as threatened by the IUCN, with at least six species having gone extinct (Le Corre & Safford 2001; Strasberg *et al.* 2005).

In terms of natural vegetation, the highest volcanic regions in the central part of Réunion are dominated by bare rock, montane shrubland, bushland, and thicket. Intermediate altitudes are dominated by moist evergreen forest and the lowlands tend to support dry evergreen forest (Cadet 1977; Strasberg *et al.* 2005).

2.1.3. Fauna

Together, the Mascarene Islands once supported one of the richest vertebrate faunas of any oceanic archipelago (Le Corre & Safford 2001). Today, however, many of these species have been exterminated as a result of human activities (Le Corre & Safford 2001; Olson 2008; Hume 2013). Indeed, it is estimated that 50 % of all vertebrates on Réunion have been driven to extinction, including more than 55 % of all bird species (Mourer-Chauviré *et al.* 1996). Over 140 vertebrate species are found on the island today including

about 91 birds, 32 mammals, and 23 reptiles. Some of these are endemic to Réunion and the Mascarenes, although the vast majority have been accidentally or deliberately introduced from elsewhere (Le Corre & Safford 2001; Safford & Hawkins 2013). These totals are preliminary, as there is ongoing taxonomic debate about a number of species and historical, paleontological, and osteological studies have revealed a number of extinct endemic fauna (Mourer-Chauviré *et al.* 1996; Le Corre & Safford 2001; Nicholls 2006; Hume 2013).

In recent years, several action plans have been implemented to conserve the remaining biodiversity and natural habitats of the island (Lagabrielle *et al.* 2011). In 2007, a marine reserve was created to preserve the coral reefs and other important coastal habitats of the west coast and a national park was created to protect most of the intact terrestrial habitats in the central parts of the island. In spite of this, most endemic species remain globally endangered as a consequence of global warming, pollution, and other large-scale human-induced perturbations (Jay *et al.* 2006; Sax & Gaines 2008). In addition, little is known about the biology and the ecology of most endemic species, which seriously impedes conservation efforts.

2.2. General methodology

2.2.1. Pre-sample collection preparation

Samples were collected from live and dead birds, although no birds were killed for the purposes of this study. A distinction was been made between three age classes of birds. Adults were sampled, but always from active nest sites thus ensuring that only breeding birds were included into this study. This controlled for differences in foraging behaviour between these individuals and prospecting adults (Pinet *et al.* 2012). Downy chicks were separated from fledglings in an attempt to understand the ways in which diet might differ

between the early and late chick-rearing stages. Downy chicks were defined as birds still in the nest, covered in down, and actively fed by adults on a regular basis. Fledglings were older, had true feathers, and were sampled around the end of April just prior to fledging. These final two ontogenetic groups are, for the purposes of this thesis, collectively referred to as pre-mature birds. Breeding is known to be highly synchronous in this species (Pinet *et al.* 2009, 2011a, 2011b, 2012), but morphometric measurements of downy chicks and fledglings were used to ensure that all individuals sampled were of similar ages (data not presented). All sampling techniques were approved by the Department of Zoology and Entomology animal ethic committee at Rhodes University, South Africa (ZOOL-01-2013). Furthermore, this study adhered to the legal requirements of France and its overseas departments and ethical clearance was received from Parc National de La Réunion and La Société d'Etudes Ornithologiques de La Réunion (La SEOR) prior to commencement.

2.2.2. Sample collection from dead birds

Approximately 587 ± 301 grounded Barau's and Mascarene Petrels, and Tropical (*Puffinus inherminieri bailloni*) and Wedge-tailed Shearwaters (*Puffinus pacificus*) are collected annually from urban areas around Réunion Island (Le Corre *et al.* 2002). In excess of 70 % of these are Barau's Petrels and almost all are fledglings, which are attracted by urban lights on their first flights out to sea (Le Corre *et al.* 2002). Collection programs are currently in place whereby fallen birds are delivered to a rehabilitation centre where they are treated (if necessary) and released out to sea (Le Corre *et al.* 2002). Less than 10 % are dead upon collection and birds with severe injuries are euthanized by qualified veterinarians – these individuals are kept frozen at -20 °C for research purposes (Le Corre *et al.* 2002). Dead birds from the years 2008, 2009, 2011, 2012, and 2013 were provided by La SEOR and

were dissected. Whole stomachs were removed and frozen in labelled 18x15 cm Ziplock™ bags until further analysis. Morphometric measurements and plumage characteristics were used to separate fledglings from adults and sex organs were used to determine gender in the adults. Furthermore, an index, based on the transverse profile of the pectoral muscles with a fat score included (Kaiser 1993; Nyeland *et al.* 2003), was used to determine body condition for all dead birds so as to ensure that these data were not skewed in any way. Scoring was as follows: C0 = no abdominal fat and keel very obvious with thin pectoral muscles making each side of the keel appear concave, C1 = small quantities of abdominal fat and keel obvious but with more pectoral muscle tissue giving the chest a triangular shape, C2 = abdominal fat present in fairly large quantities and keel not visible since pectoral muscles are large enough to make the chest profile look slightly rounded, and C3 = abdominal fat reserves well developed and the pectoral muscles are large making the chest profile look very bulky and convex (Nyeland *et al.* 2003). Most individuals had a score greater than or equal to C2 (> 90 %), whilst only a few had a score of C1 or C0 (< 10 %). All birds with a 'poorer than normal' index score (< C2) were excluded from this study.

2.2.3. Sample collection from live birds

Six separate trips were made to sample live birds at the large colony at Vallée des Deux Miches on the Grand Bénare massif. These coincided with the arrival of adult birds from the non-breeding grounds (16-17 September 2013), the return of adults from the pre-laying exodus (06-07 and 20-22 November 2013), the egg incubation (17-18 December 2013), and early (27-28 January 2014), and late (18-19 March 2014) chick-rearing periods (Pinet *et al.* 2011b, 2012). More than eighty burrows were routinely visited on each excursion. When a bird was found, it was carefully pulled (bill first) from its burrow. Many

seabird species regurgitate instinctively when threatened (Duffy & Jackson 1986; Fair *et al.* 2010) and these were opportunistically collected throughout the breeding season. The use of a reverse stomach flush was disallowed for ethical reasons, so birds were inverted and their stomachs massaged thus ensuring complete draining of stomachs when/if regurgitation occurred. Samples were stored in 18x15 cm Ziplock™ bags and were kept on ice for the duration of each field excursion. Subsequently, the birds were ringed/banded facilitating individual identification and a suite of morphometric measurements taken. These included the weight (5 g precision) and wing chord, culmen, bill depth at the maximum gonydeal expansion, maxillary unguis, and tarsus lengths (1 mm precision). A blood sample of up to 1 % (but never exceeding 1.2 ml) of the birds body weight was then extracted from the brachial vein and stored in labelled 2 ml Eppendorf tubes on ice for the duration of the field excursion. This conservative approach, as recommended by McGuill and Rowan (1989) and Voss *et al.* (2010) provides a measure of safety for the birds as explained by Fair *et al.* (2010). Prior to this, the area around the vein was sterilized with alcohol swabs and, to prevent coagulation, the BD Micro-Fine™ 1.5 ml syringes and needles were rinsed with 0.1 ml of sodium heparin. Although some debate exists, sodium heparinization is believed to have no effect on the chemical signatures received from blood (Hobson *et al.* 1997; Kurle 2002; Lemons *et al.* 2012). Three all-white auxiliary feathers were plucked from each adult and fledgling and secondary down feathers were trimmed from downy chicks, taking extreme care not to compromise the birds' flight capabilities and/or insulation. Feathers were stored in labelled envelopes. Fledglings with extremely low (score < C2) body condition index scores (Nyeland *et al.* 2003) were not sampled.

Blood samples were separated into three aliquots immediately upon arrival at the laboratory. A small amount (± 0.1 ml) was added to 0.5 ml ethanol (70 %) and stored in a

refrigerator for genetic sexing. Approximately 0.8 ml was then transferred into 1.5 ml screw neck glass vials capped with silica lined screw-top lids and frozen at -80 °C for fatty acid analysis. The remainder was frozen at -20 °C for stable isotope analysis. Unfortunately the difficulty associated with collecting samples in the field implied that blood could not be separated in time and thus whole blood was used. Stomach contents were frozen at -20 °C and feathers were stored at room temperature until further analysis.

2.2.4. Molecular sexing

Prior to further sample processing, it was necessary to sex the live adult birds using molecular techniques as described in Griffiths *et al.* (1996). This molecular method relies on the size differences between the CHD1W and CHD1Z introns located on the W and Z sex chromosomes, respectively.

DNA was extracted from the whole blood aliquots following the QIAamp Blood and Tissue Kit (Spin-column Protocol; QIAGEN 2006). A small amount of blood was added to 180 µL of Buffer ATL. Subsequently 20 µL of proteinase K was added and the solution was mixed thoroughly using a vortex. The samples were then left to incubate overnight at 56 °C on a thermomixer. On the following day, the samples were removed and vortexed for 15 seconds before the addition of 200 µL of both Buffer AL and ethanol (> 96 % concentration). After mixing, each solution was transferred into a DNeasy Mini Spin-column in a 2 ml collection tube. All were centrifuged at 8 000 revolutions per minute (rpm) for one minute and the collection tubes discarded. The spin columns were then placed into a new 2 ml collection tube and 500 µL of Buffer AW1 added. Samples were centrifuged for another minute and the collection tube discarded once more. A new collection tube was given to each and 500 µL of Buffer AW2 added to the spin-column. All were then centrifuged at

14 000 rpm and the collection tubes discarded for a third time. Extreme care was taken when removing the spin-columns so that they never came into contact with the flow-through liquid. Finally, the spin-columns were placed into labelled 2 ml microcentrifuge tubes and 200 µL Buffer AE added. Prior to centrifuging (8 000 rpm for one minute) all were incubated at room temperature for five minutes. This final step was repeated using 100 µL Buffer AE thus ensuring maximum yield. Spin-columns were then discarded as all genetic material remained in the collection tube. Next, the DNA was amplified following O'Dwyer (2004) and Griffiths *et al.* (1998), using a classic polymerase chain reaction (PCR) procedure as follows. The genetic material (40-50 ng) was added to a PCR reaction mix containing 75 mM Tris-hydrochloride (pH 8.8), 1.5 mM magnesium chloride, 0.2 µM of primers P2 and P3, 50 µM of each dNTP, and 0.75 units of Taq DNA polymerase (Red Hot DNA polymerase, ABgene). Amplifications were performed using a GeneAmp PCR 9700 system as follows: denaturation at 94 °C for three minutes, 35 cycles at 94 °C for 30 seconds, 45 °C for 30 seconds, 72 °C for 45 seconds, and final elongation at 72 °C for four minutes. During the restriction phase, 5 µL of each PCR were then digested in five units of Hae III (pRomega) in 10x Buffer C in a total volume of 10 µL at 37 °C for three hours. The PCR products were then size-fractioned (10 µL) in a 2 % agarose TBE gel stained with GelRed^{TM} Nucleic Acid Gel stain (FluoProbes) and exposed to electrolysis for three hours. Completed gels were photographed under ultra-violet light. Hae III cuts the CHD1Z introns, but not the CHD1W, so that males (ZZ) present two fragments at 65 and 45 base pairs (bp; visualised as two fragments in agarose TBE gels) whereas the females (ZW) show fragments at 45, 65, and 110 bp (visualised as a single dark band in agarose TBE gels;Figure 2.2).

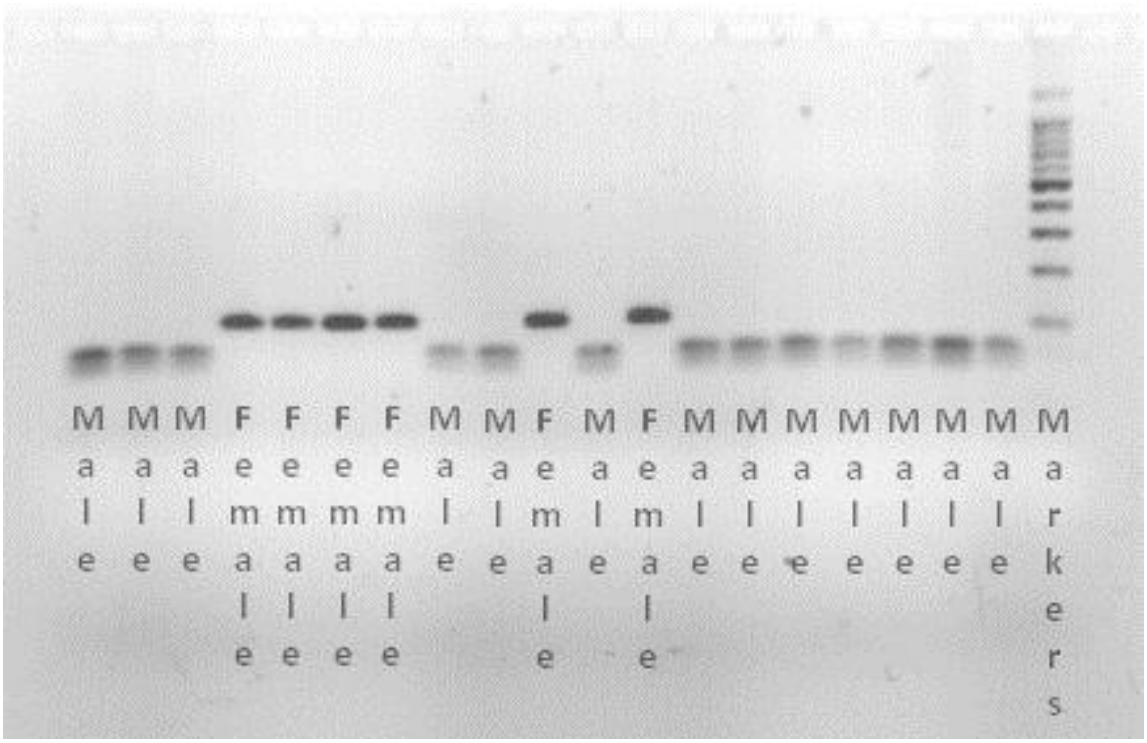

Figure 2.2: Barau's Petrel (*Pterodroma baraui*) are sexually monomorphic, implying that all adult birds needed to be sexed using molecular techniques. The restriction enzyme Hae III (pRomega) cuts the CHD1Z intron, but not CHD1W, so that males (ZZ) display two fragments at 65 and 45 base pairs (bp; seen as two separate bands) and females (ZW) show three fragments at 45, 65, and 110 bp (visualized as one dark band). Bands in the 2 % agarose TBE gel (stained with GelRed™ Nucleic Acid Gel stain, FluoProbes) were photographed under ultraviolet light.

CHAPTER 3: IDENTIFICATION OF THE KEY DIETARY RESOURCES TARGETED BY BARAU'S PETREL (*PTERODROMA BARAUI*) DURING THE BREEDING SEASON AS INFERRED FROM TRADITIONAL STOMACH CONTENT ANALYSIS.

3.1. Introduction

The nature of the resources available to marine top predators, including their quality, quantity, and distribution, influences various demographic parameters of their populations (Karnovsky *et al.* 2012). These include population size, fecundity, survival, reproductive success, and the age of first breeding (Tickell & Pinder 1975; Montevecchi *et al.* 1988; Pierotti & Annett 1990; Prince *et al.* 1994; Montevecchi & Myers 1995, 1996; Weimerskirch *et al.* 1997; Waugh *et al.* 1999a, 1999b, 1999c; Weimerskirch 2002; Baduini & Hyrenbach 2003). Not surprisingly then, diet studies form an essential and integrated component in the conservation of any species, helping to identify the principal resources (e.g. Schramm 1986; Imber 1973, 1995; Connan *et al.* 2007a; Cury *et al.* 2011; Connan *et al.* 2014) and key habitats (e.g. MacLeod *et al.* 2008a, 2008b; Louzao *et al.* 2011) on which they depend. Furthermore, effective conservations strategies imply the need to understand the ways in which resources are exploited. Key questions include how prey are located (by sight and/or using olfactory senses) and if they are hunted or scavenged (Shealer 2002). Predation has important effects on predator and prey populations, influencing their behaviour, population size, and at-sea distribution, whereas scavenging species must overcome extreme spatio-temporal variability in prey availability in order to survive and reproduce (Shealer 2002). An understanding of foraging ecology is especially important for seabirds that commute over extreme distances (> 1 000 km) between feeding areas and their breeding colonies (e.g. Pinaud & Weimerskirch 2007; Pinet *et al.* 2012), since central place foraging is synonymous

with great energetic constraints and over investment into a particular breeding episode may jeopardize future survival and reproductive output (Stearns 1992; Tavecchia *et al.* 2007; Le Bohec *et al.* 2008).

Gadfly petrels (genus *Pterodroma*) have some of the greatest dispersal capabilities of all birds, relative to their body size, and will often fly thousands of kilometres over single foraging trips during the breeding season (e.g. MacLeod *et al.* 2008a, 2008b*;* Pinet *et al.* 2012). Yet, despite the many obvious risks that this presents, their food resources are not nearly as well-known as for species in other genera from the Order Procellariiformes (e.g. *Puffinus, Diomedea,* and *Thalassarche;* Bester *et al.* 2010). Considerable attention has also been devoted to the diets of higher latitude petrels and albatrosses (e.g. Imber 1973; Schramm 1986; Harper 1987; Rodhouse *et al.* 1990; Cherel & Weimerskirch 1995; Cherel *et al.* 2004; Connan *et al.* 2007a; Richoux *et al.* 2010; Connan *et al.* 2014), whereas tropical species have mostly been neglected (some examples of studies include Harrison *et al.* 1983; Imber *et al.* 1995; Spear *et al.* 2007; Bester *et al.* 2010). Barau's Petrel is one of only a few gadfly petrels that breed in the tropical Indian Ocean (Sinclair & Lagrand 2003; Safford & Hawkins 2013). Other resident species include the Trinidade (*P. arminjoniana*), Kermadec (*P. neglecta*), and Herald (*P. heraldica*) Petrels, which have all propagated small populations on Round Island, Mauritius (refer to Figure 2.1, Chapter 2; Brown *et al.* 2011; Safford & Hawkins 2013). The feeding habits of these species have never been formally addressed in this region, although two mentions of the prey of Barau's Petrel exist in the scientific literature (Stahl & Bartle 1991; Kojadinovic *et al.* 2007). Observations made during an early ship-based survey, which investigated the at-sea distribution of the Barau's Petrel (Stahl & Bartle 1991), suggested a diet consisting mainly of fish. This was surprising as most other *pterodromas* feed on squid, while fish and crustaceans are usually of little to no importance (e.g. Prince &

Morgan 1987; Marchant & Higgins 1990; Bester 2003; Bester *et al.* 2010). Fishes have been shown to be important in at least a few other cases, however (e.g. Kermadec Petrel in the eastern topical Pacific Ocean; Spear *et al.* 2007), and the diets of gadfly petrels are known to be spatially and temporally variable (Warham 1990). A more recent study (i.e. Kojadinovic *et al.* 2007), which assessed the bioaccumulation of trace elements in the tissues of marine birds from Réunion Island, noted that this species' diet consisted mostly of cephalopods (98 %; *Sthenoteuthis oualaniensis* and *Taonius* sp.), whereas fish were only occasionally recorded (2 %). This conclusion was reached through an analysis of stomach contents of 'fallen birds' (refer to section 2.3.2), but no indication was given as to number of samples used or the body condition, and age structure of the individuals in question. This sparse and contradictory information makes it extremely difficult to understand the ways in which anthropogenic activities (e.g. industrial fishing in the south-western Indian Ocean), or other such pressures, might be influencing the demographic parameters of this already threatened species. Therefore, the topic needs to be revisited and thus, in assessing the stomach contents of Barau's Petrel, this chapter aimed to:

a) Identify the main prey species in the diet of Barau's Petrel.
b) Attempt to clarify the relative importance of the different prey groups (particularly with respect to fish and cephalopods) in this species' diet.
c) Determine, wherever possible, the size structure of the animals consumed.
d) Identify any additional threats that this species might be facing during breeding. These might include an importance of commercially valuable species or the consumption of plastics, for example.

3.2. Materials and methods

3.2.1. Sample description

For ethical reasons, stomach content samples were collected from live birds only if/when regurgitation occurred naturally during handling at the nest. So, in addition, stomachs were dissected from dead birds that had been gathered during recovery programs (see section 2.3.2). Most samples were collected from dead fledglings (March-May; n = 29), but a few were also collected from adult (n = 1), and downy chicks (n = 6) at the colony. More than half of the samples were gathered from individuals that had not fed for about two weeks (fledglings), so very few fresh items were recovered. In addition, only five samples, corresponding to some of those collected from downy chicks at the colony, contained amber-orange stomach oil. Hence, these analyses are almost entirely reliant on the accumulated hard structures in the stomachs of the birds. This method is associated with a number of inherent biases, which complicate the estimation of relative prey importance, so brief discussions about the prevalence of various fresh prey items are provided where applicable (refer to section 3.4.1). Soon after collection, the samples were frozen until further processing.

3.2.2. Stomach content analysis

Each stomach content sample was thawed overnight and subsequently weighed, thus giving an indication of wet weight (WW). Liquid contents were then gravity drained from the solid items through a 0.5 mm sieve. The remaining structures were visually examined in a 50x30 cm white sorting tray under a dissecting microscope. This ensured that all structures were removed for identification. Accumulated items were separated into nine broad prey

classes (fish, cephalopods, vegetative materials, marine insects, plastics, molluscs, crustaceans, other arthropods, and unidentified items) prior to identification.

To estimate the number of individual crustaceans in the diet of Barau's Petrel, entire carapaces and/or pairs of eyes were counted (Raya Rey & Schiavini 2005). The number of shells and legs were tallied as indicators of the frequency of molluscs and marine insects, respectively, whilst other arthropods were counted using their whole segmented bodies or sections of tough chitinous pleura (Day 1974). Vegetative material was then grouped into marine and terrestrial assemblages and then sorted by leaf and/or cushion and stem structure. Since it was not possible to differentiate between separate feeding events, the number of times plant material was consumed was assumed to equal the number of species present within each sample. Plastics were also grouped and counted on the basis of type and colour. The number of cephalopods consumed was estimated by counting the accumulated upper and lower beaks. Lower beaks show far greater morphological variation among species and are most often used in identification, but a recent study (Xavier *et al.* 2011) has necessitated the combined use of upper beaks to improve the assessment of the contribution of different cephalopods to predator diets. Only whole or slightly fragmented upper and lower beaks were retained; some stomachs contained up to 30 beaks worth of unidentifiable fragments. These whole or partially fragmented structures were paired, wherever possible, or identified separately. When pairing was not possible, the total number of cephalopods consumed was calculated as the sum of the lower beaks plus any unmatched upper beaks after identification (Cherel & Duhamel 2004). Finally, segments of vertebral columns and otoliths were counted as indicators of the number of different fish consumed by each bird (Alonso *et al.* 2013). Otoliths were preferentially used since they show far greater morphological variation among species. Two partially-digested fish were recovered,

both of which had intact heads. Both specimens were photographed and their otoliths removed for identification. Fragmented sections of vertebral column were more commonly found among the accumulated prey items with never more than one small section per stomach. Thus, these structures were used to increase the detectability of this prey class (Pierce & Boyle 1991; Pierce *et al.* 1991; Granadeiro & Silva 2000), although species identification of vertebral columns was not possible.

All samples were stored in 70 % ethanol in labelled 2 ml Eppendorf centrifuge tubes to prevent desiccation prior to identification. This biological solvent is known to dissolve otoliths and other skeletal sections (M. Smale. pers. comm.), so that fish remains were rinsed with water, oven dried at 50 °C, and stored dry in labelled 2 ml Eppendorf centrifuge tubes.

3.2.3. Prey group identification

All accumulated structures were identified to the lowest possible taxonomic levels. Otoliths were identified using Smale *et al.* (1995) and cephalopod beaks identified using three separate identification guides (Wolff 1984; Clarke 1986; Xavier & Cherel 2009). These structures were also compared with voucher specimens in the reference collection at the Port Elizabeth Museum (Bayworld), South Africa, and with photographs from the Tree of Life project (Young *et al.* 2012 and the webpages therein). Since reference material was difficult to come by, Jereb and Roper (2010) and Ménard *et al.* (2013) were used as guides for the potential cephalopod prey species in the region and all identifications were checked by a specialist (Dr. Malcolm Smale). Marine insects were also identified by means of identification guides (Herring 1961; Cheng 1975), as were all specimens from the phyla Mollusca and Arthropoda (Day 1974). Finally, terrestrial vegetative material was identified

by comparison with known voucher specimens taken from the higher mountainous slopes of Réunion Island near the breeding colonies of Barau's Petrel.

3.2.4. Data analysis

As indicators of the relative occurrence of each prey species/group, both frequency of occurrence (FO) and the averaged relative percentage occurrence (RPO) were calculated using the equations presented below:

$$FO = \left(\frac{n_i}{n}\right) \times 100$$

$$RPO = \left(\frac{\sum\left(\frac{n_{\Sigma a}}{\sum n_a n_b n_c}\right)}{n}\right) \times 100$$

Where 'n_i' is the number of stomach contents containing prey group 'i', 'n' is the total number of stomach contents, '$n_{\Sigma a}$' is the number of observations of prey group 'a' within a given stomach, and '$\Sigma n_a n_b n_c$' is the sum of the observations of all prey groups in the same stomach content sample as '$n_{\Sigma a}$'. The FO of each prey item indicates how often a consumer feeds on a certain prey species/group (Ashmole & Ashmole 1967; Hyslop 1980; Balestrieri *et al.* 2011). This method can overestimate the dietary importance of infrequently consumed prey species, however (Hyslop 1980; Balestrieri *et al.* 2011). Thus the RPO method was used to give an indication of the breadth of the diet by signifying how often a food type is encountered relative to all other food types (Hyslop 1980; O'Sullivan & Cullen 1983; Montague & Cullen 1988; Balestrieri *et al.* 2011). Insufficient numbers of samples were

collected to allow comparison among ontogenetic groups or different breeding stages and thus all data were lumped for the purposes of these analyses (see section 3.4.3 for further motivation). Results were tabulated using Microsoft Excel packages.

3.2.5. Size estimation of cephalopod prey

Using logarithmic equations, which were built from taxon specific regression analyses based on the lower rostral length (LRL; Figure 3.1) of lower beaks, it is possible to estimate the mantle length (ML) and WW of the cephalopod prey (Clarke 1986; Xavier & Cherel 2009). Measurements of LRL extended from the rostral tip (RT) to the jaw angle of each lower beak (JA; Clarke 1986; Xavier & Cherel 2009; Figure 3.1). Each beak was measured using digital callipers (0.1 mm precision) and taxon/genus/family specific equations applied thereafter (Table 3.1). Estimated WW and ML of cephalopod prey are reported as means with standard deviations and were visualised using Microsoft Excel packages.

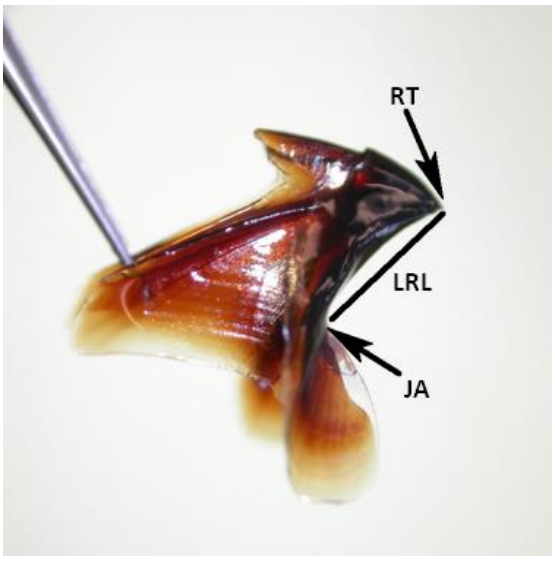

Figure 3.1: Mantle lengths and wet weights of the cephalopod prey of Barau's Petrel (*Pterodroma baraui*) were estimated using logarithmic equations based on the lower rostral

lengths (LRL) of lower beaks. LRLs were measured from the tip of the rostrum (RT) to the jaw angle (JA) using digital callipers (precision 0.1 mm; photo credit: Valerie Hartigan).

Table 3.1: Species or genus specific logarithmic equations, based on lower rostral lengths (LRL; mm) of lower cephalopod beaks, used to estimate wet weight (g) and mantle length (mm) of cephalopod prey.

Species/Genus/Family	Wet weight (g)	Mantle length (mm)	Source
Chiroteuthis sp.	$ln(m) = -0.241 + 2.7ln(lrl)$	$l = 11.4 + 24.46(lrl)$	Clarke 1980; Clarke 1986
Cycloteuthidae	$ln(m) = 1.89 + 1.95ln(lrl)$	$l = 31 \times (lrl)$	Clarke 1980; Clarke 1986
Histioteuthidae	$ln(m) = 1.594 + 2.31ln(lrl)$	$l = -13.6 + 22.21(lrl)$	Clarke 1986
Sthenoteuthis oualaniensis	$ln(m) = 0.892 + 3.0ln(lrl)$	$l = 6.98 + 3.25(lrl)$	Wolff 1983; Clarke 1986
Other Ommastrephidae*	$ln(m) = 1.834 + 2.07ln(lrl)$	$l = 52.7 + 27.61(lrl)$	Wolff 1983; Clarke 1986
Taonius sp.	$ln(m) = 0.789 + 2.19ln(lrl)$	$l = -12.3 + 61.43(lrl)$	Clarke 1986; Rodhouse *et al.* 1990

**based on Ommastrephes bartrami,* which has also been frequently observed in the diet of marine top predators from the western Indian Ocean (Ménard *et al.* 2013).

3.3. Results

3.3.1. Breadth of diet

Thirty-six stomach content samples, with an average WW of 30.81 ± 17.39 g, were collected from adult, fledgling, and downy chick Barau's Petrel. Although soft-bodied organisms may have been overlooked, this sample size seems to have been sufficient to detect most regular and rarely consumed prey since the number of taxa identified remained constant after 14 stomachs (Figure 3.2). Roughly 24 prey groups were identified including about eight cephalopods, possibly more than two fish species, three crustaceans, one gastropod, one marine insect, and four terrestrial plant species (Table 3.2). On average, 2.63 ± 2.06 prey groups were identified per stomach while only about 2 % of all accumulated structures could not be identified as they were in too far a state of digestion.

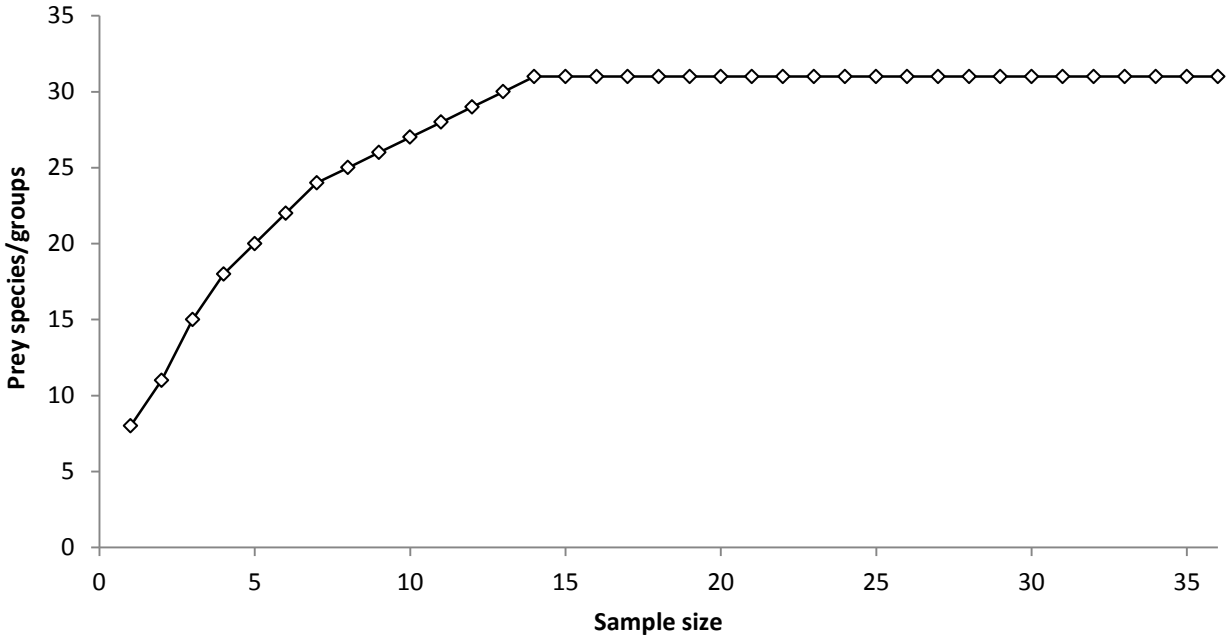

Figure 3.2: Cumulative number of identified prey species/groups in the stomachs contents of breeding (adults, fledglings, and downy chicks) Barau's Petrel (*Pterodroma baraui*). n = 36 stomach contents.

3.3.2. Diet composition

Cephalopod beaks dominated the accumulated prey items in the stomachs of Barau's Petrel, accounting for more than 50 % of all structures found (Table 3.2). The species from the families Cranchiidae (*Taonius* sp.*;* FO and RPO greater than 8.33 and 2.73 % in all cases, respectively) and Ommastrephidae (particularly *Stenoteuthis oualaniensis;* FO and RPO greater than 8.33 and 2.39 % in all cases, respectively) were found in particularly high abundance, whilst less important genera included *Chiroteuthis* (PO and RPO both 2.78 %), *Stigmatoteuthis* (FO and RPO 2.78 and 0.62 %, respectively), and *Discoteuthis* (FO and RPO 2.78 and 0.25 %, respectively; Table 3.2). Crustaceans and marine gastropods were found in fairly high abundance (FO and RPO all > 5 %), as was also the case for vegetative material

(FO and RPO 25 and 5.07 %, respectively; Table 3.2). This latter prey class was dominated by terrestrial groups including grasses (Poaceae), and the perennial shrubs *Stoebe passerinoides,* and *Hypericum lanceolatum* (Table 3.2). An unidentified broad-leafed terrestrial plant species was another noteworthy find, albeit in only one stomach (Table 3.2). Fish were also recorded (FO and RPO 16.67 and 8.06 %), with at least one genus (i.e. *Atherinomorus*) of animals present (Table 3.2). Other apparently rarer prey groups included marine insects (*Halobates;* FO and RPO 1.05 and 0.69 %, respectively), and bryopsid mosses (FO and RPO 5.59 and 3.17 %, respectively; Table 3.2). Finally, whilst these structures contributed nothing to the energy requirements of the birds, three different types of plastic were discovered in the stomach contents of Barau's Petrel (Table 3.2). The most frequently encountered types were small blue/turquoise micro-plastic (≤ 5 mm) fragments, and lengths of fishing gut (monofilaments of up to 6 cm in length; FO and RPO greater than 2 % in both cases; Table 3.2).

Table 3.2: Frequency of occurrence (FO) and relative percentage occurrence (RPO) of accumulated prey items from the stomachs of breeding Barau's Petrel (*Pterodroma baraui*). n = 36 stomach contents.

Prey group/Class:	Order:	Family:	Genus:	Species:	Stomachs containing	Total number	Frequency of occurrence	Rank order	Relative percentage occurrence	Rank order
Cephalopoda										
	Teuthida									
		Chioteuthidae	*Chiroteuthis*	unidentified sp.	1	1	2.78	9	2.78	11
			Discoteuthis	unidentified sp.	1	1	2.78	9	0.25	24
		Histioteuthidae	*Stigmatoteuthis*	*Hoylei*	1	2	2.78	9	0.62	20
				unidentified sp.	1	1	2.78	9	2.78	11
		Ommastrephidae	*Ommastrephes*	unidentified sp.	1	3	2.78	9	0.69	19
			Stenoteuthis	*Oualaniensis*	10	20	27.78	1	13.01	1
			Other		3	4	8.33	7	2.73	12
		Cranchiidae	*Taonius*	sp. 1 (upper rostral length – 5.0 ± 0.22 mm)	5	8	13.89	5	7.68	6
				sp. 2 (upper rostral length – 3.5 ± 0.51 mm)	3	3	8.33	7	2.39	13
				sp. A	7	10	19.44	3	9.38	2
				sp. B	6	7	16.67	4	8.21	5
		unidentified			9	12	25.00	2	8.55	4
				Overall:	28	72	77.78	-	59.07	-
Actinopterygii										
	Atheriniformes	Atherinidae	*Atherinomorus*	unidentified sp.	1	2	2.78	9	2.78	11
	Other				5	5	13.89	5	5.28	8
				Overall:	6	7	16.67	-	8.06	-
Gastropoda										
	Littorinoidea	Littorinidae	N/A		7	17	19.44	3	9.03	3

Table 3.2: cont.

Prey group/Class:	Order:	Family:	Genus:	Species:	Stomachs containing	Total number	Frequency of occurrence	Rank order	Relative percentage occurrence	Rank order
Insecta										
	Hemiptera	Gerridae	*Halobates*	unidentified sp.	1	1	2.78	9	0.69	19
Malacostraca										
	Decapoda (Brachyura)				1	1	2.78	9	0.56	21
	Isopoda				1	1	2.78	9	0.56	21
	Other				6	9	16.67	4	4.07	9
				Overall:	6	11	16.67	-	5.18	-
Plastics										
Blue microplastic					3	4	8.33	7	5.56	7
Fishing gut microfilaments					3	3	8.33	7	2.18	14
Thin black sheets					1	1	2.78	9	0.40	23
				Overall:	6	8	16.67	-	8.13	-
Angiospermae										
	Poales	Poaceae			2	2	5.56	8	0.54	22
	Ericales	Asteraceae	*Stoebe*	*passerinoides*	4	4	11.11	6	1.90	15
		Hypericaceae	*Hypericum*	*Lanceolatum*	1	1	2.78	9	0.25	24
	Unidentified broad leaved plant				1	1	2.78	9	0.56	21
	Other				2	2	5.56	8	1.70	16
Unidentified marine algae					1	1	2.78	9	0.93	17
				Overall:	9	11	25.00	-	5.87	-
Bryopsida					2	2	5.56	8	3.17	10
Unidentified					3	3	8.33	7	0.79	18

3.3.3. Size structure of cephalopod prey

The lower rostral lengths of cephalopods beaks varied greatly within and among taxa (Table 3.3). Based on these measurements, the estimated ML of cephalopod prey ranged between about 59 and 350 mm (average ML: 187 ± 93.10 mm). These results produced a bimodal pattern indicating that Barau's Petrel feed on two different size classes of cephalopod prey (Figure 2.3). The smaller (ML: 40-140 mm) group consisted of species from the genera *Discoteuthis*, *Chiroteuthis*, *Stigmatoteuthis*, and the dominant *Stenoteuthis*, and the second, much larger (ML: 220-340 mm), group was composed entirely of species from the genus *Taonius* (Figure 3.3).

Table 3.3: Measurements of lower rostral lengths (LRL ± SD; mm) of accumulated lower beaks from the stomachs of breeding Barau's Petrel (*Pterodroma baraui*).

Prey sp.	n	LRL ± SD (mm)
Chiroteuthis sp.	1	4.3
Discoteuthis sp.	1	5.7
Stigmatoteuthis hoylei	2	4.5 ± 1.70
Other Ommastrephidae	6	2.33 ± 0.46
Sthenoteuthis oualaniensis	12	2.51 ± 0.56
Taonius sp1.*	10	4.80 ± 0.60
Taonius sp2.*	7	5.01 ± 0.19

**Taonius sp.* lumped with one another for estimations of WW and ML because of the high degree of overlap. Presented separately here to highlight the difference in beak structure between the two species.

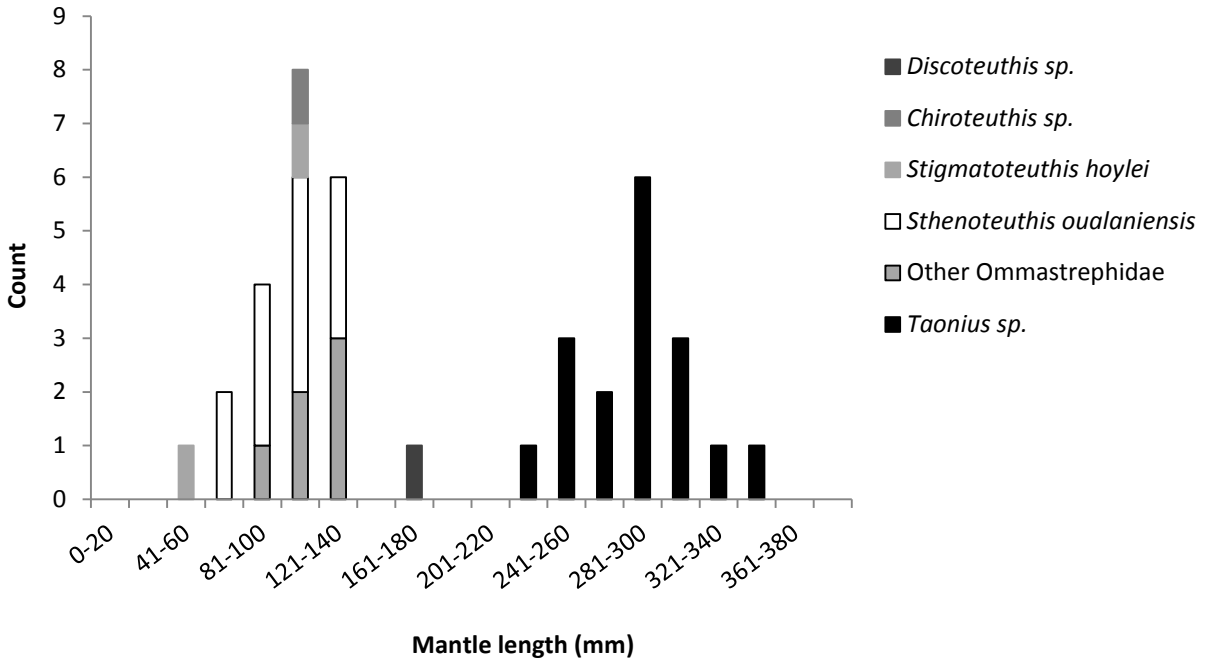

Figure 3.3: Estimated mantle lengths (mm) of cephalopod prey consumed by breeding (adults, fledglings, and downy chicks) Barau's Petrel (*Pterodroma baraui*), calculated from lower rostral lengths of lower beaks.

A different pattern was observed in terms of the estimated WW of cephalopod prey. This ranged between about 14 and 270 g (WW: 65.63 ± 47.52 g). The overall pattern was strongly skewed to the right with more than 95 % of all the animals consumed having an estimated WW of less than 120 g (Figure 3.4). Although there was great overlap, the ommastrephids (including *Stenoteuthis oualaniensis*) were among the smallest species and the cranchiids (*Taonius* sp.) dominated the larger animals (Figure 3.4). The largest species recorded were *Discoteuthis* sp., and *Stigmatoteuthis hoylei* (Figure 3.4).

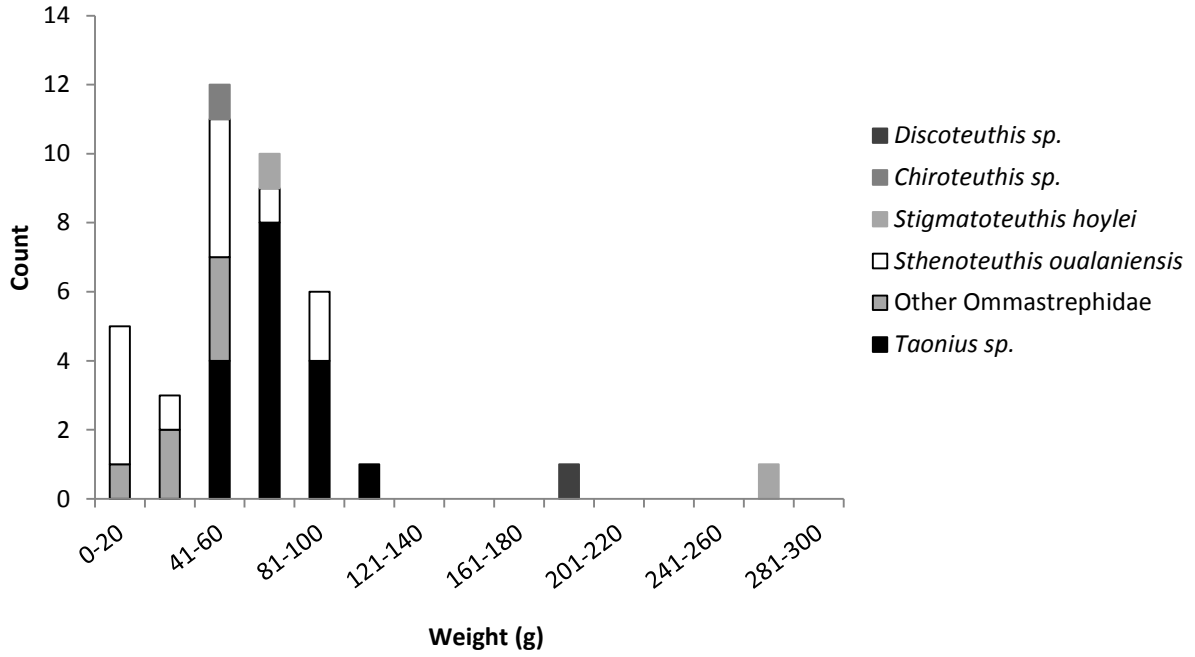

Figure 3.4: Estimated weight (g) of cephalopod prey consumed by breeding (adults, fledglings, and downy chicks) Barau's Petrel (*Pterodroma baraui*), calculated from lower rostral lengths of lower beaks.

3.4. Discussion

Aside from their central application in diet studies (Barrett *et al.* 2007), stomach content analyses have provided valuable insight into the distribution (e.g. Fahrig *et al.* 1993; Robinette *et al.* 2007; Ménard *et al.* 2013; Staudinger *et al.* 2013), and size structure of prey species (e.g. Headley *et al.* 2009), as well as in quantifying prey-predator relationships (e.g. Imber 1975; Clarke 1980; Clarke & Stevens 1980; Weimerskirch *et al.* 1994b; Peristeraki *et al.* 2005). Furthermore, stomach content analyses have improved our understanding of overall community assemblages (e.g. Caddy & Rodhouse 1998; Cherel *et al.* 2004, 2007b; Staudinger *et al.* 2013), the existing and predicted biomass of prey populations (e.g. Clarke

1983; Montevecchi 1993; Cherel & Weimerskirch 1995), interspecific relationships among predators (e.g. Shimose *et al.* 2010), and the potential impacts of commercial exploitation on ecosystem structure and function (e.g. Overholtz *et al.* 2000). Ultimately, these results have then been projected into dynamic community and ecosystem level models and have become integrated into conservation strategies for threatened and/or range restricted species (Bailey & Ainley 1982). Studying different types of predators (marine mammals, fish, and/or seabirds) has also offered opportunities to solve different problems. Highly mobile groups, such as pelagic seabirds, have proven especially valuable in that they forage over wide areas and can thus offer great quantities of assorted data. Compared with subtropical groups, the diets of tropical seabirds have received little scientific attention (some examples include Harrison *et al.* 1983; Imber *et al.* 1995; Bester *et al.* 2010). In fact, the functioning of tropical ecosystems is, in general, not nearly as well understood as those from higher latitudes. Hence, this research has far greater application than simply identifying the principal resources on which breeding Barau's Petrel rely.

3.4.1. Overall diet composition of breeding Barau's Petrel

The single most important foraging strategy of tropical seabirds is feeding in large multispecies flocks in association with subsurface predators (e.g. tuna and dolphins; Au & Pitman 1986; Ballance & Pitman 1999). These animals, when feeding, force forage fish and squid to the surface, making them available to seabirds feeding from above (Au & Pitman 1986; Harrison & Seki 1987; Ballance & Pitman 1998, 1999; Le Corre & Jaquemet 2005; Jereb & Roper 2010). Some seabirds are even regarded as 'near obligate commensals' of tuna, with more than 70 % of all feeding activity occurring in association with shoals of predatory fish (Au & Pitman 1986; Harrison & Seki 1987; Jaquemet *et al.* 2004). Barau's Petrel, along

with most other gadfly petrels (Bretagnolle 1993), are solitary feeders that only occasionally associate with other birds and subsurface predators (Del Hoyo *et al.* 1992; Jaquemet *et al.* 2004). They feed primarily through shallow-dipping and surface seizing (Pinet *et al.* 2009; pers. obs.), and it has been proposed that their great flight potential allows them to search large areas for isolated prey (Warham 1990; Ballance & Pitman 1999). These two feeding strategies are known to be unselective with regards to prey discrimination (Harper *et al.* 1985), so it would seem obvious that gadfly petrels would be strongly opportunistic in their feeding habits. Consequently, the wider variety of prey found in the diet of Barau's Petrel, compared to the earlier studies of Stahl and Bartle (1991) and Kojadinovic *et al.* (2007), was not unexpected. The possibility that at least some of this prey was ingested as secondary prey cannot be entirely excluded, however. This implies that some of the more unusual prey items (e.g. Liittorinidae, gastropods) may not have been actively/intentionally consumed by the birds themselves, but rather sourced from within the digestive tracts of other intermediate predators, such as the mesopelagic squids and fishes, which were also found in the stomachs of breeding Barau's Petrel (Cherel *et al.* 2000).

Gadfly petrels are collectively known to consume a large proportion of cephalopod prey (Prince & Morgan 1987). These animals appeared to constitute an important prey group of Barau's Petrel, with ommastrephid and cranchiid beaks dominating the accumulated prey items. This finding was not dissimilar from the conclusions drawn in an independent diet study on the Barau's Petrel (Kojadinovic *et al.* 2007), and for the diets of other marine top predators in the western Indian Ocean (Ménard *et al.* 2013). However, since the present study was based mostly on accumulated prey items, it is difficult to judge whether this prey group dominates the diet of Barau's Petrel as has previously been suggested (Kojadinovic *et al.* 2007; Pinet *et al.* 2012). It is noted though, that the colour of

procellariiform stomach oil has been correlated with diet composition; orange, amber, and red samples, such as was recorded from breeding Barau's Petrel, that contain carotenoids and esterified astaxanthin pigments, are typically linked to a crustacean and cephalopod dominated diets, whereas oils derived from fish are typically colourless or are of light shades of yellow (Lewis 1969; Warham *et al.* 1976; Connan *et al.* 2005). In addition, beaks with at least some flesh still attached were more regularly found than any other fresh prey, further suggesting dietary importance of cephalopod prey. It was also curious to note that cranchiids, not the ommastrephids, seemed to dominate the cephalopod prey of this seabird. This contrasts with what has previously been found in other diet studies on this (Kojadinovic*et al.* 2007), and other seabird species from the south-western Indian Ocean (Ménard *et al.* 2013), and may be a consequence of the methodological approach, which relied on accumulated prey items only and/or the use of upper and lower beaks in prey species identification, or as a consequence of spatio-temporal variations in prey assemblages, availability, and/or accessibility.

Fishes (particularly myctophids) have been identified as another major component in the diets of *Pterodroma* petrels in the southern Pacific Ocean (Spear *et al.* 2007), and Stahl & Bartle (1991) suggested that this might also be the case for Barau's Petrel (Stahl & Bartle 1991). Yet these results would seem to dispute that hypothesis, and rather support Kojadinovic *et al.* (2007). This was somewhat unsurprising as fishes are virtually absent from the diets of a few other *Pterodroma* petrels (Imber 1973; Schramm 1986; Warham 1996; Bester *et al.* 2010), and appear to be of little importance to other seabirds and predatory fish in the western Indian Ocean (e.g. Potier *et al.* 2007; Jaquemet *et al.* 2008; Romanov *et al.* 2008; Catry *et al.* 2009a; Jaquemet *et al.* 2011). But, to understand how both this study and Kojadinovic *et al.* (2007) differed from Stahl and Bartle (1991), one needs to scrutinize

the methodological approaches used. Like this study, the dietary conclusions made by Kojadinovic *et al.* (2007) were based on an analysis of accumulated prey items. It needs to be mentioned that fish remains (including scales and bones) are typically eroded in one to two days whereas squid and crustacean hard tissues can accumulate for weeks and sometimes months, depending on the size of the animals in question. As such, the dietary importance of fish would likely have been underestimated in this study. To further illustrate this argument, Furness *et al.* (1984) experimentally fed Sardines (*Sardinops sagax*; 300 g per day for 46 days) and 19 pairs of *Loligo* beak pairs (38 upper and lower mandibles; three days before euthanasia) to a captive Shy Albatross (*Thalassarche cauta*). Later, subsequent to euthanasia, the bird was dissected and 38 beaks were found to have accumulated in the bird's stomach. This included 19 *Loligo* and 19 Ommastrephiidae beaks; the latter structures must having had been retained in the bird's stomach from before its capture (more than about 50 days prior). No obvious fish remains were reported from the bird's excrement during the course of the study. Stahl and Bartle (1991) were, by contrast, entirely reliant on ship-based surveys in the areas to the south of Réunion Island. All of their observations were made from the unenclosed bridge wings (13 m a.s.l.) of the *RV Marion-Dufresne* during daylight hours. This constitutes a major flaw in that study since nocturnal feeding appears to be important to most Procellariiformes (Harper 1987), including for Barau's Petrel (Pinet *et al.* 2012; this study – refer to section 3.4.2). But aside from their dietary conclusions, Stahl and Bartle (1991) also noted that 98 % of all feeding behaviour occurred in association with other seabirds. A more recent study (i.e. Jaquemet *et al.* 2004) noted that Barau's Petrels were only infrequently observed alongside other seabird species when feeding in the vicinity of Réunion, however. The possibility that Barau's Petrels do behave differently when in their foraging habitats to the south of Réunion Island cannot be excluded (Pinet et al 2012), but

these inconsistencies imply that the overall reliability of Stahl and Bartle (1991) should also be questioned since they likely overestimated the importance of fishes. Ship-based surveys are recognised as being greatly misleading, especially when vessels as large as the *RV Marion-Dufresne* are used (Fair *et al.* 2010). Some seabird studies have even documented boat avoidance (Henckel *et al.* 2007), or shifts in behaviour associated with maritime traffic (Burger 1998). But, whilst it seems that fish can finally be disregarded as the most important prey group, the presence of two *Atherinomorus* specimens (tentatively identified as either *A. breviceps* or *A. duodecimalis*), which constituted the only fresh and identifiable fish remains from Barau's Petrel stomachs, was surprising. These taxa inhabit calm and shallow coastal (especially around reefs) and estuarine habitats (Anam & Mostarda 2012), neither of which are frequently used by adult Barau's Petrel during foraging exoduses (Pinet *et al.* 2012). So either they were sourced from coastal areas around the South African and Mozambique coasts (*A. breviceps*), or this prey was opportunistically gathered when leaving from or returning to Réunion Island (*A. duodecimalis*).

3.4.2. Other inferences about the foraging behaviour of breeding adult Barau's Petrel

Nocturnal feeding is a common behaviour of many of the Procellariiformes (Ballance & Pitman 1999). In a recent tracking study on Barau's Petrel, Pinet *et al.* (2012) noted that the greatest levels of activity were at night, suggesting that this was when the birds were most vigorously feeding. The results from this study do not contradict that suggestion as the estimated size structure of cephalopod prey, which is consistent with what has been found in the Providence (*Pterodroma solandri;* Bester *et al.* 2010), Grey-faced (*P. macroptera*; Imber 1973), Soft-plumaged (*P. mollis*), and Kerguelen Petrels (*Lugensa brevirostris;* Schramm 1986), as well as the presence of photopores on many of the identified prey

species, suggest nocturnal feeding as an important foraging strategy. Adult cephalopods of some species (e.g. *Stenoteuthis oualaniensis*) typically occur at depths of between 100 and 1 000 m during the day, but perform diel vertical migration to feed in surface waters at night (Jereb & Roper 2010). It is at this time that they become available to birds and it has been suggested that their bioluminescence might benefit petrels in that their prey (i.e. the squids and fish) can more easily be detected at night (Warham 1990). Conversely, planktonic postlavae and juveniles of some cephalopods perform reverse diel vertical migrations; this is to say that they reside in surface waters during daylight hours and descend to depth at night (Jereb & Roper 2010). Hence, diurnal feeding could be equally as important to Barau's Petrel since the smaller juvenile squids would most likely have been consumed during the day. Seabirds that actively scavenge the floating carcasses of other dead marine organisms are also presumed less likely to do so at night (Warham 1990). This is an important feeding strategy among procellariiform seabirds, with typical examples including the albatrosses (e.g. *Diomedea, Thalassarche, Phoebastria,* and *Phoebetria;* Ashmole & Ashmole 1967; Harper 1987; Lipinski & Jackson 1989), and fulmarine petrels (e.g. *Macronectes, Fulmarus, Thalassoica, Daption,* and *Pagodroma;* Warham 1990; Imber *et al.* 1995; Ballance & Pitman 1999; Bester 2003; Bester *et al.* 2011). So the presence of stigmatoteuthids and other very large cephalopods, which were likely scavenged since they had estimated masses (± 270 g) similar to those of adult Barau's Petrel (bird weight range: 250 – 620 g), might also suggest that the birds feed by day. Not all cephalopods groups float to the surface after death, but those that do include some of these large species that were recorded in the diets of Barau's Petrel (Dell 1952; Lipinski & Jackson 1989; Jereb & Roper 2010). An alternate, but less likely, explanation is that the birds could be capable of subduing large cephalopod prey, after which they tear the animals into manageable pieces using their sharp bills and head shakes.

This has previously been observed in the Wandering Albatross (*Diomedea exulans;* Harper 1987), and would imply that this larger cephalopod component could have been underestimated.

The hypothesis that these birds will opportunistically scavenge on floating materials was supported by the presence of vegetative material in this species' diet (Bester *et al.* 2010). Indeed, marine algae and, irrespective of their absence at high elevations on Réunion, broadleaved terrestrial plant material were found among the accumulated prey items of Barau's Petrel. The presence of *Stoebe passerinoides* and *Hypericum lanceolatum* in the diet of Barau's Petrel was equally striking as both would have been ingested around the breeding colonies where these plants dominate (Cadet 1977; Strasberg *et al.* 2005). Few other studies have ever recorded the consumption of plants by seabirds (e.g. Connan *et al.* 2007a; Bester *et al.* 2010), presumably because most birds are unable to produce cellulase, the enzyme needed to catalyse cellulose. Digestion in true-folivorous avian groups (e.g. mousebirds, Coliidae) is entirely dependent on gut flora and specialised anatomical adaptations (Downs *et al.* 2000). Seabirds apparently lack this same gut flora so that this plant material cannot contribute to the birds' nutritional requirements. So why do these petrels ingest vegetative material around the breeding colonies? Adults commence their migration before the end of the breeding season (end of March), and pre-fledglings typically remain in their burrows for an additional two weeks thereafter (Pinet *et al.* 2009). It is thought that these young birds survive on their large lipid and protein stores and the slow loss in body condition finally forces them to fledge in mid-April (Brooke 1990; Pinet *et al.* 2009). Thus, this result might signify that young birds will be consuming plant material and other such peculiar prey groups (i.e. bryopsid mosses) over periods of extended food deprivation. Alternatively, as the downy chicks grow into fledglings, they become more aware of their environment and

the nest itself. So this vegetation may not necessarily be 'actively consumed' and may just reflect them 'discovering' their surroundings. This behaviour has previously been reported for albatross chicks (M. Connan. pers. comm.), but not from petrels. The present study lacked the resolution required to separate these two theories, however.

Many species will also scavenge trawl discards behind fishing vessels and hundreds of thousands of petrels and albatrosses are entangled in purse seine nets or are caught and drowned on baited longline hooks each year (Montevecchi 2002; Anderson *et al.* 2009, 2011). Procellariiformes are highly K-selected and these increases in mortality have significant impacts on the long-term population trajectories of these species (Hamer *et al.* 2002). In fact 19 of 22 species of albatross and many petrels are now threatened with extinction, directly as a consequence of fishing mortality (ACAP 2014). But the available evidence suggests that Barau's Petrel, like most other *Pterodroma* petrels, never forage in association with fishing vessels (Del Hoyo *et al.* 1992). In fact, gadfly petrels are among the least impacted of all marine birds in terms of the effects of direct negative interactions with fisheries; analyses of bird mortality in the South African longline fishing industry have indicated that no *Pterodoma* petrels are killed in > 8 000 catches (Albatross task force. pers. comm). So the presence of monofilament lengths of fishing gut in the stomachs of this species is compelling for it suggests some unknown (likely indirect) association between the birds and line-based fishing vessels. Some of the greatest longline catches, from across the entire Indian Ocean, are taken from the south-western sector of the Indian Ocean where Barau's Petrel forage during the breeding season so this interaction may indeed be plausible (IOTC 2012; Danckwerts *et al.* in press). Other micro-plastics were also recorded in the stomachs of Barau's Petrel. This was somewhat unsurprising since a recent study has identified a large plastic sink in the south Indian Ocean around 20°S near where Barau's

Petrel forage over the breeding season (Pinet *et al.* 2012; Cózar *et al.* 2014). Furthermore, linking to their opportunistic feeding habits, plastic ingestion has been recorded in 63 % of all procellariiform species and it is estimated that more than 60 % of all individual petrels and albatrosses (all species) ingest plastics on a regular basis (Laist 1997). At high incidences, these two threats may adversely affect the survival and health of Barau's Petrels, although no obvious detrimental effects were apparent over the course of this study.

3.4.3. Limitations of stomach content analyses

Many methods can be used to study the diet of seabirds (Barrett *et al.* 2007). These are broadly separated into four different lines of approach. The first is to assess the diets of seabirds directly by collecting the accumulated items from within the digestive tract (oesophagus, crop, proventriculus, gizzard, and small intestine; Duffy & Jackson 1986; Barrett *et al.* 2007). It is also possible to collect diet information *ad hoc*. This is to say that one can observe food uptake directly, or collect the prey that has been dropped around the breeding colony (e.g. Atwood & Kelly 1984; Harris & Wanless 1985; Duffy & Jackson 1986). Alternatively, for single load-species, such as the Atlantic Puffin (*Fratercula arctica*; Baillie & Jones 2003, 2004), and Roseate Tern (*Sterna dougallii*; Ramos *et al.* 1998), it is possible to determine the composition of prey carried back to the colonies in the bills of adults for the chicks. Then, a more recent approach is the application of biochemical methods including serological, genetic, stable isotope, and fatty acid analyses (Barrett *et al.* 2007; Iverson *et al.* 2007). No 'ideal' methodology exists with which to study the diets of seabirds, as each available approach is subjected to its own suite of biases. It is therefore necessary to adapt the intensity, focus, duration, and analysis of diet studies according to the questions being asked.

When using stomach content analyses, it is essential to recognise what sample sizes are appropriate for the questions being asked (Hanson & Graybill 1956). The presence/absence of a particular prey type in the diet of a predator can, for example, be easily detected with small sample sizes (Duffy & Jackson 1986). Indeed, if a single prey species/type accounts for 10 % of some seabird's diet, then the probability of it being detected in a sample of 10 stomachs is 65 % (Duffy & Jackson 1986). Theoretically then, the sample size of stomach content analyses can be considered acceptable when the rate of accumulation of new prey species reaches an asymptote. In other words, each additional sample has a proportionally smaller chance of adding new prey types to a species' diet and the sample size is deemed appropriate when 99 % of the prey found in a particular stomach have previously been found in other samples (Korschgen 1948; Hanson & Graybill 1956). Enormous and sometimes unrealistic sample sizes are usually required to quantitatively (proportion of each prey type) estimate diet, however. This is not always logistically feasible and can be associated with strong ethical considerations (Duffy & Jackson 1986). Hanson and Graybill (1956) indicated that the sample size required to determine the relative importance 'p' of a theoretical prey species, with a 95 % chance of it being within a certain percentage 'd' of the true value, can be calculated as $n = 4p(100 - p)/d^2$. Correctly estimating the proportion of a smaller 'p' (less important prey group) requires a larger sample size; the food with 'p' closest to 50 will theoretically then determine the minimal sample size required to correctly estimate the proportion of most prey in the diet of a predator (Hanson & Graybill 1956). In this study, the number of prey species/groups in the diet of Barau's Petrel remained stable after only 14 stomachs, which indicates that most prey species/groups would have been identified. Furthermore, using cephalopods (RPO 59.53 %; n = 36) from this study, the prey proportions presented should be within less than 24 % of

their true values. Whilst this margin of error may seem large, it does support the hypothesis that cephalopod prey dominates the diet of Barau's Petrel, whereas crustaceans and fish are of lesser importance. It is noted though, that some of the obvious biases associated with stomach content analyses (i.e. differential digestion rates of prey) may have reduced the detectability of certain prey groups (e.g. scavenged animals, softer bodied organisms, and smaller groups; reviewed in Duffy & Jackson 1986, Iverson *et al.* 2004, and Barrett *et al.* 2007), whereas the importance of other groups (e.g. larger cephalopods) would likely have been overestimated (Furness *et al.* 1984; Duffy Jackson 1986; Iverson *et al.* 2004; Barrett *et al.* 2007).

Two final drawbacks to consider relate to the timing of stomach content collection. Indeed, the prey diversity identified in this study is only relevant to the spatial and temporal scope of the samples from which these conclusions were drawn. Firstly, all samples were taken from birds during the mid- to late- breeding season (December – April). Thus, these samples cannot then be used to infer diet over the early breeding stages (September – December), or over the interbreeding period (April – September) when the birds are known to forage elsewhere (Pinet *et al.* 2011b). Secondly, some Procellariiformes, including Barau's Petrel, also occupy a dual foraging strategy during the breeding season; adults alternate long foraging trips, where they feed for themselves, with numerous short trips, to sustain their young (e.g. Weimerskirch *et al.* 1993, 1994a; Chaurand & Weimerskirch 1994; Baduini & Hyrenbach 2003; Congdon *et al.* 2005; Pinet *et al.* 2012). This, combined with the fact that the present study was almost entirely reliant on the accumulated prey remains (i.e. hard structures), implies that the geographic origin of these structures, which can remain in the bird's stomach for extended periods, is not known. Fresh items from the stomachs of adult birds would have been sourced from waters immediately around Réunion for the pre-

mature birds and hence would not be a reflection of the adult diet. Similarly, accumulated prey items remain in the stomachs of adults for much longer periods and could therefore reflect theirs and not the downy chicks' or fledglings' diets.

3.4.4. Conclusions and recommendations

Two other descriptions of the diet of Barau's Petrel exist. Each contradicts the other and hence, reanalysis was necessary. Not unlike the conclusions made by Kojadinovic *et al.* (2007), these data confirm that cephalopod remains dominate the accumulated prey items in the stomachs of Barau's Petrel, therefore suggesting that these animals would constitute an important (and possibly the main) dietary component. Fish remains (i.e. bones, scales or eye lenses) also accumulate in the stomachs of seabirds, but were rarely recorded in this study and so this prey class would seem to be of lesser importance to the birds. However, it is noted that these structures are eroded much quicker than cephalopod and crustacean hard tissues and so the importance of this prey class has likely been underestimated. Numerous other prey groups (i.e. marine insects, crustaceans, vegetative material, and molluscs) were also recorded, suggesting that the diet of this species is more varied than has previously been thought (Stahl & Bartle 1991; Kojadinovic *et al.* 2007; Pinet *et al.* 2009). Some of these would certainly have been consumed as secondary prey (i.e. molluscs; Cherel *et al.* 2000) or, in the case of terrestrial vegetative material, during a critical stage at the end of the breeding season when pre-fledglings begin starving in the nest just prior to fledging (Brooke 1990; Pinet *et al.* 2009). Plastics were also recorded among the stomach contents of breeding Barau's Petrel. This is worrying for two reasons. Firstly, one of the more frequently encountered plastics was a type of fishing gut, which implies some form of undescribed interaction with line-based fisheries. The literature would suggest that these birds do not

often associate with fishing vessels (Jaquemet *et al.* 2004), and hence additional research is required to understand the nature and severity of this interaction. Secondly, recent studies have found that plastics act as vectors of heavy metals and correlations exists between plastic ingestion, the exposure and accumulation of heavy metals in tissues, and mortality in marine birds (Lavers *et al.* 2014; Lavers & Bond in press). These two newly identified threats might have severe conservation implications on the demographic parameters of Barau's Petrel in the near future.

This study has also shed light on the foraging behaviour of this enigmatic species. These data have indicated an opportunistic feeding behaviour during the breeding season, which is not unlike what has been found in many other Procellariiformes (Imber 1973; Schramm 1986; Bester *et al.* 2010). The birds appear to rely as heavily on active nocturnal predation (Pinet *et al.* 2012), on vertically migrating cephalopods, as they do on scavenging floating carcases at the surface of the water column, which would presumably happen during daylight hours. This implies some degree of resilience against prey distribution shifts and/or changes in the community assemblage of the areas to the south and east of Madagascar since these birds would adjust their diet composition in response to changes in relative prey availability and/or accessibility.

CHAPTER 4: INTRA-ANNUAL VARIATION IN THE FORAGING ECOLOGY OF A MIGRATORY, TRULY-PELAGIC SEABIRD: INSIGHTS FROM AN ANALYSIS OF CARBON AND NITROGEN STABLE ISOTOPES.

4.1. Introduction

Our knowledge of seabird biology is in its infancy (Shealer 2002). Past research has been limited in scope, typically addressing various terrestrial aspects of the birds' biology during the breeding season. It is at this time that many species congregate in large numbers to reproduce (Danchin & Wagner 1997; Coulson 2002), and, at least for most truly pelagic seabirds, this is the only time when they are accessible for study (Ballance & Pitman 1999; Shealer 2002). Far less is known about the at-sea behaviour of marine birds. During the breeding season, adults commute between their colonies and feeding areas to provision their offspring and/or to restore their own body condition (e.g. Phillips *et al.* 2005; Pinaud & Weimerskirch 2005, 2007; González-Solís *et al.* 2007; Egevang *et al.* 2010; Quillfeldt *et al.* 2010a, 2010b). This central place foraging imposes great energetic constraints on the adults, which limits their overall foraging distribution during breeding and results in significant inter-seasonal variations in their energy demands, which in turn are influenced by variations in environmental and oceanographic conditions (Stearns 1992; Pinaud & Weimerskirch 2005). Over the interbreeding or non-breeding period, defined as the time between each reproductive event when adults are no longer constrained by the energy demands of their offspring, many species disperse widely out at sea (e.g. Phillips *et al.* 2004; González-Solís *et al.* 2007). Some species even undertake long-distance migrations at this time; a behaviour that is especially pronounced in higher-latitude species (González-Solís *et al.* 2007; Egevang *et al.* 2010; Quillfeldt *et al.* 2010a, 2010b). Recent evidence suggests that a number of

tropical species also perform similar movements, sometimes over equally impressive distances (e.g. Catry *et al.* 2009b; Pinet *et al.* 2011b; Le Corre *et al.* 2012). Migration is typically a behavioural adaptation in response to seasonal changes in resource availability and/or habitat quality (Schreiber 2002). It can be especially important in helping birds restore the body condition lost after costly reproductive events, particularly in the case of marine birds, which show high levels of parental investment during breeding (Schreiber 2002). Consequently, environmental predictability or unpredictability during the interbreeding period has great potential to influence seabird population dynamics as well as their reproductive output in successive breeding seasons (Ramos 2000; Ramos & Pacheco 2003; Surman & Nicholson 2009).

Considerable scientific attention has been devoted to understanding the at-sea foraging ecology of marine birds. Early studies were almost entirely limited to ship-based surveys (e.g. Bailey 1968; Pocklington 1979; Abrams & Miller 1986; Dunlop *et al.* 1988; Stahl & Bartle 1991). This work has provided a fundamental basis on which a greater understanding of seabird foraging behaviour, habitat utilisation, and prey acquisition has since been built, but these studies were limited in scope and could often be misleading (Shealer 2002; Henckel *et al.* 2007; Fair *et al.* 2010). Recent technological advances have furthered our ability to study the foraging ecology of breeding and non-breeding seabirds, however. Animal-attached electronic sensing, particularly satellite transmitters (including GPS devices), represents one of the greatest advances in marine avifaunal research as it allows scientists to gather a wide range of information about the birds' behaviour whilst at-sea, including the precise areas/habitats that are used (Ropert-Coudert & Wilson 2005; Fair *et al.* 2010). Several limitations preclude their use, however, particularly where smaller (< 400 g) species and the interbreeding period are concerned (Fair *et al.* 2010). Smaller, but

less precise, devices known as a GLS, have recently become available that partially overcome the shortfalls of GPS transmitters (Cochran 1980; Barron *et al.* 2010; Fair *et al.* 2010). However, the cost of deploying such devices (particularly in GPS devices) is high, which sometimes limits replication. Furthermore, the deployment of GLS devices rests on the assumption that they can be recovered, only after which the data can be accessed. As a result, the statistical reliability of tracking studies can often be questionable, particularly where the unpredictability in animal behaviour reduces recovery rates of the biologgers (Ropert-Coudert & Wilson 2005). In addition to this, tracking studies are somewhat limited in what information they are able to provide; researchers are only able to speculate as to when the birds might be feeding and what this implies in terms of the species' diet composition for example. Hence, various indirect methods of studying seabird foraging ecology have been suggested, as complimentary techniques to support tracking studies, and one such approach is stable isotope analysis (SIA; Bond & Jones 2009).

Traditional SIA follows the principle that the ratios of carbon (^{13}C over ^{12}C; denoted as $\delta^{13}C$) and nitrogen (^{15}N over ^{14}N; denoted as $\delta^{15}N$) isotopes in the tissues of a consumer reflect those of its prey in a predictable manner, depending on the isotopic signatures at the base of the food web (Post 2002; Shealer 2002; Fry 2006; Barrett *et al.* 2007; Bond & Jones 2009). One of the main advantages of this is that it combines the benefits of food web (carbon isotopes as spatial tracers) and trophic level (nitrogen isotopes as dietary indicators) paradigms in marine ecological research (Post 2002; Fry 2006). In particular, the composition of particulate organic matter in the ocean is affected by various processes that regulate the $\delta^{13}C$ signatures at the base of marine food webs and the resulting spatial variability in baseline signatures is known to be incorporated into, and conserved through, marine food webs (Wallace *et al.* 2006; Cherel & Hobson 2007; Ménard *et al.* 2007; Bond &

Jones 2009). Consequently, the ratios of heavy to light carbon isotopes have the potential to characterise isotopically distinct regions (e.g. Kroopnick 1985; Post 2002; Cherel *et al.* 2006; Fry 2006; Cherel & Hobson 2007; Phillips *et al.* 2009; Militão *et al.* 2013). For example, it is possible to differentiate high versus low latitudes, inshore versus offshore, and pelagic versus benthic ecosystems using carbon isotopes (Minagawa & Wada 1984; Kroopnick 1985; Shealer 2002; Barrett *et al.* 2007; Bond & Jones 2009). $\delta^{15}N$ signatures at that base of marine food webs also vary in space and time according to the composition of particulate organic matter, which in turn is determined by the nitrogen sources available to the primary producers (nitrates, ammonium or N_2 gas; Wada & Hattori 1991). Higher trophic level organisms preferentially retain the heavier isotope from their prey, referred to as isotopic discrimination, and so are enriched (higher $\delta^{15}N$) in terms of nitrogen relative to their diet (Post 2002; Fry 2006; Bond & Jones 2009). This predictable shift between trophic levels permits quantitative diet estimation using mixing models, if the isotope signature of potential prey groups are available, and trophic niche approximation under the assumption that the isotope baseline for the particular ecosystem in question is known (Fry 2006; Bond & Jones 2009). Complementing this, the differential turnover rates of diverse tissue groups (e.g. blood, feather, and bone) give SIA the added benefit of being able to provide data on the foraging ecology of a species over varying time scales (Hobson & Clark 1992, 1993; Bond & Jones 2009). In avian isotope studies, blood and feathers are most commonly used. Blood is metabolically active and typically integrates isotopes over a period of days to weeks and feathers, which are metabolically inactive once grown, reflect the signature of the food consumed during moult (Hobson & Clark 1992, 1993; Fair *et al.* 2010). Most seabirds endure moult during the non-breeding season (e.g. Ashmole 1963, 1968; Diamond 1976), implying that well-structured SIAs, which utilise both blood and feathers, have the potential to

describe the trophic relationships of seabird species/communities over complete annual cycles (e.g. Bearhop *et al.* 2002; Cherel *et al.* 2005, 2007a; Quillfeldt *et al.* 2008b; Tierney *et al.* 2008b; Connan *et al.* 2014). However, it has to be noted that comparisons between the isotope signatures of blood and feathers cannot be made without first accounting for the prey-tissue-specific isotope discrimination factors (Cherel *et al.* 2014b). Indeed, the isotope signatures of these tissues are known to differ, with feathers being enriched in both ^{15}N and ^{13}C compared to blood (Quillfeldt *et al.* 2008a), even when feathers and blood are synthesized over the same time period (Bond & Jones 2009).

Gadfly petrels (including the genus *Pterodroma*) breed on tropical, subtropical, and sub-Antarctic oceanic islands. They are exclusively pelagic and often forage at great distances from their colonies during the breeding period (e.g. MacLeod *et al.* 2008a, 2008b; Pinet *et al.* 2012). They all then disperse even more widely out at-sea during the interbreeding period (e.g. Pinet *et al.* 2011b), but very little is known about this behaviour. Some research has been devoted to understanding the at-sea ecology of the Barau's Petrel, however. Pinet *et al.* (2011a, 2011b, 2012) applied biologgers (GPS-PTT and GLS) onto adult birds and, in doing so, identified this species' principal foraging areas during both the breeding and non-breeding seasons. Their results suggested that birds consistently used areas to the southwest of Réunion Island during the breeding season (Pinet *et al.* 2012), after which a collective migration occurred to the central Indian Ocean (Pinet *et al.* 2011b). Inferences from ship-based surveys and other sight records suggest that these birds disperse widely across the whole Indian Ocean, however (Chapman & Cheshire 1987; Dunlop *et al.* 1988; van Marle & Voous 1988; Stahl & Bartle 1991; Van Den Berg *et al.* 1991; Lambert 2001; Pinet *et al.* 2009). The exact origin/identity of these birds remains uncertain and misidentification of this species is unlikely in the region (Sinclair & Lagrand 2003; Pinet *et al.*

2009). Recently, in addition, Kojadinovic *et al.* (2008) investigated the trophic ecology of seabirds from Réunion Island, including Barau's Petrel, using SIA of feathers and hepatic tissue. This research delivered some basic information about the trophic position of this species, but gave little insight into the expected intra-annual, sexual, and ontogenetic differences in its diet and habitat use. Thus, a more complete isotope study is necessary to generate an adequate understanding of the at-sea biology of the endangered Barau's Petrel. As such, this chapter aims to use the ratios of carbon and nitrogen stable isotopes (SI) in various tissues from Barau's Petrel to:

a) Provide new data to complement the preliminary isotope work of Kojadinovic *et al.* (2008) and the tracking research by Pinet *et al.* (2011, 2012).
b) Describe the trophic position of adult and pre-mature birds during the breeding season.
c) Describe the trophic position of adults during the non-breeding period.
d) Identify any sources of temporal, ontogenetic, or sexual variation in trophic position and/or foraging grounds of this species.

4.2. Materials and methods

4.2.1. Tissue selection

Whole blood and feathers were selected for SIAs because of their ability to be easily and non-destructively sampled from birds during a single sampling session and since they provide information over very different time scales. Whole blood has a short to medium turn-over rate (± 15 days) whereas feathers reflect signatures from the time at which they were grown (Hobson & Clark 1992, 1993). In this instance, blood reflected the isotopes integrated at various stages of the breeding season whereas feathers reflected the

interbreeding period (complete moult occurs between March and September; Pinet *et al.* 2012) for adults and secondary down and true-feathers provided information on the early- and mid- growth stages in downy chicks and fledglings, respectively. Secondary down was chosen to minimise the maternal trophic effect on downy chick isotope signatures, since this tissue reflects prey eaten after hatching and not the nutrients from within the egg as would be the case for primary down (Browne *et al.* 2011).

4.2.2. Sample description

Adult birds were sampled five times throughout the breeding seasons of 2013/2014. Sampling trips roughly corresponded with the return of adult birds from the non-breeding grounds (16 September 2013), the first and second incubation shifts of the pre-laying exodus (06-07 and 20-22 November 2013), the incubation period (17-18 December 2013), and the early chick-rearing period (27-28 January 2014), as was based on Pinet *et al.* (2011b, 2012). All-white auxiliary feathers were plucked from each individual and blood samples (± 0.2ml) were taken from every bird on each excursion irrespective of whether they had been sampled earlier on in the season or not. Although moult cycles are not well-known for auxiliary feathers, this feather tract was considered more ethically appropriate since it contributes nothing to a birds' flight capabilities (as primaries do, for example) or insulation (as body feathers do, for example). Downy chicks and fledglings were each sampled once over the early (27-28 January 2014) and late (18-19 March 2014) chick-rearing period, respectively. Secondary down feathers and approximately 0.2 ml of blood were taken from each downy chick and all-white auxiliaries were taken from fledglings. Logistical constraints prevented the collection of blood samples from fledglings and hindered the possibility of separating blood components after all other sampling trips.

4.2.3. Sample preparation

Traditionally, lipids are removed from lipid-heavy tissues (e.g. liver and muscle) prior to SIA (Bond & Jones 2009). Whilst there is little doubt about the necessity of this procedure in those tissues, the situation for seabird blood is not nearly as clear. Procellariiformes typically have lipid-rich blood that usually requires lipid correction (Bond *et al.* 2010), but this is not always the case (e.g. Bond & Jones 2009; Richoux *et al.* 2010; Cherel *et al.* 2014a). Furthermore, it is now known that chemical lipid extraction can have an effect on $\delta^{15}N$ so that unnecessary use of this technique should be avoided (Murry *et al.* 2006). Instead, whole blood aliquots were oven dried at 50 °C for 24 hours and then homogenised using a mortar and pestle cleaned with absolute methanol. Ten sub-samples were then experimentally analysed to estimate lipid content and to deduce whether lipid-extraction was necessary. All exhibited low C:N ratios (< 4), precluding the necessity of lipid removal (see also the C:N ratios in Table 4.4; Bearhop *et al.* 2000; Post *et al.* 2007; Richoux *et al.* 2010; Cherel *et al.* 2014a). Many feathers were 'contaminated' by trace amounts of waterproofing oils (stomach oil and/or preen gland oils), excrement, and material from around the colony so that cleaning was essential. A single all-white feather, per individual bird, was vigorously washed in a 2:1 chloroform-methanol solution on a vortex set at high speed for two minutes. Subsequently each was then submerged in absolute methanol within a microreaction vessel and placed into an ultrasonic bath for three minutes. Next, the samples were triple bathed in type-3 distilled water before being oven dried at 50 °C for approximately 36 hours (N. Voogt. pers. comm). Feather samples were then cut into small fragments using cleaned (again with absolute methanol) stainless steel scissors prior to sample analysis.

4.2.4. Sample analysis

Subsequent to sample preparation, subsamples of between 0.8 and 1.2 mg (average 0.97 ± 0.09 mg) of each tissue sample were weighed into individual 8x5 mm tin cups. Sixty two samples, together with 34 standards, were sorted into each SI tray for analysis. Twenty nine of the standards were beet sugar and ammonium sulphate (in-house standards), and five were certified protein standard casein calibrated against International Atomic Energy Agency (IAEA) standards (IAEA-CH-6 and AIEA-N-1). Analyses were performed at the IsoEnvironmental isotope facility in the Department of Botany at Rhodes University (Grahamstown, South Africa) using an ANCA-SL analyser coupled with a SERCON 20-20 Iso Ratio Mass Spectrometer (IRMS).

SI abundances are expressed in the standard notation as the deviation from standards in parts per thousand (‰), as follows:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1\,000$$

Where 'X' is either ^{13}C or ^{15}N and 'R' is the corresponding ratio of $^{13}C/^{12}C$ (denoted as $\delta^{13}C$) or $^{15}N/^{14}N$ (denoted as $\delta^{15}N$; DeNiro & Epstein 1978; Bond & Jones 2009). The '$R_{standard}$' values were based on the Vienna-Pee Dee Belemnite (VPDB) for ^{13}C and atmospheric N_2 (air) for ^{15}N. Replicate measurements of internal laboratory standards indicated measurement errors of 0.07 ± 0.01 ‰ and 0.10 ± 0.02 ‰ for carbon and nitrogen isotopes, respectively.

4.2.5. Trophic level estimation

The naturally occurring isotopes of nitrogen undergo stepwise biomagnification along the length of the food web, tending to increase by between 3 and 4‰ per trophic level (Post 2002; Fry 2006). Thus, trophic levels were calculated from the whole blood isotopic measurements, for each grouping (sex, age, and stage), using the following equation:

$$TL = 2.0 + \frac{\delta^{15}N_{secondary/tertiary\ consumer} - \delta^{15}N_{primary\ consumer}}{3.2}$$

Where '$\delta^{15}N_{secondary/tertiary\ consumer}$' is the nitrogen signature of an individual bird, '$\delta^{15}N_{primary\ consumer}$' is the nitrogen reference baseline at the second trophic level, taken from the literature (Ménard *et al.* 2014), and 3.2 ‰ is an estimate of the average increase of $\delta^{15}N$ per trophic level (Sweeting *et al.* 2007; Cherel *et al.* 2010). Most primary producers exhibit high spatio-temporal variability in $\delta^{15}N$ signatures, which complicates their direct use in estimating isotope baselines (Post 2002). By contrast, primary consumers are known to integrate nitrogen isotopes over much longer periods and so the use of these animals as indicators of nitrogen baselines is believed to help reduce the errors associated with such calculations (Post 2002; Martínez del Rio *et al.* 2009). Little is known about the isotope composition of micronekton in the areas to the south and east of Madagascar, however. Thus, salps, which are abundant filter-feeders in the southern Mozambique Channel, near where adult Barau's Petrel forage during the breeding season (Pinet *et al.* 2012), were chosen as the primary consumers for estimating trophic levels in this study. An averaged (n = 5) nitrogen value (5.2 ‰) for *Salpa maxima* was taken from Ménard *et al.* (2014) for this purpose. This is marginally lower than what was expected, based on recent evidence of

isotope signatures of particulate organic matter from between Antarctica and the Equator in the Indian Ocean basin, for the latitudes that encompass the areas to the south and east of Madagascar (S. Jaquemet *et al.* unpub. data). Trophic level estimation was not attempted for non-breeding birds since insufficient baseline information exists about the areas being used.

Since few other attempts have ever been made to estimate the trophic level of seabirds breeding in the western Indian Ocean, the isotope signatures of seabird blood presented in Cherel *et al.* (2008) from Europa Island in the southern Mozambique Channel (for location refer to Chapter 2, Figure 2.1), were analysed alongside the Barau's Petrel signatures, thus permitting comparisons among species. All trophic levels are reported as averages with standard deviations, except for Europa's seabirds for which only an average is presented for each species.

4.2.6. Data analysis

Prior to statistical analyses, all data were checked for normality using the Shapiro-Wilk test, where normal distribution was accepted when the P-value exceeded 0.05. Furthermore, Levene's test was used to determine homogeneity of variances in cases where several univariate sample groups were compared. A correlation analysis was also performed on overall signatures as well as seasonal and sex data in an attempt to identify relationships between both $\delta^{13}C$ and $\delta^{15}N$, and bird weight and $\delta^{15}N$. Since these assumptions (i.e. normality, homogeneity of variances, relationship between $\delta^{13}C$ and $\delta^{15}N$, and weight and $\delta^{15}N$) were almost never met, a range of nonparametric tests were used to investigate differences between groups (sexes, ontogenetic, and breeding stages). This was followed, wherever necessary, by appropriate post-hoc tests.

The influence of tissue type on carbon and nitrogen isotope signatures was first investigated by means of the Wilcoxon signed-rank test. Blood samples were averaged for individuals that were sampled multiple times over the course of the breeding season. Thereafter, Mann-Whitney U tests were used to explore the effects of age and sex on the carbon and nitrogen isotope signatures. Finally, the Kruskal-Wallis test was used to investigate seasonal/intra-annual variances in the $\delta^{13}C$ and $\delta^{15}N$ signatures of whole blood from adult birds. Multiple pairwise comparisons, using Mann-Whitney U tests, and the resulting uncorrected P-values were then used to elucidate the origin of any observed differences. Whether pairwise P-values, such as these, require correction or not remains a greatly debated topic. Indeed, whilst some argue that alpha-inflation correction procedures, such as Bonferroni, are necessary to control for Type 1 errors, others stress that these procedures are over-cautious and result in the loss of biologically meaningful information (discussed in Garcia 2004). In this manner, Moran (2003) addressed a situation whereby relatively small P-values, all of which had a clear logical link, were lost after the application of the sequential Bonferroni rule. This situation was mirrored when Bonferroni correction was applied to the data from the present study (particularly those presented in Table 4.2), and the decision was made to abandon alpha-correction in place of 'following one's own logic,' as recommended by Moran (2003). All statistical analyses were performed using Past 3.01 software (Hammer 2013), and results were graphically represented using Microsoft Excel 2007.

4.3. Results

4.3.1. Influence of tissue type on the ratios of carbon and nitrogen isotopes

The paired $\delta^{13}C$ and $\delta^{15}N$ signatures of feathers and blood from downy chicks differed significantly (Wilcoxon signed-rank test: W = 28.00, P = 0.02 in both cases; Table 4.1). Feathers were enriched, relative to whole blood, by 1.45 ± 0.15 ‰ and 1.50 ± 0.96 ‰, for $\delta^{13}C$ and $\delta^{15}N$, respectively. Not surprisingly, there was also a highly significant difference in $\delta^{13}C$ between paired blood and feather samples taken from adult birds (Wilcoxon signed-rank test: W = 1953, P < 0.01; Table 4.1), with feathers being enriched relative to blood by about 3.42 ± 0.50 ‰. In this case feathers were also enriched in $\delta^{15}N$, by about 0.22 ± 0.91 ‰, but the difference was not significant (Wilcoxon signed-rank test: W = 1202.5, P = 0.11; Table 4.1). This result is confounded by the temporal variability in foraging behaviour of the adult birds, however, for which a greater investigation is provided further on in this section.

Table 4.1: $\delta^{15}N$ and $\delta^{13}C$ signatures (mean ± SD; ‰) of adult and downy chick Barau's Petrel blood and feathers with the results of the Wilcoxon signed-rank tests, altogether indicating the influence of tissue type on isotope signatures. Asterisks denote significant differences (P < 0.05).

	$\delta^{15}N$ (‰)				$\delta^{13}C$ (‰)			
Age class	Blood	Feather	W	P	Blood	Feather	W	P
Adults (n = 62)	12.71 ± 0.55	12.93 ± 0.89	1202.50	0.11	-18.50 ± 0.32	-15.09 ± 0.28	1953.00	< 0.01*
Downy chicks (n = 7)	11.58 ± 0.64	13.08 ± 0.58	28.00	0.02*	-19.39 ± 0.20	-17.94 ± 0.16	28.00	0.02*

*this table, unlike the figures presented below, includes only those individuals for which at least one blood sample and feathers were taken so that sample sizes may appear inconsistent.

4.3.2. Sex and ontogenetic related variation in the isotopic composition of whole blood and feathers

Blood samples from downy chicks were significantly different to those samples taken from adult birds over the same breeding period in terms of both $\delta^{13}C$ and $\delta^{15}N$ (Mann-Whitney U test: U = 0 and 3.5, respectively, $P < 0.01$ in both cases; Figure 4.1). Adult samples were enriched by about 0.77 ‰ about 1.08 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, compared to those of downy chicks. Similarly, the overall signatures from adult males differed from those of the females in terms of $\delta^{13}C$ (Mann-Whitney U test: U = 563.5; $P < 0.001$), but, in this case, the $\delta^{15}N$ values were almost identical (Mann-Whitney U test: U = 1022.5; P = 0.99)

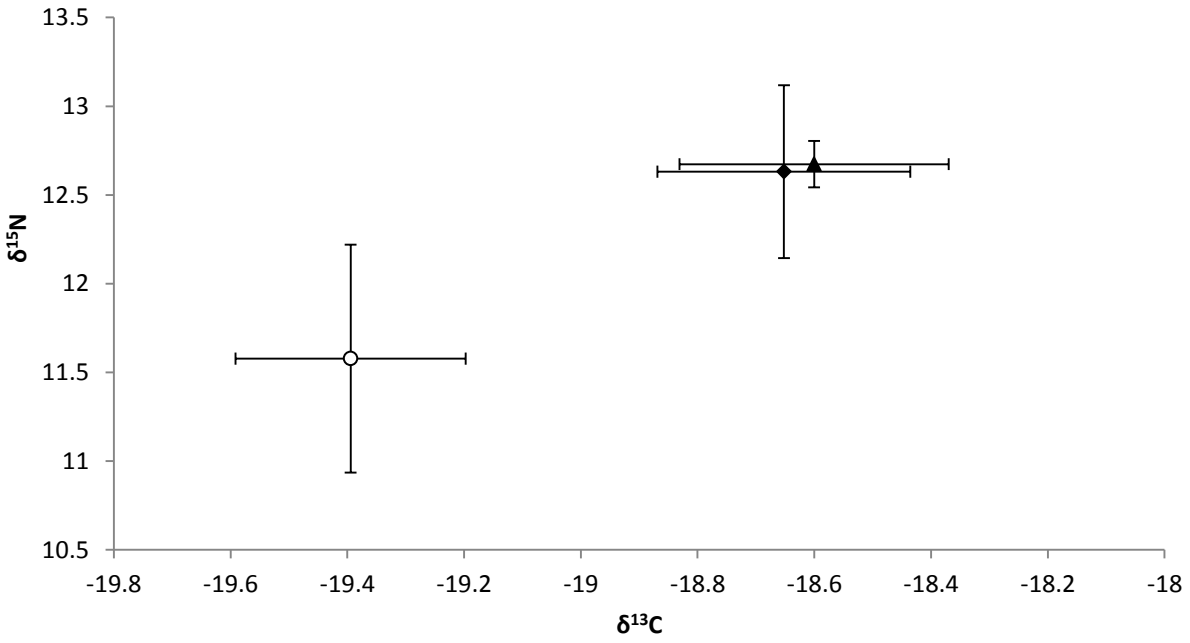

Figure 4.1: $\delta^{13}C$ and $\delta^{15}N$ signatures (mean ± SD; ‰) of whole blood from chick-rearing adult male (filled diamond) and female (filled triangle) Barau's Petrel (*Pterodroma baraui*) as are compared with those of downy chicks (open circle). Adult male n = 3, adult female n = 5, downy chicks n = 7.

Feather samples from adult birds were similar between the sexes in terms of $\delta^{13}C$ (Mann-Whitney U test: U = 474.5, P = 1.0), but significantly different with respect to $\delta^{15}N$ (Mann-Whitney U test: U = 288.5, P < 0.01; Figure 4.2). Here the samples from adult females were enriched in terms of $\delta^{15}N$, by about 0.55 ‰, relative to those from male birds. Much more variability was also observed in the adult feather samples in terms of $\delta^{15}N$ (variance 0.75 and 0.72 for males and females, respectively), compared with $\delta^{13}C$ (variance 0.06 and 0.13 for males and females, respectively; Figure 4.2). Adult feathers were significantly enriched in $\delta^{13}C$ relative to the samples taken from downy chicks and fledglings (Kruskal-Wallis test: H = 42.68, P = 5.33 x 10^{-10}), whilst no differences in $\delta^{15}N$ were detected among the ontogenetic groups (Kruskal-Wallis test: H = 0.94 P = 0.62). Secondary down and true feathers, taken from downy chicks and fledglings, during the same season, differed with respect to $\delta^{13}C$ (Mann-Whitney U test: U = 4, P < 0.01; Figure 4.2) but not in terms of $\delta^{15}N$ (Mann-Whitney U test: U = 26.5, P = 0.23; Figure 4.2). Down tended to be enriched in terms of $\delta^{15}N$ (+ 0.33 ‰) but depleted in terms of $\delta^{13}C$ (- 0.72 ‰) relative to true feathers from fledglings.

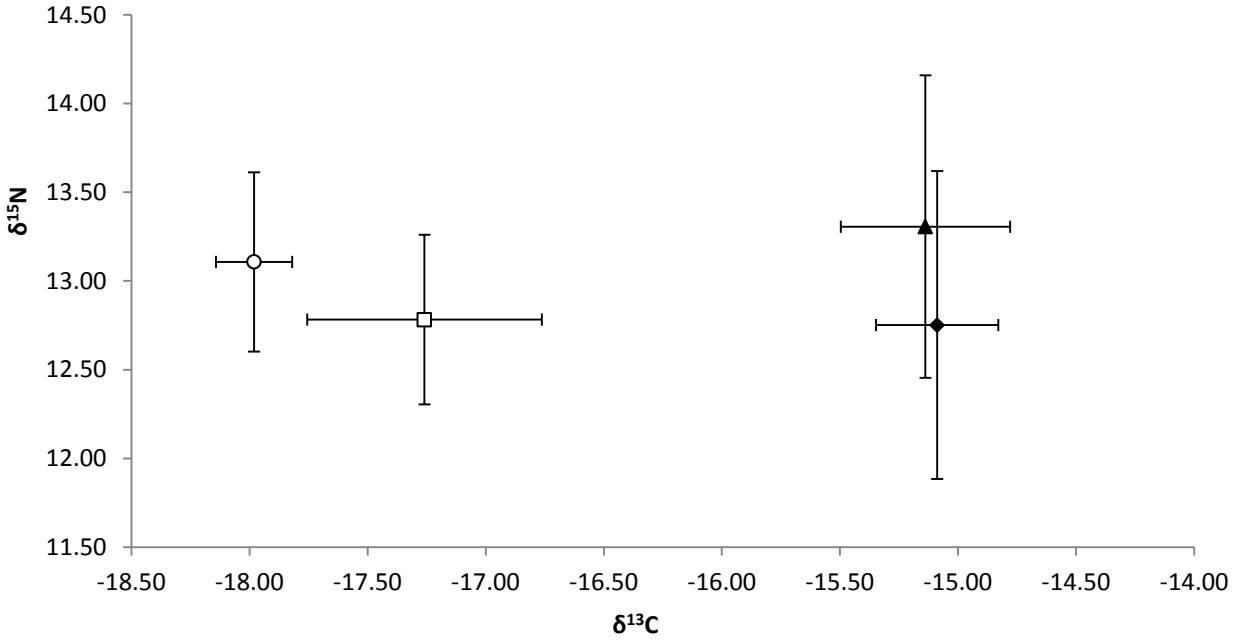

Figure 4.2: $\delta^{13}C$ and $\delta^{15}N$ signatures (mean ± SD; ‰) of feathers from adult male (filled diamond) and female (filled triangle) Barau's Petrel (*Pterodroma baraui*) as are compared with downy (open circle) and true (open square) feathers from downy chicks and fledglings. Adult male n = 38, adult female n = 25, down n = 9, fledgling feathers n = 9.

4.3.3. Intra-annual variation in isotope composition of whole blood from adult Barau's Petrel

Seasonal variations in the $\delta^{13}C$ (Kruskal-Wallis test: $H = 44.5$, $P < 0.001$) and $\delta^{15}N$ (Kruskal-Wallis test: $H = 46.4$, $P < 0.001$) signatures were detected from whole blood samples taken from adults (Figure 4.3). The signatures from breeding birds were consistently depleted in terms of both $\delta^{13}C$ and $\delta^{15}N$ relative to the samples taken from adult birds immediately upon their return from the non-breeding grounds (pairwise Mann-Whitney U test: $P < 0.01$ in all cases; Figure 4.3). Whilst $\delta^{13}C$ did vary through the breeding season, no differences were found to be statistically significant (pairwise Mann-Whitney U test: all $P > 0.40$; Figure 4.3). Significant differences in $\delta^{15}N$ were observed between the pre-laying

exodus and both the incubation and chick-rearing breeding stages (pairwise Mann-Whitney U test: both $P < 0.2$; Figure 4.3), however, whilst no significant variations in $\delta^{15}N$ existed between the incubation and chick-rearing period (pairwise Mann-Whitney U test: $P = 0.18$; Figure 4.3). Here the nitrogen signatures from the pre-laying period were depleted by about 0.43 ‰ and 0.32 ‰ compared with the signatures for the incubation and chick-rearing stages, respectively (Figure 4.3).

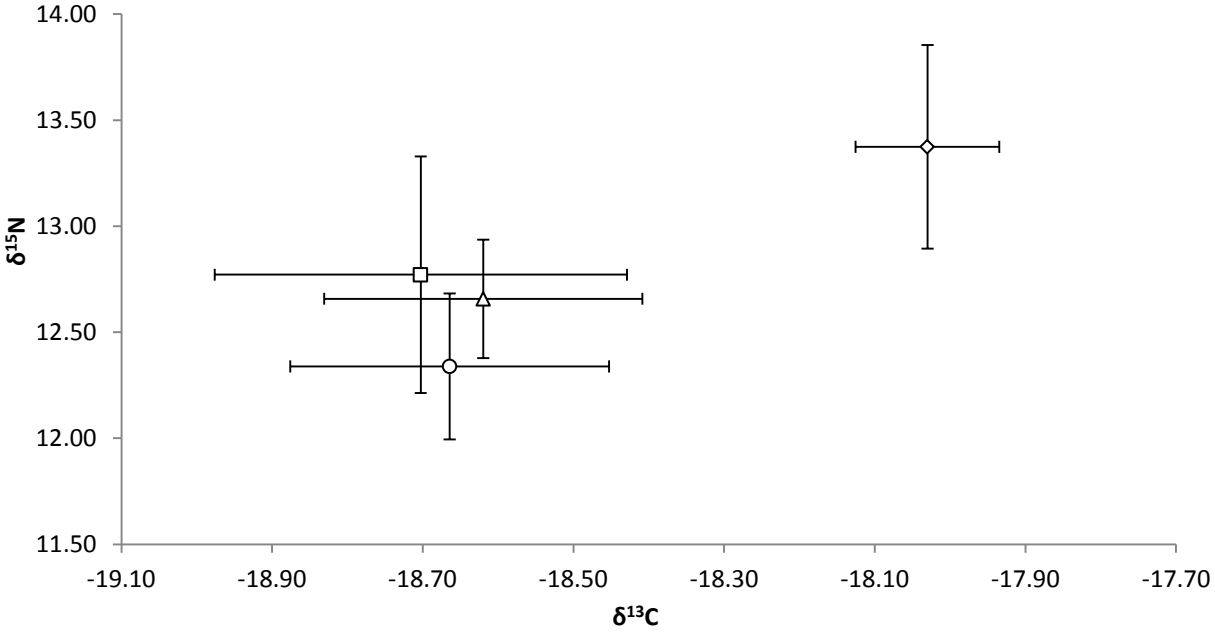

Figure 4.3: $\delta^{13}C$ and $\delta^{15}N$ signatures (mean ± SD; ‰) of whole blood from adult Barau's Petrel (*Pterodroma baraui*) during non-breeding season (diamond), pre-laying exodus (circle), incubation (square), and chick-rearing periods (triangle). Non-breeding n = 19, pre-laying exodus n = 43, incubation = 26, chick-rearing n = 8.

A number of additional differences became apparent for both $\delta^{13}C$ (Kruskal-Wallis test: $H = 56.0$, $P < 0.001$) and $\delta^{15}N$ (Kruskal-Wallis test: $H = 47.7$, $P < 0.001$) once sex was also

taken into consideration. The carbon signatures of whole blood from non-breeding males and females differed from one another (Table 4.2a; Figure 4.4). Furthermore, the carbon signatures of non-breeding birds were significantly different from those of all of the breeding stages in both sexes (Table 4.2a; Figure 4.4). Females were also always enriched in $\delta^{13}C$, by 0.16 ± 0.14 ‰, compared to the males over the same breeding stage (Figure 4.4). Male birds from the non-breeding period differed from all of the breeding stages (both sexes), in terms of the $\delta^{15}N$ composition of their blood, whilst non-breeding females differed only from breeding females (all stages) and male birds during the pre-laying exodus (Table 4.2b; Figure 4.4). It is compelling to note that all differences in $\delta^{13}C$ and $\delta^{15}N$ within the breeding season were associated with the pre-laying exodus and incubation periods (Tables 4.2a and 4.2b; Figure 4.4). $\delta^{13}C$ signatures differed between male and female birds within both the pre-laying exodus and incubation periods and each sex during the pre-laying exodus was significantly different from the other sex during the incubation period (Table 4.2a; Figure 4.4). Finally, the $\delta^{15}N$ signatures of the incubating males were very highly significantly different from the signatures of both sexes during the pre-laying exodus, whilst the pre-laying signatures (both sexes) were also dissimilar to those of females during the early chick-rearing period (Table 4.2b; Figure 4.4).

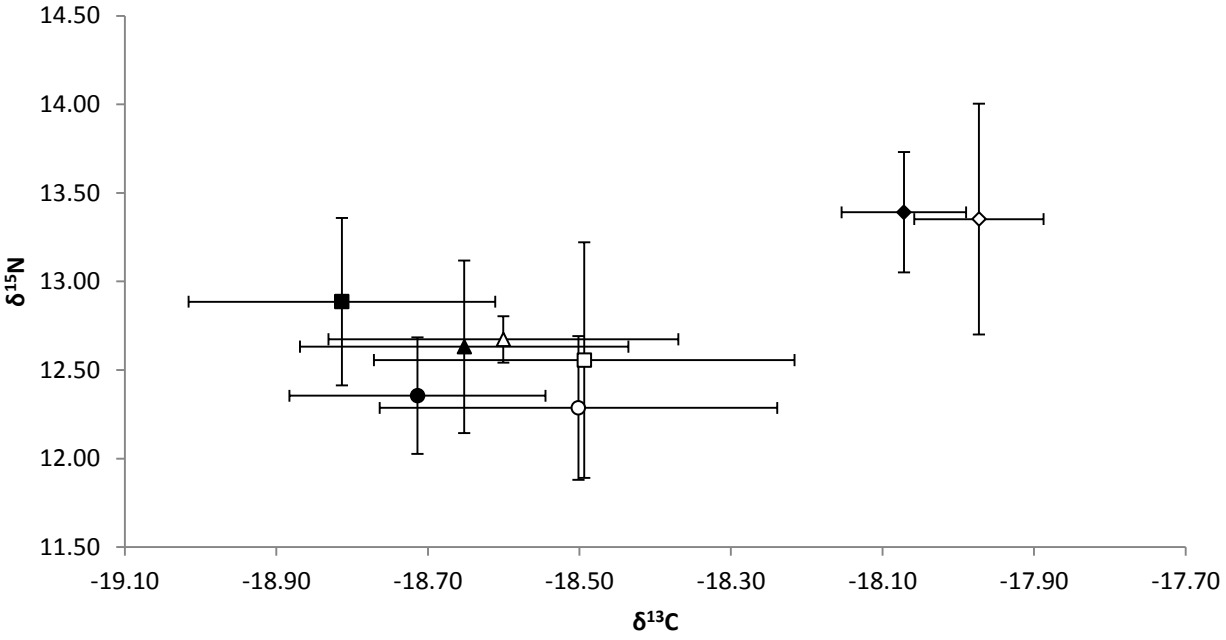

Figure 4.4: $\delta^{13}C$ and $\delta^{15}N$ signatures (mean ± SD; ‰) of whole blood from adult male (filled symbols) and female (open symbols) Barau's Petrel (*Pterodroma baraui*) during non-breeding season (diamonds), pre-laying exodus (circle), incubation (square), and chick-rearing periods (triangle). Male non-breeding n = 11, female non-breeding n = 8, male pre-laying exodus n = 33, female pre-laying exodus n = 10, male incubation n = 17, female incubation n = 9, male chick-rearing = 3, female chick-rearing n = 5.

Table 4.2: Uncorrected P-values from the pairwise Mann-Whitney U test of whole blood (a) $\delta^{13}C$ and (b) $\delta^{15}N$ signatures indicating the seasonal differences between adult male and female Barau's Petrel (*Pterodroma baraui*). Asterisks denote significant differences ($P < 0.05$).

a)		Non-breeding		Pre-laying exodus		Incubation		Early chick-rearing	
		Males	Females	Males	Females	Males	Females	Males	Females
Non-breeding	Males	-	-	-	-	-	-	-	-
	Females	0.04*	-	-	-	-	-	-	-
Pre-laying exodus	Males	9.90×10^{-07}*	1.51×10^{-05}*	-	-	-	-	-	-
	Females	2.16×10^{-04}*	4.46×10^{-04}*	0.01*	-	-	-	-	-
Incubation	Males	1.21×10^{-05}*	8.36×10^{-05}*	0.13	0.01*	-	-	-	-
	Females	8.26×10^{-04}*	6.36×10^{-04}*	0.01*	0.90	4.26×10^{-03}*	-	-	-
Early chick-rearing	Males	0.01*	0.02*	0.77	0.18	0.29	0.36	-	-
	Females	2.21×10^{-03}*	4.31×10^{-03}*	0.30	0.39	0.14	0.51	0.77	-

b)		Non-breeding		Pre-laying exodus		Incubation		Early chick-rearing	
		Males	Females	Males	Females	Males	Females	Males	Females
Non-breeding	Males	-	-	-	-	-	-	-	-
	Females	0.80	-	-	-	-	-	-	-
Pre-laying exodus	Males	9.27×10^{-07}*	2.29×10^{-04}*	-	-	-	-	-	-
	Females	2.86×10^{-04}*	3.88×10^{-03}*	0.50	-	-	-	-	-
Incubation	Males	0.01*	0.07	2.81×10^{-05}*	0.01*	-	-	-	-
	Females	1.41×10^{-03}*	0.02*	0.12	0.25	0.29	-	-	-
Early chick-rearing	Males	0.04*	0.13	0.30	0.15	0.46	1.00	-	-
	Females	2.21×10^{-03}*	0.03*	0.03*	0.05*	0.10	0.59	1.00	-

4.3.4. Trophic level estimation and interspecific comparisons

Adult Barau's Petrel occupied a relatively narrow feeding guild between the fourth and fifth trophic levels, which was slightly higher than was found for the other seabirds that breed at Europa Island in the southern Mozambique Channel (Table 4.3). The downy chicks of Barau's Petrel showed the lowest overall trophic level and, unsurprisingly then, ontogenetic differences were detected within this species (M-Whitney U-test: all $P < 0.01$; Table 4.3). Such a trend was also observed in the White-tailed Tropicbird (*Phaethon lepturus*) and Great Frigatebird (*Fregata minor)*, whereas Sooty Tern (*Onychoprion fuscatus)* displayed the reversed pattern (Cherel *et al.* 2008).

Table 4.3: Estimated trophic positions (mean ± SD) of adult and downy chick Barau's Petrel (*Pterodroma baraui*) as compared with trophic levels of seabirds breeding at Europa Island in the southern Mozambique Channel. Data for other species were adapted from Cherel *et al.* (2008).

Species	Age/Sex	Stage	n	$\delta^{15}N$ (‰)	C/N ratio	Trophic level
Barau's Petrel	Adult/Male	Pre-laying exodus	33	12.35 ± 0.33	3.53 ± 0.8	4.24 ± 0.10
		Incubation	17	12.89 ± 0.47	3.52 ± 0.06	4.32 ± 0.15
		Chick-rearing	3	12.63 ± 0.49	3.55 ± 0.06	4.40 ± 0.15
		Overall	53	12.54 ± 0.45	3.53 ± 0.07	4.29 ± 0.14
Barau's Petrel	Adult/Female	Pre-laying exodus	10	12.29 ± 0.41	3.54 ± 0.15	4.21 ± 0.13
		Incubation	9	12.56 ± 0.67	3.51 ± 0.08	4.30 ± 0.21
		Chick-rearing	5	12.67 ± 0.13	3.53 ± 0.12	4.34 ± 0.04
		Overall	24	12.47 ± 0.50	3.51 ± 0.12	4.27 ± 0.16
	Downy chicks	N/A	7	11.58 ± 0.64	3.68 ± 0.09	3.99 ± 0.20

Table 4.4: cont.

Species	Age/Sex	Stage	n	δ^{15}N (‰)	C/N ratio	Trophic level
Sooty Tern	Adult	Breeding	18	11.79 ± 0.21	3.43 ± 0.03	4.06
(*Onychoprion fuscatus*)	Juvenile	N/A	15	12.64 ± 0.17	3.46 ± 0.04	4.33
White-tailed Tropicbird	Adult	Breeding	7	12.28 ± 0.05	3.41 ± 0.05	4.21
(*Phaethon lepturus*)	Juvenile	N/A	2	11.97 ± 0.24	3.44 ± 0.04	4.12
Red-footed Booby	Adult	Breeding	21	11.59 ± 0.17	3.32 ± 0.04	4.00
(*Sula sula*)	Adult	Breeding	18	11.79 ± 0.21	3.43 ± 0.03	4.06
Great Frigatebird	Adult	Breeding	12	12.18 ± 0.26	3.35 ± 0.09	4.18
(*Fregata minor*)	Immature	N/A	11	11.90 ± 0.18	3.38 ± 0.06	4.09
	Juvenile	N/A	10	11.95 ± 0.19	3.45 ± 0.03	4.11
Lesser Frigatebird (*Fregata ariel*)	Adult	Breeding	5	12.20 ± 0.21	3.39 ± 0.04	4.19

4.4. Discussion

SIAs have furthered our understanding of seabird foraging ecology during the breeding and non-breeding seasons. They have been successfully applied in ecological studies, which have attempted to understand the foraging and migration strategies used by marine birds (e.g. Cherel *et al.* 2006; Quillfeldt *et al.* 2008b; Phillips *et al.* 2009; Militão *et al.* 2013), as well as the niche partitioning that sometimes exists within and among species (e.g. Cherel *et al.* 2008; Kojadinovic *et al.* 2008; Connan *et al.* 2014). In particular, this work has expanded on the knowledge of the endangered Barau's Petrel, which was virtually unknown until recently (Kojadinovic *et al.* 2008; Pinet *et al.* 2009). But one of the main assumptions underlying SIAs is that the isotope signatures of a consumer's tissue will reflect those of their prey and ultimately the signatures at the base of the food web (Post 2002; Shealer 2002; Fry 2006; Barrett *et al.* 2007; Bond & Jones 2009). Seabirds, with the partial exception of a few specialist species (e.g. Roseate Tern*,* at colonies south of Parguera, Puerto Rico*;* Shealer 1998), are multi-taxon predators, implying that they consume a wide range of prey species in an almost infinite number of combinations (Bond & Jones 2009). This presents a

major problem as two theoretical birds that exploit two different food webs, which have similar nitrogen and carbon baselines, may have comparable isotope signatures when diverse combinations of prey are consumed in different proportions (Bond & Jones 2009). Knowledge about the isotopic baselines in the food webs of interest, at the time of study, is thus required (Post 2002). Such patterns have been well documented at higher latitudes but are, in general, poorly known for tropical ecosystems (Kojadinovic *et al.* 2008). That research which has been done in the western Indian Ocean has demonstrated weak spatial $\delta^{13}C$ and $\delta^{15}N$ variations in predatory marine fish between Somalia and the southern Mozambique Channel (Ménard *et al.* 2007), as well as weak latitudinal variations in $\delta^{13}C$, but slightly greater differences in $\delta^{15}N$, of particulate organic matter in the sub-Equatorial (0 - 40°S) tropical waters (S. Jaquemet *et al.* unpub. data). In order to correctly interpret isotope data, one also needs discrimination factors so as to control for the ways in which isotopes from prey are integrated into different predator tissues (Bond & Jones 2009). But, whilst controlled laboratory experiments have been conducted on a number of seabird species (e.g. Hobson & Clark 1992; Bearhop *et al.* 2002; Cherel *et al.* 2005; Sears *et al.* 2009), a few recent studies have shown that these ratios are unique to each tissue-consumer-prey combination (Bearhop *et al.* 2002; Cherel *et al.* 2005; Caut *et al.* 2009). Since petrels cannot easily be kept in captivity (J. Tourmetz. pers. comm.), no such studies have ever been successfully conducted. Hence, interpreting the present isotope work in terms of the well-defined foraging zones and trophic position of Barau's Petrel was not possible and instead focus is given to overall patterns and general trends.

4.4.1. Intra-annual variations in habitat use and trophic position

From very early on, it became obvious that Barau's Petrel occupied different areas of the Indian Ocean at different times of the year (reviewed in Pinet *et al.* 2009). Land based observations at Réunion Island were almost entirely limited to the austral summer months, when the birds where presumed to be breeding (Brooke 1978; Jouanin & Gill 1987). At-sea records supported these claims, indicating seasonal variations in the distribution of this species (Stahl & Bartle 1991). More recently, tracking studies have shown an impressive migratory pattern between the central and south-western regions of the Indian Ocean (Pinet *et al.* 2011b, 2012). Preliminary isotopic investigations confirmed these seasonal differences in foraging habitats (J. Kojadinovic. unpub. data), for the signatures of adults returning from the non-breeding grounds tended to be significantly enriched in terms of carbon relative to individuals sampled over the breeding season. However, based on that work alone, it was not possible to identify the precise regions that these birds were using. The similarity of these new isotope data with other recent signatures of particulate organic matter (S. Jaquemet *et al.* unpub. data), zooplankton (De Lecea *et al.* 2011), micronekton (Ménard *et al.* 2014), and seabirds (Cherel *et al.* 2008; Kojadinovic *et al.* 2008), from the south-western Indian Ocean compliment that early work and now seem to confirm the breeding distribution of adult Barau's Petrel. But as to how the carbon signatures of the non-breeding birds might relate to other organisms that co-occur in the same areas of the central Indian Ocean during the austral winter is not certain, as the region remains understudied.

Interestingly, a difference in the carbon composition of whole blood was detected between non-breeding male and female birds and, even though many of the other differences were not statistically significant, the same enriched state of female birds was recorded across all breeding stages. This result, compelling as it may be, is difficult to

interpret. Carbon isotopes undergo minimal discrimination between trophic levels and are typically used to characterise isotopically distinct regions (e.g. Kroopnick 1985; Cherel *et al.* 2006; Phillips *et al.* 2009; Militão *et al.* 2013). Thus, the most obvious interpretation of this result is that the sexes feed in different and isotopically distinct habitats over different periods of the year. This is known to be the case for the pre-laying exodus, but not during all other breeding stages and the non-breeding season (Pinet *et al.* 2011b, 2012). It would then seem reasonable to suggest that this result is as a consequence of some behavioural, physiological, and/or metabolic difference between the sexes. Whilst this explanation might seem unrealistic, any dissimilarity in foraging areas would have been detected by the tracking studies (i.e. Pinet *et al.* 2011b, 2012). Indeed, such a pattern could not have been overlooked owing to the errors associated with the GLS trackers (Phillips *et al.* 2004), because of the degree to which the sexes overlapped over the non-breeding season (Pinet *et al.* 2011b, 2012). Further investigation using a database of isotope signatures of potential prey species from the regions in question would be invaluable in understanding this difference.

Barau's Petrels are known to exhibit complex patterns of habitat use that vary through the breeding season (Pinet *et al.* 2012). The $\delta^{13}C$ signatures from whole blood changed between the different stages of the breeding cycle but disentangling this from the above mentioned physiological and/or metabolic differences between birds, which presumably also influenced $\delta^{13}C$ (Sears *et al.* 2009), is challenging. However this result will certainly be, at least in part, related to differential habitat use between the sexes at different stages of the breeding season. Indeed, significant differences were detected between the male birds during the pre-laying exodus and female birds during both the pre-laying and incubation periods. The most probable interpretation of these results, which rests on the assumption

that the areas that each of the sexes are using are isotopically distinct from one another, is that there is spatial segregation between the sexes during the early stages of the breeding season. Pinet *et al.* (2012) first noted this when they recognised that, during the pre-laying exodus, males consistently foraged over the Mozambique Plateau while females remained in the areas over the Madagascan Ridge and Walter Shoals. But as to whether this assumption holds true or not is uncertain and further clarification requires a better understanding of spatio-temporal heterogeneity in the isotope baselines of the areas in question. However, the fact that the waters to the south and east of Madagascar have a different origin (oceanic South-Equatorial Current; Swallow *et al.* 1988; Schott *et al.* 2002) to the waters flowing through the Mozambique Channel (eddies propagating off the more coastal North East Madagascar Current; Donguy & Piton 1991; De Ruijter *et al.* 2002, 2005) implies that this may indeed be the case.

Later on in the breeding season, the at-sea distribution of male birds shifts to the east and both sexes then occupy the area immediately to the south of Madagascar (Pinet *et al.* 2012). Based on the former argument, it would seem likely that the differences that then existed between the sexes during the incubation period and between the pre-laying females and incubating males would not be attributed to differential habitat use. But, even though a dramatic increase in overlap occurs at the end of the pre-laying exodus, the similarity in core foraging distribution of adult male and female birds is only between 40 and 60 % during the incubation period (Pinet *et al.* 2012). Hence, these differences could still be, at least in part, related to spatial segregation between the sexes. Various factors other than the specificities of the habitats used will also be contributing to these differences, however. Like most other Procellariiformes, Barau's Petrel undertake an extended exodus just before egg-laying (Pinet *et al.* 2012). Males gain body mass over this period, whereas females must endure the costs

of producing a disproportionately large (relative to adult body size) and extremely nutrient rich egg (Warham 1990; Mallory *et al.* 2008). Thereafter males invariably take the first incubation shift where they remain at the colony fasting for about 17 days, while the females return to sea to restore the body condition lost during egg production (Pinet *et al.* 2012). Many studies have found a limited impact of such episodic nutritional stress on $\delta^{13}C$ (e.g. Hobson *et al.* 1993; Kempster *et al.* 2007), but there is believed to be a level above which isotope signatures become adversely affected. Seabirds, in particular, have become well-adapted to periods of extended and often extreme food deprivation (e.g. periods of incubation; Mrosovsky & Sherry 1980; Grant 1981), over which they survive exclusively on their lipid and protein reserves as the only sources of energy. Body condition noticeably decreases and the concentration of free fatty acids (particularly triglycerides, and cholesterols) in the blood sharply increases (Totzke *et al.* 1999). Blood is inherently a transport medium, implying that its composition is in a continual state of flux, and this increase in free flowing lipids depletes the overall carbon signatures of this metabolically active tissue. This is as a direct consequence of the fractionation caused by the oxidation of pyruvate to acetyl coenzyme A during lipid synthesis (DeNiro & Epstein 1977). But some authors have suggested that nutritional stress should result in higher $\delta^{13}C$ signatures (Hatch *et al.* 1995; Oelbermann & Scheu 2002). However, what is clear from these results, which is not unlike what has been found in a few other marine bird species (e.g. Cherel *et al.* 2005), is that some of the highest C:N ratios and lowest $\delta^{13}C$ values were detected when adults were suffering from the greatest levels of nutritional stress (particularly the incubation period in males).

Fewer blood samples were collected from adults over the chick-rearing period and no samples were taken just prior to the end of the breeding season. Hence, it is only possible to

determine how the foraging behaviour of males and females differed during the early chick-rearing period. Based on the acute similarity of the carbon signatures of blood from males and females during this breeding stage, it would seem likely that this period was when the greatest overlap in foraging distribution existed. This was indeed the case in the tracking studies where the overlap in core foraging distribution of both sexes was at its greatest (between 68 and 91 %) towards the end of the breeding season (Pinet *et al.* 2012). However, using these samples alone, it is not possible to determine how adult foraging distribution might have shifted over the course of this final breeding stage. But the down and true-feather samples from downy chicks and fledglings, which differed from each other in terms of $\delta^{13}C$, together indicate that the adult distribution shifted between the early and late chick-rearing periods. Pinet *et al.* (2012) recognised that the adults alternate short and long foraging trips to sustain the chicks and themselves, respectively, and trip distance away from Réunion Island (January: 1448 ± 990 km; February: 1105 ± 733 km; March: 881 ± 603 km) noticeably decreases as the energy demands of the chicks increases. The depleted nature of the feathers from fledglings is consistent with Pinet *et al.'s.* (2012) observations for, alongside what knowledge exists about isotope baselines of the region (S. Jaquemet *et al.* unpub. data), it confirms that adults are foraging closer to Réunion towards the end of the chick-rearing period. However, it is also important to note that periods of rapid growth, carryover of egg nutrients, and physiological differences between downy chicks and fledglings are all able to impact $\delta^{13}C$. Few studies have ever addressed these variables in these ontogenetic groups (e.g. Romano 2000; Sears *et al.* 2009), and hence it is difficult to understand the ways in which they might have influenced these signatures.

Differences in $\delta^{15}N$ signatures were also detected among the different breeding stages. Whilst it is tempting to speculate that these dissimilarities might have arisen as a

consequence of dietary changes, the most credible explanation is that they are also a reflection of nutritional stresses and associated changes in metabolic rate. Indeed, some of the highest overall nitrogen signatures of blood in each of the sexes were detected when the birds were subjected to the greatest levels of nutritional stress at which time body condition also dramatically decreases (pre-laying exodus for females, incubation for males). This result is in agreement with what has previously been found in Japanese Quails (*Coturnix japonica*), wild Ross's Geese (*Chen rossii*; Hobson *et al.* 1993), and King Penguins (*Aptenodytes patagonicus*; Cherel *et al.* 2005), for it suggests that these birds will 'feed on themselves' during periods of extreme nutritional stress, thus increasing their $\delta^{15}N$ signatures and apparent trophic level. Conversely though, this result differs from what Kempster *et al.* (2007) found in Song Sparrows (*Melospiza melodia*), which might imply that birds which are simply malnourished will show only slight changes to blood isotope composition (Hobson *et al.* 1993; Kempster *et al.* 2007). The rate and extent to which $\delta^{15}N$ enrichment occurs as a consequence of fasting will also be influenced by the rate of isotopic turnover in the tissues used (Hobson *et al.* 1993); metabolically active tissues (e.g. blood) are expected to show the effects of fasting far sooner than tissues with a slower isotopic turnover (e.g. bone; Hobson *et al.* 1993). A recent review has also highlighted that individuals undergoing periods of extended nutritional stress will dramatically alter their metabolic rate (Thomson *et al.* 1998; Ellis & Gabrielsen 2002; Tinbergen & Williams 2002; Shoji *et al.* in press), which in turn affects isotope discrimination. This reduces energy consumption in on-duty birds, allowing off-duty birds to stay away longer while foraging in distant, yet predictable, habitats (Shoji *et al.* in press). But, as has already been indicated (e.g. Hobson *et al.* 1999; Kempster *et al.* 2007), laboratory experiments that assess the precise levels of nutritional stress required to adversely affect bird metabolism, isotope

discrimination, and rates of tissue turnover are greatly needed. In addition, there will certainly be some influence of the spatio-temporal variability in $\delta^{15}N$ baselines on these signatures (S. Jaquemet *et al.* unpub. data), but further work is needed in the region before this interpretation can be debated further.

4.4.2. Interspecific, ontogenetic, and sex-related differences in trophic ecology

Adult and pre-mature Barau's Petrel occupy a trophic niche between the fourth and fifth trophic levels, which are not unlike those of other seabird species that breed on Europa Island in the southern Mozambique Channel (Cherel *et al.* 2008). Europa hosts globally significant breeding populations of marine birds, including tropicbirds (*Phaethon*), boobies (*Sula*), and frigatebirds (*Fregata;* Le Corre & Jouventin 1997; Le Corre & Jaquemet 2005), and constitutes one of the nearest major seabird colonies to Réunion Island. This biodiverse island is also situated in close proximity to the principal foraging areas of Barau's Petrel during the breeding season (Pinet *et al.* 2012), and there is thought to be some overlap in the foraging distribution of Barau's Petrel with at least a few species (e.g. Tropicbirds) that breed there (Weimerskirch *et al.* 2004; Jaquemet *et al.* 2005; Le Corre *et al.* 2012; Pinet *et al.* 2012). As such, the similarity in the estimated trophic niches among the seabird community of the south-western sector of the Indian Ocean was not unexpected. In fact, this species assemblage encompasses a surprisingly narrow range of $\delta^{15}N$ signatures (less than one trophic level difference), as compared with other seabird communities in temperate and polar ecosystems (e.g. Sydeman *et al.* 1997; Thompson *et al.* 1999; Forero *et al.* 2002, 2004). This is presumably a reflection of the intrinsic characteristics (i.e. weak temperature gradients) of tropical ecosystems that underlie the spatio-temporal homogeneity in their isotopic baselines (Ménard *et al.* 2007; Cherel *et al.* 2008; S. Jaquemet

et al. unpub. data), as well as the narrower range of diet in tropical seabirds (mostly tertiary consumers) compared with temperate and polar species, which include multiple feeding guilds from primary consumers to tertiary consumers (Shealer 2002).

It was also curious to note that adult Barau's Petrel apparently fed at a higher trophic level than all other co-occurring species in the southern Mozambique Channel. Whilst this could be an artefact of spatio-temporal shifts in the nitrogen baseline(s), no significant latitudinal differences have been recorded from other nearby areas (Ménard *et al.* 2014). Furthermore Kojadinovic *et al.* (2008) independently noted a similar pattern in that, among the most numerically abundant seabird species that breed on Réunion Island, Barau's Petrel had the highest hepatic $\delta^{15}N$ signatures. This is coherent with what is understood about the foraging behaviour of the seabird community of the south-western Indian Ocean. Tropicbirds and boobies are somewhat specific in their dietary habits, favouring mainly flying fish (Exocoetidae) and squids (Ommastrephidae; Diamond 1975a, 1983; Cherel *et al.* 2008), and frigatebirds are largely kleptoparasites of the former groups (Diamond 1975b). In contrast, Barau's Petrel show a scavenging behaviour (Kojadinovic *et al.* 2008; Chapter 3), and will opportunistically feed on large prey of much higher trophic levels including the floating carcasses of dead marine mammals, large fishes, and squids (Kojadinovic *et al.* 2008). Caution is applied to this interpretation, however, as this theory received only speculative support from stomach content analyses (Kojadinovic *et al.* 2008; Chapter 3).

At a finer scale then, adult Barau's Petrel differed from downy chicks on the basis of the $\delta^{15}N$ signatures of their whole blood. Dissimilarities of this nature have previously been recorded in albatrosses (Connan *et al.* 2014), Northern Rockhopper Penguins (*Eudyptes moseleyi*; Booth & McQuaid 2013), and Tropical Shearwaters (*Puffinus iherminieri bailloni*; Kojadinovic *et al.* 2008), as well as from the hepatic tissues of Barau's Petrel (Kojadinovic *et*

al. 2008). Three hypotheses may have contributed to these variations. Firstly, there are certainly metabolic and physiological differences between adult and pre-mature birds, which affect isotope discrimination (Sears *et al.* 2009). However, understanding how these factors may have influenced the signatures of Barau's Petrel is extremely difficult since prey isotope signatures and captive feeding trials were not a part of this present work. Secondly, this finding may be a reflection of spatial differences in isotope baseline. In occupying a dual foraging strategy, adults feed for themselves in the areas to the south of Madagascar whilst pre-mature birds are fed from waters closer to Réunion (Pinet *et al.* 2012). Particulate organic matter from around Réunion Island is enriched in terms of nitrogen relative to waters around south-eastern Madagascar (S. Jaquemet. *et al.* unpub. data), which might suggest that a greater amount of the pre-mature birds' food is derived from areas to the south of Madagascar than has previously been thought. However, this seems unlikely since the pre-mature birds were also depleted in terms of carbon, relative to the adults, which relates to the lower carbon baseline of waters around Réunion Island (S. Jaquemet. *et al.* unpub. data). Finally, as was the case in many of the above mentioned species (Kojadinovic *et al.* 2008; Booth & McQuaid 2013), pre-mature birds may be fed on prey from lower trophic levels compared with the prey in the diets of breeding adults. This could imply one of three things. The prey species assemblage in the diet of chick might differ from that of the adults or the adults might be feeding the chicks on size-specific prey. If either of these were the case, it becomes curious to note that most other adult seabirds provision their young with food of a similar or even higher trophic level than the prey used in self-provisioning (e.g. Cherel *et al.* 2007a; Cherel 2008; Tierney *et al.* 2008b; Richoux *et al.* 2010). This would be in accordance with the individual-optimisation theory, which suggests that precedence is given to adult survival, rather than maximising reproductive output, when conditions are not

entirely favourable for reproduction (Drent & Daan 1980; Nur 1986). This, which is similar to what has been found in a suite of other seabirds (including many Procellariiformes; Hodum & Hobson 2000; Forero *et al.* 2002; Cherel 2008; Booth & McQuaid 2013; Cherel *et al.* 2014a), could have important conservation implications for this already threatened species. The third variable to consider is the physical adaptation of most Procellariiform seabirds, which allows adults to convert fresh prey into extremely concentrated stomach oils on which the downy chicks and fledglings are fed (Warham *et al.* 1976; Warham 1977; Roby *et al.* 1997; Hamer *et al.* 2002; Baduini & Hyrenbach 2003). This prior processing of prey is thought to affect the isotope values of young birds (Thompson *et al.* 2000), although the influence on $\delta^{15}N$ (if any) is not well understood.

4.4.3. Conclusions and recommendations

The at-sea biology of the endangered Barau's Petrel was virtually unknown until recently. Ship-based and tracking studies have together shed light on the forging behaviour of this species, whilst Kojadinovic *et al.* (2008), using SIAs, provided the first insights into this species' trophic position. The results from this study are in accordance with all of this earlier work. However, this present work had a much greater resolution than the initial isotopic investigation (i.e. Kojadinovic *et al.* 2008), which has resulted in a number of additional differences in $\delta^{13}C$ and $\delta^{15}N$ signatures being detected. These included intra-annual differences as well as disparities between the sexes in adult birds. Variations among $\delta^{13}C$ signatures altogether highlighted differential habitat use among adults at different stages of the breeding season whilst $\delta^{15}N$ signatures generally indicated differences in trophic position and/or were a reflection of metabolic and physiological differences. But whilst many conclusions could be drawn from these data, it is obvious that more laboratory

experiments, which investigate specific prey-tissue discrimination factors, and marine species studies, which investigate spatio-temporal variability in $\delta^{13}C$ and $\delta^{15}N$ baselines, are needed to fully understand the assumptions and limitations of SIAs.

CHAPTER 5: INTRA-SEASONAL VARIATION IN THE DIET OF BREEDING BARAU'S PETREL (*PTERODROMA BARAUI*): INSIGHTS FROM LIPID ANALYSES OF WHOLE BLOOD.

5.1. Introduction

Fundamental concepts in developing ecosystem-based management strategies are the prediction, understanding, and resolution of the long-term impacts of global climate change on ecosystem structure, integrity, and function (Dalsgaard *et al.* 2003). Coupled with this is a necessity of recognising the influences of change on key species within marine ecosystems (Furness & Camphuysen 1997). Since autotrophic organisms, which form the basis of every food web, lay down certain structures that are subsequently transferred up through the marine food webs, any changes in ecosystem integrity are likely to be reflected at higher trophic levels (Dalsgaard *et al.* 2003; Sergio *et al.* 2005). Furthermore because local, regional, and global variations in algal distribution, diversity, and biomass are largely in response to differences in temperature, light, and nutrient availability, the use of trophic markers to monitor such long term impacts of climate change is now becoming increasingly valued by ecologists (Iverson *et al.* 2004; Barrett *et al.* 2007; Iverson 2009). An ideal trophic marker is one whose origin can be uniquely and easily identified and which is selectively incorporated by a consumer species during food uptake (Dalsgaard *et al.* 2003). It must also be metabolically stable, thus ensuring that it can be detected as it moves from one trophic level to the next (Dalsgaard *et al.* 2003). These expectations are easily met in controlled situations (e.g. Leal *et al.* 2014), but less ideal constituents must usually be used since these markers are extremely rare under natural conditions. Lipid components of animal and plant tissues are particularly valuable in this regard, in that phytoplankton (microalgae) and water weeds (macroalgae), are some of the only organisms that are able to biosynthesize a diverse

array of fatty acids (FAs) *de novo* (Dalsgaard *et al.* 2003; Iverson *et al.* 2004; Iverson 2009). These ubiquitous structures form an essential and integral part of both neutral (including triglycerides and wax esters) and polar lipids (including phospholipids) that, in turn, form the basic matrices of all cellular membranes into which cholesterol, proteins, and other cellular components are embedded (Linder *et al.* 2010). They can either be saturated (SFA), monounsaturated (MUFA), or polyunsaturated (PUFA) depending on the number of double bonds present and tend to consist of straight, even-numbered, and unbranched hydrocarbon chains between 14 and 24 carbon molecules in length, with a carboxylic acid (-COOH) group at one end and a terminal methyl ($-CH_3$) group at the other (Williams & Buck 2010; Karnovsky *et al.* 2012). Some FAs, known as 'iso' (*i*-) or 'anti-iso' (*ai*-) FAs, also contain a methyl branch on the second or third carbon atom closest to the terminal methyl group. These structures, subsequent to their bioformation, get transferred through food webs moving between each trophic level in a predictable manner (Iverson *et al.* 2004; Barrett *et al.* 2007; Williams & Buck 2010; Karnovsky *et al.* 2012). They are thus able to provide essential insight into the trophic dynamics of ecosystems (Dalsgaard *et al.* 2003; Iverson *et al.* 2004; Iverson *et al.* 2007), particularly in marine habitats where a diverse array of FAs exist (Linder *et al.* 2010; Williams & Buck 2010).

Higher trophic level species are only able to synthesize certain kinds of fatty acid (FA) *de novo* so that the total fatty acid (TFA) composition of their tissues usually reflects that of their prey (Iverson *et al.* 2004; Barrett *et al.* 2007; Williams & Buck 2010; Karnovsky *et al.* 2012). This implies that fatty acid signature analyses (FASAs) of consumer tissues are able to accurately resolve the relative importance of different prey groups in both qualitative and quantitative estimative manners (Iverson *et al.* 2004). The degree to which these analyses

are applicable to studies of feeding ecology is constrained by the degree to which the consumers alter FAs through *de novo* synthesis, however (Williams & Buck 2010). The dynamics of these factors are closely coupled with variables such as physiological status, life history stages, environmental conditions, and lipid storage types. Consequently, higher trophic level FA signatures never directly match those of their prey but the strong dietary influence does permit inferences to be made (reviewed in Budge *et al.* 2006; Williams & Buck 2010). This usually involves the use of complex statistical models that determine the closest dietary origin of the TFA signatures, taking into account the potential effects of *de novo* synthesis, metabolism, and partial breakdown on these structures (Iverson *et al.* 2004). This relies on a database of signatures from potential prey species from which quantitative diet estimations of consumer species can be made (Iverson *et al.* 2004). In many cases, particularly in instances where the organism in question commutes over long distances between breeding colonies and foraging areas, these data are unavailable and such analyses become reliant on fatty acid trophic markers to qualitatively estimate diet (reviewed in Dalsgaard *et al.* 2003). This concept is based on the observation that certain individual groups (e.g. long chain MUFAs) or combinations (e.g. ratios of one FA to another) of FAs in the tissues of a consumer are associated with particular feeding guilds and/or prey types (reviewed in Dalsgaard *et al.* 2003).

In seabird studies, FASAs have been successfully used to identify the consumption of particular prey species (e.g. Raclot *et al.* 1998; Connan *et al.* 2005; Käkelä *et al.* 2005) or prey from specific foraging guilds whilst also providing information on niche partitioning within and among species (e.g. Connan *et al.* 2014) as well as spatial and temporal differences in diet (e.g. Tierney *et al.* 2008b; Ronconi *et al.* 2010; Owen *et al.* 2013). In addition, a large

part of this work, particularly those studies on the Procellariiformes, has been done in the validation of stomach content and/or SI analyses (e.g. Connan *et al.* 2007a; Iverson *et al.* 2007; Käkelä *et al.* 2007; Karnovsky *et al.* 2008; Williams *et al.* 2008; Ronconi *et al.* 2010; Richoux *et al.* 2010; Williams & Buck 2010). The combined use of the three methods has been advised for the most accurate description of diet, however (Karnovsky *et al.* 2012). Therefore, in using FASAs of blood from adult Barau's Petrel, the objectives of this chapter are to:

a) Identify any major temporal shifts in the diet over the length of a single breeding season, whilst also examining the potential sources of their origin.
b) Provide new data on the diet of this species using FA trophic markers and, in doing so, attempt to clarify the relative dietary importance of fish and squid.

5.2. Materials and methods

5.2.1. Tissue selection

Whole blood was selected as the tissue group for FASAs as it can be non-destructively sampled (Käkelä *et al.* 2005), from birds and since it has an intermediate turnover rate. Stomach oils and adipose tissue are preferentially used in FASAs (Käkelä *et al.* 2005; Foster *et al.* 2010; Karnovsky *et al.* 2012), but insufficient fresh stomach content samples were collected in the field and gathering adipose fat samples would have proven too difficult from these protected and endangered seabirds in their high altitude nesting habitats. Furthermore, the variable ages of the dead light-strike birds and the uncertainty regarding the collection date for many of the specimens also precluded the use of their adipose tissues. Finally, whilst plasma is also valued (Käkelä *et al.* 2005; Foster *et al.* 2010; Karnovsky

et al. 2012), logistical constraints implied that the blood components could not be separated in time. Dietary changes in lipid composition of whole blood are typically recorded after five days but a full adjustment in composition can take anything up to a month subsequent to feeding (Käkelä *et al.* 2005, 2009).

5.2.2. Sample description

Adult birds were sampled four times throughout the breeding seasons of 2013/2014. Blood samples (0.5-1.2 ml) were taken from every bird found on each excursion irrespective of whether they had been sampled earlier on in the season or not. Sampling trips for adults roughly corresponded with the first and second incubation shifts of the pre-laying exodus (06-07 and 20-22 November 2013; males n = 15, females n = 8), the incubation period (17-18 December 2013; males n = 15, females n = 9), and the early chick-rearing period (27-28 January 2014; males n = 3, females n = 4), as was based on Pinet *et al.* (2012). Downy chicks and fledglings were not sampled so as to minimise the chances of nest failures and because of the sheer volume of blood required for FASAs. After collection, samples remained frozen at -80 °C for up to four months prior to lipid extraction and gas chromatography (GC) analysis. All steps used thereafter were performed under a constant flow of nitrogen gas as a precautionary measure against lipid auto-oxidation.

5.2.3. Lipid extraction

Total lipids were extracted, from whole blood samples, and purified following a modified Folch procedure (Folch *et al.* 1957; Allan *et al.* 2010). In short, each sample was added into a lipid cleaned test tube along with chloroform and butylated hydroxytoluene (concentration 0.1 % of total solvent volume). Ice cold methanol was added, with the

solvent ratio of 2:1 (v/v; chloroform/methanol), and, after thorough vortexing, all were left to extract at -20 °C for 24 hours. The lipid layer was then cleaned using potassium chloride and methanol, under the ratio of 8:4:3 (v/v/v; chloroform/methanol/water + KCl). Solvent volumes were adapted, depending on the size of the sample, but retained the same solvent/water/sample ratio to prevent selective loss of lipids. The remaining organic phases were then concentrated down to dryness before the addition of 1.5 ml dried dichloromethane. At this stage, 1 µL of internal standard (19:0; 0.5 mg/ml^{-1}) was added to each sample. Vials were capped, sealed with Teflon tape, and stored at -20 °C until the fatty acid methyl esters (FAME) step.

5.2.4. Fatty acid methyl ester (FAME) synthesis

FAME synthesis was adapted from Budge *et al.* (2006) and involved the addition of 3ml Hilditch Reagent (freshly prepared each day by adding 1.5 ml concentrated sulphuric acid to 100 ml dried methanol) into the lipid extract, which was suspended in 1.5ml dichloromethane. After transesterification (one hour of incubation at 100 °C), tubes were cooled to room temperature. Thereafter each was exposed to successive hexane washes, first with hexane and distilled water and twice thereafter with only hexane. All were then dried through anhydrous sodium sulphate and the remaining solvents (containing the final lipid extracts) transferred into lipid cleaned pre-weighed glass vials. Each was evaporated to dryness under a constant stream of nitrogen gas and subsequently reweighed (giving total lipid extract weight). Hexane was then added and vials flushed with nitrogen, sealed with Teflon tape, and stored at -20 °C before GC analysis.

5.2.5. Sample analysis

FAME composition of each sample was determined through GC analysis (Agilent Technologies, InnoVenton – The Nelson Mandela Metropolitan University Institute of Chemical Technology) in a Hewlett Packard 6850 gas chromatographer (Agilent J&W GC Capillary Column; model number 19091Z-413E; column HP-1, 30m length, 0.32 mm internal diameter, 0.25 µm film thickness) with helium as the carrier gas. One microliter aliquots were manually injected per sample and were run splitless or were split at a ratio of 2:1, 5:1, 7:1, or 10:1 according to the initial concentration of FAME (based on weight of total lipid extract) in each sample. The flame ionization detector was set at 300 °C and the oven was initially set at 70 °C. After one minute, the oven temperature was increased (+ 40 °C/min^{-1}) until it reached 170 °C. Then it rose to 250 °C at a rate of 2.5 °C/min^{-1} and was held at that temperature for four and a half minutes.

Peaks were integrated using GC ChemStation software (Agilent Technologies, version B.04.02) and identified by comparison with retention times of external standards (37 component FAME mix Supelco, marine PUFA no. 1 Supelco, menhaden oil PUFA no. 3, bacterial acid methylesters mix Supelco) as well as by comparison with mass spectrometry analysis (Agilent Technologies 7 000 GC/MS Triple Quad; Agilent Mass Hunt, version B.05.00, FA Facility at Rhodes University - NRF) using the NIST library. Each FA was measured as an averaged proportion of the total fatty acid composition (TFA ± SD) in the shorthand form *X*:*a*n-*b*, where 'X' is the number of carbon atoms in the acyl chain, '*a*' is the total number of double bonds in the chain, and '*b*' is the position of the first double bond from the methyl end of the molecule. SFAs have no double bonds (e.g. 14:0), MUFAs contain one double bond (e.g. 16:1n-7), and PUFAs have more than one double bond (e.g. 22:6n-3). Essential fatty acids (EFAs) included 20:4n-6, 20:5n-3, and 22:6n-3. Branched FAs were termed 'iso' (*i*-;

e.g. *i*-17:0) or 'anti-iso' (*ai*-; *ai*-15:0) depending on the position of the methyl molecule in relation to the terminal methyl group at the end of the carbon chain.

5.2.6. Data analysis

Differences in TFA compositions of blood were investigated using an analysis of similarities (one-way ANOSIM) based on the Bray-Curtis distance measure. Those FAs that occurred below trace levels (< 0.2 % of TFA composition) were lumped with one another (16 PUFAs separately from others) for the purposes of these statistical analyses. Furthermore, the data were not transformed in any way so to avoid overestimating the significance of those fats that contributed little to TFA composition. Conservative Bonferroni corrected P-values and the global R-value were used to assess differences between groups (sexes, breeding stages), where a large positive R (up to 1) signified strong dissimilarities. Similarity percentage (SIMPER) was subsequently used to investigate which individual FAs were primarily responsible for any observed differences (Clarke 1993), using cumulative FA specific dissimilarities rather than the overall pooled index. Finally non-metric multidimensional scaling (MDS) plots were used to graphically represent these results. The relationship between samples was considered well represented when two-dimensional stress did not exceed 0.2 (Clarke & Warwick 2001). This approach was deemed more appropriate than the principal component analysis (PCA) technique traditionally used in analysing FASA results since the number of samples per group (sex, breeding stage) was always exceeded the number of variables (different FAs) in question. All analyses were performed using Past 3.01 software (Hammer 2013), and results were graphically represented using Microsoft Excel 2007.

5.3. Results

5.3.1. Overall fatty acid composition of whole blood

In total, 56 FAs were detected in the blood of adult Barau's Petrel including 18 MUFAs, 24 PUFAs, and 14 SFAs. Of these 17 MUFAs, 17 PUFAs, and 14 SFAs were detected above trace levels (> 0.2 % of TFA composition), altogether accounting for between 96.70 % and 97.24 % of the TFA composition of the whole blood samples (Table 5.1). SFAs marginally dominated the profiles but MUFAs and PUFAs were found in almost equal abundance (Table 5.1). The dominant FAs included 18:1n-9 (TFA 22.53 ± 2.52 %), 16:0 (TFA 18.69 ± 2.40 %), 18:0 (TFA 11.18 ± 1.42 %), 20:4n-6 (TFA 9.23 ± 1.47 %), and 22:6n-3 (TFA 8.91 ± 1.19 %).

5.3.2. Temporal variation in the fatty acid composition of blood

A great deal of individual variation was recorded for certain FAs within each of the breeding stages (see for example 20:4n-3 but refer to all standard deviations in Table 5.1), and variations in TFA composition of blood were also observed among the different stages of the breeding season (ANOSIM: global-R = 0.26, $P < 0.01$; Figure 5.1). It is noted though, that the low global-R value with these significant outcomes suggests that these differences are the result of only a small proportion of the data. Profiles from the pre-laying exodus were dissimilar from those of the incubation period (ANOSIM: global-R = 0.27, $P < 0.01$), but were not unlike those of the chick-rearing period (ANOSIM: global-R = 0.10, $P = 0.53$). SIMPER indicated that this distinction between the pre-laying and incubation periods was the result of the sum of fairly large contributions from a relatively small number of FAs. The TFA composition of blood from the incubation period was also significantly different from that of the chick-rearing period (ANOSIM: global-R = 0.40, $P < 0.01$; Table 5.1), but whilst the FAs responsible for this outcome never varied, the relative importance of each did. More

than half of these dissimilarities between different breeding stages were attributed to gradual increases in the FAs 16:0, 20:5n-3, and the 16 PUFAs as well as fluctuations in 18:0, 22:6n-3, 18:4n-3, 18:1n-9, and 20:4n-6 (Table 5.1). These variations were visualised in the MDS analysis although the separation of the different reproductive stages was not clear cut (2D stress value = 0.21; Figure 5.1).

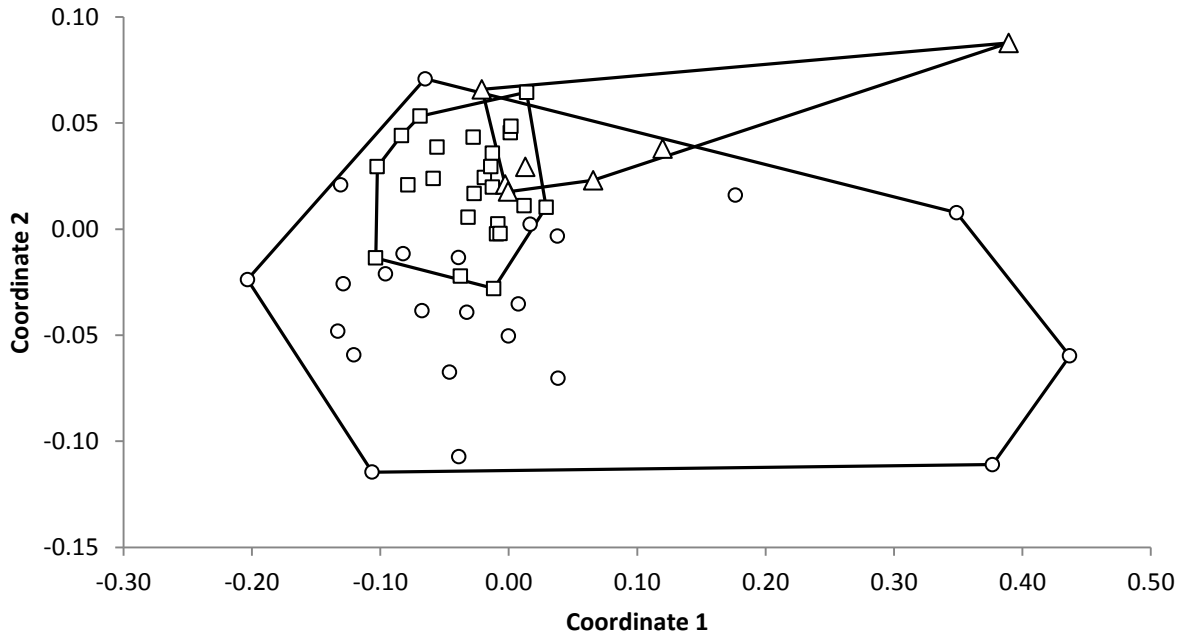

Figure 5.1: Non-metric multidimensional scaling plot (including convex hulls) indicating the relationship between total blood fatty acid profiles of adult Barau's Petrel (*Pterodroma baraui*) during pre-laying exodus (circle), incubation (square), and chick-rearing periods (triangle). Pre-laying exodus n = 24, incubation n = 25, chick-rearing n = 7. Two dimensional stress value = 0.21.

5.3.3. Sexual variation in the total fatty acid composition of blood

No differences in the TFA composition of whole blood between adult male and female birds were detected (ANOSIM: global-R = 0.06, P = 0.07). Detailed investigations into the seasonal differences between sexes revealed a number of significant differences between and within sexes, however (ANOSIM: global-R = 0.24, P < 0.01; Table 5.2). Once again, the low global-R value suggests that this is the result of only a small proportion of the data. Reproductive period had its strongest effects on male birds and all significant differences involved the incubation period (Table 5.2). Incubating males showed significant differences from pre-laying birds of both sexes as well as from incubating females (Table 5.2). In contrast, females showed no significant differences in their FA profiles among all reproductive periods (Table 5.2), nor did they differ from males in the chick-rearing phase (Table 5.2). Overall greater variability in FA profiles was recorded for both sexes during the pre-laying exodus than during either the incubation or chick-rearing periods (Figure 5.2). Furthermore, rather than reflecting a widespread central cluster, the overall spread of the lipid profiles within each breeding stage appeared to be dominated by numerous outlying individuals. These were mainly spread along the first coordinate and were generally characterised by higher abundances of long-chain (C18, 20, and 22) MUFAs and 16 PUFAs, and lower incidences of 16:0, 18:0 18:1n-9, 20:4n-6 relative to other profiles (Figure 5.2). These overall results were only moderately supported by the MDS analyses, however (2D stress value = 0.21), and were the product of the same dominant FAs (16:0, 18:1n-9, 20:4n-6, 20:5n-3, 18:0, 22:6n-3, 18:4n-3, and the 16 PUFAs).

Table 5.1: Temporal variability in the total fatty acid composition. Values indicate (mean % ± SD) for each identified fatty acid in whole blood of adult Barau's Petrel (*Pterodroma baraui*).

	Pre-laying Exodus		Incubation		Chick-rearing	
	Male (n = 15)	Female (n = 8)	Male (n = 15)	Female (n = 9)	Male (n = 3)	Female (n = 4)
14:0	0.60 ± 0.33	0.64 ± 0.21	0.52 ± 0.08	0.50 ± 0.11	0.73 ± 0.21	0.69 ± 0.20
i-15:0	0.29 ± 0.23	0.31 ± 0.30	0.23 ± 0.11	0.19 ± 0.07	0.39 ± 0.21	0.33 ± 0.15
ai-15:0	0.31 ± 0.25	0.34 ± 0.28	0.32 ± 0.17	0.25 ± 0.11	0.46 ± 0.16	0.37 ± 0.17
15:0	0.34 ± 0.10	0.34 ± 0.26	0.29 ± 0.08	0.25 ± 0.07	0.37 ± 0.21	0.36 ± 0.07
15:1	0.32 ± 0.28	0.33 ± 0.27	0.19 ± 0.06	0.22 ± 0.09	0.30 ± 0.14	0.27 ± 0.06
i-16:0	0.44 ± 0.27	0.46 ± 0.37	0.38 ± 0.09	0.36 ± 0.11	0.61 ± 0.41	0.46 ± 0.20
16:0	19.83 ± 2.71	19.48 ± 4.13	18.25 ± 0.98	18.29 ± 1.00	16.28 ± 2.10	17.24 ± 0.67
16:1n-9	0.43 ± 0.25	0.44 ± 0.20	0.29 ± 0.12	0.26 ± 0.08	0.45 ± 0.18	0.37 ± 0.18
16:1n-7	0.94 ± 0.18	0.96 ± 0.15	0.88 ± 0.13	0.83 ± 0.17	0.86 ± 0.06	1.21 ± 0.47
16:1n-5	0.29 ± 0.22	0.31 ± 0.19	0.25 ± 0.08	0.25 ± 0.10	0.20 ± 0.14	0.41 ± 0.20
i-17:0	0.21 ± 0.17	0.22 ± 0.24	0.2 ± 0.07	0.22 ± 0.08	0.45 ± 0.17	0.41 ± 0.05
17:0	0.53 ± 0.22	0.52 ± .22	0.27 ± 0.14	0.36 ± 0.22	0.29 ± 0.18	0.39 ± 0.29
17:1	0.28 ± 0.19	0.25 ± 0.14	0.19 ± 0.16	0.10 ± 0.16	0.31 ± 0.27	0.46 ± 0.15
i-18:0	0.40 ± 0.34	0.42 ± 0.42	0.26 ± 0.10	0.25 ± 0.08	0.47 ± 0.26	0.50 ± 0.36
18:0	11.27 ± 1.77	10.90 ± 2.19	11.79 ± 0.66	11.08 ± 0.52	11.37 ± 2.02	10.73 ± 0.25
18:1n-9	22.68 ± 2.56	22.71 ± 3.45	22.8 ± 1.48	23.26 ± 2.74	20.30 ± 3.25	20.86 ± 2.77
18:1n-7	2.50 ± 0.25	2.49 ± 0.60	2.38 ± 0.28	2.22 ± 0.28	2.70 ± 0.04	2.70 ± 0.48
18:1n-5	0.26 ± 0.15	0.28 ± 0.25	0.22 ± 0.05	0.23 ± 0.05	0.42 ± 0.17	0.41 ± 0.08
18:2n-9^{D}	0.26 ± 0.20	0.27 ± 0.15	0.20 ± 0.05	0.24 ± 0.08	0.37 ± 0.23	0.29 ± 0.05
18:2n-6^{D}	1.06 ± 0.19	1.05 ± 0.32	1.19 ± 0.26	1.23 ± 0.42	1.27 ± 0.24	1.37 ± 0.35
18:2n-4^{D}	0.20 ± 0.15	0.22 ± 0.12	0.15 ± 0.06	0.19 ± 0.10	0.39 ± 0.27	0.25 ± 0.09
18:3n-6^{D}	0.15 ± 0.13	0.17 ± 0.10	0.13 ± 0.06	0.16 ± 0.08	0.31 ± 0.22	0.32 ± 0.07
19:1	0.33 ± 0.30	0.37 ± 0.25	0.25 ± 0.12	0.35 ± 0.19	0.66 ± 0.46	0.60 ± 0.19
18:3n-3^{D}	0.95 ± 0.42	1.00 ± 0.72	1.18 ± 0.39	1.34 ± 0.48	1.21 ± 0.85	1.12 ± 0.38
18:4n-3^{D}	2.24 ± 1.11	2.11 ± 1.51	2.88 ± 1.12	2.91 ± 1.13	2.06 ± 1.10	1.82 ± 0.27
20:0	0.27 ± 0.17	0.27 ± 0.19	0.25 ± 0.05	0.27 ± 0.06	0.54 ± 0.45	0.37 ± 0.07
20:1n-11^{D}	0.23 ± 0.18	0.27 ± 0.17	0.60 ± 0.52	0.60 ± 0.63	0.54 ± 0.28	0.43 ± 0.16
20:1n-9^{D}	1.10 ± 0.39	1.15 ± 0.33	1.21 ± 0.55	1.16 ± 0.53	1.58 ± 0.32	2.01 ± 0.24
20:1n-7^{D}	0.08 ± 0.07	0.11 ± 0.11	0.19 ± 0.05	0.22 ± 0.08	0.46 ± 0.37	0.44 ± 0.20
20:1n-5	0.25 ± 0.20	0.27 ± 0.26	0.21 ± 0.07	0.21 ± 0.07	0.39 ± 0.30	0.42 ± 0.22
20:2n-6^{D}	0.40 ± 0.20	0.42 ± 0.19	0.34 ± 0.11	0.30 ± 0.06	0.49 ± 0.18	0.48 ± 0.10
20:3n-6^{D}	0.28 ± 0.19	0.30 ± 0.15	0.24 ± 0.10	0.28 ± 0.12	0.40 ± 0.11	0.37 ± 0.04
21:0	0.28 ± 0.19	0.29 ± 0.24	0.17 ± 0.06	0.22 ± 0.10	0.25 ± 0.09	0.23 ± 0.19
20:4n-6^{D}	8.57 ± 1.11	8.66 ± 1.71	10.31 ± 1.11	9.68 ± 1.51	8.88 ± 1.91	8.70 ± 0.77
20:3n-3^{D}	0.27 ± 0.29	0.28 ± 0.43	0.21 ± 0.12	0.21 ± 0.12	0.41 ± 0.42	0.32 ± 0.07
20:4n-3^{D}	0.34 ± 0.36	0.36 ± 0.34	0.31 ± 0.15	0.25 ± 0.09	0.55 ± 0.30	0.41 ± 0.12
20:5n-3^{D}	4.59 ± 1.76	4.67 ± 1.31	3.14 ± 0.70	2.71 ± 0.63	2.50 ± 0.89	2.93 ± 1.27
22:0	0.52 ± 0.15	0.53 ± 0.23	0.52 ± 0.08	0.56 ± 0.10	0.48 ± 0.33	0.54 ± 0.06
22:1n-11^{D}	0.52 ± 0.29	0.55 ± 0.32	0.53 ± 0.13	0.45 ± 0.16	0.66 ± 0.26	0.84 ± 0.22

Table 5.1: cont.

	Pre-laying Exodus		Incubation		Chick-rearing	
	Male (n = 15)	Female (n = 8)	Male (n = 15)	Female (n = 9)	Male (n = 3)	Female (n = 4)
22:1n-9[D]	0.30 ± 0.24	0.30 ± 0.26	0.21 ± 0.07	0.26 ± 0.09	0.54 ± 0.41	0.45 ± 0.18
22:1n-7[D]	0.26 ± 0.24	0.27 ± 0.24	0.20 ± 0.10	0.21 ± 0.12	0.55 ± 0.39	0.30 ± 0.11
22:2n-6[D]	0.20 ± 0.17	0.22 ± 0.19	0.16 ± 0.06	0.18 ± 0.08	0.37 ± 0.30	0.30 ± 0.08
22:4n-6[D]	0.44 ± 0.16	0.43 ± 0.32	0.24 ± 0.12	0.32 ± 0.22	0.53 ± 0.26	0.60 ± 0.07
22:5n-6[D]	0.75 ± 0.38	0.76 ± 0.28	0.72 ± 0.20	0.80 ± 0.18	1.16 ± 0.04	1.06 ± 0.26
22:5n-3[D]	0.56 ± 0.16	0.50 ± 0.13	0.20 ± 0.08	0.22 ± 0.08	0.40 ± 0.26	0.33 ± 0.18
24:0	0.41 ± 0.25	0.53 ± 0.12	0.89 ± 0.20	0.94 ±0.23	0.99 ± 0.49	1.01 ± 0.30
22:6n-3[D]	8.55 ± 1.59	8.49 ± 0.91	9.23 ± 0.94	9.63 ± 0.70	8.88 ± 0.89	8.70 ± 0.76
24:1n-9[D]	0.94 ± 0.36	1.01 ± 0.40	1.34 ± 0.23	1.62 ± 0.23	1.93 ± 0.80	1.83 ± 0.24
16 PUFAs[1, D]	2.47 ± 0.69	2.45 ± 1.14	2.70 ± 0.63	2.91 ± 0.63	2.99 ± 1.21	2.68 ± 0.17
OTHERS[2]	0.30 ± 0.20	0.32 ± 0.28	0.21 ± 0.09	0.28 ± 0.23	0.28 ± 0.21	0.32 ± 0.12
Total SFA	35.17 ± 2.86	35.28 ± 3.31	34.33 ± 0.81	33.73 ± 0.81	33.67 ± 1.89	33.63 ± 0.81
Total MUFA	31.90 ± 2.24	32.15 ± 0.77	32.07 ± 1.21	32.58 ± 2.33	33.14 ± 0.32	34.25 ± 2.35
Total PUDA	32.39 ± 2.98	32.57 ± 3.71	33.60 ± 1.50	33.69 ± 2.37	33.19 ± 2.15	32.13 ± 1.75

[*]Underlined FAs were responsible for the observed differences between groups (breeding stages).
[D]Fatty acids of dietary origin (Iverson *et al.* 2004).
[1]16 PUFAs all detected below trace levels including 16:2n-6, 16:2n-4, 16:3n-6, 16:4:n-3, 16:4n-1
[2]Other fatty acids detected below trace levels including 14:1, 18:3n-4, 18:2n-7, 21:5n-3

Table 5.2: Bonferroni corrected P-values from one-way Analysis of Similarities (ANOSIM; Bray-Curtis distance measure; global-R = 0.24) indicating the seasonal differences in total fatty acid composition of whole blood from adult male and female Barau's Petrel (*Pterodroma baraui*). Global-R values in parentheses and asterisks denote significant differences (P < 0.05).

		Pre-laying Exodus		Incubation		Chick-rearing	
		Female	Male	Female	Male	Female	Male
Pre-laying Exodus	Female	-	-	-	-	-	-
	Male	(0.13) 1.00	-	-	-	-	-
Incubation	Female	(0.21) 0.16	(0.16) 0.61	-	-	-	-
	Male	(0.52) < 0.01*	(0.29) < 0.01*	(0.08) 1.00	-	-	-
Chick-rearing	Female	(-0.03) 1.00	(0.24) 1.00	(0.28) 0.85	(0.57) 0.02*	-	-
	Male	(-0.03) 1.00	(0.23) 1.00	(0.24) 1.00	(0.33) 1.00	(0.00) 1.00	-

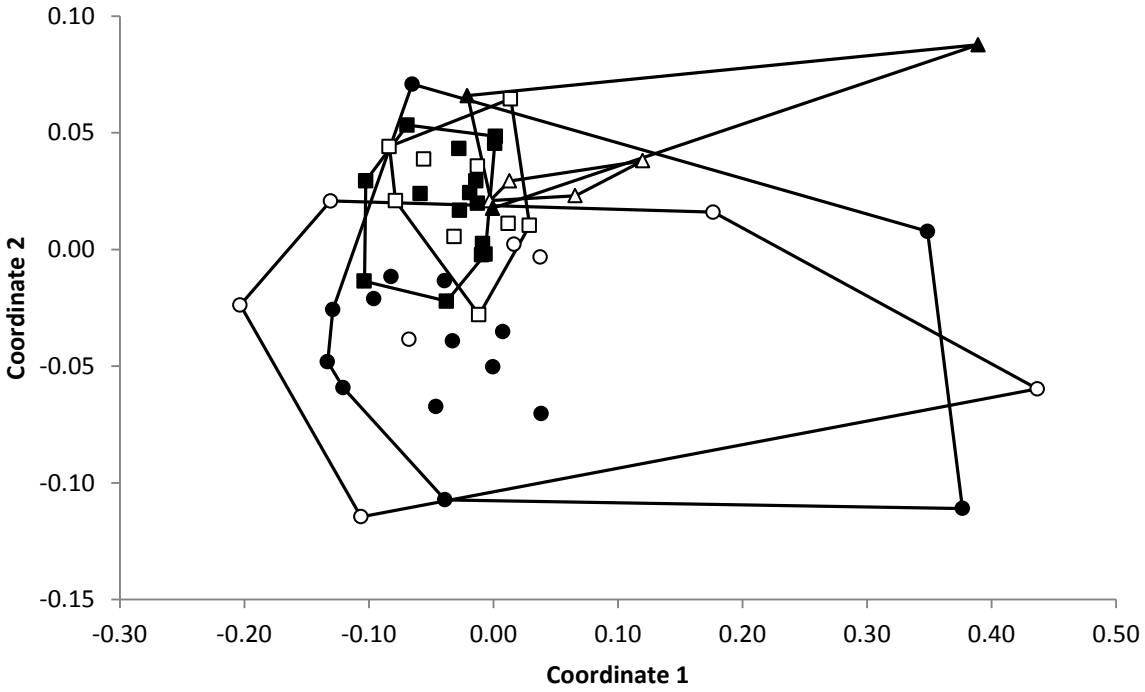

Figure 5.2: Non-metric multidimensional scaling plot (including convex hulls) indicating the relationship between total blood fatty acid profiles of adult male (filled symbols) and female (open symbols) Barau's Petrel (*Pterodroma baraui*) during pre-laying exodus (circle), incubation (square), and chick-rearing periods (triangle). Male pre-laying exodus n = 15, female pre-laying exodus n = 8, male incubation n = 15, female incubation n = 9, male chick-rearing = 3, female chick-rearing n = 4. Two dimensional stress value = 0.21.

5.4. Discussion

FAs have been widely applied as markers for tracing predator-prey relationships in marine ecosystems (e.g. Raclot *et al.* 1998; Tierney *et al.* 2008a; Iverson 2009; Käkelä *et al.* 2010; Ronconi *et al.* 2010; Richoux *et al.* 2010; Owen *et al.* 2013; Connan *et al.* 2014), as well as in the identification of key processes that are expected to affect the overall integrity of

these habitats (Dalsgaard *et al.* 2003; Iverson *et al.* 2004). Here, lipid analyses of total blood have, unsurprisingly, indicated a number of key differences between sexes and breeding stages in adult Barau's Petrel. But for now though, it is difficult to determine whether these dissimilarities are of physiological (e.g. costs of egg production and/or fasting), biochemical (e.g. differences in lipid metabolism/storage), or dietary origin. FASAs have, up until now, never been applied to tropical seabirds; past lipid analysis in the Indo-Pacific region have been entirely limited to species from the sub-Antarctic Islands, with a definite focus on stomach oil (e.g. Connan *et al.* 2007a, 2007b; Richoux *et al.* 2010; Connan *et al.* 2014). Stomach oil is derived from the mechanical breakdown of lipid-rich prey (Foster *et al.* 2010), the TFA composition of which reflects the average signature of the dominant and partially undigested prey from preceding meals (Place *et al.* 1989; Connan *et al.* 2005). It is preferentially used in FASAs since its overall FA composition remains relatively unmodified between prey and predator (Käkelä *et al.* 2005; Foster *et al.* 2010; Karnovsky *et al.* 2012). The TFA composition of blood and adipose tissue also predictably reflects the diet over ecologically relevant time scales, but feeding experiments are needed to deduce calibration factors for the transfer of FAs from prey to predator tissues (Käkelä *et al.* 2005, 2010; Williams & Buck 2010; Karnovsky *et al.* 2012). Few studies have ever addressed this constraint (e.g. Käkelä *et al.* 2005, 2010), for the obvious reason that only a few seabirds can be successfully kept in captivity for periods long enough to allow complete tissue turnover. The FASA method is also best suited to predators that feed exclusively on fatty foods so that the importance of these prey will be overestimated when consumed alongside leaner animals, such as cephalopods (Iverson *et al.* 2007). So, whilst a number of conclusions could be drawn from this study, further clarification of the diet of Barau's Petrel through FASAs would rely on additional physiological data as well as FA profiles of potential prey species,

taking into account the temporal and spatial plasticity in foraging behaviour and habitat use of this highly migratory species.

5.4.1. Total fatty acid composition of Barau's Petrel blood and its relatedness to diet

The observed differences in TFA composition of whole Barau's Petrel blood can, at least in part, be related to diet. Since there was no striking seasonal variation in TFA composition of the blood, it is unlikely that there were any major shifts in the resources targeted during the breeding season, though the TFA composition of blood varied considerably among individuals within each breeding stage. This result suggests minor population level differences but major individual variations in diet composition and nutrient uptake, which is consistent with an opportunistic feeding nature. The framework on which the interpretation of these data, and most other FASAs on seabirds, has been based was built on the extensive research conducted on marine mammals, however (Williams & Buck 2010). Iverson *et al.* (2004) identified those fats in the tissues of northern hemisphere seals (*Halichoerus grypus, Phoca groenlandica*) that were sourced from diet. The 16, 18, and 22 PUFAs (with the exception of 22:5n-3) were all directly linked to diet in the pinnipeds, as were the 20 and 22 MUFAs, 21:5n-3, and 24:1 (Iverson *et al.* 2004). What data are currently available from calibration tests on marine birds suggests that there are no major differences between mammals and seabirds, with long chain MUFAs (particularly C20 and 22) and PUFAs (particularly C16, 18, 20 and 22) typically increasing in consumer tissues subsequent to feeding (Käkelä *et al.* 2005, 2009, 2010). Interestingly, these broad patterns encompass many of the FAs (i.e. 16:2n-6, 16:2n-4, 16:3n-6; 16:4n-3, 16:4n-1, 20:4n-6, 18:4n-3; 20:4n-6; 20:5n-3, and 22:6n-3) that were consistently responsible for the observed differences in FA profiles from Barau's Petrel blood. However, Käkelä *et al.* (2005, 2009, 2010) worked on

blood plasma, confounding the present interpretations, and analyses of total blood have yet to be conducted on captive birds. Whole blood contains a mixture of both neutral and polar lipids (including phospholipids), rendering it considerably more difficult to make dietary deductions (Iverson *et al.* 2004), and the prevalence of structural lipids (e.g. 16:0 and 18:1n-9) in these samples, which is presumably an artefact of this methodological approach, would further explain the absence of any major population variation in TFA compositions of blood among the breeding stages.

FA profiles of whole blood from adult Barau's Petrel consistently showed higher proportions of PUFAs (particularly EFAs) and lower incidences of SFAs and MUFAs compared to other published summaries of the FA composition of seabird stomach oil (e.g. Connan *et al.* 2005, 2007a, 2007b; Wang *et al.* 2007). Interestingly, these patterns were also observed when comparing Barau's Petrel signatures with the plasma (Richoux *et al.* 2010), whole blood (Tierney *et al.* 2008a), and adipose tissue (Wang *et al.* 2007, 2009), of other seabirds from elsewhere. This would further suggest some relationship between the TFA composition of the birds' blood and their diet, since PUFAs are preferentially incorporated from prey into the blood and adipose tissues of marine birds (Richoux *et al.* 2010). Alternatively it may reflect the suggested increase in the degree of FA saturation with increasing water temperature (Lewis 1962, 1967; Ackman *et al.* 1968; Patton 1975; Pohl & Zurheide 1982; Reinhardt & Van Fleet 1986). The higher temperature of tropical seas supposedly increases the fluidity of membranes of the inhabiting organisms, whereas colder environments of the subtropics and poles dramatically decrease it (Hochachka & Somero 2002; Mansilla *et al.* 2004). To counter these variations, some organisms change the ratio of SFAs to MUFAs, and PUFAs in their tissues (Lewis 1962, 1967; Farkas & Herodek 1964; Ackman *et al.* 1968; Patton 1975; Hazel 1995). This increased degree of unsaturation at higher latitudes aids in

protecting the animal's cellular constituents against the near freezing temperatures by increasing the mobility of compounds across membranes, thus allowing the animals to metabolize their FA constituents more easily (Reinhardt & Van Fleet 1986; Mansilla *et al.* 2004). However, in the absence of analyses of total lipid composition of tissues from other tropical marine organisms and the required database of signatures from the potential prey species of Barau's Petrel, it is not possible to differentiate between these two interpretations.

No single FA can be uniquely assigned to any one species and, as such, qualitative diet estimation using FA trophic markers has limited scope in studies of consumer feeding ecology. It is possible to draw certain broad conclusions, based on the assumption that certain combinations of FAs are associated with different prey types, however. For example, the pervasiveness of 20:4n-6 and 18:1n-9 in the blood of Barau's Petrel (8-11 and 20-23 % of TFA composition, respectively) are consistent with a carnivorous diet as these markers tend to dominate FA profiles of higher trophic level marine organisms (Falk-Petersen *et al.* 1990; Budge *et al.* 2002; Dalsgaard *et al.* 2003). Furthermore, Käkelä *et al.* (2005) demonstrated an index of importance of pelagic versus demersal prey species in the diets of captive fed Herring Gulls (*Larus argentatus*). This index, based on the ratio of 20:4n-6 to the sum of 18:3n-3, 18:4n-3, and 20:5n-3, has since been widely applied to free-living marine birds and application of it to Barau's Petrel signatures (mean ratio 1.34 ± 0.19) indicated little dietary importance for demersal prey species and greater prominence of pelagic groups (Käkelä *et al.* 2005; Owen *et al.* 2013). More specifically, squids, which are believed to be the dominant prey group of Barau's Petrel (Kojadinovic *et al.* 2007; Chapter 3), contain the greatest proportion of total lipids in their tissues. These fats are largely restricted to the digestive gland, however, whilst the mantle and arms/tentacles are typically lean in terms of lipid

composition (Phillips *et al.* 2002). Since minimal metabolic modifications are made during the assimilation of FAs, squid FA profiles tend to reflect those of their prey rather than consisting of a unique signature (Phillips *et al.* 2002). As a result, interpretations of squid consumer profiles (like Barau's Petrel; Kojadinovic *et al.* 2007; this study) are complex and not always possible (Phillips *et al.* 2002). The situation is exacerbated because virtually no dietary information exists about the squid species found in this bioregion. Fish oil, which is different in FA composition from fish flesh and is selectively retained by piscivorous Procellariformes as stomach oil, also tends to be very rich in MUFAs including 18:1n-9, 16:1n-7, 20:1n-9, and 20:1n-11 (Nichols *et al.* 1994; Raclot *et al.* 1998; Dalsgaard *et al.* 2003). The low incidence of many of these FAs in the blood of Barau's Petrel might point toward little dietary importance of myctophids, which tend to show a unique signature (Raclot *et al.* 1998; Connan *et al.* 2007b), and other fish species. This is supported by the low occurrence (± 5 % of TFA composition) of the n-3 FA family, which are typically high in both larval and adult marine fish (Raclot *et al.* 1998; Dalsgaard *et al.* 2003), and their consumers (Raclot *et al.* 1998; Tierney *et al.* 2008a; Richoux *et al.* 2010). This interpretation is obscured, however, since the TFA composition of larval fish can be very similar to that of their microalgae and zooplankton prey (St John & Lund 1996), and the FA composition of adult fish can also be affected by diet and a suite of physiological and behavioural traits (Sargent *et al.* 1993, 1999; Anthony *et al.* 2000; Dalsgaard *et al.* 2003).

5.4.2. Seasonality in the total fatty acid composition of Barau's Petrel blood

Seasonal changes in diet are known from many seabird species worldwide and have been broadly attributed to intrinsic factors, such as the need to feed on size-specific prey, or environmental factors that might influence the timing of prey availability (Owen *et al.* 2013).

Seasonal differences are also known to occur between sexes at different stages of the breeding season (Owen *et al.* 2013). Disentangling these from variations associated with differences in physiological and biochemical pathways is challenging, as all will certainly act synergistically in determining blood FA composition. Indeed, the main assumption underlying this work is that the differences in FA signatures from predator tissues will reflect changes in diet rather than differences in FA metabolism within or among groups (Williams & Buck 2010). Thus, it is necessary to examine other hypotheses when interpreting these data as changes in physiological state associated with periodic events in a birds' life (including moult, egg production, incubation, migration, and nutritional stress) are highly energetically demanding and are also known to alter FA metabolism to some extent (Williams & Buck 2010).

Key differences in FA composition of Barau's Petrel blood were observed between the pre-laying exodus and incubation periods when the birds would have been subjected to different types of nutritional stress (Grant 1981). To date though, little scientific attention has been devoted to understanding the functional importance of and stresses associated with the pre-laying exodus in procellariiform seabirds. The degree to which these birds rely on endogenous (capital) versus exogenous (income) sources of energy varies through the breeding season and income breeding has now been proposed as the explanation for the pre-laying exodus. At this time, females are exposed to the costly demands of egg production whilst males, linked into incubation duties later in the breeding season, must attain large fat and protein (associated with increasing levels of water; Groscolas *et al.* 1991) reserves (Warham 1990, 1996; Mallory *et al.* 2008; Williams & Buck 2010). As a consequence, Mallory *et al.* (2008) identified sex-specific changes in the body of Northern Fulmars (*Fulmarus glacialis*) around the pre-laying exodus, noting that females would

preferentially select calcium-rich prey, to support egg production, whilst males gathered fat- and protein-rich prey, to support fasting during incubation. Egg production, in particular, sharply increases the concentration of dietary, modified, and *de novo* synthesized FAs in the blood, all of which are intended for yolk-production (Williams & Buck 2010). Thus, the dietary shifts, body composition changes, and suspected changes to digestive and reproductive organs (as was found in Northern Fulmars; Mallory *et al.* 2008) together indicate differences in FA acquisition and allocation strategies between the sexes during the pre-laying exodus, which would all have contributed to the observed differences in the TFA composition of Barau's Petrel blood.

Mallory *et al.* (2008) also highlighted the fact that, during the pre-laying exodus, the Northern Fulmars targeted areas where they were likely to acquire sufficient resources to endure reproduction, even though those habitats tended to be significantly further away than the closest ice-free areas. This then begs question as to why Barau's Petrel forage south of Madagascar, some 3 000 km away from Réunion Island, with males consistently using areas further away from their nesting grounds than females (Pinet *et al.* 2012). This region is characterised by strong mesoscale activity and has also been associated with predictable, seasonal, and persistent phytoplankton blooms (Srokosz *et al.* 2004; Penven *et al.* 2006; Siedler *et al.* 2006; Lutjeharms 2007). Waters closer to the colonies are less productive during early summer (Pinet *et al.* 2012), and tend to host significant numbers of seabirds (particularly petrels and shearwaters; Mannocci *et al.* 2014). As such, it can be suggested that the petrels, like the fulmars, are using distant but reliable foraging grounds where the energy to be invested in reproduction can be more easily attained and where inter-specific competition may be reduced, thus maximising reproductive output in the current breeding season (Elliott *et al.* 2010).

Subsequent to egg laying, the roles of the two sexes become similar, but males of this species invariably take the first incubation shift and will frequently fast at the nest for around 17 days (Pinet *et al.* 2012). Fasting in birds can be divided into three different physiological stages. The first is characterised by a high rate of body mass loss through the consumption of endogenous carbohydrates, which tend to have fairly low mass-specific energy contents (Totzke *et al.* 1999). As these reserves are depleted, the rate of body-mass loss slows and energy is supplied mostly through the oxidation of lipids (some proteins are catabolized), at which time the concentration of free FAs (particularly triglycerides and cholesterols) in the blood sharply increases. Finally, as the fat reserves are exhausted, the birds catabolize bodily proteins as the only source of energy at which time body-mass loss increases once more (Totzke *et al.* 1999). This entire process, as shown by Groscolas (1990) and Totzke *et al.* (1999), has a great influence on the FA composition of seabird tissues and was most likely responsible for the observed differences in FA signatures associated with male Barau's Petrels during incubation. Male Northern Fulmars are also known to attain more body fat during the pre-laying exodus than females; the average mass of male birds increases by between 5 and 14 % during the pre-laying exodus (Hatch 1990), equating to around 6 000 kJ of energy, which has the capability of supporting them over ± 11 days of incubation (Mallory *et al.* 2008). Upon returning from the pre-laying exodus, male Barau's Petrel are also significantly heavier than females (≤ 535 g and ≤ 480 g for males and females, respectively; Pinet *et al.* 2012; this study). Most of these additional reserves are lost before the end of the breeding season in both species (Mallory *et al.* 2008), directly as a consequence of fasting, providing additional support for this hypothesis.

Male and female birds share parental duties during chick-rearing and spend significantly less time fasting at the nest when compared with the earlier breeding stages.

Foraging trips at this time are, on average, shorter that the exoduses during the pre-laying or incubation periods, but adults alternate numerous short trips, to sustain chicks, with longer trips where they gather food for themselves (Pinet *et al.* 2012). It is not surprising then that the least variation in signatures, from a physiological and biochemical perspective, between males and females was observed during this period. This also suggests that both sexes target the same resources over this final breeding stage – a result that has been supported by the SI values (Chapter 4). On the other hand, differences in FA composition of whole blood were expected between this period and both the incubation and pre-laying exoduses as birds forage closer to their nesting grounds on Réunion Island (Pinet *et al.* 2012). Indeed, it is not unreasonable to speculate that there may be differences in the lipid composition of conspecific prey from the different areas, since latitudinal differences in FA composition of micronekton, reflecting gradients in temperature, have been described from elsewhere (Ackman *et al.* 1968; Patton 1975; Pohl & Zurheide 1982; Reinhardt & Van Fleet 1986). In addition, it was expected that adult birds would ingest a greater proportion of size specific prey (juvenile fish and squid with which seabirds sometimes feed their young), which differ in FA composition from mature individuals (Sargent *et al.* 1993, 1999; Anthony *et al.* 2000; Dalsgaard *et al.* 2003). These differences may well have become apparent if a greater number of samples had been collected over this final breeding stage, however.

5.4.3. Conclusions and recommendations

Although preliminary, this work on the FA composition of the whole blood of Barau's Petrel is at the forefront of FASAs in tropical ecosystems. The results have indicated a number of key differences in TFA composition of adult birds over the course of the breeding season and these have broadly been attributed to dietary, physiological, and/or biochemical

differences between male and female birds. Nutritional stresses associated with egg production and fasting (associated with incubation) were thought to be the principal factors shaping the observed patterns, particularly where incubating males were concerned. Whilst it was extremely difficult to make conclusions about diet, it would appear that fish (particularly myctophids) are of little importance in the diet of this species. This result contrasts with Stahl and Bartle (1991) but supports Kojadinovic *et al.* (2007) and was not surprising as these groups are virtually absent from the diets of other tropical seabirds (e.g. Jaquemet *et al.* 2008; Catry *et al.* 2009a) and surface feeding predatory fish (e.g. Potier *et al.* 2007; Romanov *et al.* 2008; Jaquemet *et al.* 2011) in the western Indian Ocean. Myctophids have been identified as an important prey group for tropical gadfly petrels in the eastern Pacific Ocean, however (Spear *et al.* 2007), but the diets of these opportunistic birds are known to be spatio-temporally variable (Warham 1990). Clarification of the prominence of squid in this species' diet is, for the moment, not possible since the FA composition and dietary relationships of key cephalopod species from the region are not well understood. Further analysis would benefit from the inclusion of potential prey species to allow more in-depth quantitative discussion on the origins of the observed differences. The use of adipose tissue would also be advantageous, as problems are inherent in the interpretation of FA signatures from blood (Williams & Buck 2010). Also of interest were the generalised parallels that exist between this work and Mallory *et al.* (2008), which highlighted the importance of the pre-laying period in the Procellariiformes and indicated that this species' reproductive output may be highly dependent on predictable physical features in the south-western sector of the Indian Ocean. This could have profound conservation implications on this already threatened species, although further work is needed to clarify whether this is indeed the case.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

6.1. Managing for the biphasic lifestyle of Barau's Petrel

The Mascarene Islands (Réunion, Mauritius, and Rodrigues) once supported one of the richest and most extraordinary vertebrate faunas of any oceanic archipelago (Le Corre & Safford 2001). They have suffered from an unparalleled extinction rate, however, and most indigenous vertebrate species have now been eradicated (Cheke 1987; Mourer-Chauviré *et al.* 1996; Le Corre & Safford 2001; Olson 2008; Hume 2013). This includes the local extirpations of at least two seabird species: Abbott's Booby (*Papasula abbotti*) is now regionally extinct (Feare 1978), and the Tropical Shearwater has become locally extinct on Mauritius (Dumont *et al.* 2010). Réunion Island remains unique, however, in that it is the only tropical island in the world that supports two endemic species of extant gadfly petrel (Pinet *et al.* 2009; Shirihai *et al.* 2014). Like many of the other remaining vertebrates, each of these species has an unfavourable conservation status. Extinction is almost inevitable for the Mascarene Petrel since its active breeding colonies have not yet been discovered, which precludes research and conservation initiatives. The situation is not nearly as severe for Barau's Petrel as the first active breeding colonies were discovered in 1995 (Probst 1995; Probst *et al.* 1995, 2000; Pinet *et al.* 2009). Most of this species' major threats on land have since been studied and protection measures have been implemented wherever possible (Le Corre *et al.* 2002; Le Corre 2008; Falquier *et al.* 2009; Pinet *et al.* 2009; Dumont *et al.* 2010). Various authors have highlighted the remaining knowledge gaps for this species and the management priorities needed to prevent further declines in its population (Pinet *et al.* 2009, 2011b, 2012; Russell & Le Corre 2009; Dumont *et al.* 2010). In particular, the conservation action plan for Barau's Petrel has indicated the need to study their at-sea

ecology so to assess and control for any additional threats they face when foraging (Salamolard 2007). This is no easy task, since recent tracking studies have shown that adults feed in oceanic areas thousands of kilometres away from their breeding colonies (Pinet *et al.* 2011b, 2012). In this regard, the combination of dietary techniques is extremely valuable as the information they provide extends over many temporal scales, offering far more insight about the at-sea behaviour of a species than just the food resources targeted. Thus, in combining three complimentary procedures (stomach content, SIA and FASA), this present thesis aimed to provide new data on the diet and foraging habits of Barau's Petrel so as to overcome many of the shortcomings of the previous studies (i.e. Stahl & Bartle 1991; Jaquemet *et al.* 2004; Kojadinovic *et al.* 2007, 2008; Pinet *et al.* 2011b, 2012). This approach helped to minimise the shortfalls of each individual method (reviewed in Duffy & Jackson 1986, Barrett *et al.* 2007, Bond & Jones 2009, and Williams & Buck 2010), allowing greater certainty in conclusions.

6.2. Overall diet composition, trophic ecology and foraging behaviour

A few mentions of the at-sea ecology (Stahl & Bartle 1991; Jaquemet *et al.* 2004; Kojadinovic *et al.* 2007, 2008), and diet composition of Barau's Petrel (Stahl & Bartle 1991; Kojadinovic *et al.* 2007, 2008) exist in the literature (reviewed in Pinet *et al.* 2009). This information has always been presented in papers with other objectives, not specifically related to this species' foraging behaviour, and the data that exists are all somewhat contradictory. Based on these accounts alone, it has not been possible to determine the resources on which this species' reproductive output is dependant and how these birds might be threatened when at sea (Pinet *et al.* 2009). The present stomach content results are similar to those of Kojadinovic *et al.* (2007), in that they highlighted cephalopods as an

important prey group of breeding Barau's Petrels. Hard structures from fish were less frequently encountered among the accumulated prey items, suggesting their dietary importance may be less than that of cephalopod prey. It is important to acknowledge that fish eye lenses, scales, and bones have faster erosion rates than cephalopod beaks inevitably resulting in some underestimation of the importance of this latter prey group. Linked to this, the FA results could not confirm cephalopods as a primary dietary constituent, but the low incidence of n-3 FAs and long-chain MUFAs in the blood of breeding Barau's Petrel further supports the conclusion that myctophid and other fish species are not important dietary constituents of the birds (Nichols *et al.* 1994; Raclot *et al.* 1998; Dalsgaard *et al.* 2003). These results contrasted with the at-sea feeding observations made by Stahl and Bartle (1991). However, it seems that that study was flawed in that their observations were all made during the day, while the available tracking evidence (Pinet *et al.* 2012), and the species composition, behaviour, and morphology (size structure, presence/absence of photopores) of the cephalopod prey identified in this study, together suggest that Barau's Petrel mostly feed at night. Indeed, nocturnal feeding is common among the Procellariidae, particularly the gadfly petrels (Harper 1987; Warham 1990; Ballance & Pitman 1999).

Numerous other prey groups (i.e. marine insects, crustaceans, vegetative material, and molluscs) were also recorded among the accumulated prey items, which suggest that these birds are strongly opportunistic and that the species' diet is more varied than has previously been thought (Stahl & Bartle 1991; Kojadinovic *et al.* 2007). Such diverse feeding habits have also been recorded for other gadfly petrels including the Providence (*Pterodroma solandri;* Bester *et al.* 2011), Grey-faced (*P. macroptera*; Imber 1973), Soft-plumaged (*P. mollis;* Schramm 1986) and Kerguelen Petrels (*Lugensa brevirostris;* Schramm 1986). Opportunism implies some degree of resilience against environmental unpredictability as these birds

should exploit different types of prey as their relative abundance/availability changes (Stahl & Bartle 1991; Kojadinovic *et al.* 2007; Montevecchi *et al.* 2009). Daytime scavenging, which is closely linked to opportunism, is another important feeding technique among procellariiform seabirds (Ashmole & Ashmole 1967; Harper 1987; Lipinski & Jackson 1989; Warham 1990; Imber *et al.* 1995; Ballance & Pitman 1999; Bester 2003; Bester *et al.* 2010). Evidence for this in Barau's Petrel came from the presence of the beaks of large cephalopods, including *Stigmatoteuthis hoylei*, among the other accumulated prey items in the stomachs. These are believed to have become available to these surface-feeding birds as they floated to the upper levels of the water column after death (Dell 1952; Lipinski & Jackson 1989; Jereb & Roper 2010). The high trophic level of these cephalopods is also believed to have raised the nitrogen stable isotope ratios of whole blood from breeding adult Barau's Petrels, which were higher than the values of seabirds breeding at a large colony nearby (i.e. Europa Island; Cherel *et al.* 2008; Pinet *et al.* 2012). Kojadinovic *et al.* (2008) independently noted a similar pattern in that, among the most numerically abundant of seabird species that breed on Réunion Island, Barau's Petrel had the highest hepatic $\delta^{15}N$ signatures. Their results were also discussed in terms of the differential foraging behaviour of seabirds from the western Indian Ocean; most tropical seabird species (boobies, frigatebirds, tropicbirds) feed on flying fishes (Exocoetidae) and flying squids (Ommastrephidae; Diamond 1975a, 1975b, 1983; Cherel *et al.* 2008), but Procellariiformes (including Barau's Petrel) will also regularly scavenge on diverse prey including animals from high trophic levels (Ashmole & Ashmole 1967; Harper 1987; Lipinski & Jackson 1989; Warham 1990; Imber *et al.* 1995; Ballance & Pitman 1999; Bester 2003; Bester *et al.* 2010). It is noted too, that latitudinal differences in $\delta^{15}N$ baseline will also have had an effect on

these signatures although insufficient information currently exists from the region to determine how so.

6.3. At-sea distribution

The available information on the at-sea distribution of Barau's Petrel is also somewhat contradictory. Inferences from ship-based surveys and other sighting records suggest that these birds disperse widely across the whole Indian Ocean (Chapman & Cheshire 1987; Dunlop *et al.* 1988; van Marle & Voous 1988; Stahl & Bartle 1991; Van Den Berg *et al.* 1991; Lambert 2001; Pinet *et al.* 2009). Pinet *et al.* (2011b, 2012) have, more recently, shown that adults employ complex patterns of habitat use that vary through the breeding period. Adults then collectively migrate to a large area in the central Indian Ocean around the Ninety East Ridge during the interbreeding period. Carbon SI signatures of tissues (whole blood, feathers) have the potential to validate/contradict these findings (Kroopnick 1985; Post 2002; Cherel *et al.* 2006; Cherel & Hobson 2007; Phillips *et al.* 2009; Militão *et al.* 2013), although the information they provide is never as precise. In order to accomplish this, knowledge about the isotopic baselines in the food webs of interest, at the time of study, is required (Post 2002). This was outside the scope of this study and that research which has been done demonstrated only very weak latitudinal variations in the $\delta^{13}C$ landscape of the Indian Ocean (Ménard *et al.* 2007; S. Jaquemet. *et al.* unpub. data). Hence, from these data alone, it is not possible to elucidate exactly where the adult birds were foraging. However, the observed differences in $\delta^{13}C$ between sexes and among breeding stages closely match this species' intra-seasonal variability in habitat use, as described by Pinet *et al.* (2011b, 2012). For example, significant differences in $\delta^{13}C$ were observed between male and female birds during the pre-laying exodus, suggesting differential habitat use. Indeed, Pinet *et al.*

(2012) noted a 2 % overlap in the foraging distribution of adult male and female birds at this time; males foraged closer to South Africa over the Mozambique Plateau whereas females remained in a large area immediately to the south of Madagascar. Upcoming research cruises in these regions (2015-2016) may shed light on this missing baseline information, allowing further discussion into this species' at-sea movements during the breeding season. Significant differences in $\delta^{13}C$ of whole blood and feathers (adult versus downy chick/fledgling feathers) were also observed between the breeding and non-breeding periods, highlighting the migratory behaviour of adult Barau's Petrel. Once again, however, too little information exists about the isotope baselines in the central Indian Ocean, particularly around the Ninety East Ridge, to identify the areas used through analyses of $\delta^{13}C$.

6.4. Nutritional stress, life history, and individual-optimisation concepts

Although the fasting effect on whole blood seems to be low, the SI and FA results, together, suggest that adult Barau's Petrel suffer significant levels of nutritional stress during various stages of the breeding season. During the pre-laying exodus, females are exposed to the costly demands of egg production whilst males, committed to incubation duties later in the breeding season, must attain large fat, protein, and water reserves (Warham 1990, 1996; Groscolas *et al.* 1991; Mallory *et al.* 2008; Williams & Buck 2010). Over periods of extended nutritional stress, such as long incubation shifts, the birds meet their energy demands by oxidizing endogenous lipids and catabolizing muscle proteins (Mrosovsky & Sherry 1980; Totzke *et al.* 1999). This process sharply increases the concentration of free FAs (particularly triglycerides and cholesterols) in the blood, which acts to deplete $\delta^{13}C$ and raise C:N ratios. This seems to have been reflected in these isotope

results, since some of the highest C:N ratios and lowest carbon values were observed in breeding stages when the birds were suffering from the greatest levels of nutritional stress. Initially, the birds meet their energy requirements by oxidising endogenous lipid reserves and catabolizing muscle proteins, but eventually, as the lipids become depleted, they survive exclusively on their muscle proteins. By 'feeding on themselves,' they also increase the $\delta^{15}N$ of their blood, raising their apparent trophic level (Hobson *et al.* 1993; Cherel *et al.* 2005), as was inferred from this study. These hypotheses are supported by high rates of loss of body condition during the pre-laying exodus and incubation periods for females and males, respectively.

Nutritional stress also affects the FA composition of their blood, although the interpretation of these results proved more difficult and a causal relationship could not be established. Differences in the TFA composition of blood were detected between the pre-laying and incubation periods and all differences involved incubating males. Fasting, during incubation, has a great influence on the FA composition of metabolically active tissues (Groscolas 1990; Totzke *et al.* 1999), such as whole blood (Käkelä *et al.* 2005, 2009), and was believed to have been principal in shaping the observed differences involving incubating males. Regarding females, egg production sharply increases the concentration of dietary, modified, and *de novo* synthesized FAs in the blood, all of which are intended for yolk-production (Williams & Buck 2010). In order to withstand these costs of reproduction, females are believed to remain closer to Réunion Island during the pre-laying exodus, thus minimising the energetic costs of flight (Pinet *et al.* 2012). They also select calcium rich prey, to support egg production (Mallory *et al.* 2008), and depart on a second foraging exodus, to restore the body condition lost during egg production, immediately after laying (Pinet *et al.* 2012). Males must travel to more distant and predictable areas during the pre-laying exodus

(Pinet *et al.* 2012), where they accumulate proteins and large non-structural lipid reserves. These reserves are used to endure the first incubation shift, which is always the longest (Pinet *et al.* 2012). If the males' body condition is not sufficient to withstand this fasting then they will likely abandon the nest. Indeed, more than 90 % of breeding failures occur during the first incubation shift (P. Pinet. pers. comm.). This is in accordance with life history theory (Stearns 1992), which suggests that both sexes contribute in complementary ways to parental care, thus increasing their survival and/or reproductive potential in current and successive breeding episodes (Elliott *et al.* 2010). Since this species' reproductive output is dependent on distant resources, it is plausible to suggest that precedence would rather be given to adult survival, than to maximising reproductive output, when conditions are not entirely favourable for reproduction (Drent & Daan 1980; Nur 1986).

6.5. Management priorities for an updated conservation action plan

The management action plan (Salamolard 2007) should soon be revised based on this, and other (i.e. Kojadinovic *et al.* 2007, 2008; Pinet *et al.* 2011a, 2011b, 2012), new information. At-sea protection is vital to the future survival of all seabirds and, whilst ecological, logistical, and economic limitations exist (Game *et al.* 2009), various tools are now available that facilitate ecosystem based management in an effective manner (Pikitch *et al.* 2004; Game *et al.* 2009). The most commonly employed method is that of Marine Protected Areas (MPAs; Wood *et al.* 2008). These have been implemented across the entire western Indian Ocean, with the primary aim of enhancing fish stocks and conserving coastal marine biodiversity (Buxton *et al.* 2006; Turpie *et al.* 2006; Wood *et al.* 2008; Worm *et al.* 2009). A single pelagic MPA has been delineated around the Chagos Archipelago (British Indian Ocean Territory; Koldewey *et al.* 2010), but all other oceanic communities remain

unprotected in this region. Indeed, pelagic ecosystem protection has been recognised as one of the major global conservation shortfalls (Wood *et al.* 2008; Game *et al.* 2009). Recent research (Le Corre *et al.* 2012), based on the at-sea distribution of seven seabird species (including Barau's Petrel), has attempted to identify other areas of the Indian Ocean that may be suitable for this kind of protection, however. Five hotspots were identified including (1) the Seychelles Basin, (2) the southern Mozambique Channel, (3) the Walter Shoals and other areas to the south of Madagascar, (4) the Mascarene Archipelago including Tromelin Island, (5) and an area in the central Indian Ocean near the Ninety East Ridge. These, barring the Seychelles Basin area, would directly benefit Barau's Petrel because (a) this species breeds on an island in the Mascarene Archipelago, (b) chicks are fed on resources from immediately around Réunion Island, (c) adults forage for themselves in a large area to the south and east of Madagascar, and (d) adults migrate to an area around the Ninety East Ridge during the interbreeding period (Pinet *et al.* 2009, 2011b, 2012). Pelagic MPAs over the Walter Shoals and in the central Indian Ocean would be especially valuable in promoting population recovery of Barau's Petrel since, as Pinet *et al.* (2011b, 2012) have recently demonstrated, these areas are very important foraging areas for this species. Furthermore, complimenting Pinet *et al.'s* (2011b, 2012) interpretations, the present results have proposed that these regions may also be important for the maintenance and replenishment of adult body condition during and after breeding. These seamounts are characterised by important upwellings and local nutrient enrichments (Machu *et al.* 2002; Lévy *et al.* 2007), have high biotic endemism (Collette & Parin 1991 and the references therein; Rogers 2012; IUCN 2013), may represent important breeding grounds for pelagic organisms (e.g. pelagic squids; M. Le Corre. pers. comm.), and attract many other marine top predators (Rogers 2012; IUCN 2013). This implies that the benefits of these pelagic MPAs would extend far

beyond the Barau's Petrel. Recent at-sea observations of the Mascarene Petrel suggest that they might also forage in productive areas south west of Réunion Island (Shirihai *et al.* 2014), so these MPAs may also benefit that species too.

6.6. Conclusions and recommendations for future work.

Following the recommendations made by Salamolard (2007) and Pinet *et al.* (2009, 2011b, 2012), this thesis addresses the uncertainty regarding the marine component of the ecology of Barau's Petrel, which currently prevents assessment and control of the threats that it faces out at sea. This work provides a fruitful preliminary investigation into the subject but a number of critical questions still remain, such as the origin/identity of the birds that have been sighted in northern and eastern Indian Ocean between Somalia and Java (reviewed in Pinet *et al.* 2009). These would need to be addressed before the species' food and feeding ecology can be properly understood.

This is the first study of its nature from a tropical setting, constituting the only in depth investigation into the diet of an Indian Ocean *Pterodroma* petrel, and the results have highlighted the value of such an integrated approach to diet determination. Many of the conclusions are speculative, however, necessitating the need for further study. Though it is difficult to recommend additional research, because of the threatened nature of this species, further work would benefit from an enhanced knowledge of the TFA composition and isotope signatures of prey species, the incorporation of FASAs in stomach oils, the quantification of blood cortisone levels to determine the influence of nutritional stress on SI and FA signatures, and the separation of sampling events for long and short foraging trips by adults during the chick-rearing period. Further at-sea observational research would also be advantageous to clarify the degree to which these birds associate with subsurface marine

predators (e.g. tunas and marine mammals) and other bird species as well as to determine this species' reliance on active predation versus scavenging. It is also necessary to quantity the precise degree to which these birds are threatened as a consequence of direct and indirect interactions with industrial fisheries, particularly longlining activity near eastern South Africa (Danckwerts *et al.* in press), and through the consumption of plastic debris. These newly identified threats are not known to account for significant annual mortalities in *Pterodroma* petrels but, depending on their nature and severity, have the potential to severely impact this species' adult survival, both having already accounted for significant decline in other seabird groups elsewhere (Brothers 1991; Laist 1997; Montevecchi 2002; Baker *et al.* 2007; Croxall 2008; Anderson *et al.* 2009, 2011; Lavers *et al.* 2014; Lavers & Bond in press). Finally, whilst this has previously been attempted without success (M. Le Corre. pers. comm.), it is necessary to document post-fledging dispersal. These individuals must develop flight skills and refine foraging techniques without the guidance of experienced adults and it is unclear where and how they achieve this. This is worrying since mortality rates of procellariiform seabirds are highest during the first few years of life at sea (Fisher 1975; Warham 1990), and estimates of recruitment are fundamental to understanding population trajectories. Age specific identification of this species at sea is not easily achieved so that this work should be based on Argos/GPS-PTT devices. These large, solar-powered devices are within the appropriate size limit for this species (± 9.5 g, 2.3 % of body weight at the time of fledging; Fair *et al.* 2010), having already been fitted onto adult Barau's Petrel during the breeding season (Pinet *et al.* 2012), and have the benefits of high accuracy and real-time data recovery over the internet (via satellites).

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