TROPHIC ECOLOGY OF BREEDING NORTHERN ROCKHOPPER PENGUINS, *EUDYPTES MOSELEYI*, AT TRISTAN DA CUNHA, SOUTH ATLANTIC OCEAN

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Abstract

Northern Rockhopper penguin populations, *Eudyptes moseleyi*, are declining globally, and at Tristan da Cunha have undergone severe declines (> 90% in the last 130 years), the cause(s) of which are unknown. There is a paucity of data on this species in the South Atlantic Ocean, therefore their trophic ecology at Tristan da Cunha was studied, specifically focusing on diet, using stomach content analysis and stable isotope analysis (SIA), in conjunction with an analysis of diving behaviour, assessed using temperature-depth recorders. In order to evaluate the influence of gender on foraging, a morphometric investigation of sexual dimorphism was confirmed using molecular analysis. Additionally, plasma corticosterone levels were measured to examine breeding stage and presence of blood parasites as potential sources of stress during the breeding season.

Northern Rockhopper penguins at Tristan da Cunha displayed a high degree of foraging plasticity, and fed opportunistically on a wide variety of prey, probably reflecting local small-scale changes in prey distribution. Zooplankton dominated (by mass) the diet of guard stage females, whereas small meso-pelagic fish (predominantly Photichthyidae) dominated diet of adults of both sexes in the crèche stage, with cephalopods contributing equally in both stages. Adults consistently fed chicks on lower-trophic level prey (assessed using SIA), probably zooplankton, than they consumed themselves indicating that the increasing demands of growing chicks were not met by adults through provisioning of higher-quality prey. SIA also indicated that adults foraged in different oceanic water masses when feeding for self-maintenance and for chick provisioning, thus temporally segregating the prey consumed for different purposes. It is possible that adults 'selected' these higher-quality prey for themselves, or this may be a reflection of opportunistic behaviour.

At Tristan da Cunha sexual dimorphism was observed in culmen dimensions (length, depth, width), with males having larger beaks and feeding on larger individuals of squid and fish than females. No sexual segregation in terms of foraging habitat (i.e. different water masses, based on $\delta^{13}C$) or trophic level ($\delta^{15}N$) during the breeding season or pre-moult period was revealed through SIA, and stomach content analysis revealed no sexual differences in prey species targeted. The results of SIA of feathers indicate that during the pre-moult period birds foraged in different water masses than during the breeding period. The fact that throughout the breeding season birds foraged in similar habitats suggests no intra-specific competition, despite both sexes feeding on the same prey.

Birds were generally diurnal, daily foragers (12 – 16 hr trips), with extended trips (maximum duration 35.5 hours) and nocturnal diving recorded in a few individuals. Birds dived well within their physiological limits, predominantly utilising the upper 20m of the water column, employing two different strategies to target different prey items. Long, deep (30 – 40 m), energetically costly dives were performed when targeting energy-rich prey (fish), and a greater number of shorter, shallower (5 – 20 m), energy-efficient dives were performed when targeting prey with a lower energy content (zooplankton).

More than half of the sampled study population were infected with the intra-cellular blood parasite *Babesia*, but infection showed no relationship to body mass, corticosterone levels or breeding success. Fasting birds showed no signs of elevated corticosterone levels, suggesting they had acquired sufficient fat reserves prior to breeding. Failed breeders did not exhibit elevated corticosterone levels. Tristan skuas, *Catharacta antarctica hamiltoni*, were observed to be a significant cause of egg and chick mortality.

The absence of sex-based differences in foraging, and the absence of any signs of stress in relation to body mass, presence of *Babesia* or breeding stage, suggest that there are

no obvious signs of high levels of stress or food limitations during breeding at Tristan da Cunha.

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Chapter 1

General introduction

1.1 Optimal foraging theory

Optimal foraging theory (Emlen 1966; MacArthur and Pianka 1966; Hughes 1980) predicts that natural selection shapes patterns of foraging behaviour, and therefore animals should forage in a manner that maximises energy gain, thus promoting fitness. Optimal foraging theory can be applied to searching behaviour, exploitation of food resources and selection between different food items (Stephens & Krebs 1986), and can be expanded to include predator avoidance while foraging (Sih 1985). Animals should primarily accept the most energetically profitable food type available, and only accept less profitable food when encounter rates with the former fall below critical levels (Emlen 1966; MacArthur & Pianka 1966). Essentially, when food is scarce, animals should, theoretically, be less selective in their diet compared to periods when food is abundant (Stephen & Krebs 1986). The complexity of foraging behaviour, however, is much greater than initially presented in these papers, and contemporary foraging theory and models incorporate factors such as nutrient requirements, social interactions, sensory limitations, reproductive state, competition and predation risk during foraging (Lebrun & Feener 2007; Focardi *et al.* 2009; Bolnick *et al.* 2010).

Intra-specific foraging plasticity enables animals to exploit locally abundant food sources, and is observed within species of many groups of animals, including marine invertebrates (Vilas *et al.* 2008), reptiles (Perry 1996; Greeff & Whiting 2000), primates (Janson 1988), marine mammals (Estes *et al.* 2003; Spitz *et al.* 2006), and mammalian carnivores (Gittleman & Harvey 1982). The diet of a predator is

primarily influenced by what prey the animal encounters, whether it chooses to eat it, and whether the animal can successfully capture and consume the prey. Preference for a certain prey type is indicated by higher consumption of this prey than would be expected based on encounter rates (Chesson 1983), and may be influenced by factors including changing environmental conditions, such as changes in the extent of sea-ice (Ainley *et al.* 2003), prey behaviour, as some prey may be easier to capture for example (Lima & Dill 1990), competition for food reducing availability of certain prey (MacArthur & Levins 1967; Abrams 1983), or predator breeding stage, when energy requirements are increased (Williams & Rothery 1990).

The necessity for a predator to exploit available food efficiently is perhaps most pronounced within marine ecological systems, in which resources are spatially and temporally heterogeneous (Croxall *et al.* 1984; Fauchald 1999), thus distribution of animals within these systems is generally aggregated rather than random (Weimerskirch *et al.* 1997, 2005; Fauchald *et al.* 2000). Prey distribution in the open ocean is affected by biological and physical processes on a range of spatio-temporal scales, and, consequently, marine top predators are concentrated in areas where prey abundance is enhanced (Pakhomov & McQuaid 1996), for example large-scale frontal zones (Hull *et al.* 1997; Bost *et al.* 2009; Scheffer *et al.* 2010).

1.2 Central-place foragers

Optimal foraging theory should apply more strictly to central place foragers, such as breeding seabirds (Orians & Pearson 1979), which are restricted in the duration and range of their foraging trips away from their colonies due to the necessity to return frequently to provision young (Wilson *et al.* 1995). During the breeding period, seabirds are challenged to meet the nutritional requirements of

rapidly growing chicks, plus their own individual energetic requirements, whilst being restricted in time and space (Williams 1966; Stearns 1976; Ydenberg *et al.* 1994). The balance between the energy required to search for and handle prey must be balanced carefully with the energy content of prey, to maximise net energy gain (Stephen & Krebs 1986). When different types of prey are available, offering different levels of energy, a trade-off between time spent travelling to prey patches and time spent foraging may be observed (Elliot *et al.* 2008a). On an evolutionary scale, seabirds have developed specific K-selected life-history traits, such as low fecundity, delayed sexual maturity and high adult survivorship (Pianka 1970; Stearns 1992) to cope with the variability in food availability in the marine environment (Hunt *et al.* 1999), and recruitment only occurs if chicks survive to several years of age (Pianka 1970; Saether & Bakke 2000). Thus, population increase is slow, and populations will be harmfully affected by any factor that acts to increase adult mortality (Furness 2003), so on a life-time scale, foraging plasticity is an essential attribute of seabirds (Burger & Piatt 1990).

Seabirds are important consumers of marine resources (Croxall *et al.* 1984), which are typically highly mobile and patchily distributed (Weimerskirch 2007), and their population dynamics are strongly regulated by food availability (Anderson *et al.* 1982; Hunt & Schneider 1987; Monaghan *et al.* 1989), as this can constrain growth, reproductive success and survival (Stearns 1992). Whilst volant seabirds are able to travel further in search of prey, flightless central place foragers such as penguins are even more constrained during the breeding period, as they are unable to increase foraging range greatly. They must therefore be highly adaptable to changing environmental conditions to ensure their own survival and successful breeding. For example, a shift in prey to localities where there are no suitable breeding grounds (e.g.

African penguins, Crawford *et al.* 2008a), and fluctuation in abundance of preferred prey (e.g. anchovy in the Benguela upwelling system, Crawford & Dyer 1995), have a strong influence on the reproductive output of penguins (Furness & Cooper 1982; Crawford & Dyer 1995; Crawford *et al.* 2006). Intra-specifically, some penguins have shown the ability to vary foraging behaviour in terms of foraging location (Lyver *et al.* 2011), diving patterns (Tremblay & Cherel 2000; Miller & Trivelpiece 2008; Pichegru *et al.* 2011), foraging trip duration (Wilson *et al.* 2005), and diet (Polito *et al.* 2011). These behavioural responses, which are attributed, at least partly, to a change in prey availability, act to maximise foraging efficiency in specific environments (Lescroël & Bost 2005; Wilson *et al.* 2005; Miller & Trivelpiece 2008; Zimmer *et al.* 2011). Factors such as sex (Forero *et al.* 2002), breeding stage (Williams & Rothery 1990) and inter-annual variation (Kato *et al*. 2003) may also be responsible for this observed plasticity in penguin foraging behaviour.

The study of seabirds in their natural environment presents a significant challenge, as they predominantly forage below the sea surface. The development of animal-attached miniaturised loggers has revolutionised studies in spatial ecology, behaviour and physiology of many vertebrate groups including seabirds (Rutz & Hays 2009). However, patterns of vertical movement of marine vertebrates through the water column have remained a primary area of interest, and miniaturised time-depth recorders (TDRs) play a key role in the these studies (Wilson *et al*. 2002).

In this thesis, TDRs were used to investigate, for the first time, the diving patterns of Northern Rockhopper penguins at Tristan da Cunha. Two complementary techniques were used to investigate the diet of penguins: conventional stomach content analysis, through stomach flushing, and stable isotope analysis (SIA). Direct stomach content analysis can provide useful detailed taxonomic data (Hobson &

Clark 1992), whereas SIA provides broad-scale time-integrated information on diet. In particular, changes in these aspects of foraging ecology were examined over the energetically demanding breeding season when birds are constrained to foraging close to their colonies to determine if, and the manner in which, foraging plasticity enables them to meet the increasing demands of chick growth.

1.3 The Principle of Allocation

Studies of feeding and food preference are important aspects of ecology, as the ability of parents to procure resources affects their fitness, in terms of provisioning current offspring (growth and condition), and the chance of future reproduction (parental condition) (Hughes 1993). The 'Principle of Allocation' forms the basis of life-history theory (Levins 1968); if an animal allocates energy to one process, another will consequently receive less energy (Cody 1966; Stearns 1992). Resources such as time and energy are limiting, and can only be spent once, therefore trade-offs between life-history traits occur at the expense of one another (Rose 1983; Stearns 1989).

In many animals, the 'Principle of Allocation' (Levins 1968) is evident as a trade-off between reproductive effort and survival, known as the 'cost of reproduction' (Williams 1966). Resources allocated to different fitness-related activities lead to the different life-history patterns we observe amongst species (Boggs 1992). Long-lived (K-selected) animals, such as seabirds, will theoretically allocate more energy to their own survival and future reproduction, whereas short-lived (R-selected) animals will allocate energy towards current reproductive investment (Stearns 1992). This trade-off is closely linked to foraging ecology, as an organism's

resource intake, in terms of nutrients and energy, is determined by its foraging patterns (Boggs 1992).

Long-lived 'prudent parents' (Drent & Daan 1980) should theoretically alter foraging effort under different environmental conditions so that current reproduction is maximised at no cost to future reproduction or adult survival (Stearns 1992). However, seabirds are known to alter reproductive strategy to ensure their own survival over that of their chicks, conforming to the 'individual optimisation hypothesis' (Nur 1986), in periods of altered prey availability (Goodman 1974; Perrins & Moss 1975; Chaurand & Weimerskirch 1994; Erikstad *et al.* 1997). Adelie penguins, *Pygoscelis adeliae*, for example, are known to regulate their own body mass at the cost of reduced chick provisioning (Watanuki *et al.* 2002; Ballard *et al.* 2010), conforming to the life-history strategy of long-lived animals favouring maintenance of body condition over current breeding success (Monaghan *et al.* 1989).

The balance between self-provisioning and chick-provisioning may be refined by animals provisioning their young with different food than that which is used for self-feeding (Hegner 1982; Kacelnik 1984; Swihart & Johnson 1986). Certain seabird species have demonstrated selective provisioning of chicks with higher quality prey items (more enriched in protein or lipid) than those that they themselves ingest (Bradstreet & Brown 1985; Davoren & Burger 1999), and this can strongly affect chick growth rates (Dahdul & Horn 2003). Certain seabirds have been found to provision young with higher trophic level prey than they consume themselves (e.g. Black-legged kittiwakes, *Rissa tridactyla,* and Thick-billed murres, *Uria lomvia,* Hobson 1993; Magellanic penguins, *Spheniscus magellanicus*, Forero *et al.* 2002; Adelie penguins, Cherel 2008). However, still other seabird species demonstrate no

segregation in prey provisioned to chicks, and used for self-feeding (e.g. Northern fulmars, *Fulmarus glacialis*, Hobson 1993; Emperor penguins, Cherel 2008).

1.4 Stress and the emergency life history stage

The breeding period is a stressful one as it places additional energetic demands on parents. Stress can influence both adult survival and reproductive success and has been defined by Busch & Hayward (2009) as an individual's perception that energy must be focused on short-term survival, rather than on long-term energetic investment such as growth, reproduction, territorial defence and immune defence. Potential stressors for wild animals can include deterioration of foraging conditions, severe weather, human disturbance, interactions with predators and con-specifics, injury and disease (Harvey *et al.* 1984; Astheimer *et al.* 1992; Vleck *et al.* 2000; Wingfield & Sapolsky 2003). According to life history theory, when under stress long-lived species, such as seabirds, should allocate more energy to self-maintenance to secure future breeding opportunities rather than provisioning their current offspring (Stearns 1992). Stress in the context of this thesis is defined as events that pose a long-term threat to an individual, in addition to those imposed by the natural life cycle, and which elicit physiological and behavioural responses (McEwen & Wingfield 2003).

Physiological and behavioural responses to environmental change, or stressors, are thought to be mediated by endocrine mechanisms (Ricklefs & Wikelski 2002). The development of minimally-invasive sampling techniques to measure hormone levels has enhanced physiological studies of wild animals (Wasser *et al.* 1997; Berger *et al.* 1999; Millspaugh & Washburn 2004). Vertebrates secrete glucocorticosteroids (GCs, corticosterone and cortisol), from the adrenal cortex, in response to acute stress (Sapolsky *et al.* 2000). The measurement of basal circulating

GCs has proven to be useful in monitoring stress and health of individuals within wild populations (Wingfield *et al.* 1997; Cockrem 2006).

At low to moderate levels, GCs are responsible for energy regulation: acquisition, mobilisation and deposition; they influence feeding behaviour, and are involved in maintaining circulating levels of glucose and fatty acids (Landys *et al.* 2006). In response to predictable changes in the balance between energy availability in the environment and required energy expenditure of an individual, circulating GC levels can be adjusted on a daily and seasonal basis (Dallman *et al.* 1993; Sapolsky *et al.* 2000; Landys *et al.* 2006). McEwen and Wingfield (2003) termed this difference allostatic load, and basal GC levels tend to rise with increasing allostatic load (Landys *et al.* 2006).

Allostatic overload, or stress, is induced when the energetic demands of an individual are greater than the amount of energy available to that individual (McEwen & Wingfield 2003), such as times of food deprivation (Dallman *et al.* 1993), inclement weather and exposure to predators (Scheuerlein *et al.* 2001). If stress is short term, increased GC levels are beneficial. Physiologically, GCs promote gluconeogenesis, immune system regulation, night restfulness and cerebral blood flow (Wingfield & Ramenofsky 1997; Wingfield *et al.* 1998; Sapolsky *et al.* 2000). From a behavioural perspective, GCs promote behaviours which improve the chance of survival, and therefore fitness, such as escape behaviour and increased foraging behaviour (Astheimer *et al.* 1992; Wingfield & Ramenofsky 1997; Wingfield *et al.* 1998; Sapolsky *et al.* 2000). Together these responses to short-term stress have been termed the 'emergency life-history stage' (Wingfield *et al.* 1998). This stage, activated and mediated by GCs, allows vertebrates to re-establish their normal homeostatic state during periods of stress and to cope with high energetic demand,

particularly during the breeding period, and assist in survival (Wingfield *et al.* 1998; Wingfield & Kitaysky 2002). However, when circulating levels of GCs are high, and stress is prolonged (chronic stress), detrimental effects on health and fitness are observed (Busch & Hayward 2009). These include effects on cognition in mammals (Sapolsky *et al.* 2000), suppression of reproductive behaviour, growth and the immune system, and neuronal cell death, protein loss and hypertension (Wingfield & Ramenofsky 1997; Wingfield *et al.* 1998; Sapolsky *et al.* 2000).

1.5 Northern Rockhopper penguins in the South Atlantic Ocean

Eudyptes (crested) penguins have a circumpolar distribution, and have undergone enormous population declines over much of their range in the last 60 years (Cunningham & Moors 1994; Bingham 1998; Ellis *et al.* 1998; Crawford *et al.* 2003; Putz *et al.* 2003). Whilst *Eudyptes* penguins are the most abundant, in both number of individuals and species (Tremblay *et al.* 1997), ecological research on this group is limited in comparison to their larger relatives in the genera *Aptenodytes* (e.g. King penguins, Charassin *et al.* 1998; Charassin & Bost 2001; Halsey *et al*. 2010), *Pygoscelis* (e.g. Adelie penguins, Volkman *et al.* 1980; Ainley *et al.* 1998; Tierney *et al.* 2009) and *Spheniscus* (e.g. Magellanic penguins, Radl & Culik 1999; Wilson *et al.* 2005; Boersma *et al.* 2007).

Rockhopper penguins, *Eudyptes chrysocome* (Forster 1781), are the smallest of the *Eudyptes* penguins, and the taxonomy of this group is contentious (Banks *et al.* 2006). Three subspecies, distinguished by slight morphological and behavioural differences, were previously recognised: Eastern Rockhoppers breeding on islands in the eastern southern Indian Ocean, Northern Rockhoppers breeding in the South Atlantic and southern Indian Ocean, and Southern Rockhoppers breeding around

South America and the Falkland Islands (Banks *et al.* 2006). Recently, the eastern and southern sub-species have been grouped together, and the northern sub-species has been re-classified as a separate species (Banks *et al*. 2006). In this thesis the classification of Banks *et al.* (2006) is used throughout, and I refer to two species: Northern Rockhoppers, *Eudyptes moseleyi*, and Southern Rockhoppers, *Eudyptes chrysocome*, (incorporating the Eastern and Southern sub-species). Breeding success of both species of Rockhopper is low compared to their *Eudyptes* relatives (Putz *et al.* 2001; Cuthbert & Sommer 2004; Hull *et al.* 2004), and populations are generally in decline (Ellis *et al.* 1998; Guinard *et al.* 1998; Kirkwood *et al.* 2007; Cuthbert *et al.* 2009). Northern Rockhopper penguins have undergone dramatic population declines over recent years, and with an estimated 168,000 pairs left, have been classified as Endangered on the IUCN Red List (Birdlife International 2009).

The majority of the world's population of Northern Rockhopper penguins (> 80%) reside in the South Atlantic at the Tristan da Cunha archipelago (Cuthbert *et al.* 2009), with the remainder being found on Amsterdam and St Paul Islands in the Indian Ocean. Tristan da Cunha is also an important breeding location for the Atlantic petrel, *Pterodroma incerta,* Spectacled petrel, *Procellaria conspicillata,* Great shearwater, *Puffinus gravis,* Little shearwater, *Puffinus assimilis,* Atlantic yellow-nosed albatross, *Thalassarche melanophrys*, Sooty albatross, *Phoebetria fusca*, Broad-billed prion, *Pachyptila vittata,* and Tristan skua, *Catharacta antarctica hamiltoni* (Ryan 2007). Direct human impacts on these seabird populations are now limited, particularly at the uninhabited islands of the archipelago. Tristan Island currently has a permanent human population of around 270, and historically (19th and first half of 20th century) penguin populations there (adults and eggs) were heavily exploited for food and decorative head plumes, which likely contributed to the large

population declines (Hilton *et al.* 2008). Restrictions now exist, however, to protect the species on Tristan (under the Tristan da Cunha Conservation Ordinance 1976), and eggs are harvested in small numbers from Nightingale Island only (Hilton *et al.* 2008).

It is estimated that Northern Rockhopper populations on mainland Tristan have declined by more than 90% over the last 130 years (Cuthbert *et al.* 2009), and this cannot be attributed to human impact alone, as populations on uninhabited Gough Island have similarly declined. There is evidence that an estimated two million pairs were present on Gough Island in the mid 1950's, and that the population has since declined by > 90% (Cuthbert & Sommer 2004; Cuthbert *et al*. 2009). The causal factors behind these more recent population crashes are unknown. Hilton *et al.* (2006) found no consistent pattern, in relation to marine productivity in a study examining stable isotope signatures of penguin feathers from this region over the period 1873 – 2001. Although inter-specific competition with Sub-Antarctic fur seals, *Arctocephalus tropicalis*, which have increased since sealing ceased in the early 20th century, may have reduced penguin prey availability during the breeding season, there is no evidence to support this theory (Cuthbert *et al.* 2009).

Development of species and ecosystem conservation measures relies critically on baseline knowledge of foraging ecology against which to compare change. Currently information on the diet of Northern Rockhoppers at the Tristan da Cunha archipelago is restricted to stable isotope analysis of feathers (Hilton *et al.* 2006), and a taxonomic investigation of diet by Klages *et al.* (1988) at Gough Island, while diving behaviour is unknown.

This thesis is an integrated investigation of the trophic ecology of breeding Northern Rockhopper penguins during the breeding season. Primarily this thesis

addresses foraging behaviour, using TDRS, stomach content analysis and SIA, after an investigation of sexual dimorphism to compare foraging behaviour of males and females. Since the breeding season is potentially a period of high stress, effects of breeding stage and presence of blood parasites, on both sexes, were examined using GCs.

1.6 Study site

The Tristan da Cunha archipelago (37º11'S 12º28'W) consists of the three closely grouped main islands of Tristan da Cunha, Inaccessible and Nightingale, with Gough Island (40º31'S 9º93'W) lying 350km to the south-east (Figure 1.1a). The group lies approximately 2800km from South Africa and 3200km from South America in the middle of the South Atlantic Ocean. The islands are volcanic in origin (Baker *et al.* 1964), originating from the Mid-Atlantic Ridge, and are surrounded by oceanic waters; they produce no significant freshwater run-off (Otley & Cuthbert 2008). The Tristan da Cunha archipelago and Gough Island are separated by the sub-tropical front (STF), which is the subtropical gyre-Antarctic Circumpolar Current boundary, with the Tristan group lying to the north in the warmer sub-tropical zone of the South Atlantic and Gough Island to the south in the cooler sub-Antarctic zone (Figure 1.1a; Otley & Cuthbert 2008). The archipelago is affected by both the Antarctic Circumpolar westerly currents, and the anti-clockwise current of the South Atlantic gyre (Otley & Cuthbert 2008). The marine macro-plankton faunal composition around the islands reflects a warm sub-tropical influence (Miller 1982; Miller & Tromp 1982).

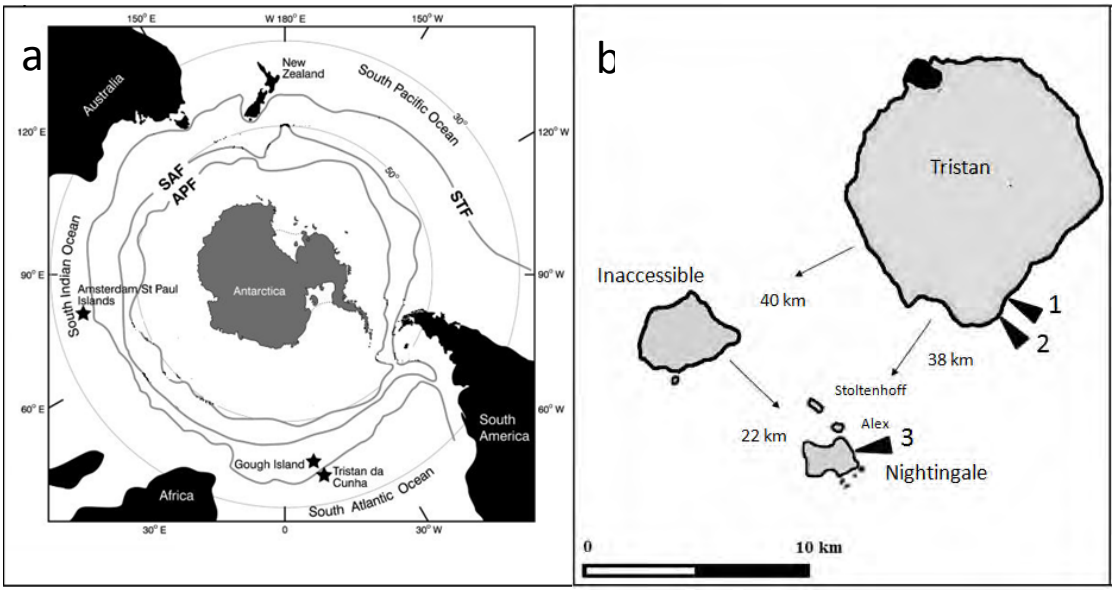

Figure 1.1– a) Map showing the location of Northern Rockhopper penguin breeding sites (*), the Tristan da Cunha archipelago and Gough Island (South Atlantic Ocean) and Amsterdam and St Paul Islands (South Indian Ocean), in relation to the sub-tropical front (Adapted Hilton *et al.* 2006 b) Distances between the islands in the Tristan da Cunha archipelago; study site locations are indicated (1 – Stony Beach; 2 – Stony Hill; 3 – Penguin Rock, adapted from Cuthbert *et al.* 2009).

Fieldwork was carried out between September and December 2010 at Stony Beach on Tristan Island. This period was chosen in accordance with previous population censuses confirming main breeding activity at this time of year. Two sub-colonies occur at Stony beach, the Stony Hill sub-colony (37º 09' 54'' S, 12º 16' 18'' W), with an estimated 281 breeding pairs in 2010, and the Stony Beach sub-colony (37º 09' 36'' S, 12º 16' 06'' W), with an estimated 262 breeding pairs in 2010 (Figure 1.1b). These colonies were selected due to the comparative ease of access to the beach by boat, and because birds nest on open hillsides, using only one path to leave and return to sea, and could therefore be easily monitored and recaptured. Birds were also

sampled for blood and feathers at Penguin Rock on the north-eastern corner of Nightingale Island (Figure 1.1b).

1.7 Northern Rockhopper penguin breeding cycle

At Tristan da Cunha, adult Northern Rockhopper penguins typically return to their breeding colonies in the winter (late July-August), and eggs are laid at the beginning of September (Ryan 2007). This commences the incubation stage, which lasts 32-34 days, and is shared by both adults. Both birds remain at the nest for the first 12 days; males then depart to sea to feed for the next 12 days, whilst females incubate eggs, and when males return they incubate eggs until hatching whilst females forage at sea. *Eudyptes* penguins, like many bird species, lay more eggs per clutch than they can rear to independence, and display brood reduction (Ricklefs 1965; Lack 1966). Typically two eggs are laid which exhibit reversed egg size dimorphism (Slagsvold *et al*. 1984), and a unique reversed hatching synchrony (St Clair 1996). A first (A) egg is laid, and after four days a much larger second (B) egg, which hatches prior to the smaller A-egg (Warham 1975; Williams 1995). Rarely are two chicks reared per clutch; the B egg receives greater parental investment and is treated preferentially (Warham 1963). If both eggs hatch, the A-chick usually dies within days of hatching from starvation (Lamey 1990). Of 262 nests monitored at the study colonies, only four were identified with two chicks, and in none of these nests did both chicks survive beyond the guard stage (personal observation).

Foraging patterns within the *Eudyptes* genus are similar (Warham 1975; Williams 1995). During the early chick-rearing period, which commences mid October to early November at Tristan, males fast ashore and brood the chicks for 24-26 days, whilst females forage, returning daily to provision the chick; this is referred

to as the guard stage. This biases foraging studies during early-chick rearing of *Eudyptes* penguins towards the female birds. After this brooding period, when thermally emancipated, chicks form loose crèches, referred to as the crèche stage, and are provisioned by both parents (Williams 1995). At Tristan da Cunha chicks fledge from the end of December and throughout January (Ryan 2007).

Chapter 2

Sexual dimorphism of Northern Rockhopper penguins, *Eudyptes moseleyi*: a comparison of morphometric and molecular gender determination

2.1 Introduction

Many seabirds are monomorphic; therefore it can be difficult to ascertain the sex of individuals in field studies. In penguins, sex-linked dimorphism is not easily distinguishable and plumage characteristics are sexually monochromatic (Davis & Spiers 1990). Males are typically larger and heavier than females (Warham 1972; Agnew & Kerry 1995; Williams 1995); however observations based on this rule are not always accurate. Age can confound attempts to discern the sex of monomorphic birds, as males must grow through the female range of sizes before reaching the male threshold (Bertellotti *et al.* 2002); hence males may be incorrectly identified as female. Conversely, large females may be incorrectly sexed as males. Similarly, the use of body mass is not reliable in discerning sex as it is confounded by various factors related to fasting and chick provisioning, and is highly variable within and between years (Scolaro *et al.* 1983; Amat *et al.* 1993).

For monomorphic birds, various methods have been employed to determine the sex of individuals; in penguins these include cloacal examination (Samour *et al.* 1983), vent measurements (Boersma & Davies 1987), observation of breeding behaviour (Scolaro *et al.* 1990), examination by ultrasound (Hildebrandt *et al.* 1996), and molecular sexing (Bertellotti *et al.* 2002). With the discovery of a suitable sex-linked marker (Ellegren 1996), DNA now

provides a useful tool to discriminate male and female birds. In comparison with mammals, birds have a reversed sex chromosome system, with females being heterogametic (ZW) and males homogametic (ZZ) (Ellegren 1996). The chromobox-helicase-DNA-binding gene (CHD-W) is the only avian W chromosome gene to have been discovered, and can be used to sex most species of non-ratite birds (Griffiths *et al.* 1998).

These methods of sex determination have shortcomings, because they are either costly, require the use of specialised equipment, or are restricted to the breeding period. It is useful in field situations to be able to make a simple measurement to determine sex, preferably measurements that require no technical expertise, and most importantly that are non-invasive. Discriminant function analysis (DFA), a mathematical approach, based on morphological measurements has been shown to be an effective method of sexing monomorphic bird species, and has been used on gulls (Hanners & Patton 1985), shearwaters (Genovart *et al.* 2003), petrels (Weidinger & van Franeker 1998) and terns (Ackerman *et al.* 2008). By combining several morphometric variables of known-sex individuals, a function is generated that best distinguishes these birds, allowing the prediction of sex of other individuals of unknown sex (Sokal & Rohlf 1981). DFA, incorporating variables such as culmen measurements, head length and flipper length, has been used to sex Pygoscelid penguins (Kerry *et al.* 1992; Amat *et al.* 1993; Renner *et al.* 1998), Magellanic penguins, *Spheniscus magellanicus* (Bertellotti *et al.* 2002), Little penguins, *Eudyptula minor* (Arnould *et al.* 2004) and Yellow-eyed penguins, *Megadyptes antipodes* (Setiawan *et al.* 2004) accurately.

Many penguin species show considerable geographical variation in body size (Williams 1995), including Rockhopper penguins (Tremblay & Cherel 2003). Thus, discriminant functions developed to identify gender in these species are generally restricted in their use to the geographic areas where they were derived. With the recent division of

Rockhopper penguins into three species, the Northern Rockhopper *Eudyptes moseleyi*, the Southern Rockhopper *Eudyptes chrysocome* and the Eastern Rockhopper penguin *Eudyptes filholi* (Banks *et al.* 2006, Jouventin *et al.* 2006, de Dinechin *et al.* 2009), it is important to develop discriminant functions separately for each of these populations. Poisbleau *et al.* (2010) recently derived a discriminant function which had a high accuracy, 96.2%, in sexing adult Southern Rockhopper penguins, *Eudyptes chrysocome*.

A fundamental requirement of the present ecological study was knowledge of the sex of the study animals. Male and female *Eudyptes* penguins exhibit distinct behavioural differences during the breeding season (described in detail in Chapter 1), and are the most dimorphic of all penguins groups (Croxall 1995), therefore this is potentially an important source of variation in foraging behaviour and diet. Molecular methods were employed to verify the sex of all birds sampled in order to analyse this variation. Additionally, a discriminant function was developed to assign gender to Northern Rockhopper penguins breeding at Tristan da Cunha, from a group of birds that were positively sexed using molecular methods, allowing the accuracy of this discriminant function to be tested.

2.2 Methods

2.2.1 Blood sample collection and morphometric measurements

Blood samples and morphometric measurements were taken from a total of 133 Northern Rockhopper penguins from sub-colonies at Stony Beach (n = 87; 54 of which formed pairs), and Stony Hill (n = 37) on Tristan da Cunha and from Nightingale Island (n = 8). Approximately 0.1 ml blood was drawn from the tarsal vein of birds using a 25-gauge

needle and syringe, stored on ice and centrifuged within 4 hours in the field. Red blood cells were immediately frozen at – 20 ºC, and stored in the laboratory at - 80 ºC.

The following morphological measurements were taken for each bird in accordance with Warham (1972, 1975): mass, using a 5 kg Salter spring balance with 25 g precision; flipper length (tip of junction with the thoracic wall when the flipper was held perpendicular to the sagittal plane of the body) and tarsus – toe length, using a 30 cm stainless steel ruler; head length (back of cranium to tip of culmen), culmen depth (at a point proximal to the tip of the triangular inter-ramal feather patch), culmen width (maximum width of the culmen) and culmen length (length of exposed culmen) using Vernier callipers precise to 0.1 mm (Figure 2.1).

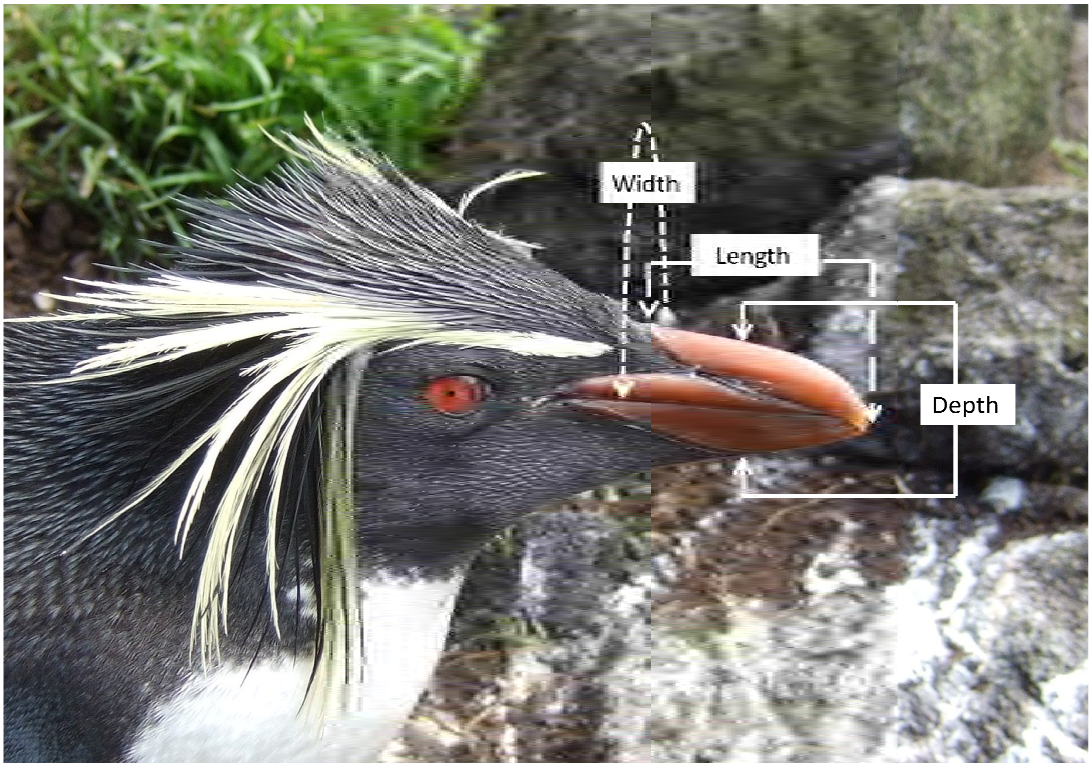

Figure 2.1 - Northern Rockhopper penguin showing positions of culmen measurements: length, depth and width.

2.2.2 DNA-based sexing

The molecular sexing method was first tested on 54 birds of known sex (27 males, 27 females) from the guard stage, after birds were assigned a gender based on behavioural characteristics, specifically attendance pattern at the nest, and observations of females provisioning chicks. Molecular methods were then used to sex all birds from the incubation and crèche stages.

In the laboratory, total genomic DNA was extracted from a small amount (50 – 100 µl) of blood using a QIAGEN DNeasy® Blood & Tissue Kit, following the manufacturer's instructions. The duration of the heating step was adapted to 4 hours at 56 °C. Extracted DNA was stored at -20 °C. A multiplex Polymerase Chain Reaction (PCR) was performed to amplify DNA using the existing primers 2945F (5' – AGAAAAAGATGGTGTTAGAT – 3'), cfR (5' – CATAACTCCTTACCACATAT – 3') and 3224R (5' – TTGAACTGTGAAAGCAACTC – 3') according to Ellegren (1996). Primer 3224R, when used in conjunction with primer 2945F, generates a 630 bp fragment in both sexes, whilst the combination of primers cfR and 2945F generates a 210 bp fragment in females only. When used together in a multiplex PCR, these primers amplify a single DNA fragment in males, but two different fragments in females, allowing the sexes to be easily distinguished via electrophoresis of PCR products.

PCR amplification reactions (0.05 µl Bioline BioTaq™ DNA polymerase, 2 µl 10x NH_4 reaction buffer (Bioline), 0.16 mM of each deoxyribonucleotide triphosphate (dNTP), 4 mM $MgCl_2$, 0.4 µM each primer and 2 µl DNA template) were made up to a total volume of 20 µl. The PCR thermal profile comprised a single cycle of 94 °C for 3 min (denaturation), 55 °C for 30 s (annealing) and 72 °C for 60 s (extension), followed by 34 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s. The program was completed with a final extension

step of 72 °C for 5 min. 5 µl PCR product was bound with SYBR® Green and analysed by electrophoresis on a 1.5% agarose gel. Products were examined under UV light for the presence of bands, one band in males and two in females.

2.2.3 Statistical analysis

Prior to analyses, all sex-grouped data were log-transformed to meet the assumptions of normality and homogeneity for analysis of variance and DFA (Kolmogorov-Smirnov tests $p > 0.05$, Cochran's tests $p > 0.05$ in all cases), as well as the known geometric effects of allometry (Platt & Silvert 1981; Blackstone 1987; Strass 1987). After confirmation of sex from molecular analyses, morphological measurements between the sexes were compared using independent sample t-tests. Body mass was compared using a two-factor ANOVA between the fixed factors sex and breeding stage. Dependent sample t-tests were used to compare morphological measurements between males and females of a group of known breeding pairs from Tristan Island (Stony beach sub-colony). Two DFA were performed to determine the accuracy of sexing birds based on, firstly, all morphological variables, and secondly, a forward stepwise analysis to determine which measurements were the most reliable for assigning gender. For the stepwise analysis, a criterion based on an F-test of Wilks' lambda was used at each step to include the variable which contributed the most discriminatory power to the model at the 0.15 level of significance (the confidence level for variable retention was increased from the default of 0.05 to ensure entry of important variables; Constanza & Afifi 1979). Body mass was excluded from the DFA due to the large intra-seasonal variation in this variable. All analyses were performed using Statistica 8.0.

2.3 Results

2.3.1 Molecular sexing

Using the primer set 2945F, cfR and 3224R, fragments were successfully amplified from Northern Rockhopper penguins, allowing gender determination. A 680 bp fragment was obtained from males and females in every case, and a second 210 bp W-specific fragment obtained from females only. Bands were resolved by gel electrophoresis, confirming this as a simple and reliable method for sexing Northern Rockhopper penguins. All known sex birds were correctly identified using this molecular method. Of 133 birds sampled, 76 were assigned as female and 57 as male.

2.3.2 Morphometrics

Within a group of 27 breeding pairs, males were significantly larger than females in culmen length ($t_{52} = 8.3923$) and culmen depth ($t_{52} = 6.1400$) only (Figure 2.2a, $p < 0.0001$ in both cases). There was no significant difference in flipper length ($t_{52} = 0.0521$), head length ($t_{52} = 0.3668$), foot length ($t_{52} = 0.3150$) or culmen width ($t_{52} = 1.1708$) between males and females of breeding pairs (Figure 2.2b, $p > 0.05$ in all cases). Within the 27 breeding pairs measured, in 26 of these pairs male birds had longer beaks than their female partners, and in 24 pairs males had deeper beaks than their female partners.

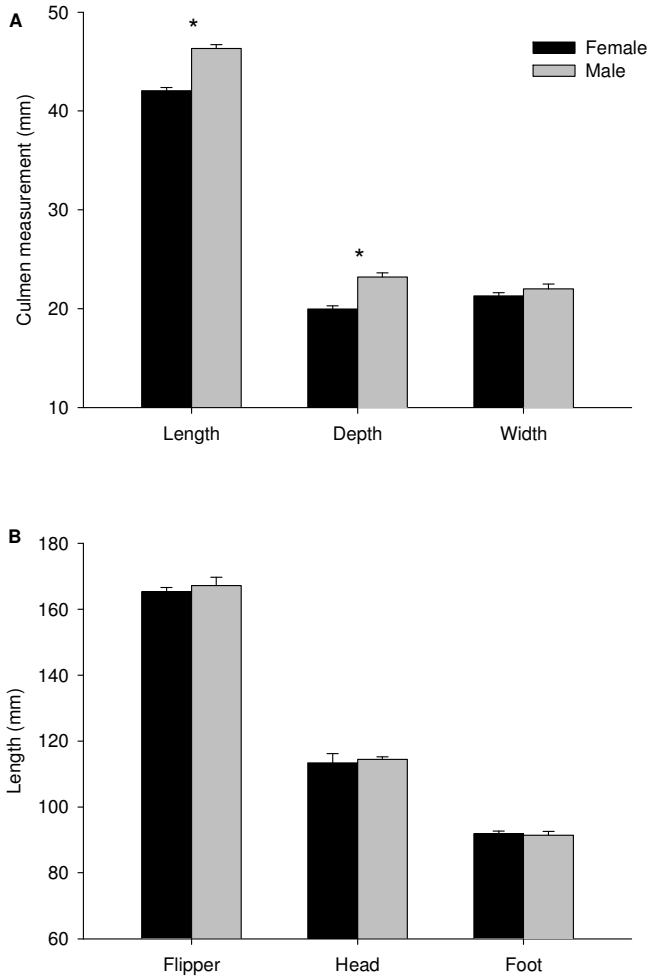

Figure 2.2 – (A) Culmen length, width and depth and (B) flipper, head and foot length of breeding pairs of Northern Rockhopper penguins (n = 27). Bars indicate means with standard error, significant results are indicated by an asterix (*).

Morphometric measurements of all male and female Northern Rockhopper penguins are presented in Table 2.1. Of the six morphological characteristics analysed, only culmen measurements differed significantly between sexes, with males being larger in terms of culmen depth, culmen width and culmen length (Table 2.1). No significant effects of sex on flipper length, head length or foot length were observed (Table 2.1).

Table 2.1 - Body measurements of male and female Northern Rockhopper penguins from Tristan da Cunha during the 2010 breeding season, sexed by molecular procedures. Values are means with standard error, with sample sizes in brackets. Results of t-tests are presented, significance was assumed at $p < 0.05$.

	Male	Female	$t_{(54)}$	p
Flipper length (mm)	162.11 ± 4.5 (n = 39)	164.44 ± 0.84 (n = 62)	0.0966	> 0.05
Foot length (mm)	94.21 ± 1.27 (n = 39)	95.77 ± 0.81 (n = 62)	0.6123	> 0.05
Head length (mm)	114.69 ± 0.73 (n = 39	110.39 ± 2.12 (n = 62)	0.8095	> 0.05
Culmen length (mm)	46.54 ± 0.32 (n = 57)	42.31 ± 0.31 (n = 76)	8.5300	< 0.0001
Culmen width (mm)	21.76 ± 0.29 (n = 57)	20.15 ± 0.19 (n = 76)	3.2954	< 0.01
Culmen depth (mm)	23.64 ± 0.29 (n = 57)	21.66 ± 0.19 (n = 76)	3.8428	< 0.001

Body mass was significantly affected by both sex and breeding stage (Table 2.2). Females had a smaller body mass than males in all breeding stages (Table 2.2, Figure 2.3). Guard birds had the smallest body mass during the breeding season (SNK $p < 0.05$), and crèche birds the greatest mass (SNK $p < 0.01$, Table 2.2, Figure 2.3).

Table 2.2 - 2-way factorial ANOVA to test the effect of the fixed factors sex (male (M), female (F)) and stage (incubation (I), guard (G) and crèche (C)) on Northern Rockhopper penguin body masses (n = 8). SNK was used to compare means when there were significant effects.

Source	d.f.	M.S.	F	P
Stage	2	1016422	24.33	< 0.0001
Sex	1	422813	10.12	< 0.0028
Stage*sex	2	111219	2.663	0.08152
Error	42	41769		
SNK		Stage: G < I < C		Sex: F < M

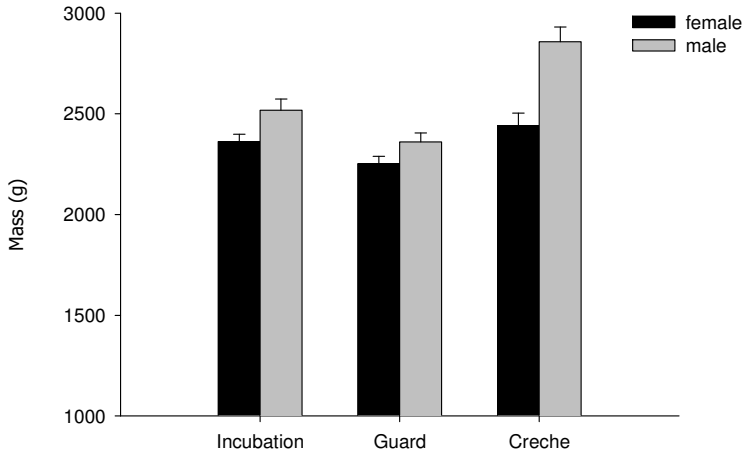

Figure 2.3 – Body mass of male and female Northern Rockhopper penguins during incubation (males n = 8, females n = 31), guard (males n = 29, females n = 28) and crèche (males n = 18, females n = 16) stages. Bars indicate means with standard error.

In the model produced by the DFA, three canonical variables were significant: culmen length (DF = -0.5685, p = 0.0011), culmen width (DF = -0.3745, p = 0.0224) and flipper length (DF = -0.3227, p = 0.0411), and these best separated male and female birds (Wilks' λ= 0.555, eigenvalue = 0.799, $F_{6, 94}$ = 12.52, p < 0.0001). Culmen depth, head length and foot length had lower canonical loadings from the DFA, which indicates that these variables contributed less to the accurate sexing of birds. As culmen length gave the best single factor correlation (canonical correlation value -0.5685), a DFA using this variable only was performed, and classified 82.5% of birds correctly. However, a model incorporating all variables produced the most accurate discriminant function, correctly sexing 82.1% of males, and 85.5% of females (overall accuracy 84.2%) sampled:

DF = 0.30(logfootlength) - 0.57(logculmenlength) - 0.37(logculmenwidth) - 0.32(logflipperlength) - 0.28(logculmendepth) - 0.14(logheadlength)

A forward step-wise discriminant analysis was then performed to determine the most useful variables in sex determination (Wilks' lambda = 0.561, eigenvalue = 0.783, $F_{5, 95}$ = 14.88, p < 0.0001), producing the following DF. The DFA did not incorporate head length as this was the least useful variable:

DF = 0.58(logculmenlength) + 0.39 (logculmenwidth) + 0.32(logflipperlength) – 0.30(logfootlength) + 0.28 (logculmendepth)

Although a high percentage of birds were sexed accurately, there was a large amount of overlap in discriminant score distributions (Figure 2.4). There is a clear outlier in the canonical variables plot (Figure 2.4). It is possible that the male with the high discriminant score, grouped with the females, was identified incorrectly in the genetic test due to sample contamination, or, more likely, was a juvenile male. Factor 1 represents culmen and flipper measurements, with birds with negative values being larger in these measurements, hence the

distinct male cluster to the left of the axis (Figure 2.4). Females are spread over a much larger range and it is not easy to distinguish these birds from males, with more being incorrectly classified by the model (9 females incorrectly classified compared to 7 males) as they were large birds. Factor 2 represents head and foot length, with birds with positive values having a larger head length, and smaller feet than those with negative values.

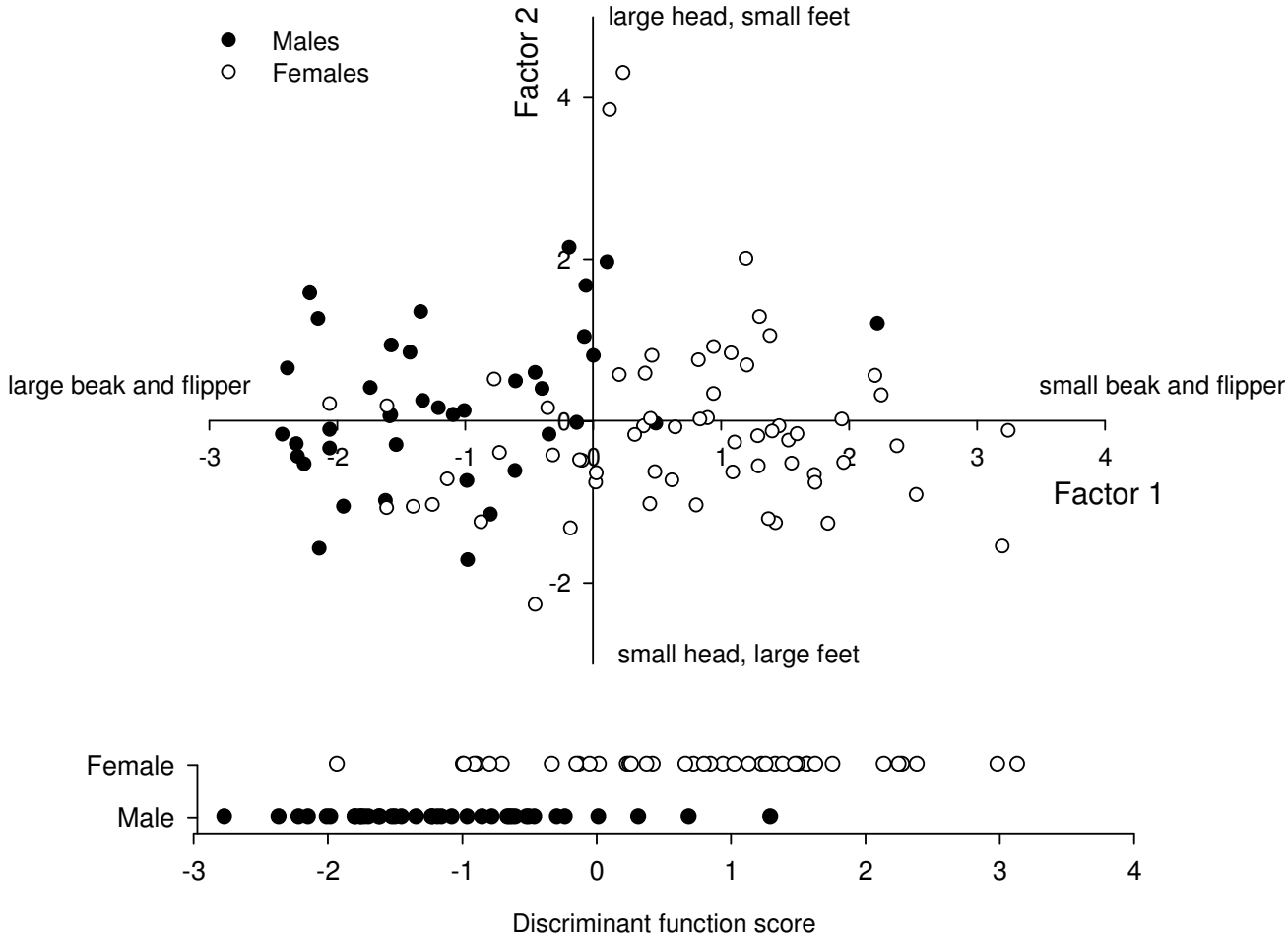

Figure 2.4 – Discriminant function canonical values, from a model incorporating all body measurements, for male and female Northern Rockhopper penguins. Distributions of discriminant function scores for these birds are presented on the lower axis.

2.4 Discussion

Alternative sexing techniques are necessary to determine the sex of penguins due to the absence of easily distinguishable differences in plumage or body size (Agnew & Kerry 1995). Molecular techniques are the most accurate method (Renner *et al.* 1998, Bertellotti *et al.* 2002, Wallace *et al.* 2008); however these are expensive and time-consuming, and certain studies necessitate knowledge of the sex of birds in the field. Other techniques such as behavioural observation and size of the cloaca are reliable in some species, but restricted to the breeding period. The most convenient method is discriminant function analysis of morphometric variables measured in the field.

A degree of sexual dimorphism is exhibited by penguins (Ainley & Emmison 1972; Warham 1975; Davis & Spiers 1990; Amat *et al.* 1993), and this has been demonstrated here, with males being the larger sex within Northern Rockhopper penguins. This study shows that, at Tristan da Cunha, this species can be sexed non-invasively using five morphometric measurements with accuracy of 84.2%; these measurements are culmen length, culmen width, culmen depth, flipper length and foot length. However, it is interesting to note that the measurement of culmen length alone accurately sexed 82.5% of birds. Whilst in Magellanic (Bertellotti *et al.* 2002) and Little penguins (Arnould *et al.* 2004), bill depth was shown to be the best single measurement to predict sex (> 90% accurate), bill length is the most accurate measurement for sexing Humboldt penguins, *Spheniscus humboldti* (Zavalaga & Paredes 1997) and significantly, the closely-related Southern Rockhopper penguins *E. chrysocome* (Poisbleau *et al.* 2010, Table 2.3). The DF derived here does not sex Northern Rockhopper penguins as accurately as that derived by Hull 1996 and Poisbleau *et al.* (2010) for the southern species, which sexed 93.2% and 96.2% respectively of adults correctly, using a function incorporating bill length and depth. However, the accuracy obtained in this study

was similar to that reported for Humboldt (Wallace *et al.* 2008), Little (Arnould *et al.* 2004) and Gentoo penguins, *Pygoscelis papua* (Renner *et al.* 1998).

Body mass was not included in the DFA as a predictor of sex as it is highly variable in *Eudyptes* penguins (Warham 1975; Hull *et al.* 2004). However, males were significantly heavier in all three breeding stages, including the guard stage when males fast (Warham 1972). Hull *et al.* (2004) also found Southern Rockhopper males at Macquarie Island to be consistently heavier throughout the breeding season. Body mass was lowest during the guard stage, which was expected as males are fasting, and females are foraging at a high frequency to provision young chicks. Body mass was greatest overall in the crèche stage, when males return from feeding trips to replenish stores lost during the guard stage, and chicks are provisioned less frequently, allowing females to dedicate more time to self-feeding rather than travelling between foraging areas and the colony. Observations by Hull *et al.* (2004), and Tremblay & Cherel (2003), support this, they found body mass of Southern and Northern Rockhopper female penguins provisioning young chicks to decrease daily during the guard stage as their endogenous reserves are depleted whilst making short, frequent foraging trips.

The fact that the DF model derived here is less accurate at predicting sex than those developed for other Rockhopper species (Table 2.3), indicates that it is necessary to derive separate discriminant functions for each of these recently re-classified species, and ideally for each colony as the extent of variation among colonies is unknown. Northern Rockhoppers are slightly larger in body size than the southern species (Tremblay & Cherel 2003; Banks *et al.* 2006). Geographic variation is an important consideration and whilst this DF is accurate for birds sampled at Tristan Island, it should be used to sex birds in different colonies with caution. It was expected that errors in this model would arise from young males being classified incorrectly as females, but it appears that, in this population, a considerable number of large, male-sized females are present, and the error was skewed towards incorrectly

identifying females. Unless a sample of known-sex birds can be obtained to confirm the accuracy of the DF being used, it is suggested that initial DNA testing is performed in previously unstudied colonies to recalibrate the DF. Hart *et al.* (2009) similarly found a large amount of overlap in culmen measurements between sexes of Macaroni penguins, *Eudyptes chrysolophus*, within the same genus as the Rockhopper penguin, which are thought to be the most sexually dimorphic penguins (Croxall 1995), and they suggest DNA testing in birds that fall into the region of overlap.

Table 2.3 – Summary of studies using morphometric measurements and discriminant functions to determine sex in penguin populations. The morphometric variables incorporated in the discriminant function models, and their accuracy in sex determination, are presented.

Species	Location	DF variables	Accuracy (%)	Study
Royal penguins (*Eudyptes Schlegeli*)	Macquarie Island	Culmen length and depth	97.1	Hull 1996
Southern Rockhopper penguins (*E. chrysocome*)	Macquarie Island	Culmen length and depth	93.2	Hull 1996
	New Island, Falkland Islands	Culmen length and depth	96.2	Poisbleau *et al.* 2010
Northern Rockhopper penguins (*E. moseleyi*)	Tristan Island, Tristan da Cunha	Culmen length	82.5	This study
		Culmen length, depth, width, flipper, foot, head length	84.5	This study
Humboldt penguins (*Spheniscus humboldti*)	Chile	Culmen length and depth	87	Wallace *et al.* 2008
Magellanic penguins (*S. magellanicus*)	Argentina	Culmen length and depth	97	Bertellotti *et al.* 2002
Yellow-eyed penguins (*Megadyptes antipodes*)	New Zealand	Head and foot length	93	Setiawan *et al.* 2004
Little penguins (*Eudyptula minor*)	Philip Island, Australia	Culmen depth	91	Arnould *et al.* 2004

Genetic testing using the 2945F, cfR and 3224R primer set was successful in all birds sampled, with the possible exception of one individual, and Ellegren's method (Ellegren 1996) is particularly useful as no 'false-positive' results can be obtained, as a second band must be visible for positive female identification. However, this method is costly, time-consuming, and requires technical expertise, equipment and an accessible laboratory. If DFA is to be used for assigning gender to a monomorphic species, it is important to consider carefully the morphometric variables to be analysed. Stable characters should be chosen; body mass was excluded here as this can fluctuate dramatically within and between breeding seasons. Culmen measurements remain the most useful determinants; culmen length and width were the most accurate predictors of sex in this study, suggesting that a similar accuracy in sex classification can be obtained by measuring these characteristics alone in this species, which would minimise handling time. This would be particularly useful in studies with very large sample sizes. It is also important that the same observer records all measurements due to observer bias; the differences between sexes are very slight, so small inconsistencies in measurement may have a significant effect on gender classification. Within pairs of breeding *Eudyptes* penguins, male gender is typically assigned to the larger bird within the pair (Warham 1975). It should be mentioned that, within breeding pairs in the present study, only in the measurements of culmen length and culmen depth were males larger than females.

In summary, whilst the DF developed here accurately sexed 84.2% of Northern Rockhopper penguins at Tristan da Cunha, some birds (9 females and 7 males out of 133 birds), would have been classified incorrectly without molecular analysis. It is therefore recommended to confirm morphometric measurements with genetic analysis. However, at sensitive times of the breeding period, such as incubation, or young chick stages, minimisation of handling time is an essential requirement of field studies, and morphology

will provide a reasonable idea of the sex of birds, potentially even the single measurement of culmen length.

Chapter 3

Plasticity in foraging behaviour of Northern Rockhopper penguins, *Eudyptes moseleyi*, over the chick-rearing period at Tristan da Cunha

3.1 Introduction

Theory suggests that animals should adjust patterns of prey searching to maximise their foraging efficiency, and thus energy gain, within their environment (MacArthur & Pianka 1966; Fretwell & Lucas 1970; Fauchald 1999). Thus, diving patterns are a fundamental aspect of the foraging ecology of deep-diving marine predators, such as penguins, as they influence prey encounters and efficient exploitation of prey (Croxall *et al.* 1988; Elliot *et al.* 2008a). Seabirds forage in a spatially and temporally heterogeneous environment (Hunt *et al.* 1999). During the breeding season, they are central place foragers and therefore their foraging strategies are constrained by the necessity to return frequently to the colony to provision chicks (Lack 1968; *sensu* Orians & Pearson 1979). As discussed in Chapter 1, due to these restrictions, if food is not available close to breeding colonies, breeding success is threatened (Furness & Cooper 1982; Monaghan *et al.* 1989; Weimerskirch *et al.* 1994; Crawford & Dyer 1995; Croxall & Davis 1999).

As a consequence of their flightless lifestyle, penguins have a very limited foraging range during breeding, compared to volant birds (Wilson 1985; Davis & Cuthbert 2001). In particular, the mobility, and changing distribution of prey within the water column, invokes the necessity for some degree of plasticity in their foraging behaviour (Croxall *et al*. 1988; Wilson 1995; Charassin & Bost 2001; Ropert-Coudert

et al. 2006; Elliot *et al*. 2008b). Intra-specific foraging plasticity, largely assumed to be a reflection of dietary shifts, has been observed in most penguin species, including Adélie, *Pygoscelis adeliae* (Ropert-Coudert *et al.* 2002), King, *Aptenodytes patagonicus* (Charassin *et al.* 1998), Gentoo, *Pygoscelis papua* (Lescroël & Bost 2005), Chinstrap, *Pygoscelis antarcticus* (Miller & Trivelpiece 2008), Macaroni, *Eudyptes chrysolophus* (Green *et al.* 2005) and Southern, *E. chrysocome*, and Northern, *E. moseleyi*, Rockhopper penguins (Tremblay & Cherel 2003). For example, plasticity in diving behaviour of Macaroni penguins has been recorded in the non-breeding season, in response to changing availability of target prey under different environmental conditions (Green *et al*. 2005). Gentoo penguins exhibit foraging plasticity closely related to local oceanographic conditions, exploiting locally abundant food sources, and adopting different strategies for the capture of demersal fish and swarming crustaceans (Croxall & Prince 1980; Bost & Jouventin 1991; Lescroël & Bost 2005; Lynch *et al.* 2009).

Foraging behaviour of penguins may also be affected by variable environmental conditions, such as light availability (Wilson *et al*. 1993), presence of sea-ice (Watanuki *et al*. 1993), and/or breeding cycle (Charassin *et al*. 1998), which are not extricable from prey availability. On a daily temporal-scale, foraging behaviour of higher-trophic level marine predators may be influenced by diel vertical migration of zooplankton and certain groups of fish (Wilson *et al.* 1993; Hays 2003). Certain seabird predators have co-evolved to exploit this movement, and adjust their behaviour accordingly (e.g. Common murres, *Uria aalge*, Regular *et al.* 2010), and Chinstrap penguins are known to increase nocturnal foraging to take advantage of an alternative prey source, Myctophids, when size-classes of krill, their primary prey source, are small (Miller & Trivelpiece 2008).

On a longer temporal scale, foraging may vary within the breeding season. While changing prey distribution and availability plays an important role in intra-seasonal variation in foraging behaviour, it is almost certainly linked to the high energetic requirements of breeding (Davis *et al.* 1989; Gales & Green 1990). Diving is an energetically expensive activity, and foraging strategy is expected to be adjusted to maximise energy gain (Fretwell & Lucas 1969). Increases in foraging effort, associated with the increased energetic demands of chick growth, have been recorded in Royal, *Eudyptes schlegeli*, and Rockhopper penguins (Hull 1999), Gentoo (Williams & Rothery 1990) and Little penguins, *Eudyptula minor* (Zimmer *et al*. 2011), for example an increase in foraging trip duration, meal size brought ashore and an increase in diving effort in terms of the amount of time spent underwater.

Intra-specific foraging plasticity may be most pronounced between sexes, particularly in sexual size-dimorphic species. Driving mechanisms behind sexual dietary differences are thought to be linked to a reduction in intersexual competition (Selander 1972; Gonzalez-Solis *et al.* 2000; Kato *et al.* 2000; Forero *et al.* 2002). Schoener (1970) proposed that a reduction in inter-sexual competition, through trophic or spatial segregation, induces a fitness benefit (the niche divergence hypothesis). This is expected to be important amongst colonial diving seabirds, such as penguins, as intra-specific competition is likely to be high due to the penguin's limited foraging range during breeding (Bearhop *et al.* 2006). In times of reduced prey availability, this may act to increase the chances of at least one member of a breeding pair finding sufficient food to provision chicks. Adelie penguins display a degree of niche partitioning, and sex differences in the duration of foraging trips, foraging locations and diet of breeding birds have been found (Clarke *et al.* 1998). In species such as Gentoo, Adelie and Magellanic, *Spheniscus magellanicus*, penguins,

males are known to prey more on fish than females, which has been attributed to their larger bill (Volkman *et al.* 1980; Forero *et al.* 2002; Tierney *et al.* 2009).

Understanding how predators modify diving behaviour with respect to prey type is complex (Ropert-Coudert *et al.* 2002), and this is particularly true in generalist predators, such as Rockhopper penguins, which rely on a wide-spectrum of prey and lack definite behavioural search and capture strategies (Davoren *et al*. 2003; Wilson *et al*. 2005). However, the combined use of time-depth recorders (TDRs), with subsequent detailed analysis of dives and stomach content analysis has proven to be useful in attempting to disentangle the relationship between dive patterns and prey selection in penguins (Croxall *et al*. 1988; Deagle *et al*. 2008). It has been shown that diving strategies (Elliot *et al*. 2008a,b) and dive shapes (Bost *et al*. 2007) of seabirds are affected by prey type. Additionally, analysis of wiggles, (the 'wiggles technique', Bost *et al.* 2007; Halsey *et al.* 2007), whilst originally believed to indicate prey pursuit in penguins (Kirkwood & Robertson 1997; Rodary *et al*. 2000), has recently been shown to correlate with prey capture (Simeone & Wilson 2003; Takahashi *et al.* 2004; Bost *et al.* 2007; Hanuise *et al.* 2010).

The aim of this study was to describe the foraging behaviour of Northern Rockhopper penguins at Tristan da Cunha, specifically to obtain information of diet composition and diving patterns of birds foraging to provision chicks during the guard and crèche stages. Chick size, breeding stage and adult sex were examined as potential sources of dietary variation; whilst diet, chick size and breeding stage were examined as sources of variation in diving behaviour. Previous studies of Rockhopper penguins at Amsterdam and Staten Islands have found a high degree of plasticity in foraging behaviour (e.g. Tremblay & Cherel 2003; Schiavini & Raya Rey 2004), and

it was expected that Northern Rockhopper penguins at Tristan da Cunha would also display a degree of foraging plasticity.

3.2 Methods

3.2.1 Stomach flushing

Stomach contents were obtained using the water-offloading technique (Wilson 1984; Gales 1987), from female birds at Stony Beach during the guard stage, between 2nd and 8th November 2010 (n = 31), and both sexes at Stony Hill during the crèche stage, between the 25th November and 2nd December 2010 (males n = 20, females n = 22). In the guard stage, stomach contents were taken from birds returning to the colony with TDRs (n = 17), however, frequently birds had already regurgitated to chicks when captured. For this reason, and also to obtain stomach contents from a larger colony-wide sample, an additional 14 random birds returning from sea were stomach flushed. During the crèche stage, stomach contents were only obtained from 3 female birds equipped with TDRs (one of these devices had a technical failure), therefore 39 additional random birds returning from sea were stomach flushed. It is possible that random birds stomach flushed, in both stages, were not breeding.

Ambient sea-water was passed down a funnel connected to a plastic catheter (external diameter 5mm: internal diameter 3.5 mm), inserted down the oesophagus to the base of the stomach. Water was introduced until it flowed from the mouth. At this point, the catheter was carefully removed, and the bird inverted over a bucket and gentle pressure applied to the base of the stomach whilst the throat was massaged to dislodge food. Birds were only flushed twice, to minimise stress to the bird, and for this reason meal mass was not analysed as the complete stomach contents may not have been obtained. Stomach contents were drained through a 0.5 mm sieve in the

field (Hull 1999; Deagle *et al.* 2008) and stored in sealed zip-loc bags. Stomach samples were frozen at – 20 ºC until analysed.

3.2.2 Stomach content analysis

In the laboratory, stomach samples were thawed, drained again through a 0.5 mm sieve, and weighed to obtain a total wet weight sample mass from which to calculate the contribution of prey categories to diet by mass. Samples were sorted into the categories macrozooplankton, cephalopods and fish, and then within these categories into fresh (whole) material and digested material. The total sample was examined, and sorted, under a dissecting microscope to ensure all otoliths, squid beaks and crustacean exoskeletons were removed for identification. Whole prey items of the crayfish *Jasus tristani*, amphipod *Themisto gaudichaudii*, fish *Phosichthys argenteus*, an ommastrephid squid. and octopus *Ocythoe tuberculata* were retained for stable isotope analysis (see Chapter 4).

To allow for the biases of different types of analysis, samples were described in terms of percentage by both number and mass (Duffy & Jackson 1986) and percentage frequency of occurrence in stomach samples:

*Percentage number (% N) = (Ni/Nt) * 100*

Where, Ni = Number of prey items in prey group 1, Nt = total number of prey items

*Percentage mass (% M) = (Mi/Mt) * 100*

Where, Mi = Mass of prey category, Mt = Mass of sample

*Percentage frequency of occurrence (% O) = (Oi/ Ot) * 100*

Where, Oi = Number of stomachs containing prey type I (frequency of occurrence), Ot = total number of stomachs containing food.

The fresh and digested portions of each prey category were weighed to obtain an estimate of proportion by wet mass in the diet (Cherel *et al.* 2007). To estimate the number of individuals of crustaceans, entire bodies and pairs of eyes were counted, (Raya Rey & Schiavini 2005). The number of cephalopods consumed was estimated by counting the number of lower beaks, either free or in buccal masses (Raya Rey & Schiavini 2005). Otoliths were separated into left and right, and the number of fish consumed was taken to be equal to the number of left or right otoliths, depending which was greater (Silva 1999). Cephalopod beaks, fish otoliths and crustaceans were identified to the lowest taxonomic group possible using published references (Clarke 1986; Smale *et al.* 1995; Campana 2004; Tuset *et al.* 2008). The otolith specimens which were identified as *Vinciguerria* sp., *Phosichthys argenteus* and *Maurolicus muelleri* are closely related, and very similar in appearance. The following key features (Smale *et al.* 1995) were used to differentiate between the three: *Vinciguerria* sp. - flat dorsal margin, ventral margin of rostrum irregular, a well-developed crista superior; *Phosichthys argenteus* - flat, rounded dorsal margin, ventral margin of rostrum serrate, small rounded antirostrum; *Maurolicus muelleri* - straight posterior margin, straight crista superior, antirostrum absent. Otoliths and beaks were compared with material in the reference collection at the Port Elizabeth Museum, South Africa and specimens were confirmed by an expert (Dr Malcolm Smale).

Lengths of whole specimens of crustaceans (anterior edge of eyeball to telson tip), otolith diameters (OD), lower rostral lengths (LRL) of squid beaks and hood lengths of octopod beaks were measured using a binocular microscope and eyepiece graticule. I was unable to source a regression equation relating hood length to mantle length for the octopus *Ocythoe tuberculata* and there are no specific regression equations for relating LRL to mantle length (ML) of ommastrephid squids. Therefore,

the following equation was used to estimate the ML of ommastrephid squid specimens (ML = -11.3 + 41.36 LRL, Clarke 1986). A regression equation relating OD to total length (TL) for *Phosichthys argenteus* (lnTL = ln3.9374 + 0.9549 lnOD, Smale *et al*. 1995) was used to calculate estimated total length (TL) of this fish.

Some cephalopod beaks and otoliths were highly eroded and these had likely been in the stomach for longer than the crustaceans, as they are highly resistant to mechanical and chemical digestion (Heezik & Seddon 1989; Gales *et al.* 1990), leading to an overestimation of the contribution of cephalopods and fish to penguin diet (Heezik & Seddon 1989). These highly eroded items were excluded from analysis.

3.2.3 Temperature-depth loggers (TDRs) and deployment

G5 CEFAS temperature-depth recorders (TDRs; G5; Cefas Technology Limited, UK) are cylindrical and weigh 1.3 g in seawater, approximately equivalent to 0.05% of the mean Northern Rockhopper penguin body mass. They have maximum dimensions of 8mm diameter x 31mm length and a memory of 1 MB to register data on hydrostatic pressure and temperature over time using defined sampling intervals. TDRs were programmed, using the software G5 Host (Cefas Technology Limited), to continuously record at 1-second intervals during the guard stage, and 2-second intervals during the crèche stage when trips were expected to be longer, to maximise data capture. TDRs were deployed in the evening and set to start recording temperature and depth (pressure) approximately 5 minutes after attachment to the birds, as it was not known when birds would depart to sea. Data were stored on a 1 MB flash drive within the device. Equipped birds were recaptured after 1 to 2 days (to record a minimum of one foraging trip). The recorded data were downloaded onto a

laptop for subsequent analysis. The depth sensor of the G5 CEFAS tags had a resolution of < 0.4 m, and the temperature sensor had an absolute temperature accuracy of ± 0.1 ºC.

TDRs were attached to 30 guard stage females between November 1st and November 11th 2010 at the Stony Beach sub-colony, and 13 crèche stage birds (males n = 7, females n = 6) between November 22nd and December 3rd 2010 at the Stony Hill sub-colony (approximately 500 m apart). Within each breeding stage, data were collected over approximately 10 days, in order to minimise confounding effects, such as temporal changes in prey availability. To reduce stress to individual birds, and to provide statistically independent data, different groups of birds were studied during each breeding stage. Birds were identified by a unique mark made with waterproof animal marker, which was still visible after ten days, and nests were marked with a painted number on a rock close to the nest to avoid pseudo-replication. Full permission was granted by the Tristan da Cunha government to access the penguin colonies and conduct the research. Animal ethics approval was granted by Rhodes University Zoology Department Ethics Committee (ZOOL-17-2010).

During the guard stage, birds were captured at the nest site, typically around dusk when provisioning chicks, to ensure the devices were deployed on female birds. During the crèche stage, it was not possible to capture birds at the nest site, so they were caught departing from the colony for a foraging trip. TDRs were attached to a leg band (constructed of a cable tie and highly flexible plastic cable) using amalgamating tape and a cable tie (Figure 3.1). Bands were then attached to the leg of the bird, ensuring the band was not too tight but tight enough to prevent it moving down over the foot to hinder the bird or be lost. Whilst many studies attach external devices to the bird's back, or tail feathers, to ensure the birds are stream-lined (as

recommended by Bannasch *et al*. 1994), the methodology used in this study was adopted due to the small size of the logger and thus to minimise the risk of losing devices. This methodology has been successfully used many times on other seabird species, such as Brünnich's guillemots, *Uria lomvia* (Elliot *et al.* 2008a), Common murres, *Uria aalge* (Regular *et al.* 2010), White-chinned petrels, *Procellaria aequinoctialis* (Peron *et al.* 2010) and Macaroni penguins, *Eudyptes chrysolophus* (Bost *et al.* 2009), with no deleterious effects on foraging behaviour observed.

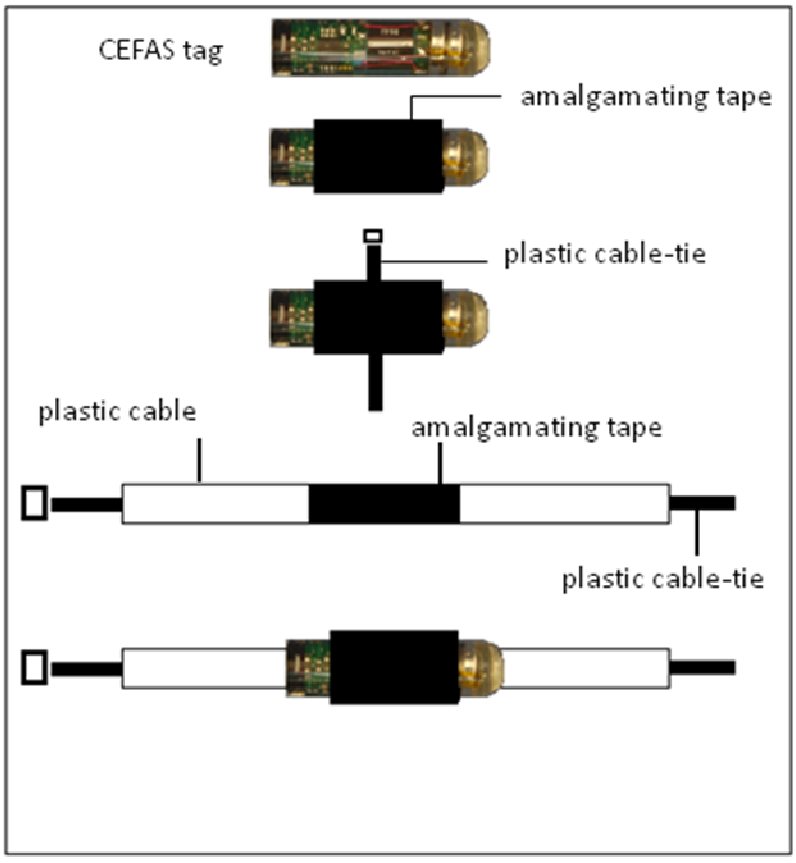

Figure 3.1 – Schematic diagram displaying the method of attaching CEFAS G5 TDRs to plastic leg bands.

Handling for device attachment was restricted to 3 minutes to minimise stress to the bird and reduce any detrimental effects of handling; all TDR-equipped birds returned to their nests. The path used by the birds to return to their nests was

monitored continuously throughout daylight hours, during the whole fieldwork season, until approximately 22:00 hrs, and then sporadically during the night to maximise capture of returning birds, which spent only a few hours at the nest in the middle of the night. Morphometric measurements of TDR-equipped birds were taken and birds sampled for blood for molecular sexing, stable isotope analysis and plasma corticosterone analysis upon return to the colony (See Chapters 2, 4 and 5 for respective details).

3.*2.4 Dive analysis*

MULTITRACE (Jensen software systems, Kiel, Germany) was used to analyse sequential dives. All data were corrected for a drifting surface level (recorded depth is adjusted so that the surface level is maintained at 0 m, countering any effects of wave action, Hagihara *et al.* 2011), and a 3 m dive threshold was set (Chappell *et al*. 1993; Tremblay & Cherel 2000, 2003). The software extracted the following parameters for each dive: dive duration, descent speed, ascent speed, surface time between dives, descent time, bottom time, ascent time, average depth at bottom phase of dive, maximum depth of dive, dive profile (U, V, W, Y) and mean temperature of dive (Figure 3.2). Y-shaped dives contain a bottom phase with a singular deep peak. W-shaped dives were dives with undulations in the bottom phase (Miller *et al.* 2009). Classification of dives as U- or V- shaped is related to the threshold for the bottom phase which determines if the dive has a bottom phase (U-shaped) or not (V-shaped). The bottom phase was defined as the time between the first and last points below 75% of maximum dive depth (following Tremblay *&* Cherel 2000 for Southern Rockhopper penguins, Figure 3.2).

MULTITRACE also calculated the following indexes: depth range (range in depth of the bottom phase/ maximum depth), symmetry (time in the bottom phase when maximum dive depth is reached/ bottom duration) and broadness (bottom duration/ dive duration). Each wiggle (undulations in the bottom phase of the dive profile, Figure 3.2) was analysed, using a > 2 m amplitude threshold, and a minimum of one wiggle per dive. From each dive, which contained wiggles, the following parameters were extracted: number of wiggles, duration of wiggles, minimum and maximum depth of wiggles and the mean amplitude of wiggles. From these data, wiggle rate (number of wiggles during the bottom phase/ duration of wiggles, Halsey *et al*. 2007), and the degree of 'raggedness' of the bottom phase (sum of the ranges in depth of wiggles during the bottom phase/ bottom duration, Halsey *et al*. 2010) were calculated. Dives with a higher raggedness index have more wiggles, with a greater change in depth associated with them.

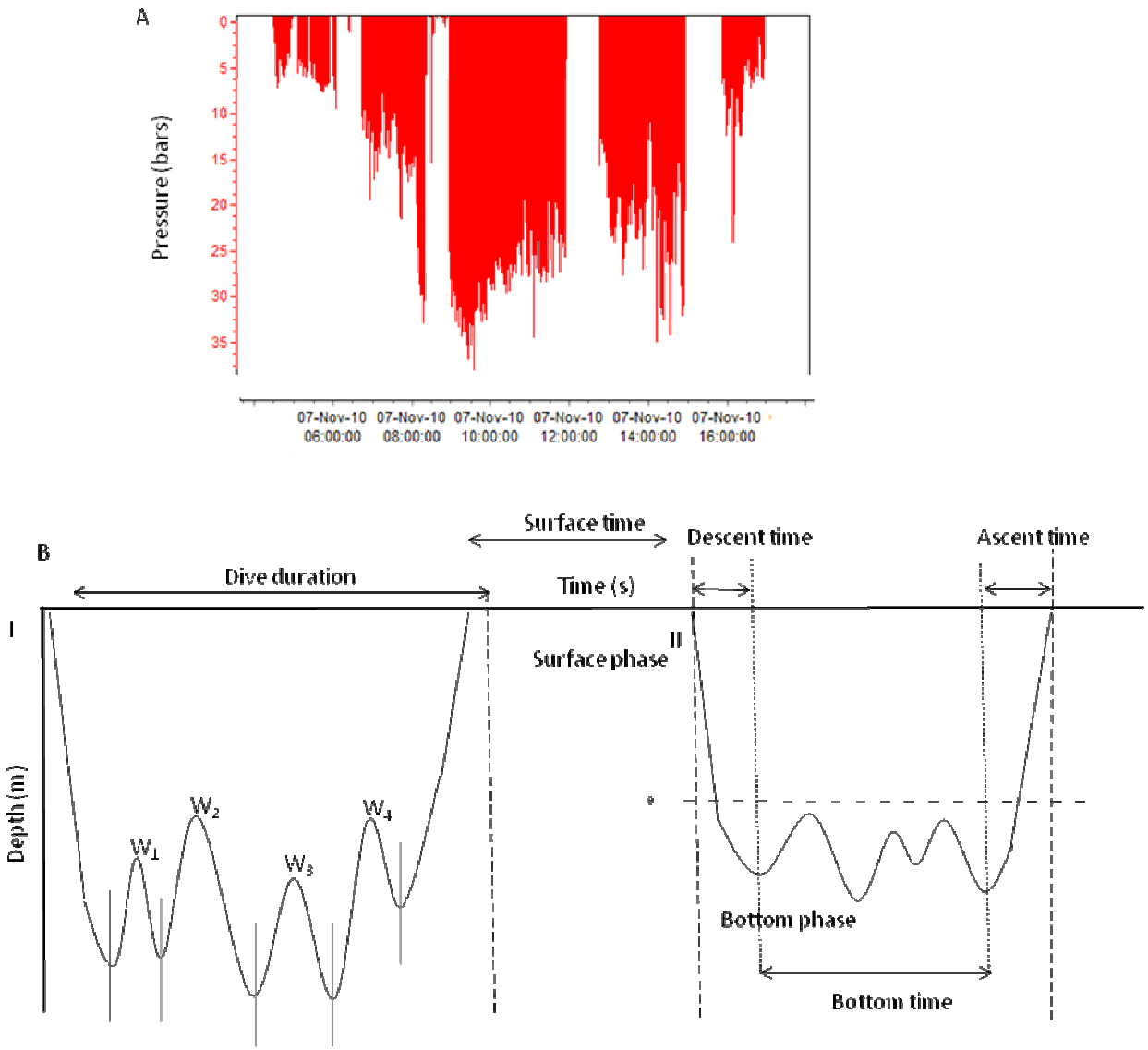

Figure 3.2 – **(A)** Example of a daily foraging trip of a female Northern Rockhopper penguin during the guard stage. **(B)** Schematic diagram of two consecutive foraging dives of a Northern Rockhopper penguin illustrating the points within a dive which separate the descent, bottom, ascent and surface phases (* - 75% of maximum depth of dive). W_1 – W_4 represent four consecutive wiggles within the bottom phase of the dive. Dive I has a higher degree of raggedness, with wiggles of a greater depth range than Dive II.

When classifying dives as day or night-time foraging dives, the nautical definition of dawn and dusk was used (when the sun is 12 ° below the horizon). At

sunrise, luminosity is already high, and again after sunset, so dawn was recorded as 45 minutes before sunrise, and dusk 45 minutes after sunset (Cherel *et al*. 1999), and night dives categorised as those occurring between dusk and dawn. These times were calculated for each day using actual daily sunrise and sunset data kindly provided by weather station staff at Gough Island.

For each bird a foraging trip was defined as all dives between the first and last dive, excluding travelling dives (< 3 m). Some birds were not recaptured after 24 hours as they were not spotted in the colony, and so consecutive trips were recorded over several days. In these cases, trips were separated when there was a gap in diving activity of more than 4 hours (Pichegru *et al*. 2011). The following foraging trip characteristics were calculated: departure and arrival time from and to the colony, trip duration (the time elapsed between the first and last dive recorded), vertical travel distance (VTD), total number of dives, dive rate (number of dives per hour of trip), dive time (as a % of trip), number of W-, Y-, V- and U-shaped dives, and distance travelled to foraging grounds (DTF). VTD was defined as the sum of the maximum dive depths for all dives during a trip, multiplied by two (Horning & Trillmich 1997). DTF was calculated as the time elapsed between the initial dive after entering the water and the 1st of at least three consecutive dives greater than 10 m depth (after Cherel *et al*. 1999), which is based on the assumption that penguins travel in a straight line at a constant speed of 7.4 km h $^{-1}$ (Brown 1987), and reflects absolute distance travelled, both vertical and horizontal.

3.2.5 Statistical analyses

Statistica 8.0 and Primer 6.1 were used to carry out all statistical testing. Percentage by mass diet data were arc-sin transformed to meet the assumptions of

normality and homogeneity (Kolmogorov-Smirnov tests $p > 0.05$, Cochran's tests $p > 0.05$ in all cases). Independent sample t-tests were used to compare mass of cephalopods, fish and zooplankton in: a) guard female stomach contents which were provisioning small (< 600 g) and large (> 600 g) chicks, b) male and female stomach contents during the crèche stage, and c) guard and crèche stage birds. Non-parametric Mann Whitney U-tests were used to compare lengths of the common ommastrephid squid and the fish *Phosichthys argenteus* found in stomach contents of a) guard and crèche stage females, and b) male and female birds.

Homogeneity of variances was confirmed using Levene's test ($p > 0.05$), and normality of distribution using the Kolmogorov-Smirnov test ($p > 0.05$) for foraging trip variables, with the exceptions of trip duration, dive rate and arrival time, which conformed after log-transformation. Independent sample t-tests were used to compare the means of all foraging trip variables between: a) guard stage females with small (< 600 g) and large (> 600 g) chicks (size groups were chosen arbitrarily) b) guard and crèche stage females, and c) guard females with a crustacean-dominated diet and those with a mixed cephalopod – crustacean diet. Correlation analysis was used to determine relationships between distance travelled to foraging grounds and trip duration of birds during the guard and crèche stage. Two 1-way ANOVAs were used to compare the mass of cephalopods, fish and macrozooplankton in stomach contents of birds foraging on different dates, firstly within the guard stage, and secondly within the crèche stage.

Analysis of dive characteristics was subject to two statistical problems. Firstly, successive dives are partially autocorrelated, as the maximum depth a bird may attain in a dive is influenced by the depth of previous dives, resulting in temporal pseudo-replication (Hurlbert 1984). Secondly, each bird did not have an equal statistical

weighting, due to the varying numbers of dives per trip and trips per bird. Following Tremblay & Cherel (2003), a partial autocorrelation was performed, which verified that maximum dive depth failed to correlate after four successive dives. Every fifth dive from each foraging trip was extracted for further analysis; the minimum number of independent dives recorded for a bird was 144, so for each bird 144 dives were randomly selected, and this dataset of dives was used for all statistical analyses and graphical presentation unless otherwise stated. When comparing foraging trip and dive parameters between birds, to avoid pseudo-replication and to ensure birds had an equal statistical weighting, the bird was considered as the sample unit rather than the foraging trip (Schiavini & Raya Rey 2004). In cases where birds performed multiple trips, means of each trip and then a grand mean for each bird were calculated.

Dive parameters were analysed in several ways. As the assumptions of normality or homogeneity were not met, non-parametric Mann-Whitney U tests were used to compare mean dive parameters between a) guard and crèche stage females, and b) birds with small and big chicks. Characteristics of the two types of foraging dives, dives that contained wiggles, U- and W- shaped dives were compared using PERMANOVA. This analysis is robust to the violation of assumptions as the sample size was very large (Underwood 1997). SIMPER analysis was used to determine on which parameters dives were most dissimilar, and Mann Whitney U tests were then used to compare the means of those selected dive parameters that contributed the greatest weighting to dissimilarity.

In cases when stomach contents were obtained from birds equipped with TDR devices, foraging dives were analysed to examine the effect of diet using a Model I factorial analyses of variance (ANOVA) (5 levels: zooplankton > 90% by mass, fish > 90% by mass, mixed cephalopod/ fish ~ 50/50% by mass, mixed cephalopod/

zooplankton ~ 50/50% by mass, mixed fish/ zooplankton ~ 50/50% by mass). Analysis was assumed to be robust to violating the assumptions of homogeneity and normality due to the large sample size (Underwood 1997). An equal number of dives were selected randomly from foraging dives made on the last day of foraging trips, as these dives were assumed the most likely to relate to prey captured that was still in the stomach. Student-Newman-Keuls (SNK tests) were used to compare means when sources of variation were significant. Principal Component Analysis (PCA) was performed on all dive variables calculated for birds feeding on different prey categories to discriminate differences among dives performed that targeted different prey. Mann-Whitney U tests were used to compare the mean dive parameters of dives made during the night (between nautical dusk and dawn) and day (between nautical dawn and dusk).

All mean values are presented with standard error; significance was assumed at $p < 0.05$ in all cases.

3.3 Results

3.3.1. Diet composition

In total 3340 prey items were identified from 73 Northern Rockhopper penguin stomach contents (31 guard females, 22 crèche females, and 20 crèche males), comprising 1373 individual fish, 348 individual cephalopods and 1619 crustaceans. A list of prey species, with the index number, frequency of occurrence and percentage frequency of occurrence are presented in Tables 3.1 and 3.2.

Table 3.1 – List of fish species (listed alphabetically) found in Northern Rockhopper penguin stomach contents (n = 73) during the breeding season 2010 at Tristan da Cunha. Number = number of individuals, F.O = frequency of occurrence in samples, and % FO = percentage frequency of occurrence.

Fish	**Number**	**F.O**	**% F.O**
Cheilodactylidae			
Unknown species	6	4	5.5
Myctophidae			
Diaphus sp.	22	4	5.5
Lampichthys procerus.	8	7	9.6
Unknown species A	21	4	5.5
Unknown species B	1	1	1.4
Unknown species (juveniles)	14	4	5.5
Paralepididae			
Lestidiops affinis	42	10	13.7
Lestidiops sp.	14	5	6.8
Magnisudis priomosa	4	4	5.5
Photichthyidae			
Phosichthys argenteus	386	25	34.2
Vinciguerria sp.	649	28	38.6
Unknown species (juveniles)	193	18	24.7
Sternoptychidae			
Maurolicus muelleri	13	5	6.8

Table 3.2 – List of cephalopod and crustacean species (listed alphabetically) found in Northern Rockhopper penguin stomach contents (n = 73) during the breeding season 2010 at Tristan da Cunha. Number = number of individuals, F.O = frequency of occurrence in samples and % F.O = percentage frequency of occurrence.

Cephalopods	**Number**	**F.O**	**% F.O**
Ocythoidae			
Ocythoe tuberculata	39	22	30.1
Ommastrephidae			
Unknown species A	232	44	60.3
Unknown species B	13	8	11.1
Unknown species (juveniles)	50	13	17.5
Unidentified cephalopods			
Unknown species A	14	12	16.4
Crustaceans	**Number**	**F.O**	**% F.O**
Decapoda			
Jasus tristani	48	15	20.5
Unidentified decapod shrimp	2	2	2.7
Amphipoda			
Hyperiid:			
Themisto gaudichaudii	68	24	32.9
Unknown Gammariid	4	4	5.5
Euphausiacea			
Euphausia sp. and *Thysanoessa* sp.	1484	57	78.1
Copepoda			
Unknown species	13	9	12.3

Although the macrozooplankton retrieved from stomach contents were highly digested, it was still possible to determine, from semi-digested body parts, the broad assemblages, and these differed between the guard and crèche stage. In the guard stage, the zooplankton component consisted of copepods, euphausiids, amphipods and

a large number of fish larvae. In the crèche stage, no fish larvae were observed, and the greatest constituent was euphausiids, with smaller numbers of amphipods and copepods. As these two categories did not co-occur temporally, and for ease of analysis, they are both referred to as macrozooplankton, but their different compositions should be remembered when considering differences between breeding stages.

No difference in diet composition, in terms of percentage by mass, was observed between crèche stage males and females (fish $t_{32} = -0.7188$, $p = 0.4775$; cephalopods $t_{32} = -0.2604$, $p = 0.7962$; zooplankton $t_{32} = 0.6755$, $p = 0.5042$), so these data were pooled. Guard stage female diet was dominated by zooplankton by mass ($t_{63} = 9.6865$, $p < 0.0001$), and crèche stage male and female diet was dominated by fish by mass ($t_{63} = -15.0929$, $p < 0.0001$; Figure 3.3a). Birds in both stages consumed a similar proportion of cephalopods by mass ($t_{63} = 1.4426$, $p = 0.1541$; Figure 3.3a). Within the guard stage, no difference in proportions (by mass) of different prey types was found in adult female stomach contents which were provisioning small (< 600 g) and big (> 600 g) chicks (fish $t_{14} = -1.7256$, $p = 0.1064$; cephalopods $t_{14} = -0.9391$, $p = 0.3636$; zooplankton $t_{14} = -1.6573$, $p = 0.1197$). Cephalopods were found to contribute to more than 50% mass in the stomach contents of only three birds; however they are clearly a very important prey item, contributing to diet of guard and crèche birds throughout the whole study period (Figure 3.3)

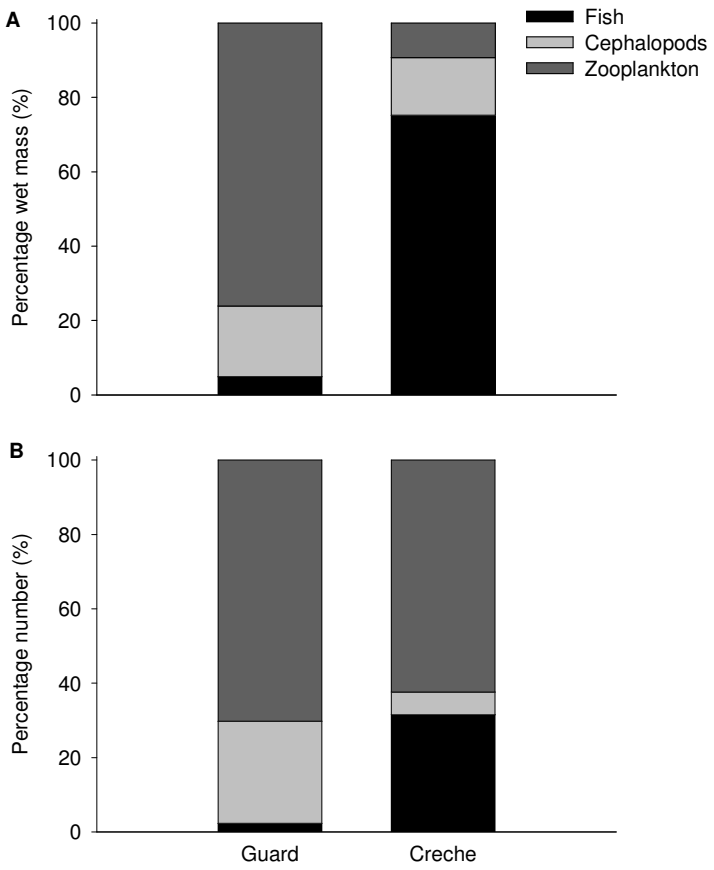

Figure 3.3 – Composition of Northern Rockhopper penguin stomach contents, by **(A)** percentage of total wet mass and **(B)** percentage of total number of prey items, at Tristan da Cunha during the guard (females n =31) and crèche (females n = 22, males n = 20) stages in 2010.

It should be mentioned that identification of the majority of crustacean specimens retrieved from stomach contents was hindered by the advanced state of digestion. In the case of cephalopod beaks and fish otoliths, reference material was used to aid identification wherever possible. However, identification to a low taxonomic group was often not possible as many specimens obtained from stomach contents were juveniles, and much of the reference material from adult specimens, and both beaks and otoliths undergo significant ontogenetic morphological change (Smale *et al*. 1995). Additionally, otoliths for a number of fish species known to occur

around Tristan da Cunha (Andrew *et al*. 1995), that are potential penguin prey species, are not found in otolith Atlases (Smale *et al*. 1995; Campana 2004; Tuset *et al.* 2008), highlighting the paucity of reference material for the South Atlantic Ocean. All identifications to species are tentative; however identifications to family and in most cases genus level were made with confidence.

Fish representing five families and at least eleven species were identified during analysis of stomach contents. Myctophids were the best represented family of fish, whilst photichthyids were the numerically dominant family (Table 3.1). *Vinciguerria sp.* and *Phosichthys argenteus* were the most important fish items by number and frequency of occurrence (Table 3.1). Photichthyid fish were the most important prey group for crèche stage birds and were found in 74% of stomach samples. Fish was not an important prey item for guard stage birds (Figure 3.3). Crèche stage birds consumed a greater proportion of fish by number than guard birds (Figure 3.3b). These number data were not analysed for statistical significance as samples were highly digested so that macrozooplankton were likely under-represented.

The most important cephalopod consumed, by number and frequency of occurrence was a squid belonging to the Family Ommastrephidae), which was found to occur in a similar proportion of guard and crèche stage stomach contents (Table 3.2, Figure 3.4). The octopus *Ocythoe tuberculata* also occurred similarly in guard and crèche diet (Figure 3.4). Guard stage birds consumed a greater proportion of cephalopods by number than crèche stage birds (Figure 3.3b).

Euphausiids were important prey items for birds in both breeding stages (Figure 3.4), and were the most numerically abundant crustaceans found in stomach contents (Table 3.2). Euphausiacea comprised the species *Euphausia spinifera*,

Thysanoessa sp. and *Euphausia lucens*. Notably the crayfish, *Jasus tristani*, was found in greater numerical abundance, and frequency of occurrence, in crèche bird's stomach contents compared to guard birds (Figure 3.4). Whilst crustaceans were found to occur more frequently in crèche bird stomach contents, this may be an artefact of the high state of digestion in guard samples, which may have prevented crustaceans being identified and counted.

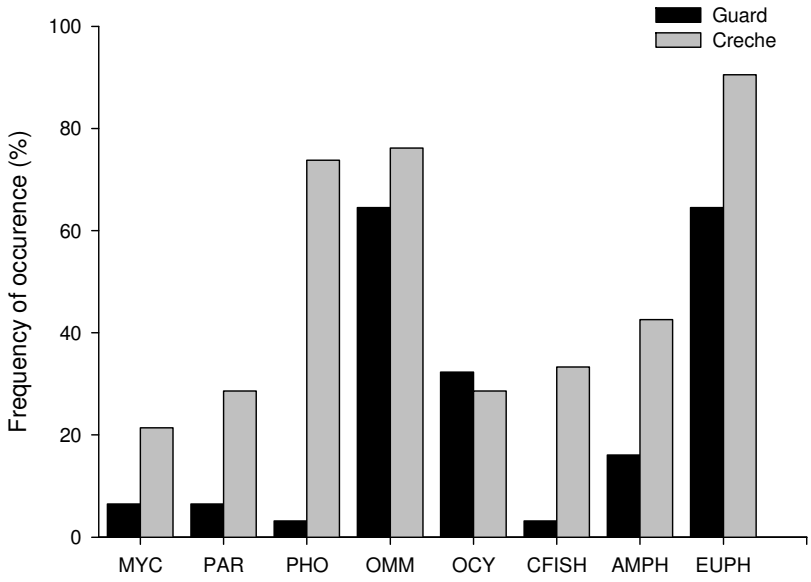

Figure 3.4 –Frequency occurrence (%) of the main prey types found in stomach contents of guard (n = 31) and crèche (n = 42) stage adult Northern Rockhopper penguins during the breeding season 2010 at Tristan da Cunha (Fish: MYC = Myctophids, PAR = Paralepidids, PHO = Photichthyids; Cephalopods: OMM = Ommastrephids, OCY = *Ocythoe tuberculata*; Crustaceans: CFISH = *Jasus tristani*, AMPH = amphipods, EUPH = Euphausiids).

The size-class distribution of the fish *Phosichthys argenteus* showed that individual fish with a total body length ranging from 35.6 – 57.9 mm were preyed upon (Figure 3.5a). No *Phosichthys argenteus* were consumed in the guard stage. Crèche male birds fed on a larger size classes of *Phosichthys argenteus* than crèche females (t_{290} = 2.7409, p = 0.007; Figure 3.5a). The size-class distribution of the

squid, unknown ommastrephid A, which was the most common type of cephalopod consumed, both in actual number and frequency of occurrence in stomach samples, showed that individuals with a mantle length ranging from 17.6 – 195.5 mm were preyed upon (Figure 3.5b). Breeding stage data for females was pooled as females in the guard and crèche stage consumed ommastrephid squids of similar lengths (17.6 – 166 mm, $U_{72} = 543.50$, $p = 0.1765$). Male birds fed on larger size classes of this squid than females ($U_{140} = 1964.0$, $p = 0.0428$; Figure 3.5b).

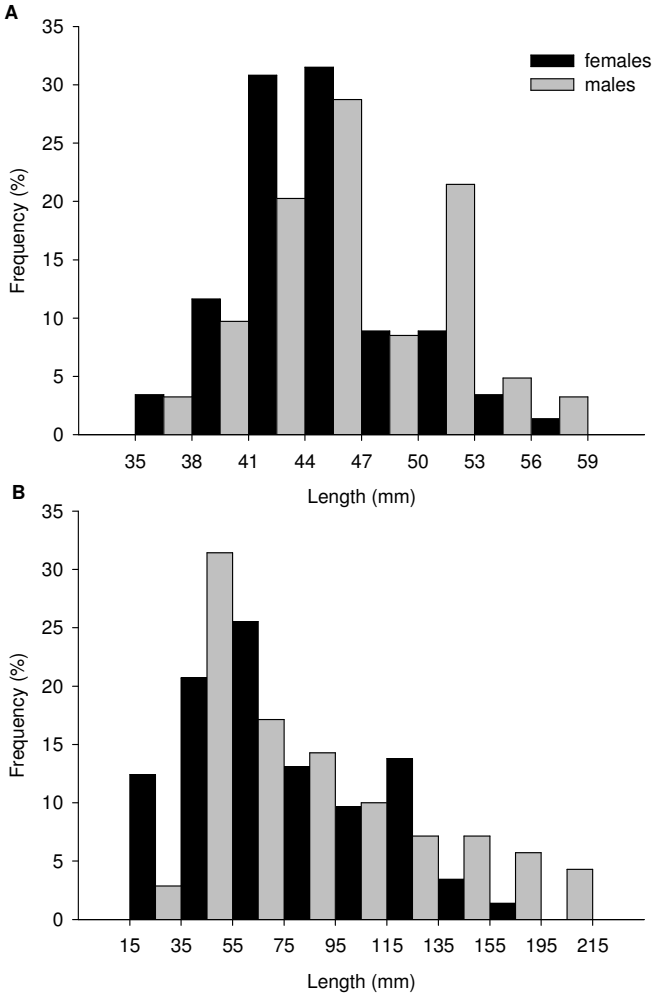

Figure 3.5 – Size class distribution of **(A)** the total length *Phosichthys argenteus* (n = 393, Family Photichthyidae) and **(B)** the mantle length of ommastrephid squid A. (n = 215) found in stomach contents of crèche stage male (n = 22) and female (n = 20) Northern Rockhopper penguins during the breeding season 2010 at Tristan da Cunha.

Within each breeding stage, diet composition was analysed to determine temporal changes in prey targeted by birds. Within the guard stage, foraging date had no effect on the percentage by mass fish ($F_{3, 29}$ = 0.1661, p = 0.9183), cephalopods ($F_{3, 29}$ = 1.1077, p = 0.3621) or zooplankton ($F_{3, 29}$ = 0.9476, p = 0.1877) contributed to diet (Table 3.3). Within the crèche stage, foraging date had a significant effect on the percentage by mass cephalopods contributed to diet ($F_{4, 34}$ = 3.7645, p = 0.0122), although these dates did not form separate homogenous groups, and there was no pattern of increase or decrease over the breeding season (Table 3.3). Foraging date had no effect on percentage mass of fish ($F_{4, 34}$ = 1.740, p = 0.1641) or zooplankton ($F_{4, 34}$ = 0.6460, p = 0.6335; Table 3.3).

Table 3.3 – 1- way ANOVAs comparing diet composition of Northern Rockhopper penguins, in terms of percentage mass of fish, cephalopods and zooplankton, across days within the guard and crèche stage. Significant results are highlighted in bold.

	Guard (n = 33)				**Crèche (n = 32)**			
	d.f.	**M.S**	**F**	**p**	**d.f.**	**M.S**	**F**	**p**
Fish								
Date	3	0.3965	0.1661	0.9183	4	1.2251	1.74	0.1641
Error	29	2.3867			34	0.7042		
Cephalopods								
Date	3	2.3334	1.1077	0.3621	4	8.0679	3.7645	**0.0122**
Error	29	2.1066			34	2.1432		
Zooplankton								
Date	3	0.9476	1.706	0.1877	4	1.5354	0.646	0.6335
Error	29	0.555			34	2.3766		

3.3.2 Foraging trip characteristics

One TDR-equipped bird during the guard stage, and five during the crèche stage, did not return to the colony during the fieldwork period. Based on beak measurements, the crèche stage birds were thought to be male birds that may have been on extended foraging trips after their guard fast. Five trips during the guard stage were excluded from analysis as complete trips were not recorded, and some data were lost through technical failure of devices in the crèche stage (including the two returning males). Complete foraging trips were recorded from 27 guard stage females (51 trips) and 6 crèche stage females (11 trips).

Birds departed for foraging trips throughout the day and night, with departure from the colony peaking at dawn, around 04:00 (Figure 3.6). The majority of birds returned to the colony at dusk, around 20:00, however birds also arrived throughout the day (Figure 3.6). No significant differences in the arrival (t_{10} = 0.5891, p = 0.5689) or departure times (t_{10} = -1.1745, p = 0.2674) of foraging trips between the guard and crèche stage were observed (Table 3.4).

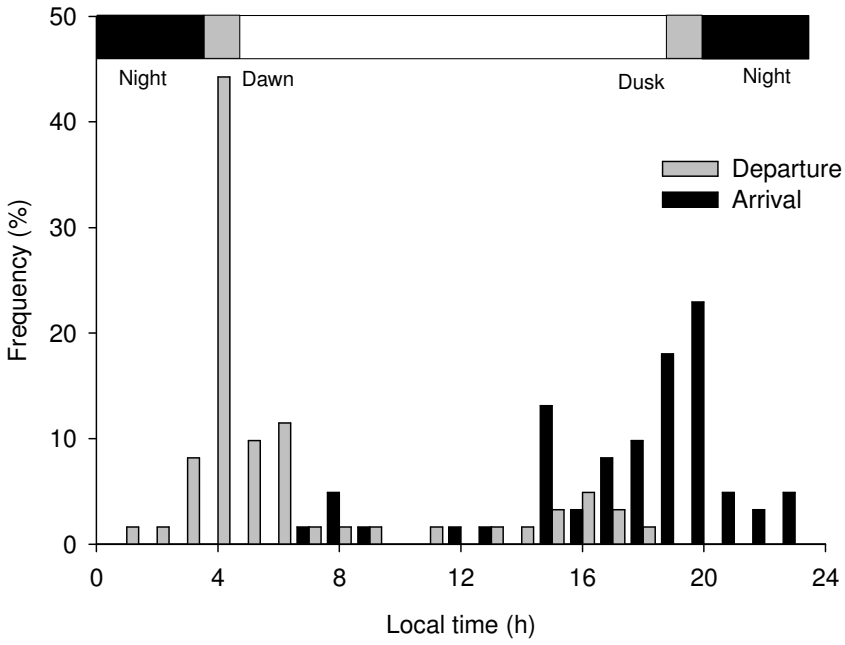

Figure 3.6 – Frequency distribution of the departure and arrival times of daily foraging trips made by Northern Rockhopper penguins at Tristan da Cunha during the guard (n = 51) and crèche (n =11) stages at Tristan da Cunha.

Table 3.4 – Mean dive characteristics, calculated on each trip (± SE) of Northern Rockhopper penguins at Tristan da Cunha during the breeding season 2010, comparing the guard (n = 51) and crèche stages (n = 11), with results of independent sample t-tests. DTF = distance to foraging grounds, VTD = vertical travel distance. Significant results are highlighted in bold.

	Guard (n = 51)	**Crèche (n = 11)**	**$t_{(1435)}$**	**p**
Trip duration (hr)	13.1 ± 0.8	15.4 ± 4.2	-0.3983	0.6988
Dive rate (dives h-1)	51.3 ± 3.3	34.2 ± 5.2	-2.1483	0.0572
DTF (km)	8.26 ± 1.02	8.77 ± 2.6	-0.9134	0.3825
VTD (km)	19.9 ± 1.1	13.3 ± 2.1	-2.3746	**< 0.05**
Total dive time (% of trip duration)	79.1 ± 2.7	74.6 ± 15.3	-1.361	0.2034
Mean temperature of dives (ºC)	14.5 ± 0.1	15.1 ± 0.1	-13.6987	**< 0.0001**
Symmetry index	0.38 ± 0.01	0.42 ± 0.02	-3.4951	**< 0.001**
Depth range index	0.52 ± 0.01	0.37 ± 0.03	11.1152	**< 0.0001**
Broadness index	0.47 ± 0.01	0.39 ± 0.01	3.3999	**< 0.001**
Average surface time (s)	8.4 ± 0.7	14.7 ± 3.8	-1.1373	0.2556
Average dive duration (s)	66.7 ± 4.1	78.6 ± 7.1	-4.7129	**< 0.0001**
Average descent time (s)	14.2 ± 0.7	21.7 ± 3.9	-4.8639	**< 0.0001**
Average bottom time (s)	38.3 ± 3.1	35.4 ± 3.3	0.5958	0.5514
Average ascent time (s)	14.2 ± 0.8	21.5 ± 4.1	-8.9026	**< 0.0001**
Average descent speed (ms^{-1})	1.16 ± 0.06	1.04 ± 0.08	-0.3366	0.7365
Average ascent speed (ms^{-1})	1.08 ± 0.05	0.94 ± 0.05	1.7139	0.0878
Average depth of dive (m)	12.2 ± 0.7	20.5 ± 5.2	-4.8299	**< 0.0001**
Average maximum depth (m)	15.5 ± 0.9	16.0 ± 2.9	-7.5059	**< 0.0001**

No significant difference in the trip duration of guard and crèche birds was observed ($p > 0.05$, Table 3.4). The majority of foraging trips were 12 – 16 hours in duration (Figure 3.7). Two birds made trips greater than 24 hours in duration, one guard bird (27.4 hours) and one crèche bird (35.5 hours) (Figure 3.7). Several birds made short trips of less than 4 hours (Figure 3.7), the shortest being 1.6 hours. No relationship was found between trip duration and distance travelled to foraging grounds (guard stage $r^2 = 0.0263$, crèche stage $r^2 = 0.2311$, $p > 0.05$ in both cases; Figure 3.8). Distance travelled to foraging grounds (DTF) did not differ between stages ($p > 0.05$, Table 3.4). Vertical travel distance was significantly higher in guard stage foraging trips ($p < 0.05$, Table 3.4).

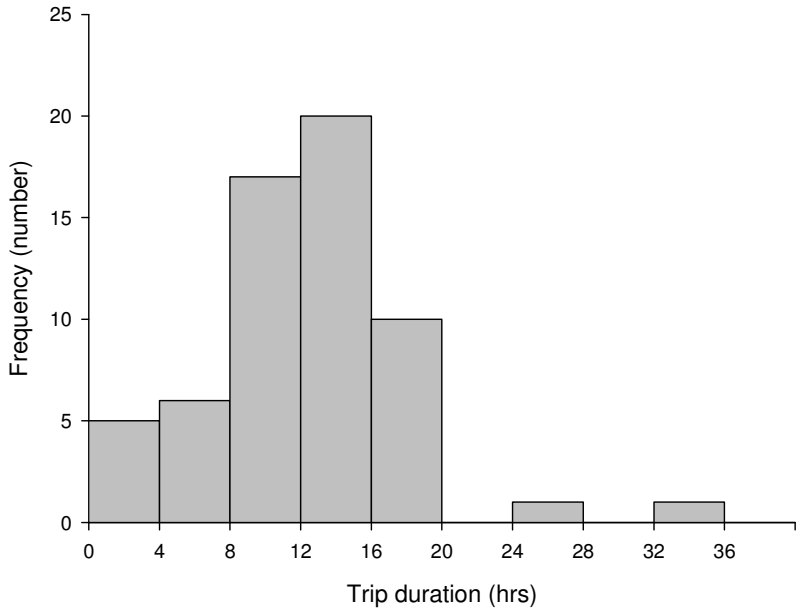

Figure 3.7 - Frequency histogram of the duration of foraging trips made by Northern Rockhopper penguins provisioning chicks at Tristan da Cunha during the breeding season 2010 (n = 62).

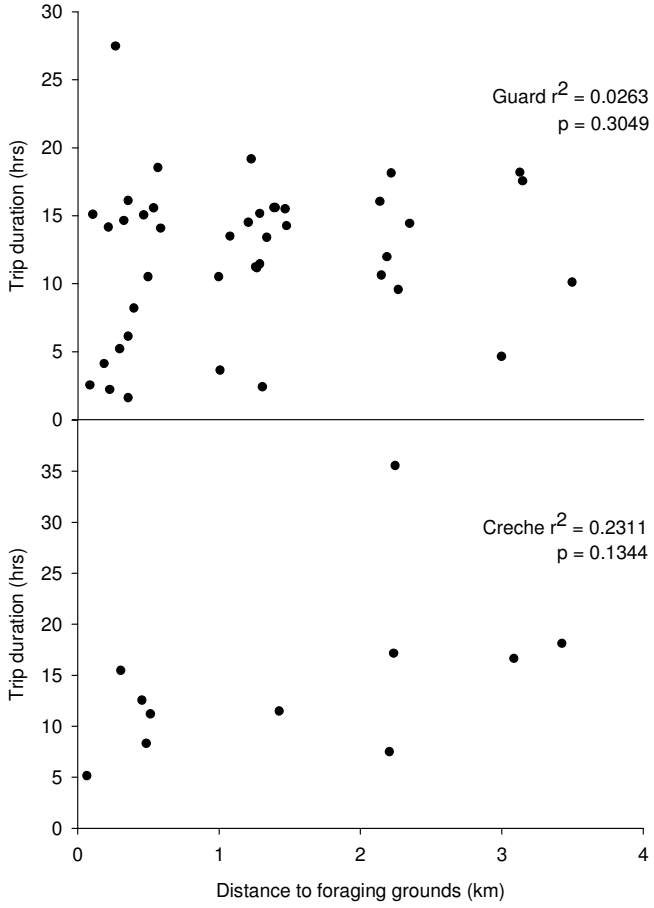

Figure 3.8 - Relationship between distance travelled by Northern Rockhopper penguins to foraging grounds and total foraging trip duration during the guard (n = 42) and crèche (n = 11) stages of the breeding season 2010.

No significant effect of stage was observed on dive rate, or dive time ($p > 0.05$, Table 3.4). No significant effects of chick size within the guard stage were observed on dive rate, trip duration, departure and arrival times of foraging trips, vertical travel distance, dive time, or distance travelled to foraging grounds ($p > 0.05$ in all cases, Table 3.5).

Table 3.5 – Independent sample t tests to compare mean foraging trip characteristics of guard female birds with small (< 600g) and big (> 600g) chicks, and guard females which fed on zooplankton (> 90% by mass) and a mixture of cephalopods and zooplankton.

	Chick size		**Diet**	
	$t_{(12)}$	**p**	**$t_{(12)}$**	**p**
Dive rate	-0.3287	0.7481	-1.2079	0.2504
Trip duration	0.9679	0.3522	-0.2272	0.8241
Departure time	1.4157	0.1823	-0.0837	0.9347
Arrival time	1.1356	0.2783	0.8471	0.4135
VTD	1.1702	0.2646	1.838	0.0909
Total dive time	-0.7658	0.4586	-1.4715	0.1669
DTF	-0.1818	0.8588	-0.499	0.6268

3.3.3 Dive characteristics

U-shaped dives contributed more than 60% of the total proportion of dives in both breeding stages during both day and night dives (Figure 3.9). In the crèche stage, at night 92% of dives were U-shaped. Y- shaped dives made up less than 2% of dives in both breeding stages (Figure 3.9).

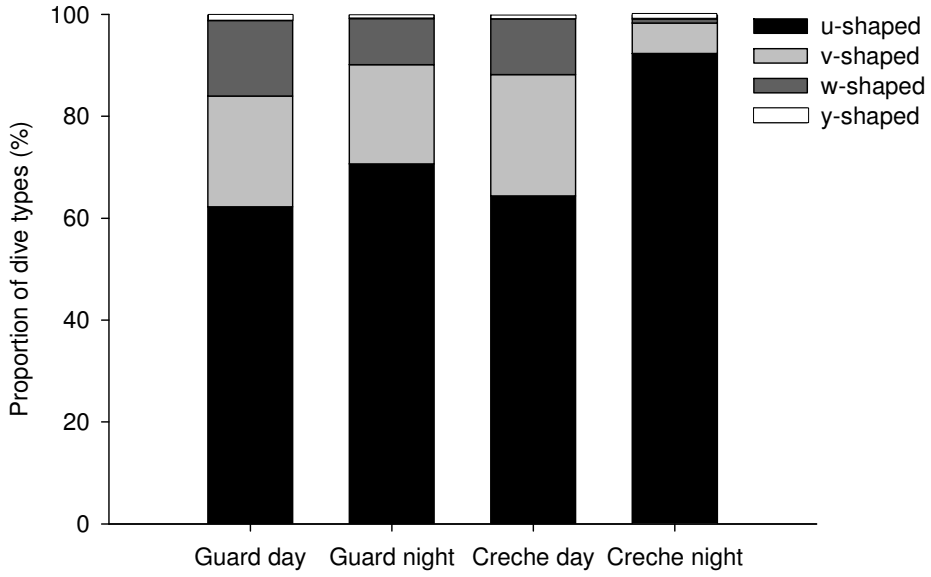

Figure 3.9 – Proportion of U-, W- ,V- and Y- shaped dives as a percentage of the total number of dives made by guard and crèche birds during the day and night. Guard day: U n = 20294, W n = 4826, V n = 7087, Y n = 390; Guard night: U n = 2122, W n = 271, V n = 584, Y n = 25; Crèche day U n = 3527, W n = 598, V n = 1306, Y n = 49; Crèche night: U n = 108, W n = 1, V n = 7, Y n = 1.

Crèche birds made longer dives, in terms of ascent, descent and total dive durations, and deeper dives, in terms of average and maximum depth, than guard birds ($p < 0.05$, Table 3.4). No significant effect of stage was observed on surface time between dives or bottom time, or on descent and ascent speed ($p > 0.5$, Table 3.4).

Guard birds made more dives in colder water than crèche birds ($p < 0.05$, Table 3.4). Crèche birds' dives had a higher symmetry index ($p < 0.05$, Table 3.4) compared to guard birds' dives. Guard birds had a higher broadness index and depth range index ($p < 0.05$, Table 3.4) compared to crèche birds dives.

Within the guard stage, birds with larger chicks had higher descent times and total dive durations than those with small chicks ($p < 0.01$, Table 3.6). Birds with smaller chicks had greater maximum and mean dive depths ($p < 0.01$, Table 3.6). Birds with larger chicks had a greater depth range and broadness indices ($p < 0.0001$), ($p < 0.05$; Table 3.6). Birds with smaller chicks made more dives in colder water than birds with large chicks ($p < 0.0001$, Table 3.6).

No effect of chick size was observed on surface, ascent, or descent time, and thus dive duration, or ascent speed, broadness or symmetry of dives ($p > 0.05$, Table 3.6).

Table 3.6 – Mann Whitney U test comparisons of dive parameters between guard birds with small (< 600g) and big (> 600g) chicks. Significant results are highlighted in bold.

	Chick size	
	U $_{(n = 1008)}$	**p**
Surface time	493108.5	0.2862
Dive duration	475281.5	**0.0149**
Descent time	471776.5	**0.0069**
Bottom time	499710	0.5751
Ascent time	486718.5	0.1197
Descent speed	498294.5	0.5035
Ascent speed	490213.5	0.1977
Maximum depth	477106.5	**0.0219**
Mean depth	295936	**< 0.0001**
Broadness	480069.5	**0.0389**
Depth range	224179.9	**< 0.0001**
Symmetry	279384.5	0.5739
Mean temperature	353705	**< 0.0001**

In summary, crèche stage birds made longer, deeper, less efficient dives, with a greater symmetry index in warmer water than guard stage birds. Guard stage birds with large chicks made longer, shallower, more efficient dives, with a greater depth range and broadness index in warmer water than those with small chicks.

3.3.4 Foraging strategies in relation to diet

All feeding dives (16252), classified as those with wiggles, were U- (13 226) or W-shaped (3 026), V- and Y- shaped dives did not have wiggles and therefore were not considered as feeding dives; U- and W- dives were significantly different from each other (PERMANOVA d.f 999, $p < 0.001$). SIMPER analysis showed that the variables dive duration, bottom time and maximum depth of dive contributed to 72.1% of the dissimilarity between U- and W- shaped dives. U-dives were significantly longer in duration than W-shaped dives ($t_{6041} = 4.6418$, $p < 0.0001$; Figure 3.10), with a longer bottom phase ($t_{6041} = 3.9247$, $p < 0.0001$; Figure 3.10). U-dives were significantly deeper than W-dives ($t_{6041} = 13.7341$, $p < 0.0001$; Figure 3.10).

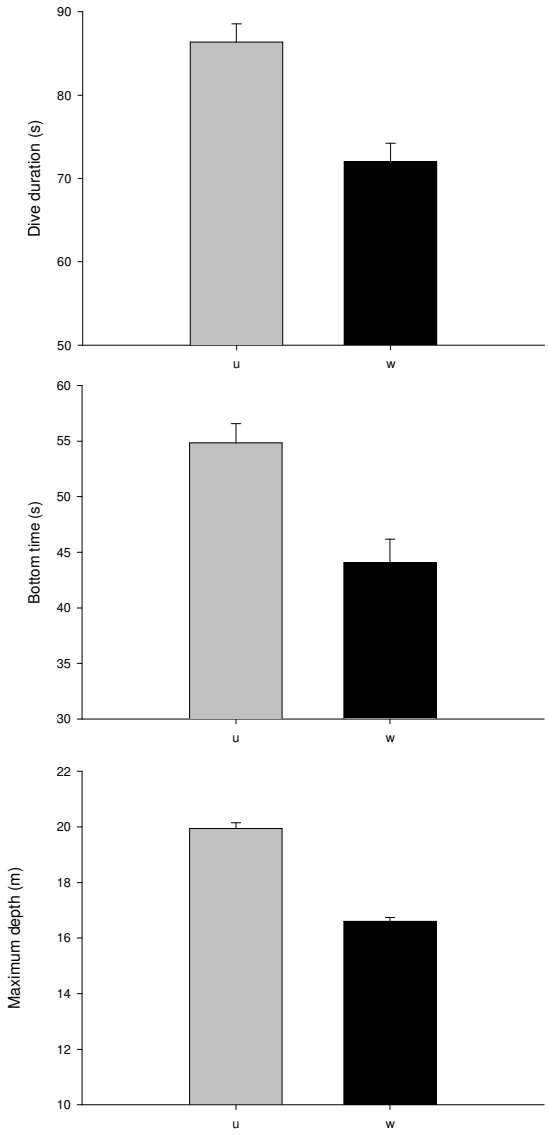

Figure 3.10 - Comparison of the mean (± SE) maximum depth, bottom time and dive duration of U- and W-shaped foraging dives (dives with wiggles) made by chick-rearing Northern Rockhopper penguins at Tristan da Cunha (U n = 13 226, W n = 3 026).

Foraging dive characteristics of birds from which stomach contents were obtained, were analysed to compare diving strategies of birds targeting the five main diet compositions: zooplankton (Z), fish (F), mixed fish/ zooplankton (FZ), mixed cephalopod/ zooplankton (CZ) and mixed cephalopod/ fish (CF). A significant effect

of diet was observed on all dive parameters ($p < 0.01$, Table 3.7) with the exception of ascent speed ($p = 0.1223$, Table 3.7).

Table 3.7 – 1- way ANOVA multiple independent group analysis of diet (5 levels: Z, CF, CZ, F, CF)) on dive parameters of all feeding dives of birds from which stomach contents were obtained. Significant results are highlighted in bold.

	M.S.	**$F_{(4, 1169)}$**	**p**
Surface time	3090.9	66.9	**< 0.0001**
Dive duration	87368	14.81	**< 0.0001**
Descent time	8399.2	78.45	**< 0.0001**
Bottom time	28153	5.6023	**< 0.001**
Ascent time	9349.4	812.1	**< 0.0001**
Descent speed	0.0821	5.73	**< 0.001**
Ascent speed	0.445	1.822	0.1223
Maximum depth	16585.9	110.58	**< 0.0001**
Broadness	0.388	23.1	**< 0.0001**
Depth range	3.393	96.22	**< 0.0001**
Symmetry	0.4557	4.654	**< 0.001**
Mean temperature	3.4	27	**< 0.0001**
Number of wiggles	97.36	3.8857	**< 0.01**
Wiggle amplitude	13195.5	12.02	**< 0.0001**
Duration of wiggles	18629	3.9471	**< 0.01**
Wiggle rate	0.1256	8.753	**< 0.0001**
Minimum wiggle depth	16961.6	161.11	**< 0.0001**
Maximum wiggle depth	161110.7	109.74	**< 0.0001**
Raggedness index	1.9395	42.79	**< 0.0001**

A principal component analysis (PCA) was performed on 21 variables calculated for dives of birds that had different diet compositions, so that the foraging strategies of birds targeting different prey could be characterised and compared.

Variables were reduced to two principal components, which together accounted for 60.4% of the total variance in the data and compared (PC1 contributed to 36.2% of the variance and PC 2 contributed to 24.2% of the variance in the data). PC1 was associated, primarily, with the variables maximum and average depth, minimum and maximum depth of wiggles, and ascent and descent time of dive (Table 3.8). Thus, dives with a high value on PC1 were characterised as being deeper, with feeding activity also occurring at greater depths, with greater travel times descending and ascending to depth (Figure 3.11). PC2 was associated, primarily, with the variables bottom time, dive duration, and the duration, amplitude and number of wiggles (Table 3.8). Thus, dives with a high value on PC2 were characterised as having longer bottom phases with more feeding activity (related to the number and duration of wiggles) (Figure 3.11). The distribution of principal component values for each dive allowed groups of dives to be distinguished, although there was a lot of overlap. Dives at the positive end of PC2 were dives made to capture FZ or CZ (Figure 3.11), and dives at the positive end of PC1 were dives made to capture fish (Figure 3.11).

Table 3.8 - Principal component loadings on the first two principal components of 19 variables of dives associated with different diet composition. Variables accounting for the majority of the variation in each principal component are highlighted in bold.

	PC 1 (36.2%)	PC2 (24.2%)
Surface time	0.6106	-0.0646
Dive duration	0.2329	**0.9397**
Descent time	**0.8293**	0.261
Bottom time	-0.0234	**0.9669**
Ascent time	**0.8521**	0.1437
Descent speed	0.3386	-0.0659
Ascent speed	0.1789	-0.0581
Maximum depth	**0.9596**	0.1035
Average depth	**0.9732**	0.0146
Broadness	-0.7529	0.3372
Depth range	-0.6988	0.2289
Symmetry	-0.024	0.0047
Temperature of dive	-0.5096	-0.0381
Number of wiggles	-0.0516	**0.9225**
Wiggle amplitude	0.0069	**0.9438**
Duration of wiggles	-0.0212	**0.9741**
Wiggle rate	-0.2104	-0.3031
Minimum wiggle depth	**0.9494**	-0.0356
Maximum wiggle depth	**0.9546**	0.1221
Raggedness	0.055	0.1959

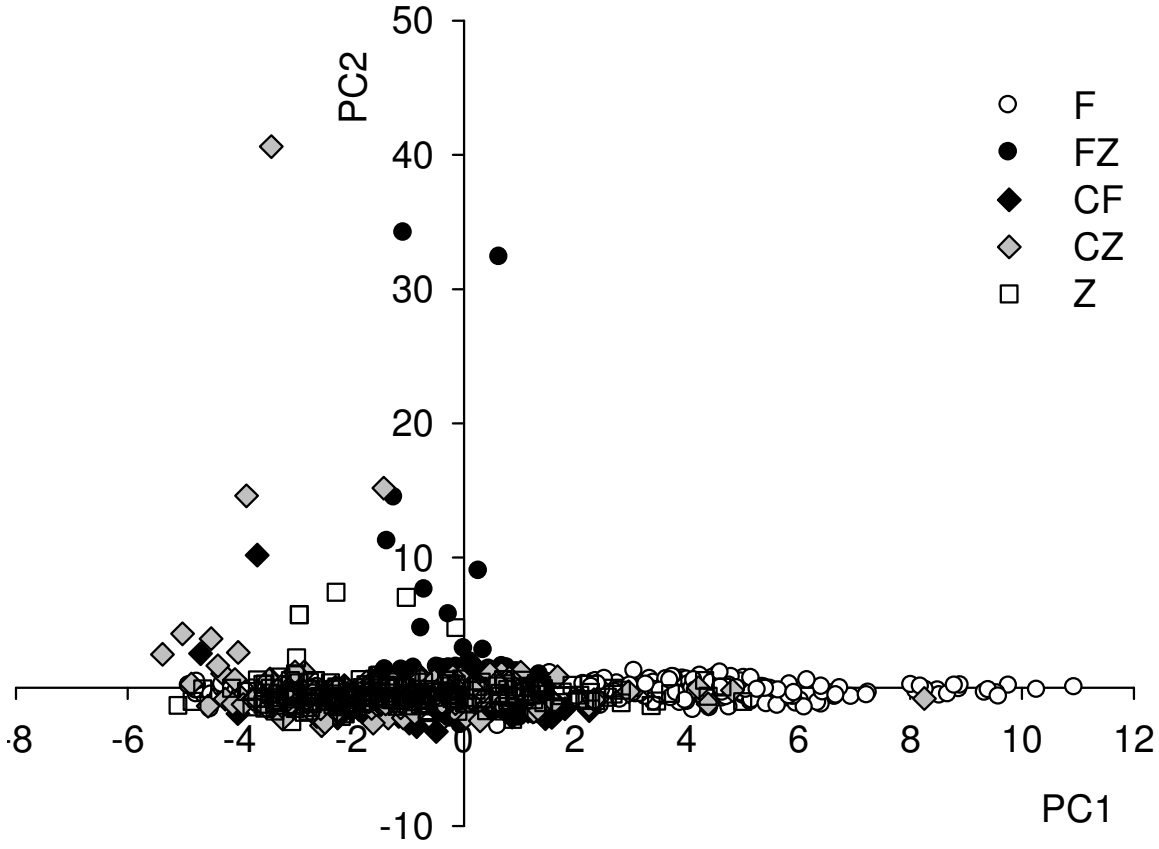

Figure 3.11 – Principal component analysis of feeding dive parameters of Northern Rockhopper penguins targeting different prey items during the guard and crèche breeding stages at Tristan da Cunha (n = 235). F = fish, FZ = fish/ zooplankton, CF = cephalopods/ fish, CZ = cephalopods/ zooplankton, Z = zooplankton.

Dives of birds that captured fish had a higher maximum depth, minimum and maximum wiggle depth (Table 3.7; SNK < 0.05, Figure 3.12), and dives of birds which had a mixed cephalopod/ zooplankton diet had the lowest minimum and maximum dive depths and maximum wiggle depths (Table 3.7; SNK < 0.05, Figure 3.12). Birds feeding on fish had dives with the longest ascent and descent times (SNK < 0.05, Figure 3.12, Table 3.7), whereas birds feeding on a mixed cephalopod/ zooplankton diet had the shortest ascent and descent times (SNK < 0.05, Figure 3.12, Table 3.7).

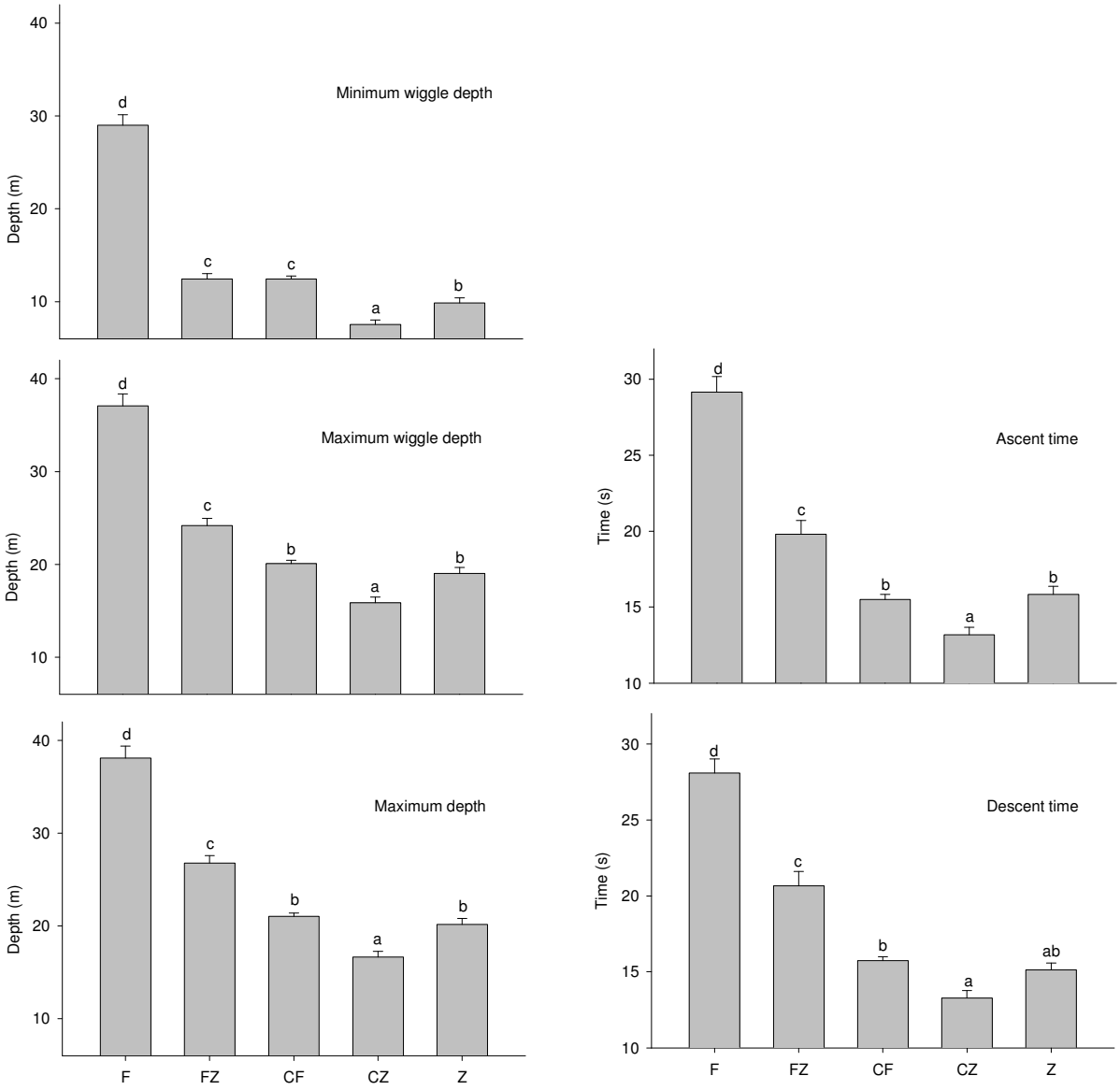

Figure 3.12 - Comparison of the diving parameters, which had the highest eigenvectors on Principal Component 1, of birds with different diets (F = fish, FZ = fish/ zooplankton, CF = cephalopod/ fish, CZ = cephalopods/ zooplankton, Z = zooplankton). Letters above bars represent SNK homogenous groups ($p < 0.05$).

Birds feeding on a mixed fish/ zooplankton diet performed dives with more wiggles and over a longer period of time in each dive, and a greater amplitude than birds feeding on other prey categories (Table 3.7; SNK < 0.05, Figure 3.13). Birds

feeding on a mixture of fish/ zooplankton, and fish only, had longer dive durations (Table 3.7; SNK < 0.05, Figure 3.13).

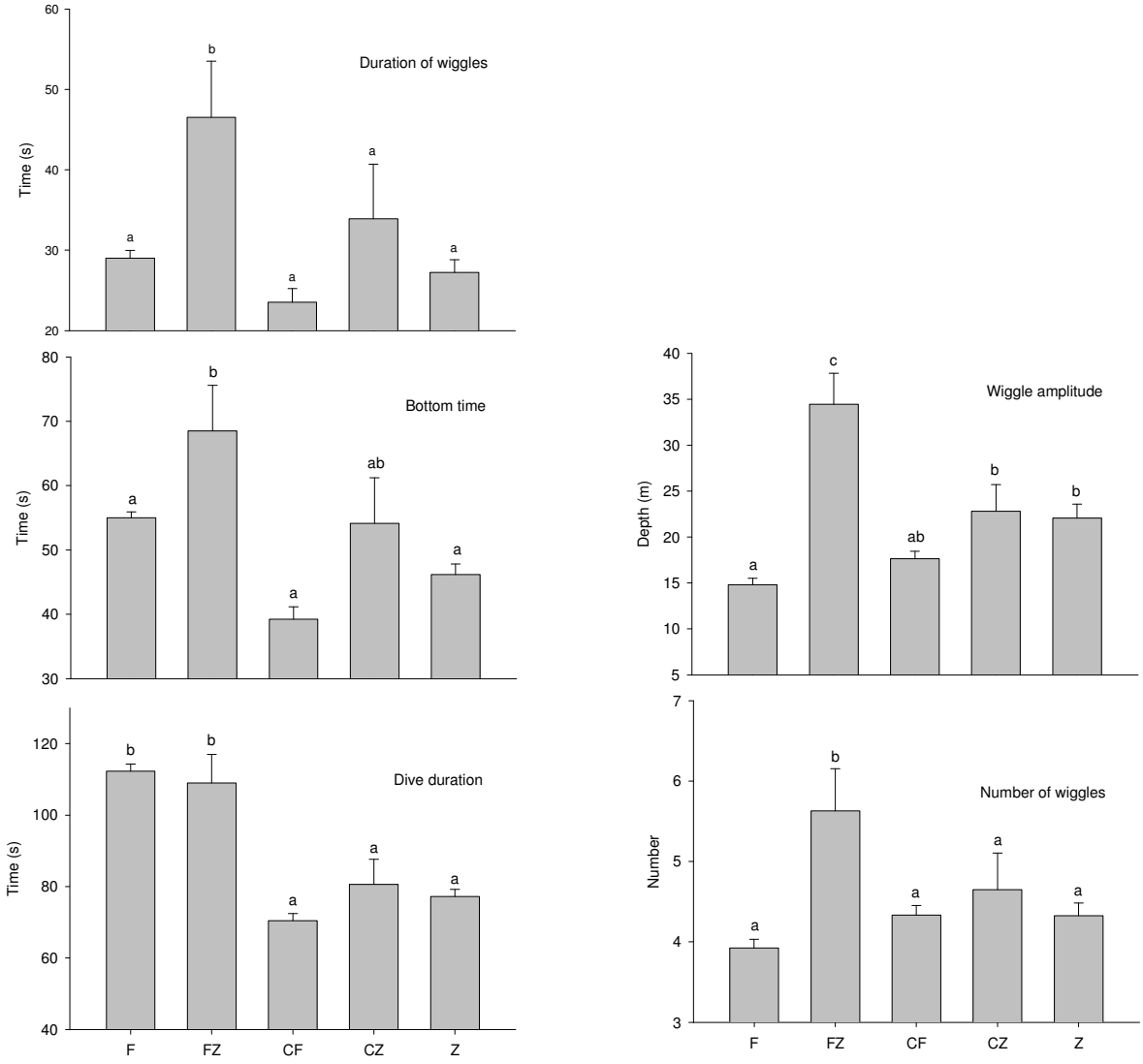

Figure 3.13 – Comparison of the diving parameters, which had the highest eigenvectors on Principal Component 2, of birds with different diets (F = fish, FZ = fish/ zooplankton, CF = cephalopod/ fish, CZ = cephalopods/ zooplankton, Z = zooplankton). Letters above bars represent SNK homogenous groups ($p < 0.05$).

No significant effects of diet were observed on dive rate, trip duration, departure and arrival times of foraging trips, vertical travel distance, dive time, or distance travelled to foraging grounds ($p > 0.05$ in all cases, Table 3.5).

In summary, birds which fed on fish made the deepest dives with the deepest feeding activity (wiggles), which were associated with longer descent and ascent times. Bird which fed on a mixture of fish and zooplankton made the longest dives with more wiggles performed per dive. Birds which fed on a mixture of cephalopods and zooplankton made the shallowest dives with the shallowest feeding activity, which were associated with shorter descent and ascent times.

3.3.5 Temporal changes in foraging strategies

The majority of guard and crèche stage dives took place within the top 14 m of the water column, during both day and night, and the number of dives decreased with maximum depth (Figure 3.14). The depth range of dives was greater during the day for crèche birds, which did not dive deeper than 20 m at night (Figure 3.14). However guard birds continued to forage at depth during the night, although in much smaller numbers (Figure 3.14). Mean foraging dive depths of guard stage birds were similar throughout the day and night, with mean depth peaking between 20:00 and 00:00 hours (Figure 3.15), which reflects overnight foraging trips of two guard stage birds. Mean dive depths of crèche stage birds show a distinct pattern; mean foraging dive depth increased with daylight, peaking at mid-day, and dive depth decreased towards dusk (Figure 3.15).

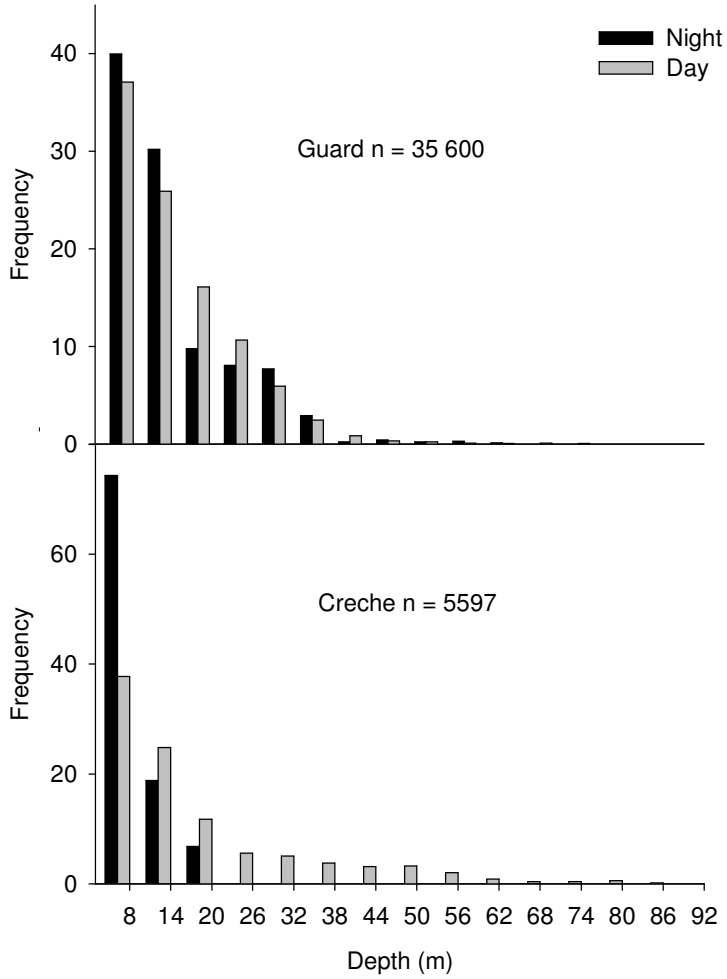

Figure 3.14 – Frequency distribution of all dives greater than 3 m made by guard and crèche stage Northern Rockhopper penguins on foraging trips during the day and night during the breeding season 2010.

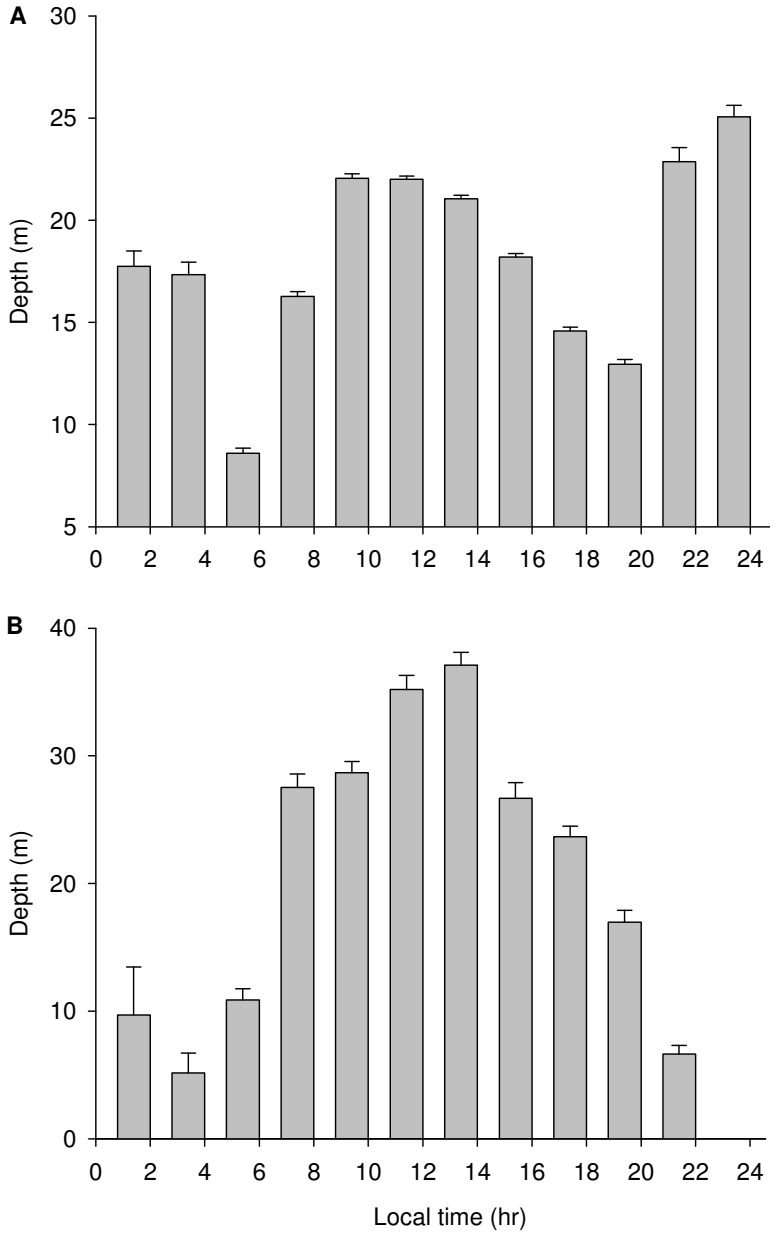

Figure 3.15 - Mean depth (± SE) of feeding dives made by Northern Rockhopper penguins during the (A) guard stage (n = 14 957) and (B) crèche stage (n = 1596) throughout the day and night.

Foraging dives were compared between day and night; it was not possible to incorporate breeding stage or diet into this analysis as not all groups were present within each factor. Diving after dusk was characterised by significantly longer dive durations, descent times and surface pauses between dives compared to dives that took place during daylight ($p < 0.05$, Table 3.9). Dives at night were also deeper with greater ascent speeds ($p < 0.05$, Table 3.9). Day dives were characterised by greater symmetry, raggedness and depth range index; they had a greater duration, number and rate of wiggles and wiggle amplitude ($p < 0.05$, Table 3.9).

Table 3.9 – Comparison of the diving parameters of all foraging dives made during the day and between nautical dusk and dawn (Mann-Whitney U tests). Significant results are highlighted in bold.

	U (n = 832)	**p**
Surface time	7.4147	**< 0.0001**
Dive duration	318608	**< 0.01**
Descent time	306901	**< 0.001**
Bottom time	327135.5	0.0833
Ascent time	329620.5	0.1396
Descent speed	325901	0.0631
Ascent speed	314864.5	**< 0.01**
Maximum depth	318047.5	**< 0.01**
Broadness	341014	0.7571
Depth range	319801.5	**< 0.05**
Symmetry	273212	**< 0.0001**
Mean temperature	259121.5	**< 0.0001**
Number of wiggles	286668.5	**< 0.0001**
Wiggle amplitude	294728	**< 0.0001**
Duration of wiggles	320878.5	**< 0.05**
Wiggle rate	314696.5	**< 0.01**
Minimum wiggle depth	187157.5	0.1994
Maximum wiggle depth	192367.5	0.6285
Raggedness index	272320	**< 0.0001**

3.4 Discussion

Fundamentally, the foraging behaviour of a diving vertebrate is limited by physiology and morphology (Schreer & Kovacs 1997; Costa & Sinervo 2004), and it is within these constraints that foraging plasticity may be observed. Diving capabilities of penguins are strongly related to body mass, with larger penguins being able to make longer deeper dives (Kooyman & Kooyman 1995). Rockhopper penguins are amongst the smallest of penguins (Williams 1995), yet they have been recorded diving to unusually great depths for their size (Northern Rockhoppers 109 m Amsterdam Island, Tremblay *et al.* 1997). They also recover relatively quickly from dive bouts, spending short periods at the surface replenishing oxygen supplies, compared to larger species, which is linked to their large heart: body mass ratio (Drabek 1989; Drabek & Tremblay 2000). Their enhanced physiological capabilities undoubtedly influence their diving behaviour, and likely contribute to their ability to feed opportunistically on a wide range of prey in very diverse habitats (Tremblay & Cherel 2003).

3.4.1 Rockhopper penguin diet

Northern Rockhopper penguins feeding around islands in the Atlantic and Indian Oceans are obligatory oceanic foragers during breeding, a feature which is unique to this species amongst *Eudyptes* penguins, as they breed on volcanic islands which lack a peri-insular shelf (Tremblay *et al.* 1997). Thus, oceanic species are available to birds relatively close to the colony; birds are not restricted to feeding on coastal species and can forage in water more than 1000 m deep within approximately 5 km from the island (Figure 3.16). Birds in this study were found to travel as far as

an estimated 25 km to foraging grounds, so it is highly likely that birds were foraging in deep water.

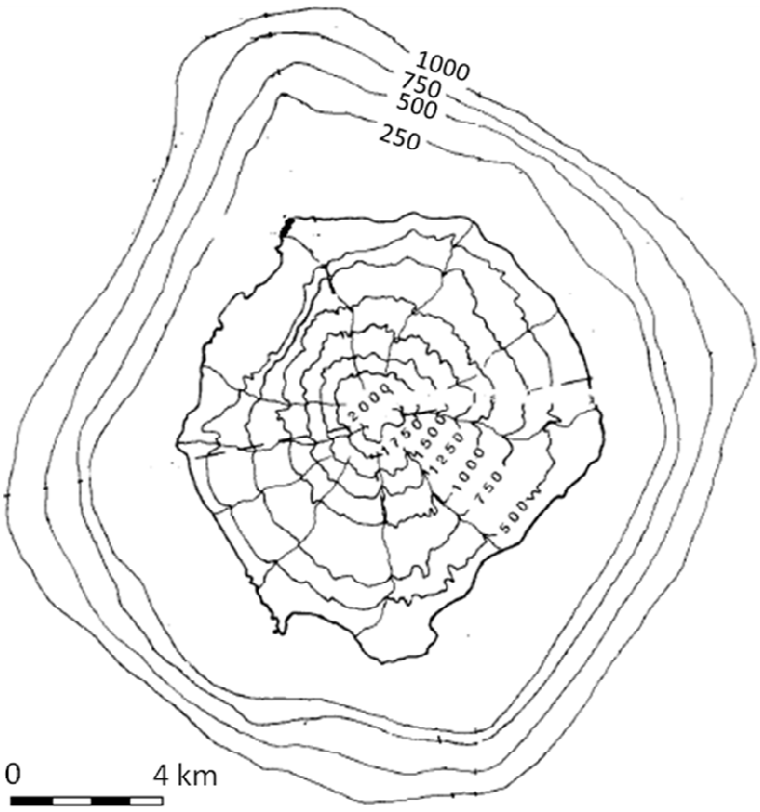

Figure 3.16 - Bathymetric map of Tristan Island displaying the close proximity of deep oceanic waters to the island. Adapted from Chevallier & Verwoerd (1987).

In accordance with the opportunistic feeding nature of Rockhopper penguins recorded at other locations (Table 3.10), birds at Tristan fed on a mixture of fish, cephalopods and macrozooplankton. The contributions of these three broad prey categories vary with geographic location, which has been attributed to differences in local prey abundance and distribution. Northern Rockhopper penguins at Gough Island, the other breeding location for this species in the Atlantic Ocean, were found in three consecutive years to feed predominantly on crustaceans, with fish and cephalopods contributing only a small amount, by mass, to diet in the guard stage

(Table 3.10, Klages *et al.* 1988). Euphausiids dominate Rockhopper diet, by mass, at several locations (Table 3.10; Brown & Klages 1987; Hindell 1988; Klages *et al.* 1988; Hull 1999; Putz *et al.* 2001; Tremblay & Cherel 2003). *Thysanoessa* and *Euphausia* species were found in stomach contents in the present study. *Thysanoessa gregaria,* which has a circumpolar distribution (Mauchline & Fisher 1969), contributes largely to the diet of Northern Rockhoppers at Amsterdam Island (Tremblay *et al*. 1997; Cherel *et al*. 1999) and Gough Islands (Klages *et al*. 1988).

Table 3.10 – Summary of diet data (percentage by wet mass) collected from Southern and Northern Rockhopper penguins at various geographic locations. Replicate data for each prey category indicate data collected in different years.

Species	Location	Diet composition	Study
Northern Rockhopper	Gough Island	cephalopods: 4, 1, 1%; crustaceans: 94, 92, 90%; fish: 2, 6, 9%	Klages *et al.* (1988)
Northern Rockhopper	Amsterdam Island	cephalopods: 50%; crustaceans: 40%; fish: 10%	Duroselle & Tollu (1977)
		cephalopods: 44, 15%; crustaceans: 31, 21%; fish: 25, 64%	Tremblay *et al.* (1997)
		cephalopods: 44%; crustaceans: 44%; fish: 12%	Tremblay & Cherel (2003)
Southern Rockhopper	Falkland Islands	cephalopods: 53%; crustaceans: 45%; fish: 2%	Croxall et al. (1985)
		cephalopods: 50%; crustaceans: 49%; fish: 1%	Cooper et al. (1990)
		cephalopods: 29%; crustaceans: 53%; fish: 18%	Pütz et al. (2001)
Southern Rockhopper	Heard Island	cephalopods: 1%; crustaceans: 60%; fish: 29%	Hull (1999)
Southern Rockhopper	Staten Island	cephalopods: 74, 87, 67%; crustaceans: 26, 14, 33%; fish: mass not estimated	Raya Rey & Schiavini (2005)
Southern Rockhopper	Kerguelen Archipelago	cephalopods: 1%; crustaceans: 95%; fish: 4%	Tremblay & Cherel (2003)
Southern Rockhopper	Marion Island	cephalopods: 1, 3%; crustaceans: 100, 91%; fish: 0, 6%	Brown & Klages (1987)

Fish typically contribute only a small amount to Rockhopper diet at most of its breeding locations (Table 3.10). At Amsterdam Island, however, Cherel *et al*. (1999) found photichthyid fish, and Tremblay *et al*. (1997) found coastal and epi-pelagic fish, to contribute more to the diet of Northern Rockhopper penguins. By far the predominant fish group preyed upon by penguins at Tristan Island were meso-pelagic photichthyids, namely *Phosichthys argenteus* and *Vinciguerria* sp. *Vinciguerria attenuate* was found in Rockhopper penguin diet at Amsterdam Island (Cherel *et al*. 1999). Andrews *et al*. (1995) reported a specimen of *Vinciguerria poweriae* floating dead on the sea surface around Tristan; specimens of *Vinciguerria* found in stomach contents in the present study were unidentifiable to species, but quite possibly belong to this species which has a circum-global subtropical distribution (Schaefer *et al.* 1986). *Maurolicus muelleri*, which is distributed globally in oceanic and shelf waters (Gjøsaeter & Kawaguchi 1980), contributed to the fish diet of study birds, as did several myctophid species. Of interest was the presence of a number of paralepidid species. These are slender, elongated fish that are meso- to bathypelagic, living below 500 m during the day, but migrate to surface waters at night (e.g. *Paralepis* sp. Cornejo & Koppelmann 2006). The cheilodactylid specimens could not be identified further than family level, as the specimens were not found in the otolith atlases used (Smale *et al.* 1995; Campana 2004; Tuset *et al.* 2008), however, *Acantholatris monodactylus* is the dominant species in terms of biomass in coastal waters around Tristan da Cunha (Andrews *et al.* 1995), and this species may have been consumed.

Cephalopods have been shown to contribute approximately 50% by mass to Northern Rockhopper penguin diet at Amsterdam Island (Duroselle & Tollu 1977; Tremblay & Cherel 2003) and Southern Rockhopper penguin diet in the Falkland Islands (Croxall *et al*. 1985). At Tristan Island, cephalopods were found to be an

important component of diet in both breeding stages. The pelagic octopus, *Ocythoe tuberculata*, was the only species of octopod found in stomach contents, and has been reported in the diet of several species of albatrosses and petrels (Imber 1992). Ommastrephid squids have been reported in the diet of many seabirds, including Black-browed albatrosses, (Cherel & Weimerskirch, 1995), Grey-headed albatrosses, (Cherel *et al.* 2002), Southern Rockhopper penguins (Tremblay & Cherel 2003) and Atlantic petrels (Klages & Cooper 1997).

Interestingly the larval Tristan Rock-Lobster, *Jasus tristani*, was found to occur in 30% of crèche birds' stomachs, but only 1 individual occurred in stomach contents in the guard stage. Tristan Rock-Lobsters are highly abundant in coastal waters around Tristan, and are fished commercially. Duroselle and Tollu (1977) found the closely related *Jasus paulensis* to occur in Northern Rockhopper penguin stomach contents from Amsterdam Island. Klages *et al.* (1988) also found no larval Rock-Lobster during their sampling in the early chick-rearing period at Gough Island. Rock-lobsters in the area spawn from September to November (Trevor Glass pers. comm.). The guard birds were sampled in early November and crèche birds in late November/ early December, so it is likely the presence of small individuals in crèche stage stomach contents, and their absence in guard stage diets, is a result of this prey item being available at a certain time of year, and opportunistic consumption by birds.

3.4.2 Northern Rockhopper penguin foraging behaviour

Although penguins were able to dive to depths greater than 90 m, they rarely did so, and dives were concentrated in the upper 20 m of the water column. Diving to shallow depths is consistent with the behaviour of Northern Rockhopper penguins at Amsterdam Island, which, although capable of diving to 109 m, mostly fed around 18

m (Cherel *et al.* 1999). Theoretically, the behavioural strategy of a diver should be based on optimal use of oxygen reserves (Kramer 1988), but obviously the distribution of prey within the water column will strongly affect these strategies (Wilson *et al*. 2002; Ropert-Coudert *et al*. 2006; Elliot *et al*. 2008a). Using allometric equations (Wilson 1995; Schreer & Kovacs 1997), Cherel *et al.* (1999) predicted Northern Rockhopper penguins, with a body mass of 2.3 kg, would have a maximum dive duration of 124 to 176 s and a maximum dive depth of 77 to 89 m. It is reasonable to assume that a diving air-breathing vertebrate would predominantly forage at depths within their aerobic dive limit, and not at its physiological limit, which is not only energetically expensive, but also increases recovery time at the surface between dives (Chappell *et al.* 1993; Wilson *et al.* 2003). Indeed, in the present study, birds were found to dive well within this predicted dive limit, with short surface intervals between dives.

Northern Rockhopper penguins in the study population generally conformed to the diurnal foraging pattern of their conspecifics at Amsterdam Island (Cherel *et al*. 1999; Tremblay & Cherel 2003), with the majority of birds making daily foraging trips of 13 to 15 hours in duration, departing at dawn and returning at dusk. Within this general pattern, a large amount of inter-individual variability was observed, with trip duration ranging from 2 to 35 hours. Very short foraging trips have also been recorded in Southern Rockhopper penguins (Tremblay & Cherel 2005). Although birds that made these short trips did display evidence of prey capture, with wiggles recorded, it is not possible to determine if these trips were chick provisioning or self-feeding trips.

Trip duration, total dive time, number and frequency of dives and VTD are often used as indices of foraging effort in studies of diving animals (Williams &

Rothery 1990; Horning & Trillmich 1997; Ainley *et al.* 1998; Tremblay & Cherel 2003; Schiavini & Raya Rey 2004). The studied birds exhibited a very high foraging effort in both stages of breeding (more than 70% of time at sea was spent diving). A similarly high foraging effort was shown by Northern Rockhopper penguins at Amsterdam Island (69% of time at sea, Cherel *et al.* 1999). This may be related to low quality or patchily distributed prey, which requires a large diving effort to encounter enough profitable prey patches. Whilst some birds started to forage almost immediately after entering the water, the average distance travelled to the start of foraging was approximately 8 km (this distance incorporates both vertical and horizontal travel), and it is likely birds spend time travelling and searching for prey patches, and therefore travel would not necessarily have been linear, but more likely tortuous

3.4.3 Sexual dietary segregation

Although no sexual differences in dietary composition were observed in the Rockhopper penguin population in this study, males tended to prey on larger size classes of squid and fish. Unfortunately, five male birds equipped with TDRs did not return to the breeding colony during the fieldwork period, and the two recovered devices attached to males had technical failures, so it was not possible to determine if there were any inter-sexual differences in foraging strategies. However, it is unlikely that any sexual segregation in foraging habitat would have been observed, in terms of depths attained when diving as found by Tremblay *et al*. (1997) at Amsterdam Island.

Male Northern Rockhopper penguins at Tristan have larger beaks than females (see Chapter 2), it is therefore not surprising that they were able to feed on larger prey, and this is observed in other seabirds (Agnew & Kerry 1995; Kato *et al.* 2000;

Forero *et al.* 2002). Competition theory suggests that males may select larger prey to reduce intra-specific competition, and therefore induce a fitness benefit in accordance with the niche divergence hypothesis of Schoener (1970). I suggest that rather than niche partitioning, males selected larger prey items opportunistically, based on their ability to handle larger prey, as they were observed to feed predominantly on the same size classes of prey as females, in addition to selecting some larger prey within prey groups. The high mobility of penguin prey is an important consideration when discussing intra-specific competition. For example, Wilson (2010) found pygoscelid penguins apparently avoid inter-specific competition in areas where they exist sympatrically, demonstrating niche segregation by foraging in different water masses. However, as they feed on the same, highly mobile, prey competition may still arise.

3.4.4 Energy requirements of chick rearing

Daily energy requirements of growing Rockhopper penguin chicks increases from approximately 211 kJ d^{-1} during the first week after hatching to approximately 1170 kJ d^{-1} half-way through the growth period (Brown 1987), decreasing afterwards. This is a rapid increase and must be met by parents in some manner, either through an increase in meal-size, frequency of provisioning, or selection of higher energy-content prey.

Many penguin species exhibit an increase in foraging effort over the chick-rearing period, and this is frequently manifested in increased foraging trip duration as in Adelie (Lyver *et al*. 2011), Macaroni (Barlow & Croxall 2002; Deagle *et al.* 2008) and Southern Rockhopper (Schiavini & Raya Ray 2004) penguins. In the present study, no increase in trip duration was observed either between guard females rearing small or big chicks in the guard stage, or between the guard and crèche stage.

Similarly, Lescroël and Bost (2005) found no increase in trip duration with chick age in Gentoo penguins. However, a greater foraging effort, in terms of vertical distance travelled (total dive depths), was exhibited by guard stage females in comparison with crèche stage females. Adelie penguins have also been shown to have a greater dive rate and vertical travel distance per hour in the guard stage, in comparison with the crèche stage (Lyver *et al*. 2011). The ways in which penguin parents cope with the increased energetic demands of chick rearing are clearly highly variable, and are likely tightly coupled with local prey abundance. It is probable that an increase in diving effort is linked to increasing encounters with prey patches to maximise meal sizes (Zimmer *et al*. 2011).

Meal mass was not assessed in this study, as birds were only stomach flushed twice, so the possibility of crèche birds provisioning chicks with larger meals cannot be ruled out. In the guard stage the stomach content mass obtained from birds ranged from 16 to 255 g, and in the crèche stage 13 to 325 g. Adult Little and Southern Rockhopper penguins are known to increase meal mass with increasing chick age in the guard stage (Hindell *et al.* 1988; Tremblay & Cherel 2003; Chiaradia & Nisbet 2006). However, Northern Rockhopper penguins displayed no evidence of increasing meal sizes with chick growth at Amsterdam Island (Tremblay & Cherel 2003). Bearing in mind that foraging effort is doubled in the crèche stage, as both parents are foraging once the chick is thermally emancipated, it is quite possible that this increasing provisioning demand is fulfilled without an increase in individual adult foraging effort.

The shift from a zooplankton dominated diet in the guard stage to a euphausiid/ fish dominated diet in the crèche stage was distinct. Although euphausiids were not well represented by mass, they were an important dietary item by number.

Crèche birds fed predominantly on small photichthyid fish and euphausiids, whereas guard birds fed predominantly on zooplankton, comprising fish larvae and other crustaceans. Cephalopods were equally important as prey items in both breeding stages. A switch from provisioning chicks with prey of low-calorific content during early chick-rearing to prey with a higher calorie content (fish) in late chick-rearing has been observed in Adelie penguins (Lyver *et al*. 2011). Myctophid fish are enriched in nutrients compared to euphausiids (Van de Putte *et al*. 2006), and enriched in energy by 17-72% per unit mass compared to gravid female krill (Ichii *et al*. 1996). Myctophids only contributed to a small proportion of fish diet, but are closely related to photichthyids, which may have a similar nutritional quality. Cephalopods have a lower nutritional value than crustaceans and fish (Heath & Randall 1985), and this may be the underlying reason for no evidence of birds feeding solely on cephalopods, despite their presumably high abundance throughout the breeding season (based on frequency of occurrence).

Whilst it is logical that birds target more energy-rich prey later in the breeding season to meet higher energetic demands of larger chicks, energy requirements are at their greatest towards the end of the guard stage (Brown 1987). Thus, if birds switched diets to select high energy content prey for chicks, we would have expected to see this switch within the guard stage, between small and big chicks, and there was no evidence of any change in diet in this period. This suggests that the energetic requirements of chick-rearing are not primarily responsible for the dietary shift observed between breeding phases in Northern Rockhopper penguins in this study. More likely, a change in local prey abundance is primarily responsible for the shift in diet.

3.4.5 Foraging strategies and prey capture behaviour

The association of dive shape with the behaviour of a marine predator is complex and in penguins V-shaped dives have been thought to represent travel (Chappell *et al*. 1993; Kirkwood & Robertson 1997), exploration (Kirkwood & Robertson 1997; Ropert-Coudert *et al*. 2002; Lescroël & Bost 2005) and, most likely, pelagic foraging (Tremblay & Cherel 2000; Schreer *et al*. 2001). There were no wiggles associated with V-shaped dives, suggesting that in the study population V-shaped dives are related to travel and exploration. U-shaped dives have a less contentious purpose, and are widely thought to be foraging dives in penguins (Kirkwood & Robertson 1997; Putz & Cherel 2000; Tremblay & Cherel 2000). This interpretation is supported by this study, as nearly all feeding dives were U – shaped, or, to a lesser extent, W- shaped. U-shaped dives were deeper and longer than W-shaped dives. Although U-shaped dives were dominant in birds targeting all prey groups, birds feeding on zooplankton did make proportionally more W- dives (~20% of dives) than birds feeding on other prey.

Whilst dive depth and depth of feeding events are related to the distribution of prey within the water column, prey behaviour may influence the duration and efficiency of dives (Lescröel & Bost 2005). For example, dives that are made to feed on highly mobile and patchily distributed prey may have longer bottom phases (i.e. U-shaped), so a predator can maximise energy obtained from the prey patch before it is lost. Although these dives are less efficient, and result in longer surface times between dives, energy gain may be maximised overall by foraging in this manner. In contrast, prey species that are located in denser aggregations are likely to be easily re-located, and thus more efficient short dives may be a better diving strategy.

Both of these strategies were observed in the foraging behaviour of the study population, and were found to be closely coupled to prey type, with birds targeting fish (> 90% mass in diet) displaying longer, deeper dives, and those targeting macrozooplankton (> 90% mass in diet) displaying shorter, shallower dives. Birds feeding on mixed diets showed diving strategies intermediate between these. Macaroni penguins are known to make deeper dives to feed on fish compared to those made when feeding on crustaceans (Deagle *et al.* 2008). Similarly, Lescroël & Bost (2005) found that Gentoo penguins feeding on fish spent more time at the bottom phase of a dive, whereas those feeding on crustaceans had a high frequency of pelagic dives. Macrozooplankton, such as euphausiids, form dense swarming aggregations close to the surface (Mauchline 1980), therefore frequent shallow dives are the most efficient diving strategy for birds targeting this prey type. Theoretically, a diver should spend more time in a patch of good quality (Mori 1998). Fish are more energetically valuable than macrozooplankton (Ainley *et al.* 2003), however foraging strategies associated with capture of this prey item are more energetically costly, and these deeper dives are less efficient (Pichegru *et al.* 2011). The number of wiggles during a dive has been shown to correlate with beak-opening events (Simeone & Wilson 2003; Hanuise *et al*. 2010), as well as number of ingestions in King penguins (Bost *et al*. 2007) and can be used as a proxy for prey capture success (Zimmer *et al*. 2010). Birds that captured fish performed wiggles between 30 and 40 m, whereas birds that captured a mixture of cephalopods and zooplankton performed wiggles at comparatively shallow depths between 5 and 20 m. This is indicative of the depths at which these prey are found within the water column.

3.4.6 Crepuscular and nocturnal foraging

Penguins are visual predators (Wilson 1995); foraging at night is typically reduced, and concentrated at shallow depths, as low light levels reduce successful prey capture (Wilson *et al*. 1993). However, as certain prey groups such as euphausiids, myctophids and photichthyids, which contribute significantly to penguin diet, exhibit diel vertical migration, from deep, dark waters during the day to shallow waters at night (Mauchline 1980; Perissinotto and McQuaid 1992; Hays 2003), co-evolution of behavioural strategies to exploit these migrating prey has occurred, and this may directly influence the foraging behaviour of predators such as penguins (Croxall *et al*. 1988; Scheffer *et al.* 2010; Pichegru *et al*. 2011).

Several birds during the guard stage did make feeding dives during the twilight period, which is likely linked to the diel vertical migration of zooplankton found in their diet. During the crèche stage, foraging data from only six birds were retrieved, and in these trips nocturnal feeding dives did not feature significantly. Yet, the two birds in the crèche stage from which dive data were obtained fed predominantly on photichthyid fish, and did not make any foraging dives between dusk and dawn. The only explanation for this is that this prey remains within depths available to Rockhopper penguins for at least some time after dawn. Cornejo & Koppelmann (2006) found that part of the population of *Vinciguerria lucetia*, in the Humboldt Current Region off Peru, remained in surface waters during the daytime, and this behaviour has also been observed in *V. nimbaria* and *Maurolicus muelleri* (Armstrong & Prosch 1991; Marchal & Lebourges 1996). This would explain how apparent diurnal foragers were feeding on fish which display DVM and are typically found at depths out of reach of these birds during the day.

Although diving activity in most birds was reduced at night, comparison of dives made during twilight and night, rather than the day, revealed unexpected foraging strategies. Birds made longer, on average 2 seconds, and deeper, on average 1 m, foraging dives at night, when diving is expected to be limited to shallow dives, compared to the day, although feeding activity was still greater during daylight hours. This result may have been biased by the fact that the majority of night dives that formed the data set for the analysis were from two birds that foraged overnight, and these birds may have displayed unusual or idiosyncratic behaviour. Stomach contents were obtained from these two birds, one of which fed on zooplankton (90% by mass) and the other on a mixture of cephalopods and zooplankton. As these prey categories were not found, in the present study or other studies, to be associated with deep dives, diet cannot be explained by deep dives (Croxall *et al*. 1988; Miller & Trivelpiece 2008). Although, it is possible that these birds fed on very small fish whose otoliths were digested before the birds returned to the colony. These deep dives were unlikely to be prey-searching dives as visibility at great depths would be reduced during the night, and these birds foraged at a new moon, so moonlight would not have increased visibility (Kirkwood & Robertson 1997).

Closer examination of these specific birds' trips did reveal that the majority of these deeper dives were made closer to dawn and dusk rather than the middle of the night, yet one bird consistently made dives around the 30m depth throughout the night. A link with diel vertical migration of prey would explain overnight foraging (as has been observed in Macaroni penguins, Pichegru *et al.* 2011), but not deep dives, as at this time prey is closer to the surface. Unexpected deeper diving at night was also observed in Chinstrap penguins (Miller & Trivelpiece 2008), which was attributed to the possibility of the birds being able to detect the bioluminescence of Myctophids

(also thought to regulate King penguin foraging, Martin 1999), or stronger night vision. As these two birds were not feeding on fish, the latter seems more likely.

3.4.7 Conclusions

Externally attached loggers are known to affect the behaviour of penguins (Wilson *et al*. 1986, 1989; Bannasch *et al.* 1994; Ropert-Coudert *et al*. 2000, 2007), and inappropriate handling may result in stress which can lead to atypical behaviour (Wilson & Culik 1992), or nest desertion (Wilson *et al.* 1989). All birds were handled carefully and stress to the bird was minimised as much as possible; no nest desertion was observed in this study. Devices attached were only 0.05% of the mass of birds, so were unlikely to affect the diving behaviour of birds greatly. However, it is recognised that device attachment may have had some effects on behaviour, and it was assumed that all comparisons were meaningful, as any potential deleterious effect of the device should have affected all birds in an equal and equivalent manner.

Using stomach contents to link diving behaviour with diet is rather tenuous without the use of animal attached video devices, as used by Takahashi *et al.* (2008) on Gentoo penguins to verify krill-feeding behaviour. Nevertheless, this is a functional method of establishing this link. A key problem is the differential rate of digestion of prey items; macrozooplankton may have been underestimated in their contribution to diet. On the other hand it is unlikely that fish were over-estimated, as penguins are able to digest whole fish to otoliths and bones within 10-16 hours (Gales 1987), approximately the duration of foraging trips of study birds at Tristan. Although birds were flushed only twice, it is unlikely that birds may have fed on prey that were not revealed in stomach content analysis. Additionally, without flushing the same individual repeatedly over time, which would be extremely detrimental to the bird, it

is not possible to determine if the prey capture strategies observed represent specialisations of certain birds, or are representative of opportunistic feeding and would vary temporally.

Northern Rockhopper penguins at Tristan da Cunha display very similar foraging behaviour, and the same level of plasticity, as both their conspecifics in the Indian Ocean, and Southern Rockhopper penguins at various geographic locations. Their opportunistic feeding behaviour and ability to modify diving behaviour dramatically in respect to prey type allow them to take advantage of presumably locally abundant prey. Interestingly, evidence of nocturnal foraging to relatively deep depths (30 – 40 m) was also revealed. Males were found to select larger size classes of prey; however they fed on the same species as females, suggesting males opportunistically exploit larger prey items.

A recurrent conclusion of foraging behaviour studies, and also presented here, is the necessity for independent sampling of prey availability. Without this, it is not possible to determine if birds actively select prey types, either to provision themselves or chicks with higher-quality prey, or if they feed on what is locally abundant and available to them. The integration of foraging location data would greatly assist interpretation of results in this respect. Northern Rockhopper penguins at the Stony Beach colony on Tristan Island exhibit a very high foraging effort. This may be an indication of unpredictable distribution of prey patches, low-quality prey or unprofitable patches, necessitating a high foraging effort. As time spent at sea searching for prey is in itself energetically costly, it is possible that this population of Rockhoppers would not be able to increase foraging effort any further. This study provides the first comprehensive description of the diving behaviour of Northern Rockhopper penguins foraging in the South Atlantic. The results obtained support the

foraging plasticity observed in other Rockhopper penguin populations in the Indian Ocean, with birds modifying their behaviour over the course of the breeding season. Northern Rockhopper populations have been in dramatic decline for decades; combining colony-level information on foraging effort with breeding success and chick growth would allow the identification of populations that are particularly vulnerable, and may help identify general mechanisms behind global population declines.

Chapter 4

A stable isotopic investigation of dietary variation in Northern Rockhopper penguins, *Eudyptes moseleyi,* at Tristan da Cunha, South Atlantic Ocean

4.1 Introduction

Stable isotope analysis (SIA) is an increasingly useful tool in ecological studies (Hobson 1999; Kelly 2000). In particular, analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes can augment dietary data obtained from conventional techniques, as ratios of stable isotopes in the tissues of a consumer reflect food which has been assimilated rather than just ingested (Hobson *et al.* 1997). Stable isotopes are employed widely in the study of food webs; trophic level, contribution of different prey to diet, and foraging habitat can all be revealed through SIA (Quillfeldt *et al.* 2005; Cherel *et al.* 2006).

Organisms have different carbon and nitrogen compositions compared to the organisms on which they feed, due to a difference in the retention of the heavy isotope and preferential excretion (N) or respiration (C) of the light isotope, referred to as isotopic discrimination (deNiro & Epstein 1981; Minagawa and Wada 1984; Michener & Schell 1994). Organisms are typically enriched, by a reasonably predictable discrimination factor, in the heavier isotope compared to their diet (Tieszen *et al.* 1983; Bearhop *et al.* 2002). For nitrogen, this stepwise enrichment of consumer tissues relative to diet is approximately 3.0 to 4.0‰ (DeNiro & Epstein 1981; Minagawa & Wada 1984; Peterson & Fry 1987), and so nitrogen can provide an indication of the trophic level at which animals are feeding (Hobson & Welch

2002; Cherel & Hobson 2005). For carbon, stepwise enrichment is not as large; consumer tissues are enriched by approximately ~ 1‰ relative to diet (DeNiro & Epstein 1978; Hobson & Clark 1992; Bearhop *et al.* 2002), but enrichment of tissues can be used to determine the carbon sources in a food web (Cherel & Hobson 2005). Carbon ratios can reveal inshore versus offshore foraging, and benthic versus pelagic feeding (Hobson *et al.* 1994; Cherel *et al.* 2000), as $\delta^{13}C$ of pelagic phytoplankton is more depleted than that of benthic and inshore phytoplankton (Kelly 2000; Cherel & Hobson 2007).

Isotope signatures reflect diet over the period of tissue synthesis (Hobson & Clark 1992; Bearhop *et al*. 2002). Therefore, unlike conventional diet sampling, i.e. stomach contents, dietary information over different time scales can be revealed through SIA by examining different tissues in an organism. Tissues have different metabolic, and consequently isotopic, turnover rates (Fry & Arnold 1982, Tieszen *et al*. 1983, Hobson & Clark 1992, Vanderklift & Ponsard 2003). Those with a high isotopic turnover rate provide information on recent diet, e.g. blood plasma, whereas those with a longer turnover rate, e.g. bone, provide dietary information that is integrated over a longer time (Hobson & Clark 1992; Hobson & Clark 1993). This allows specific tissues from the same individual to be targeted, depending on the aims of the study (Tieszen *et al.* 1983).

Feathers and blood are the preferred avian tissues for SIA, as they can be sampled non-destructively and the same individual can be re-sampled over time (Bearhop *et al*. 2002). Whilst blood integrates dietary information over 3 to 4 weeks, feathers integrate diet over the period of feather growth, and are metabolically inert, and therefore fixed in their isotopic composition (Hobson & Clark 1992; Bearhop *et al.* 2002). New feathers are synthesised during the moulting period, so isotopic

composition of feathers reflects diet during this time (Mizutani *et al.* 1990, 1992; Hobson & Clark 1992). As penguins fast ashore during the period of moult and feather growth, interpretation of diet from SIA of penguin feathers has been a contentious area (Hobson & Clark 1992). It is now widely accepted that amino acids for keratin synthesis are derived from protein catabolism from skeletal muscles, and thus indirectly from food obtained whilst feeding intensively prior to the moult (Cherel *et al.* 1994).

An important component of studies of foraging ecology is intra-specific variation; this may be observed in particular between sexes. Many animals display biparental care; however this is perhaps most widely displayed in birds, with around 90% of species exhibiting this trait (Lack 1968). Biparental species typically show a conflict over division of parental effort between the sexes (Davies 1989; Slagsvold & Lifjeld 1994), with one parent compensating for any variation in the provisioning effort of the other to ensure a stable cooperation (Chase 1980). Sex-specific foraging and diet have been recorded in many sexually size-dimorphic birds (reviewed in Thaxter *et al*. 2009), and more recently in size-monomorphic birds (Wiggins & Morris 1987; Bradley *et al.* 2002; Fraser *et al.* 2002; Hamer *et al.* 2005). Life-stage is another source of intra-specific variation in diet; differences have been observed in the type of food that adults select for chick provisioning and the food that they consume themselves (Hobson 1993; Hodum & Hobson 2000; Cherel 2008). Cherel *et al.* (2007) found that the $\delta^{13}C$ and $\delta^{15}N$ values of King, *Aptenodytes patagonicus*, Southern Rockhopper, *Eudyptes chrysocome*, and Macaroni, *Eudyptes chrysolophus*, penguin chick and adult blood were very similar, which suggests that adults foraged on similar prey in similar areas for self-maintenance and chick-provisioning. Similarly, Tierney *et al.* (2008) found that adult Adélie penguins, *Pygoscelis adeliae*,

fed chicks on similar food to that which they consumed themselves, although $\delta^{13}C$ values indicted that food for chicks was obtained closer inshore than food used for self-maintenance. This is contrary to studies of Black-legged kittiwakes, *Rissa tridactyla*, and Thick-billed murres, *Uria lomvia* (Hobson 1993), Southern giant petrels, *Macronectes giganteus* (Forero *et al.* 2005), Magellanic penguins, *Spheniscus magellanicus* (Forero *et al.* 2002), and Grey-headed albatrosses, *Thalassarche chrysostoma* (Richoux *et al.* 2010), where SIA revealed dietary differences between adults and chicks. In these studies adults frequently fed chicks on prey items with a higher calorific content.

Diet may also vary temporally in terms of foraging areas and trophic position (Dahl *et al.* 2003; Olive *et al.* 2003; Morrison & Hobson 2004). Within the breeding season, opportunistic foragers, such as many penguin species, may exhibit dietary changes (e.g. Rockhopper penguins, Tremblay & Cherel 2003); however, this is likely to be a reflection of changes in local prey abundance and availability (Gales *et al.* 1990; Lescröel & Bost 2005; Clarke *et al.* 2006; Miller & Trivelpiece 2008; Boersma *et al.* 2009). We may expect temporal variation in diet and foraging areas to be more prominent over a longer time scale i.e. between the breeding and non-breeding seasons, when birds are able to increase their foraging range. The winter diet of seabirds is often not well documented as dietary studies tend to focus on the breeding season for practical reasons, as birds are more accessible (Cherel *et al.* 2007). It is possible that birds may switch to a different trophic level when not constrained to a breeding site, or between a specialist and generalist diet (Hobson *et al.* 1994). Cherel *et al.* (2007) used SIA to document seasonal changes in trophic niches of Sub-Antarctic penguins and fur seals at the Crozet archipelago, finding that in winter, when animals are no longer restricted by central place foraging, trophic niches

widened, and larger inter-individual variations were found in carbon isotope signatures.

Direct and indirect techniques for diet determination complement each other (Hobson & Clark 1992), and have been successfully combined in a number of studies (e.g. Hobson *et al.* 1994; Sydeman *et al.* 1997; Ainley *et al.* 2003; Karnovsky *et al.* 2007), providing a great deal of time-integrated and taxonomic dietary information. SIA was used in this study to complement the dietary information obtained for guard and crèche adults in Chapter 3, and to supplement the scarce data which exist on Northern Rockhopper penguins, by obtaining information on diet composition throughout the pre-moult and breeding seasons. Sex, age and breeding stage were examined as potential sources of dietary variation.

4.2 Methods

4.2.1 Blood sampling

Blood sampling has no detrimental long or short term effects on birds (Hoysak & Weatherhead 1991). According to the American Ornithologist's Union (1988), the volume of blood collected from an individual should not exceed more than 10 - 20% of total blood volume, which is typically 6-8 ml per 100 g body mass (Sturkie 1986). As Northern Rockhopper penguins weigh over 2 kg, withdrawal of a maximum of 2 ml should have no detrimental effects on body weight or mortality rate (Stangel 1986). The smallest chick sampled weighed 50 g, and only 0.5 ml blood was withdrawn from chicks.

Blood samples were taken from adult birds during the three breeding stages: incubation, 27th and 29th September 2010 (39 females), guard, between 1st and 11th November 2010 (27 females, 27 males) and crèche between 22nd November and 3rd

December 2010 (18 females, 19 males). In addition, eight incubating males at Nightingale Island were opportunistically sampled for blood on 21st October 2010. Fasting behaviour of birds during the incubation stage, and of males during the guard stage, may result in $\delta^{15}N$ enrichment of blood of these birds (Hobson *et al.* 1993; Cherel *et al.* 2005a). As this enrichment is most pronounced in plasma, whole blood was analysed. Whole blood has a C/N ratio very close to that of red blood cells, which contain more organic matter than plasma, thus the effect of fasting on isotope signatures can be minimised (Cherel *et al*. 2005a).

0.5 ml of blood was collected from the tarsal vein of each bird with a 25-gauge needle and 3 ml syringe containing no additives, as heparin affects the isotope signature of blood (Cherel *et al.* 2007). Whole blood was transferred to eppendorf tubes and frozen at -30 ºC in the laboratory, within 6 hours of collection, until analysed (Tierney *et al.* 2008). In addition to sampling adults, blood was also taken from the chicks of guard study adults (n = 27) at the end of the guard stage, using a 26-gauge needle, and prepared for isotope analysis as for adult blood. As it was not possible to capture adult birds at the nest during the crèche stage and sample both parents and their chicks, 32 randomly selected crèche chicks, weighing between 1.1 and 1.7 kg, were sampled for blood. It is important to control for digestive state as far as possible; when animals are still in an absorptive state, dietary lipid in plasma may influence whole blood isotope signatures (Bearhop *et al.* 2002). The majority of female birds returned around dusk (Chapter 3) and were sampled for blood on return to the colony as it was not known if these birds could be recaptured. However all chicks were blood sampled between the hours of 09:00 and 12:00, and it was assumed they would have been fed the previous evening as females had always returned to sea by this time.

4.2.2 Feather sampling

Feather samples were taken from the adults that were sampled for blood during the incubation (39 adults) and guard stages (27 males, 27 females). In addition, feather samples were taken from 64 incubating birds on Nightingale Island to determine if birds from the two islands have different diets outside the breeding season. Five feathers were plucked from the back of each bird's body, and placed in a sealed plastic bag until analysed (Bearhop *et al.* 2006; Tierney *et al.* 2008). Only black feathers were used so that results could be compared with other studies which used black feathers from the back of the bird; white and black feathers have been shown to have different carbon isotope signatures due to the influence of melanin (Michalik *et al.* 2010). To compare stable isotope values of blood and feathers, values should be corrected as they have different protein turnover rates, and therefore diet-tissue isotopic discrimination factors (Hobson & Clark 1992). Cherel et al. (2005b) published discrimination factors for SRP fed a fish-diet in captivity, but in the wild, Rockhopper penguins have a mixed diet and the authors proposed these discrimination factors should only be applied to piscivorous birds. In this study, the mean blood isotopic discrimination rates of marine birds (obtained from Caut *et al.* 2009) that Denhard *et al.* (2011) applied to their study of SRP were used: diet - blood $\delta^{13}C = 0.6‰$, $\delta^{15}N = 2‰$; diet – feathers $\delta^{13}C = 2‰$, $\delta^{15}N = 4‰$.

4.2.3 Preparation of samples for SIA

In preparation for SIA, surface contaminants and lipids were removed from feathers with 2:1 chloroform: methanol rinse, at room temperature, in a sonicating water bath, followed by two methanol rinses, and a final rinse in distilled water (Jaeger & Cherel 2011). Feathers were dried at 50 ºC in an oven, and then cut into

small fragments (all feathers from an individual bird were analysed together) with stainless steel scissors (Hilton *et al.* 2006). It is not necessary to remove lipids from whole blood for SIA, due to its low lipid content (Cherel *et al.* 2005b). Blood was dried in an oven at 60 ºC (Cherel *et al.* 2000, 2007), and then ground to a fine powder with a pestle and mortar. Specimens of crayfish, *Jasus tristani*, photichthyid fish and cephalopods, *Ocythoe tuberculata* and an ommastrephid squid, retrieved from stomach contents were treated to remove lipids and carbonates prior to SIA. Carbonates were removed from crayfish, *Jasus tristani*, with 1 mol $HCL.1^{-1}$ (Cherel 2008), and specimens were then rinsed with distilled water. Lipids were removed from all powdered prey items using 2:1 chloroform: methanol soak and rinse and air dried in a fume hood to control for differences in lipid content (Bearhop *et al*. 2002; Hobson & Cherel 2006).

Relative abundances of stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined from 0.6 mg sub-samples of homogeneous samples by combustion in a Thermo 1112 Elemental Analyser via a Thermo Conflo III to a Thermo Delta XP Plus stable light isotope mass spectrometer in the Light Stable Isotope Laboratory in the Archaeology Department, University of Cape Town. Samples were run against in-house reference materials and the results normalised against and reported relative to the international standards (Pee Dee Belemnite for carbon, atmospheric air for nitrogen). $\delta = \{(R_{sample} - R_{standard})/ R_{standard}\} \times 1000$, where R_{sample} is the isotopic ratio of the sample and $R_{standard}$ is the isotopic ratio of the relevant international standard. Replicate measurements of internal laboratory standards indicate measurement errors of approximately 0.9‰ for $\delta^{15}N$ and 0.13‰ for $\delta^{13}C$.

4.2.4 Statistical analysis

Statistica 8.0 was used to carry out all statistical testing. Homogeneity of variances was confirmed using Levene's test ($p > 0.05$), and normality of distribution using Kolmogorov-Smirnov test ($p > 0.05$). Model I factorial analyses of variance (ANOVA) were used to compare, firstly $\delta^{15}N$ and $\delta^{13}C$ values (corrected for tissue discrimination) of blood and feathers between the fixed factors breeding stage (4 levels: moult, incubation, guard and crèche) and sex (2 levels: male and female); and secondly to compare $\delta^{15}N$ and $\delta^{13}C$ values of blood between the fixed factors breeding stage (2 levels: guard and crèche) and age (2 levels: adult and chick). Independent sample t-tests were used to compare $\delta^{15}N$ and $\delta^{13}C$ values of male and female feathers from Tristan (sex of Nightingale birds was unknown and could not be analysed), and $\delta^{15}N$ and $\delta^{13}C$ values of feathers between Tristan and Nightingale. Samples from the same individual were used to compare $\delta^{15}N$ and $\delta^{13}C$ values of firstly, the mantle and tentacles of squid, and secondly the effect of removing the outer skin layer on the tentacles of squid using t-tests. Finally, 1-way ANOVA was used to compare $\delta^{15}N$ and $\delta^{13}C$ values of firstly, three size classes of ommastrephid squid: small, mantle length < 30mm; medium, mantle length 60 – 80mm; large, mantle length > 120mm; and secondly, the prey items crayfish, photichthyid fish and the three size classes of squid Student-Newman-Keuls (SNK) tests were used to compare means when sources of variation were significant. Significance was assumed at $p < 0.05$; all mean values are presented with standard errors.

4.3 Results

Mean carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures of adult and chick Northern Rockhopper penguin blood and feathers are presented in Table 4.1

Table 4.1 - $\delta^{15}N$ and $\delta^{13}C$ signatures (means ± SE and range, ‰) of adult and chick Northern Rockhopper penguin blood and feathers in each stage of breeding and the moulting period, 2010, from Tristan and Nightingale (N. Gale) islands (n = number of birds).

Colony	Age	Stage	Sex	Tissue	*n*	$\delta^{15}N$	Range $\delta^{15}N$	$\delta^{13}C$	Range $\delta^{13}C$	C/N mass ratio
Tristan	Chicks	Guard	Unknown	Blood	27	8.80 ± 0.11	7.68 to 9.87	-19.69 ± 0.04	-20.16 to -19.33	3.6 ± 0.02
		Crèche	Unknown	Blood	32	8.57 ± 0.04	8.06 to 8.97	-19.52 ± 0.04	-20.26 to -19.23	3.5 ± 0.02
	Adults	Incubation	Female	Blood	39	12.49 ± 0.10	11.19 to 13.74	-18.73 ± 0.07	-19.99 to -18.02	3.5± 0.01
		Guard	Female	Blood	27	10.72 ± 0.08	10.08 to 12.03	-19.02 ±0.04	-19.33 to -18.59	3.3 ± 0.01
			Male	Blood	27	11.65 ± 0.15	9.78 to 13.07	-18.83 ± 0.09	-19.76 to -18.02	3.4 ± 0.01
		Crèche	Female	Blood	18	9.60 ± 0.13	8.69 to 11.09	-18.83 ± 0.05	-19.28 to -18.5	3.4 ± 0.01
			Male	Blood	19	9.98 ± 0.13	8.75 to 11.32	-18.67 ± 0.04	-18.95 to -18.22	3.4 ± 0.01
		Moult	Female	Feathers	70	12.17 ± 0.04	11.56 to 12.99	-17.98 ± 0.03	-18.59 to -17.42	3.2 ± 0.01
			Male	Feathers	37	12.11 ± 0.11	10.61 to 13.04	-18.03 ± 0.05	-18.77 to -17.58	3.2 ± 0.01
Nightingale	Adults	Incubation	Male	Blood	8	10.96 ± 0.13	10.37 to 11.43	-18.94 ± 0.07	-19.21 to -18.73	3.4 ± 0.01
		Moult	Unknown	Feathers	64	15.17 ± 0.07	14.26 to 16.55	-16.15 ± 0.05	-16.79 to -15.34	3.1 ± 0.01

4.3.1 Comparison of $\delta^{13}C$ and $\delta^{15}N$ signatures of adult females and chicks

A highly significant effect of both age and stage was observed on $\delta^{13}C$ values of blood (Table 4.2). Crèche chicks were significantly enriched in $\delta^{13}C$ compared to guard chicks (Table 4.2, Figure 4.1). Crèche females were significantly enriched in $\delta^{13}C$ compared to guard females (Table 4.2, Figure 4.1). Crèche adult females were significantly enriched in $\delta^{13}C$, compared to crèche chicks, and guard adult females were similarly significantly enriched in $\delta^{13}C$ compared to their chicks (Table 4.2; Figure 4.1). Thus females were always enriched relative to chicks and crèche stage individuals were enriched relative to guard stage individuals.

A significant interaction of stage and age was observed for $\delta^{15}N$ values of blood (Table 4.2). Guard females were enriched in $\delta^{15}N$ compared to crèche females (SNK $p < 0.01$, Table 4.2). In both the guard and crèche stage, adult females were enriched in nitrogen compared to chicks (SNK $p < 0.01$, Table 4.2). In the guard stage, there was a difference of one trophic level between adults and their chicks (~ 1.9‰, Table 4.2; Figure 4.1). $\delta^{15}N$ signatures of chicks in the crèche and guard stages were not significantly different from each other (SNK $p > 0.05$, Table 4.2). Thus, as with $\delta^{13}C$, females were enriched relative to chicks in both stages, but stage had no effect on chicks and its effect for females was the opposite to $\delta^{13}C$, guard individuals were relatively enriched.

Table 4.2 – 2-way factorial ANOVA to test the effect of the fixed factors breeding stage (guard, crèche) and age (chick, adult) on $\delta^{15}N$ and $\delta^{13}C$ signatures of adult and chick Northern Rockhopper penguin blood from Tristan da Cunha. Whilst both males and females provision chicks in the crèche stage, analysis was performed on females only to remove the confounding effect of adult sex. SNK was used to compare means relative to the hypotheses of interest: Chicks = Ch, Adults = Ad, Guard = G, Crèche = C. Stage (age) = interaction of age within stage; age (stage) = interaction of stage within age.

	Nitrogen				Carbon			
Source	d.f.	M.S.	F	p	d.f.	M.S.	F	p
Stage	1	7.438	30.96	< 0.0001	1	0.8	17.7	< 0.0001
Age	1	38.67	160.97	< 0.0001	1	8.66	193.2	< 0.0001
*Stage*Age*	1	4.25	17.69	< 0.0001	1	0	0	0.8831
Error	68	0.24			68	0.04		

SNK Stage(age)	Ch: C = G, Ad: C < G	Stage: G < C
SNK Age(stage)	G: Ch < Ad, C: Ch < Ad	Age: Ch < Ad

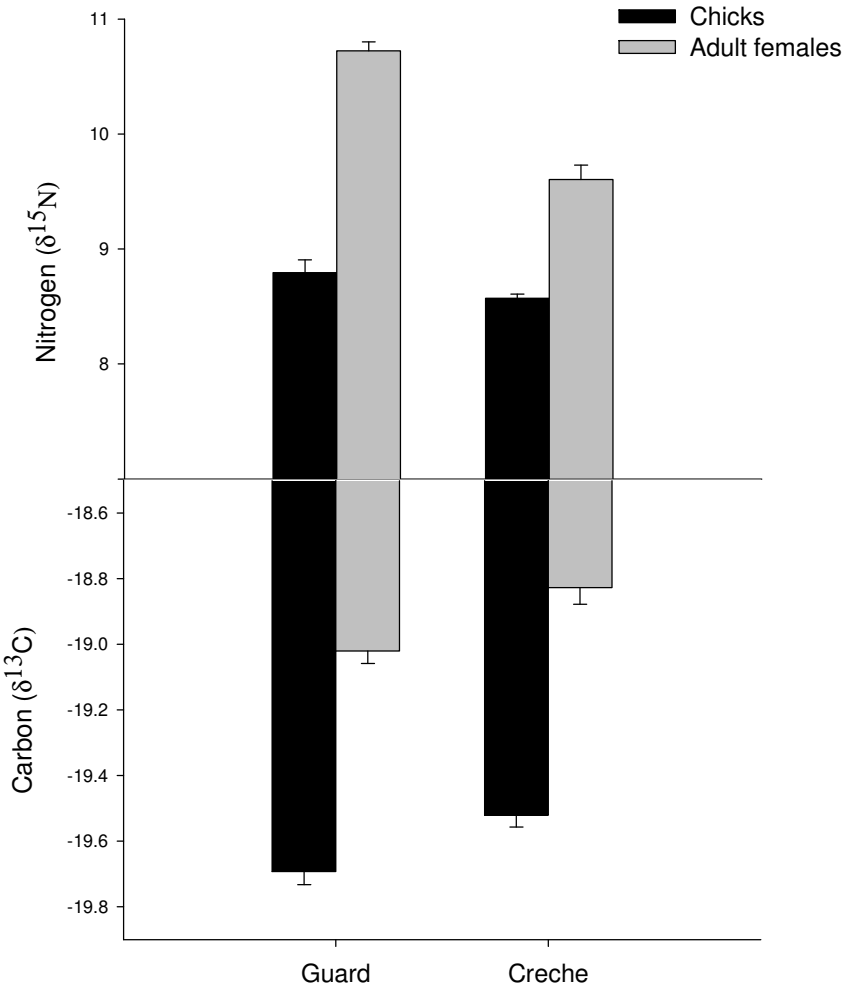

Figure 4.1 - δ^{15}N and δ^{13}C signatures of adult and chick Northern Rockhopper penguin blood from Tristan da Cunha during the guard and crèche stage. Values are presented as means with standard errors. Guard females n = 27, crèche females n = 18, guard chicks n = 27, crèche chicks n = 32.

4.3.2 Temporal variation in $\delta^{13}C$ and $\delta^{15}N$ signatures of adult blood and feathers

A significant interaction of stage and sex was observed in carbon and nitrogen isotope signatures of adult penguins (Table 4.3). Specifically, moulting male birds were more enriched in $\delta^{13}C$ than males in all other stages (SNK $p < 0.01$, Table 4.3). Moulting females were significantly enriched in $\delta^{13}C$ (SNK $p < 0.01$, Table 4.3), and guard females significantly depleted in carbon, compared to all other female birds (SNK $p < 0.05$, Table 4.3). All adult blood (representing the diet during the breeding season) fell within a narrow $\delta^{13}C$ range, -19.02‰ (guard females) to -18.67‰ (crèche males) (Table 4.1, Figure 4.2). Guard males were more enriched and crèche males more depleted in $\delta^{15}N$ than male birds in all other stages (SNK $p < 0.01$, Table 4.3, Figure 4.3), and incubating females were enriched in $\delta^{15}N$ compared to all other female birds (SNK $p < 0.01$, Table 4.3, Figure 4.3).

Males were significantly enriched in $\delta^{15}N$ and $\delta^{13}C$ in the guard stage compared to guard females (SNK $p < 0.01$, Table 4.3, Figure 4.3). During incubation, females were enriched in $\delta^{13}C$ and $\delta^{15}N$ compared to males (SNK $p < 0.01$, Table 4.3, Figure 4.2). No differences in $\delta^{15}N$ and $\delta^{13}C$ of males and females were observed in the crèche and moult stages (SNK $p > 0.05$, Table 4.3).

Table 4.3 – 2-way factorial ANOVA to test the effect of the fixed factors breeding stage (moult, incubation, guard, crèche) and sex (male, female) on $\delta^{15}N$ and $\delta^{13}C$ signatures (corrected for tissue discrimination, *4.2.2*) of adult Northern Rockhopper penguin blood and feathers from Tristan da Cunha. SNK was used when sources of variation were significant, relative to the hypotheses of interest: males = M, females = F, incubation = i, guard = g, crèche = c, moult = m. Stage (sex) = interaction of sex within stage; sex (stage) = interaction of stage within sex.

	Nitrogen				Carbon			
Source	d.f.	M.S.	F	p	d.f.	M.S.	F	p
Stage	3	12.21	35.38	< 0.0001	3	20.5	242.3	< 0.0001
Sex	1	0.001	0.002	0.9637	1	0.1	1.2	0.279
*Stage*Sex*	3	5.166	14.97	< 0.0001	3	0.56	6.7	0.0006
Error	56	0.345			56	0.04		

SNK Stage(sex)	M: c < m = i < g; F: c = m = g < i	M: i = c = g <m; F: g <c = i < m
SNK Sex(stage)	i: M< F; g: F<M; c: M= F; m: M= F	i: M<F; g: F<M; c: M = F; m: M = F

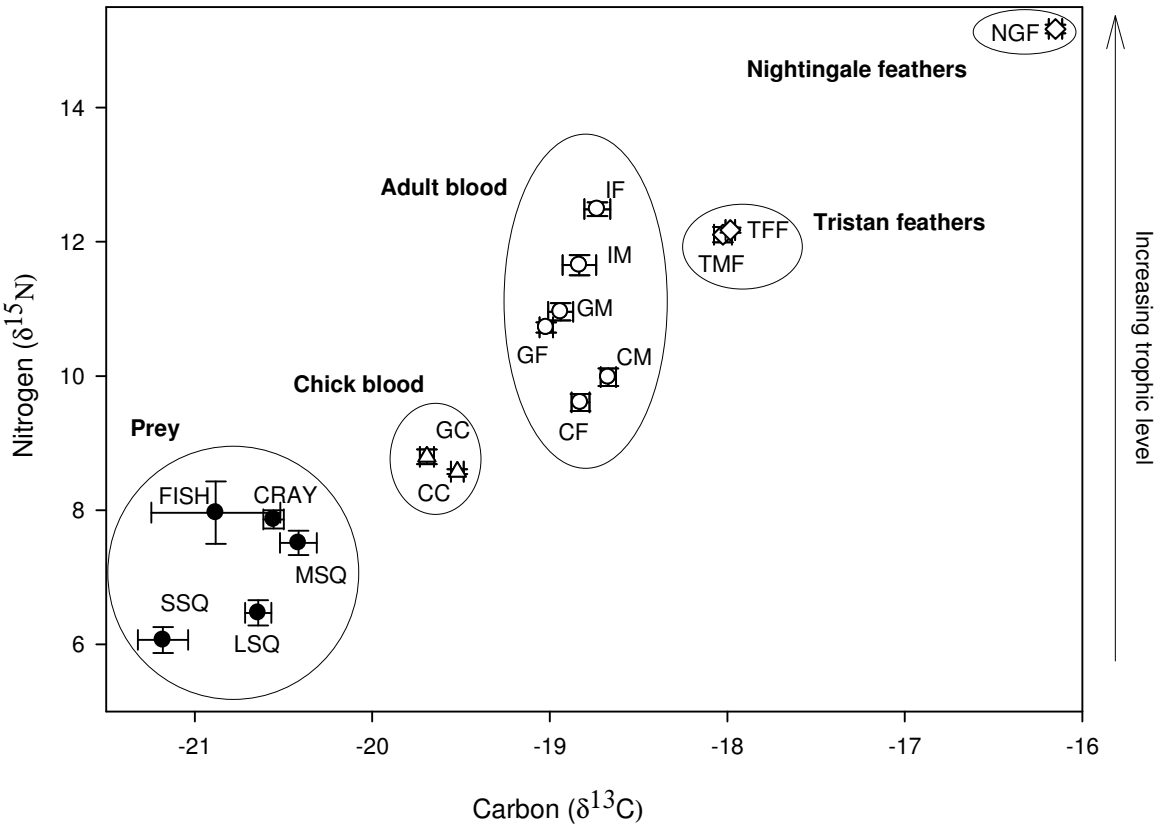

Figure 4.2 – $\delta^{15}N$ and $\delta^{13}C$ values (means ± SE) of Northern Rockhopper penguin adult blood and feathers and chick blood. Abbreviations (with sample sizes in brackets): GC = guard chick blood (28); CC = crèche chick blood (32); NGF = Nightingale Island feathers (64); TMF = Tristan male feathers (37); TFF = Tristan female feathers (70); IF = incubating female blood (39); IM = incubating male blood (8); GF = guard female blood (27); GM = guard male blood (27); CF = crèche female blood (18); CM = crèche male blood (19). All samples from Tristan Island, except NGF and IM. Prey groups from stomach contents also represented: CRAY = crayfish (20); FISH = Photichthyid fish (3); LSQ = large squid (10); MSQ = medium squid (14); SSQ = small squid (7).

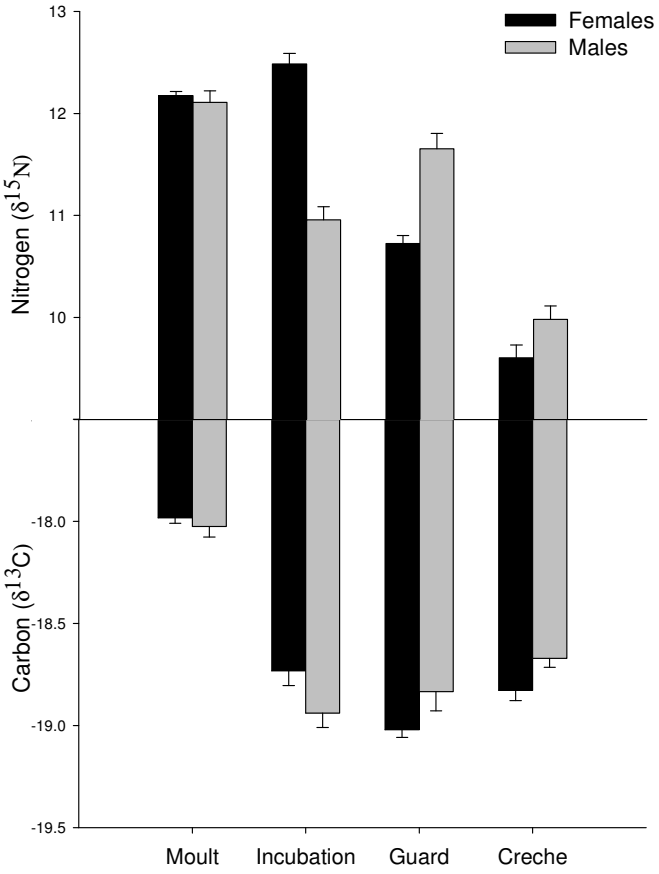

Figure 4.3 - δ^{15}N and δ^{13}C signatures of adult Northern Rockhopper penguin blood (incubation, guard and crèche stages) and feathers (moult) from Tristan da Cunha during the breeding and non-breeding (moult) season. Values are presented as means with standard errors. Incubation: females n= 39, males n = 8 (Nightingale colony); Guard: females and males n = 27; Crèche: females n = 18, males n = 19; moult: females n = 70, males n = 37.

As there was no significant effect of sex on δ^{13}C (t_{104} = -0.1612) or δ^{15}N (t104 = -0.3510; $p > 0.05$ in both cases) of feathers from Tristan Island birds, these data were pooled and an inter-colony comparison performed between Tristan and Nightingale Island. Feathers from Nightingale birds were enriched in both carbon (t_{168} = -35.7891) and nitrogen (t_{168} = -31.6620; $p < 0.0001$ in both cases) compared to feathers from Tristan birds. Feathers from Tristan were enriched in δ^{13}C by an

average of 0.8‰ compared to Tristan blood, and Nightingale feathers were enriched in $\delta^{13}C$ by 0.9‰ compared to Nightingale incubating male blood (Table 4.2; Figure 4.2). Nightingale feathers had the highest $\delta^{15}N$ signatures of all tissues sampled (15.2‰, Table 4.2; Figure 4.2).

4.3.3 Comparison of $\delta^{13}C$ and $\delta^{15}N$ signatures of prey items

$\delta^{15}N$ and $\delta^{13}C$ isotope analysis was performed separately on the mantle and tentacles of individual cephalopods (*Todarodes* sp.). A significant effect of body part was found, with the mantle being significantly more enriched in $\delta^{15}N$ ($t_{52} = -3.6261$, $p < 0.001$) and $\delta^{13}C$ ($t_{52} = -5.514$, $p < 0.0001$) than tentacles. Cephalopod prey items were also tested for the effect of removing the outer skin layer. This was found to have a significant effect on $\delta^{13}C$ isotope values ($t_{26} = 2.8728$; $p < 0.01$), but no effect on $\delta^{15}N$ ($t_{26} = 0.5031$; $p > 0.05$). As penguins consume whole prey items, un-skinned whole cephalopod material was analysed for isotopes, to avoid any effect of body part in the overall analysis. A significant effect of body size (measured as mantle length) was found for the $\delta^{15}N$ of *Todarodes* tissue, ($F_{2, 29} = 16.21$, $p < 0.0001$), with medium sized individuals being significantly more enriched in $\delta^{15}N$ (SNK $p < 0.05$) compared to small and large individuals (Figure 4.2). A significant effect of body size was also found for the $\delta^{13}C$ of *Todarodes* tissue, ($F_{2, 29} = 12.30$, $p < 0.001$), with small sized individuals being significantly more depleted in $\delta^{13}C$ compared to small and large individuals (SNK $p < 0.05$; Figure 4.2).

Whilst there was a significant effect of prey item on $\delta^{15}N$ ($F_{4, 10} = 2.27$, $p < 0.05$), with small *Todarodes* sp. individuals being depleted and photichthyid fish more enriched in $\delta^{15}N$ than other prey items (Figure 4.2), the SNK did not separate prey items into distinct homogenous groups. There was a significant effect of prey item on

$\delta^{13}C$ ($F_{4, 10} = 3.70$, $p < 0.05$), with small *Todarodes* individuals being depleted and medium *Todarodes* sp. individuals more enriched in $\delta^{13}C$ than other prey items (Figure 4.2), but again the SNK did not separate these prey items into homogenous groups.

4.4 Discussion

Understanding of marine ecosystem dynamics can be greatly enhanced by knowledge of spatial and temporal dietary variability of top predators. Carbon and nitrogen isotope signatures of blood and feathers can identify seasonal trophic segregation in foraging strategies of birds and intra-specific niche partitioning, specifically amongst breeding stages and age classes (Forero *et al.* 2002; Bearhop *et al.* 2006; Cherel *et al.* 2007; Karnovsky *et al.* 2008; Thiebot *et al.* 2011).

4.4.1 Isotopic niches of Northern Rockhopper penguins

Based on the results of this study, there appears to be trophic segregation between the guard and incubation stages of breeding, and spatial segregation between the breeding and pre-moult period in both colonies. Isotope signatures of prey items taken directly from stomach contents were obtained. However, it is known that some of the birds fed on larger fish (Paralepididae and Myctophidae) of a higher trophic level, for which isotope signatures could not be obtained (Chapter 3). Larger size classes of fish tend to occupy higher trophic levels (Lindsay *et al.* 1998), and it is likely that these form a substantial part of the diet of incubating birds and birds from Nightingale Island outside of the breeding season. Guard and crèche birds broadly appear to feed on a combination of the prey sources analysed, and when integrated

with analysis of stomach contents (Chapter 3), this would seem to be true. Notably absent from the prey sources analysed are zooplankton, which were highly digested. Two specimens of *Themisto gaudichaudii* obtained from stomach contents had mean carbon and nitrogen isotope values of -21.7 and 5.1‰ respectively, which gives a general indication of the lower trophic level of the crustaceans in penguin diets.

Prey items were obtained from stomach contents, and although they were intact specimens, digestion may have affected isotope signatures. Cherel (2008) found that carbon and nitrogen values were reduced in digested *Euphausia superba*, attributing this to an induced change in biochemical composition. Specifically, Cherel (2008) speculates that this change is a result of an increased relative importance of pure chitin; exoskeleton is more resistant to digestion than the soft tissues of crustaceans, and has a higher C/N ratio than protein and whole crustaceans from which lipids have been removed (Smyntek *et al.* 2007). It should also be considered that by treating the samples to remove lipids and carbonates, the isotope signatures may have been altered. Stenroth *et al.* (2006) found lipid extraction resulted in higher carbon isotope values, while acidification resulted in lower carbon and nitrogen isotope values for whole body analysis of crayfish. Overall, given the implications of treatment of specimens, and their digestive state, the measured nitrogen values of prey may have been artificially low. As squid were not treated for removal of carbonates, and as these specimens were relatively undigested, these isotope signatures are likely to be comparable to those of fresh specimens. Rockhopper penguins are known to be opportunistic feeders, and whilst the literature reports them to be mixed feeders taking predominantly crustaceans, with cephalopods and fish to a lesser extent, this is based on analysis of stomach contents in a narrow chick-rearing period (Klages *et al.* 1988;

Brown & Klages 1987; Cooper *et al.* 1990; Putz *et al.* 2001; Tremblay & Cherel 2003). Knowledge of broad-scale dietary changes in Northern Rockhopper penguin diet is sparse in comparison with the southern species, particularly in the Atlantic Ocean, and this is the first study to use stable isotope analysis of blood to look at diet within the breeding season. The breeding season isotope signatures obtained in this study (Table 4.1) are much lower than those obtained by Denhard *et al.* (2011) for Southern Rockhoppers, but comparable to values obtained on the Crozet Islands (Cherel *et al.* 2007), which may suggest that Northern Rockhopper penguins have a similar diet during the breeding season to these birds, which were found to feed on crustaceans, and this is supported by analysis of stomach contents (Chapter 3).

4.4.2 Selective chick provisioning

Isotopic differences between seabird adults and their chicks have been linked to concentration of blood uric acid, a waste product of protein catabolism, which, compared to body protein, is depleted in nitrogen (Bearhop *et al.* 2000). As chicks have increased catabolism, and should have lower urea concentrations in their blood than adults, a reduction in $\delta^{15}N$ may be induced in chick blood, which may confound dietary interpretation. Whilst earlier studies have based conclusions on the assumption that age does not affect isotopic fractionation (Minagawa & Wada 1984; Hobson & Welch 1995; Hodum & Hobson 2000), Sears *et al.* (2009) found nitrogen isotope signatures of Rhinoceros auklet, *Cerorhinca monocerata,* chicks (derived from red blood cells) to be depleted in response to rapid growth, and moderate nutritional stress, the combined effect of which resulted in a decrease by ~0.92‰ in $\delta^{15}N$. In the present study, crèche chicks were slightly (~0.23‰), but not significantly, depleted in

nitrogen compared to guard chicks, and chicks in both stages were significantly depleted in nitrogen compared to their respective parents (guard ~1.9‰ and crèche ~ 1.2‰). It appears from SIA that chicks in both breeding stages are fed on similar trophic level prey, which does not wholly accord with the results of analysis of stomach contents, where birds appeared to provision chicks with a greater proportion, by mass, of fish, which are more enriched in nitrogen (Figure 4.2) than zooplankton, (Quillfeldt *et al.* 2005) which dominated stomach contents brought ashore to provision guard chicks (Chapter 3). In Rockhopper penguin chicks, growth peaks at the end of the guard stage, so one would expect any physiological effects of growth to be most pronounced at this time (Sears *et al.* 2009). No relationship between the body mass and nitrogen signatures of chicks was observed ($r = -0.2208$, $p = 0.0988$, $n = 57$), but body mass of chicks sampled in the crèche stage would suggest these chicks were in the early stages of crèche, and it is possible that the observed reduced nitrogen signatures are a result of the physiological effects of growth (Sears *et al.* 2009).

Whilst guard females appear to feed at a higher trophic level for themselves compared to the trophic level at which they provision chick, crèche adults and their chicks, were not separated trophically to such a great degree. Carbon isotope signatures of chicks were similar, but depleted compared to their parents, which suggests that adults foraged in different oceanic water masses for provisioning purposes in both breeding stages. It is likely that the only way penguins are able to select food for provisioning purposes is through temporal segregation, i.e. feeding for self-maintenance at the beginning of a foraging trip, and for chicks towards the end. Foraging trips were on average 15 to 18 hours (Chapter 3), and penguins are able to digest fish to otoliths and bones within this time (Gales 1987). As the digested components of the food brought ashore were squid and zooplankton in the guard stage

and photichthyid fish in crèche stage (Chapter 3), these results would suggest that these are the items on which adults are feeding. Less digested material brought ashore was predominantly squid in the guard stage and squid and crustaceans in the crèche stage (Chapter 3), suggesting these were the food items selected towards the end of the foraging trips for chick provisioning. Photichthyid fish and medium sized squid had very similar nitrogen isotope signatures, which may explain the similarity in signatures of crèche adults and chicks, despite the apparent difference in diet. The elevated nitrogen signature of guard females compared to their chicks may be linked to the size of cephalopod prey items, as this was observed to affect nitrogen signatures.

The feeding of chicks on lower trophic level prey in the guard stage was unexpected, as seabirds commonly provision chicks with higher, or similar, trophic level prey. For example, Antarctic fulmarine petrels, *Fulmarus glacialoides*, Thick-billed murres, Black-legged kittiwakes, Magellanic and Adelie penguins are known to feed their chicks on higher trophic level prey (a greater proportion of fish) than they consume themselves (Hobson 1993; Hodum & Hobson 2000; Forero *et al*. 2002; Cherel 2008). Observed similarities in trophic level between adults and their chicks have been recorded in King, Emperor, *Aptenodytes forsteri*, Macaroni and Rockhopper penguins (Cherel *et al*. 2007; Cherel 2008). Many studies have reported that the higher nutritional demands of early growth stages are associated with more selective provisioning by parents (Moreno & Sanz 1996). Chick growth rate is influenced by calorific content of prey (e.g. African penguins, Heath & Randall 1985; Yellow-eyed penguins, van Heezik & Davis 1990), and fish have higher lipid, calcium and calorific values compared to squid and crustaceans (Clarke & Prince 1980; Cherel & Ridoux 1992). Brown & Klages (1987) found that Southern

Rockhoppers on Marion Island had an altered diet over the breeding season, with an increase in the amount of fish and squid in the diet in the mid to late chick rearing period, which they attributed to an increase in foraging range with increasing chick demand. If the nitrogen isotope signatures obtained in this study (Table 4.1) are compared with those of Northern Rockhopper penguin chicks at Amsterdam Island (δ15N = 9.2 ± 0.3‰, mean ± SD, Cherel & Hobson 2007), which similarly forage in subtropical oceanic waters, Northern Rockhopper penguins at Tristan da Cunha appear to provision chicks on lower trophic level prey than their conspecifics.

4.4.3 Temporal and sexual variation in stable isotope signatures

Pre-moult birds at Nightingale Island appear to have a different foraging strategy to both incubating males from the same colony and birds at Tristan Island during the breeding and pre-moult period, feeding on prey of a higher trophic level and in waters where prey are carbon enriched during the pre-moult fattening trip. Birds are spatio-temporally restricted to the vicinity of their breeding sites, where they return to moult, during the 2 – 3 week pre-moult fattening trip, but not to the same extent as during chick-rearing. However, SIA indicates that in the pre-moult period birds from both colonies foraged in different oceanic water masses than during the breeding season. Considering the proximity of the two islands, it is unusual that Tristan Island birds did not also display this large inter-seasonal difference in foraging strategy. Although birds were able to disperse away from their breeding colonies during the pre-moult period, inter-individual variation was negligible, suggesting that they fed in the same oceanic water masses.

No sexual segregation in foraging strategy, in terms of prey selection (trophic level) or foraging area (based on SIA) was observed in the crèche or moult stages in the Tristan da Cunha Rockhopper penguin population. Sexual differences were expected to be most pronounced, in particular, during the pre-moult stage, as birds are not restricted to foraging close to the colony, and are able to take advantage of a presumably wider variety of prey. However, Denhard *et al.* (2011) similarly found no differences in foraging strategy between male and female Southern Rockhopper penguins (based on SIA analysis of feathers), in the south-west Atlantic Ocean. Certain sexually dimorphic pelagic seabirds, such as Cory's shearwater, *Calonectris diomedea*, Black-browed, *Thalassarche melanophrys*, and Grey-headed albatrosses, display no spatial or trophic segregation during the breeding period (Philips *et al.* 2004; Navarro *et al.* 2009), and, whilst Waved albatrosses, *Phoebastria irrorata*, display no sexual trophic segregation during chick-rearing, spatial segregation is apparent (Awkerman *et al.* 2007).

Nitrogen isotope signatures of incubating females were higher than for all other groups of breeding birds at Tristan da Cunha. Like pre-moult individuals, incubating birds are similarly not restricted to the colony as feeding trips during the incubation stage are approximately ten days (Williams 1995). Although no large differences in blood carbon signatures were revealed amongst breeding penguins at Tristan, the possibility of their foraging in different areas cannot be excluded due to the absence of a longitudinal $\delta^{13}C$ isoscape within the Southern Ocean (Cherel & Hobson 2007; Jaeger *et al*. 2010) Incubating males at Tristan Island were not sampled; however, isotopic signatures of males during the guard stage will reflect food consumed during the incubation stage; during the guard stage males fast, and metabolise their endogenous fat reserves built up during incubation feeding trips. The

results suggest that incubating males do not feed on such high trophic level prey as incubating females. The physiological status of guard males should be mentioned as an effect of fasting, in the form of nitrogen enrichment, may occur in these birds (Cherel *et al.* 2005a), and if this were the case, the sexes would be separated even further trophically in the incubation stage. Similar carbon signatures suggest males and females were foraging in similar habitats so the same prey species were presumably available to both. It could be possible that the elevated nitrogen signatures observed in incubating females are a result of their physiological status linked to the high energetic costs of egg formation (Astheimer & Grau 1985).

In the crèche stage, males were only slightly enriched in nitrogen compared to females. It was expected that males may feed at a higher trophic level, based on the sexual size dimorphism hypothesis, which predicts sexual segregation based on males and females having different energy requirements, with the larger sex, typically males, consuming prey of higher energy content, or a greater amount of low energy prey items (Clutton-Brock *et al.* 1987; Nagy 1987; Mysterud 2000). Within seabird species, males commonly forage at a higher trophic level both in and outside of the breeding season, and this has been linked to their enhanced physiological capabilities and their ability to dive deeper and target prey of a higher trophic level (e.g. Gentoo penguins, Macaroni penguins and South Georgian shags, *Phalacrocorax georgianus*, Volkman *et al*. 1980; Bearhop *et al.* 2006). For size-dimorphic penguins, such as Magellanic penguins, there is evidence that males, with their larger beaks, are able to capture larger prey (Forero *et al.* 2002; this study, Chapter 3). However, with evidence from monomorphic species (e.g. Northern Gannets, *Morus basanus,* Lewis *et al*. 2002) showing trophic segregation in the absence of morphological differences,

it seems unlikely body size alone plays a role. Thiebot *et al.* (2011) similarly found no effect of sex on isotopic signatures of the closely-related Macaroni penguin.

In summary, stable isotope analysis revealed spatial differences in foraging areas of Northern Rockhopper penguins between the breeding and non-breeding periods, with very little sexual variation within breeding stages. The most pronounced dietary differences were between birds from Tristan and Nightingale Islands outside of the breeding season. Within the breeding season, no spatial segregation was observed between adult birds, however there was pronounced trophic segregation between incubating and crèche females, with elevated nitrogen signature of incubating birds indicative of feeding at a higher trophic level. Selective provisioning was observed throughout the chick-rearing period, with adults consistently feeding chicks on lower trophic level prey than they consumed themselves, although this was more pronounced in the guard stage. Interpretation of diet was impaired by the lack of isotopic baseline data and this study highlights the need to obtain isotope signatures from a wide range of potential prey items of marine predators in the mid-Atlantic Ocean.

Chapter 5

Stress and parasitism in breeding Northern Rockhopper penguins, *Eudyptes moseleyi*, at Tristan da Cunha

5.1 Introduction

Physiological mechanisms that maintain homeostasis (allostasis) are involved in the ability of an organism to adapt to its environment, and are regulated by gluco-corticosteroids (GCs) (Romero *et al.* 2009). The principle GC in birds is corticosterone, synthesised from cholesterol, and, like other GCs, it is released into blood plasma from the hypothalamic-pituitary-adrenal (HPA) axis in response to various physical and psychological stressors (Siegel 1980; Harvey *et al.* 1984; Landys *et al.* 2006). Corticosterone mobilises energy stores (promotes glucogenesis and muscle breakdown) and regulates physiology (Gray *et al.* 1990; Wingfield *et al.* 1998; McEwen & Wingfield 2003; Landys *et al.* 2006). Thus, behavioural and physiological traits are altered to maximise the survival of individuals within a changing environment (Landys *et al*. 2006). There is evidence that both basal and stress-induced GC concentrations can be modulated seasonally in many wild species (review in Romero 2002). The term basal is used in this thesis rather than baseline, as "baseline" concentrations implies that the animals are unstressed prior to collection and this cannot be known (Romero 2002).

According to life history theory, long-lived animals should not jeopardise the chance of their own survival and future breeding for current breeding, and should, therefore, redirect energy from parental investment to self-maintenance in times of environmental stress and/or poor body condition (Nur 1986). The physiological and behavioural changes associated with the emergency life history stage act to increase the survival of adults in the face of stress, to

secure future breeding opportunities, with concomitant fitness, and promote abandonment, or reduced parental care, for current offspring (Wingfield *et al.* 1998). Inhibition of energy allocation towards current reproduction, in favour of self-maintenance, associated with an increase in basal corticosterone, has been observed in breeding birds (Silverin 1986; Wingfield & Kitaysky 2002; Wingfield & Sapolsky 2003; Landys *et al.* 2006). More recently, in a study of Black-legged kittiwakes, *Rissa tridactyla*, Angelier *et al.* (2009a) found that increased levels of corticosterone resulted in a reduced expression of parental behaviour (specifically nest attendance and motivation to return to nest after stressful events). As GCs are important in the mobilisation of energy resources, concentrations should be at their greatest during times of the year that are energetically costly, and the breeding season carries very high energetic demands (Williams & Rothery 1990). Indeed, in 72% of 29 studies of avian species, basal levels of GC peaked in the breeding season (Romero 2002).

Elevated basal GC levels are associated with low energy availability (Landys *et al.* 2006), and correlations between basal GC levels and body condition have been reported in many studies, with animals in poorer body condition exhibiting higher basal levels of GC (Williams *et al*. 2008; Spee *et al.* 2011). Essentially basal corticosterone should theoretically be elevated in individuals that are energetically challenged, for example during periods of fasting or low food availability (McEwen & Wingfield 2003; Angelier *et al*. 2007; Romero *et al.* 2009).

Fasting is known to induce an increase in circulating plasma corticosterone levels in many birds (Lynn *et al*. 2003; Angelier *et al*. 2007; Groscolas *et al*. 2008). However, in penguins, for which fasting is part of their natural life-cycle, this is not necessarily physiologically stressful (Hood *et al.* 1998). During fasting periods, such as when moulting or incubating, penguins obtain most of their energy from accumulated fat reserves. When these stores are depleted, increased corticosterone levels induce gluconeogenesis, and protein

reserves, primarily from skeletal muscle, are mobilised (Cherel *et al.* 1988a; Holberton *et al.* 1996). Thus, fasts do become stressful if individuals do not have sufficient fat reserves (Hood *et al.* 1998). Vleck *et al.* (2000) found that corticosterone levels in fasting Adélie penguins, *Pygoscelis adeliae*, only altered when the fast exceeded 40 days (longer than usual), and the energy reserves of the individual were almost exhausted. This is in accordance with previous studies of Magellanic, *Spheniscus magellanicus*, and King penguins, *Aptenodytes patagonicus* (Cherel *et al.* 1988b; Hood *et al.* 1998), in which corticosterone levels only increased once fat stores had been depleted and protein was utilised as a primary energy source.

Individuals that are infested with parasites may also be energetically challenged, as immune function to combat disease will compete for resources which could be allocated to other processes. Egg-laying female birds appear to be more susceptible to parasite infections, a possible manifestation of a trade-off between reproduction and immune function (Gustafsson *et al.* 1994; Oppliger *et al.* 1997). Whilst infestation with parasites can induce stress, prolonged elevated corticosterone levels can have a deleterious effect on immunity (Landys *et al.* 2006). Intracellular blood parasites, such as Haemosporidia (genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*), Haemogregarinidae (genus *Hepatozoon*) and Piroplasmida (genus *Babesia*) are prevalent in many groups of birds (Bennett *et al*. 1994), though certain groups, such as seabirds, are less affected (Quillfeldt *et al*. 2011). For example, Chinstrap penguins, *Pygoscelis antarcticus* (Merino *et al.* 1997), Storm petrels (Merino & Minguez 1998) and Crested auklets, *Aethia cristatella* (Engstrom *et al.* 2000) all show low levels of parasitism.

Globally, populations of Northern Rockhopper penguins are declining (Chapter 1), and while it is possible that 'at-sea' factors, such as changing prey availability, have and continue to contribute to this decline, the results of this study (Chapter 3 and 4) indicate that

food for breeding penguins around Tristan Island is not limiting. During periods when birds are able to extend their foraging range to take advantage of presumably more abundant, profitable food types, they do not do so and remain close to the colony (Chapter 4). I suggest it is possible that factors such as stress and the presence of blood parasites, which may affect body condition, could contribute to breeding failure, and population declines. To test this hypothesis, plasma corticosterone levels were measured in a group of incubating and guard stage Northern Rockhopper penguins at Tristan da Cunha, and blood smears were examined for the presence of parasites in this population. It was expected that fasting birds (incubating adults and guard stage males) might display elevated plasma corticosterone levels compared to birds that were continually replenishing energy reserves (guard stage females), if sufficient fat reserves had not been accumulated prior to breeding. It was also expected that there would be an interaction between the presence of blood parasites and plasma corticosterone levels. Finally, if reproductive failure was linked to physiological stress, then birds that did not successfully rear chicks would be expected to have elevated plasma corticosterone levels compared to their conspecifics that were successful breeders.

5.2 Methods

5.2.1 Blood sampling

To determine plasma concentrations of corticosterone, 0.5 ml of blood were collected from the tarsus vein with a 25-gauge needle and heparinised 3 ml syringe and stored on ice. Whilst it is generally accepted that blood should be collected within three minutes of the time from initial disturbance (Wingfield *et al.* 1982; Holberton *et al.* 1996; Pravosudov *et al.* 2001), as corticosterone levels start to increase from the stress of handling after this period (Wingfield *et al.* 1982; Kitaysky *et al.* 1999), it was not possible to collect sufficient blood in

this time. Samples were collected within five minutes of initial disturbance to the bird, defined as elapsed time from first approaching the bird (Vleck *et al.* 2000). In certain species, such as Adelie penguins no increase in basal corticosterone is observed within five minutes of initial disturbance (Vleck *et al*. 2000).

All blood samples were centrifuged for ten minutes, within one hour of collection (Groscolas *et al.* 2008), using a 2000g Labnet Spectrafuge (Model C1201). Plasma was extracted with a Pasteur pipette and transferred to eppendorf tubes and frozen at – 30 ºC (Angelier *et al.* 2009a).

5.2.2 Radioimmunoassay for Corticosterone

Corticosterone analysis was performed in the RIA Hormone Laboratory in the Department of Production Animal Studies at Onderstepoort, University of Pretoria, South Africa. The concentration of corticosterone in blood plasma was tested, in duplicate, using the radio immune assay method with a Coat-A-Count Rat Corticosterone kit (Siemens), which can be used for tests in various species. The minimal detectable corticosterone level was approximately 1.8 ng ml^{-1}. Three samples fell below this limit and were thus excluded from further analysis.

5.2.3 Blood smears and parasites

As it was not possible to make smears in the field, due to inclement weather, blood was stored in heparinised eppendorf tubes to prevent clotting, and smears were made in duplicate in the laboratory within 6 hours of collection. Small drops of whole blood were placed on the end of a frosted, labelled microscope slide, and spread to one cell layer thick with a spreader slide (Butler *et al.* 2009). The slides were air-dried for 24 hours, and then fixed in absolute methanol for 3 - 4 minutes (Butler *et al.* 2009). Once dry, the slides were

wrapped in tissue paper and stored in zip-loc bags. In the Bayworld Aquarium laboratory (Port Elizabeth, South Africa), blood smears were stained with Giemsa stain (Vleck *et al.* 2000), and examined at a magnification of 100x under a compound microscope with oil immersion. Approximately 760 fields were examined per slide, presenting approximately 92 erythrocytes per field and approximately 70 000 erythrocytes per slide. Blood smears from all study birds in the incubation and guard stages were made, but unfortunately many smears were too thick to examine. A total of 76 slides were examinable (some were replicates) comprising blood smears from 9 guard stage females, 6 guard stage males, 28 incubating females and 6 incubating males.

5.2.4 Statistical analysis

Correlation analysis was performed to test for relationships between 1) adult body mass and plasma corticosterone levels; 2) guard male plasma corticosterone levels and the body mass of the chicks they were guarding; 3) guard female plasma corticosterone levels and the body mass of their chicks.

Plasma corticosterone concentration data were log transformed to meet the assumptions of normality and homogeneity (Kolmogorov-Smirnov tests $p > 0.05$, Cochran's tests $p > 0.05$ in all cases). 2-way factorial ANOVA was used to test the effects of the fixed factors breeding stage (incubation, guard) and sex (male, female) on plasma corticosterone concentrations of birds. An independent sample t-test was used to test the effect of breeding success (failed or successful) on plasma corticosterone concentrations of incubating female birds. A 3-way factorial ANOVA was used to test the fixed effects Babesia (presence, absence), breeding stage (incubation, guard) and sex (male, female) on body mass and plasma corticosterone levels of adult birds.

5.3 Results

5.3.1 Plasma corticosterone levels

Inter-individual variability in plasma corticosterone levels of penguins was large, ranging from 1.88 to 100.6 ng. ml^{-1}. No relationship between adult body mass and plasma corticosterone concentration was found ($r^2 = 0.0061$, $p = 0.5489$, Figure 5.1). No relationship was found between the body mass of guard females and their chicks ($r^2 = 0.0002$, $p = 0.9532$, Figure 5.2a) or guard males and their chicks ($r^2 = 0.1546$, $p = 0.1064$, Figure 5.2b).

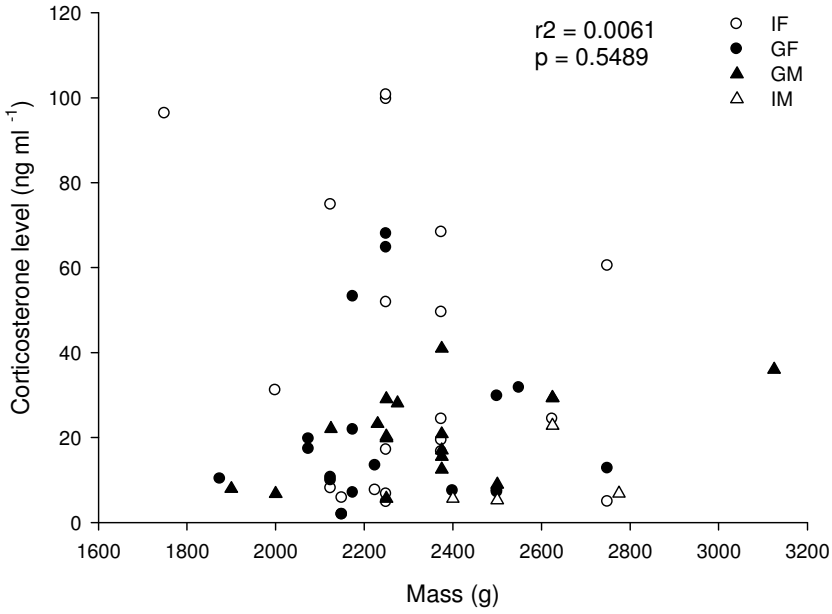

Figure 5.1 – Relationship between Northern Rockhopper penguins adult body mass and blood corticosterone level (n = 61). IF = incubating females (n = 22); GF = guard females (n = 17); GM = guard males (n = 18); IM = incubating males (n = 4).

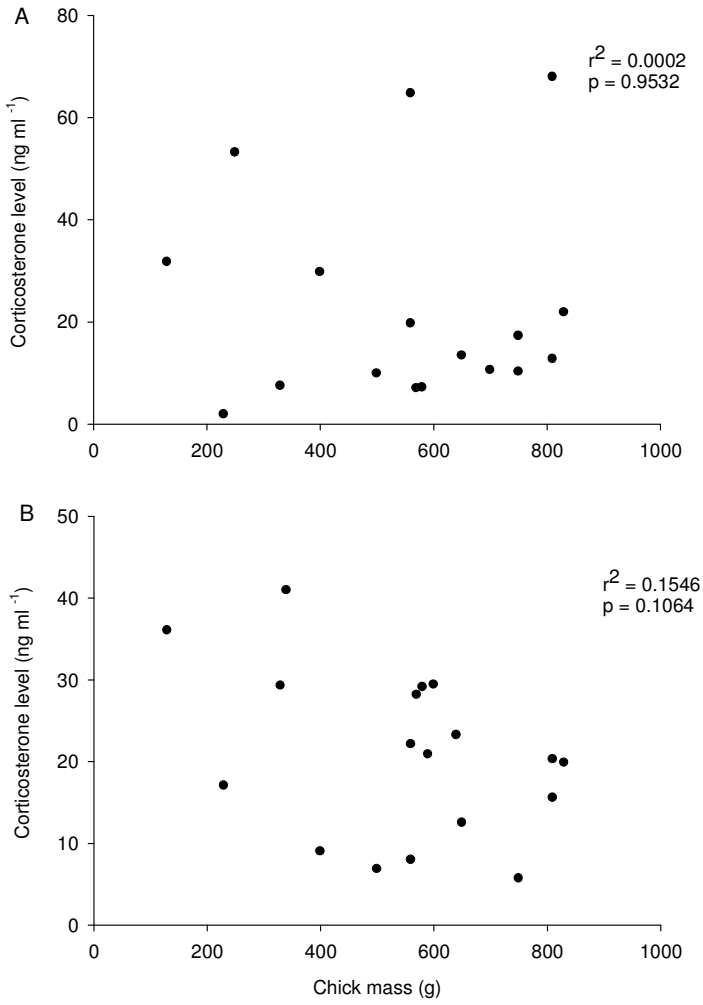

Figure 5.2 – Relationship between Northern Rockhopper penguin chick body mass and (A) female (n = 17) and (B) male (n = 18) blood corticosterone level.

No significant effect of sex or stage was observed on the plasma corticosterone levels of male and female birds during the incubation and guard stages ($p > 0.05$ in both cases, Figure 5.3, Table 5.1). The interaction between sex and stage was close to significance ($p = 0.09$, Table 5.1). While the effect was non-significant, males and females had similar plasma corticosterone levels in the guard stage, with females having much higher levels than males

during incubation (Figure 5.3). Incubating females that were successful breeders had similar plasma corticosterone levels to those that were failed breeders ($t_{19} = 1.2181$, $p = 0.2382$).

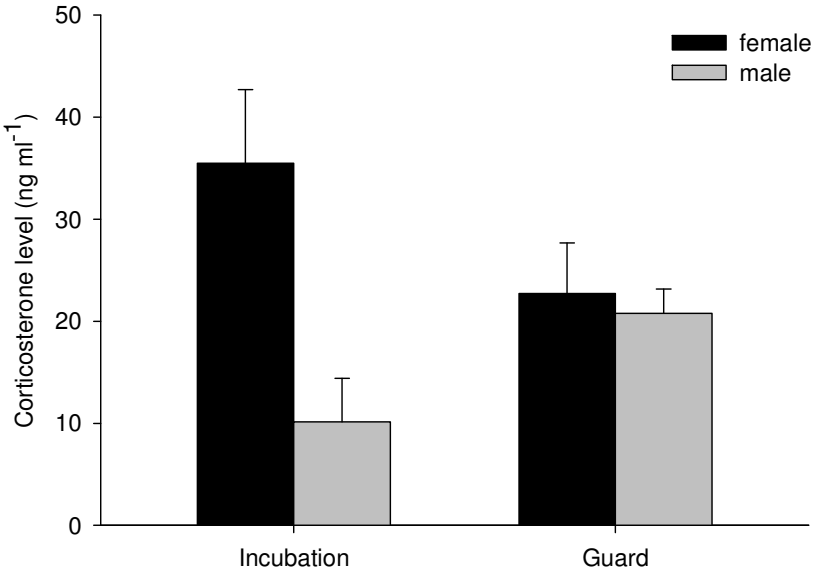

Figure 5.3 – Mean (± SE) blood corticosterone levels of incubating males (n = 4), incubating females (n = 22), guard stage males (n = 18) and guard stage females (n = 17).

5.3.2 Babesia

Babesia was the only parasite observed in blood smears. 61% of all birds examined presented with *Babesia* infestation, and 39% of birds presented no signs of parasitism. Within each sex by stage group, a greater percentage of birds presented with parasite infestation than not (Table 5.2). As no slide yielded complete development of the parasite, it was not possible to identify it to species, however it may have been *B. peircei* (pers. comm. Albert Schultz).

Table 5.2 – Presence and absence of *Babesia* in male and female Northern Rockhopper penguins blood smears.

		Babesia present	*Babesia* absent	Sample size
Incubation	Males	80%	20%	5
	Females	57%	43%	28
Guard	Males	67%	33%	6
	Females	56%	44%	9

All effects and interactions of infestation with parasites, sex or stage on body mass or plasma corticosterone levels were non-significant ($p > 0.05$ in all cases, Table 5.3).

Table 5.3 – 3-way factorial ANOVA testing the effect of the factors: *Babesia* (present, absent), sex (male, female) and breeding stage (incubation, guard) on body mass and blood corticosterone levels of adult Northern Rockhopper penguins.

	Body mass				Corticosterone			
Source	d.f.	M.S.	F	P	d.f.	M.S.	F	P
Babesia	1	0.0044	0.4635	0.4998	1	0.0471	0.0453	0.8337
Sex	1	0.0292	3.0914	0.0862	1	2.2542	2.1644	0.1568
Stage	1	0.0248	2.6185	0.1133	1	0.4639	0.4454	0.5121
Babesia*Sex	1	0.0003	0.0346	0.8533	1	0.0033	0.0031	0.9560
Babesia*Stage	1	0.0000	0.0014	0.9700	1	0.3418	0.3282	0.5731
Sex*Stage	1	0.0293	3.0951	0.0860	1	0.2669	0.2563	0.6182
Babesia*Sex*Stage	1	0.0164	1.7341	0.1952	1	0.3588	0.3445	0.5638
Error	41	0.0095			20	1.0415		

5.4 Discussion

The role of hormones in vertebrate physiology and behaviour is highly complex, and is mediated by numerous factors, making it difficult to extricate the effects of single factors. It should be mentioned that basal plasma corticosterone levels in this study may have been elevated due to a stress response induced by human presence and handling. Whilst Vleck *et al.* (2000) reported no effect of a five minute handling time on basal corticosterone levels of Adélie penguins, these responses are likely species-specific, as elevated corticosterone is observed in Magellanic penguins after 2 – 3 minutes of initial disturbance (Hood *et al.* 1998). Moreover, responses likely show a high level of intra-specific variability, and this may explain the very large inter-individual variability in basal plasma corticosterone concentration in this study.

5.4.1 Does food acquisition/deprivation mediate corticosterone levels?

Corticosterone is responsible for variation in the allocation of energy resources to reproduction and body maintenance (Wingfield *et al.* 1998). One of the ways corticosterone can act is to modulate foraging behaviour (Kitaysky *et al.* 2001; Wingfield & Kitaysky 2002; Angelier *et al*. 2007). In turn, intake of energy can affect the secretion of corticosterone, with fasting increasing, and feeding decreasing the secretion of corticosterone (Lynn *et al*. 2003; Lanctot *et al.* 2003; Angelier *et al.* 2007). However this is not always the case, and *et al.* (2009b) found that acquisition of food at sea did not affect basal corticosterone levels of King penguins, which supports the results of this study. Northern Rockhopper penguins at Tristan da Cunha that were continually replenishing energy reserves (guard stage females), had similar plasma corticosterone levels to both guard males, that had been fasting for up to 24 days, and incubating males and females that had probably been fasting for at least ten days.

Critical energy depletion in birds, such as penguins, that fast during incubation and chick brooding, is one of the factors which may promote the redirection of parental behaviour from breeding to food searching and self-maintenance (Olsson 1997; Robin *et al*. 1998; Groscolas *et al*. 2000). However, this is typically associated with a transition from phase II to phase III of fasting, which involves an increase in catabolism of body protein concomitant with an increase in the level of plasma corticosterone (Cherel *et al*. 1988b; Robin *et al*. 1998). In Emperor penguins, *Aptenodytes forsteri*, this involves an increase in locomoter activity and in King and Adélie penguins, the abandonment of eggs (Groscolas *et al*. 2008; Spée *et al.* 2010). *Eudyptes* penguins, although they do not fast for such extended periods as their larger *Aptenodytes* relatives, undergo similar mass-specific losses in body weight (Williams *et al.* 1992). Williams *et al*. (1992) found that Macaroni penguins, *Eudyptes chrysolophus*, at the end of their moulting and incubating fasts did not enter phase III of fasting, and continued to use lipid reserves towards the end of their fasts. Presumably fasting birds had sufficient fat stores, and and, although protein reserves would have been mobilised at a low level, which is necessary for the synthesis of glucose,an increase in corticosterone level was not induced (Cherel *et al*. 1988b, b; Hood *et al*. 1998; Vleck *et al*. 2000). This is supported by the general absence of a relationship between adult body mass and plasma corticosterone levels in this study, and the absence of a negative relationship between chick body mass and guard male plasma corticosterone level, which one would expect to decrease with duration of fast and increasing chick size.

5.4.2 Prevalence of Babesia

Babesia is prevalent within the Northern Rockhopper penguin population at Tristan da Cunha, being found in more than half of the birds sampled for blood (61%). Penguin populations in the Antarctic and sub-Antarctic generally are free from blood parasites, and

this is attributed to the lack of suitable vectors (Jones & Shellam 1999). However, populations in more temperate regions, for example African penguins, *Spheniscus demersus*, in South Africa, and Little penguins, *Eudyptula minor*, in Australia (Peirce 2000) have presented with *Babesia*, transmitted by ixodid ticks. The colonial nature of breeding seabirds provides parasites, and ticks in particular, with a suitable habitat to aggregate, and a reliable host resource for the duration of the breeding season (Danchin 1992). Ticks are also able to survive between breeding seasons while awaiting the arrival of new hosts (Dietrich *et al.* 2011), and so populations of these ecto-parasites can thrive in seabird colonies. The presence of *Babesia* in some individuals from Tristan da Cunha indicates that suitable vectors are present in the breeding colonies on the island, and the absence of signs of parasitism in some breeding birds may be indicative of these individuals having better immunological capability (Ricklefs, 1992; Merino & Minguez, 1998; Tella *et al*., 1999). The immune system acts against pathogens, including viruses, bacteria and ecto-parasites, and is energetically costly for vertebrates, thus energy allocation towards immunity occurs as a trade-off with other processes (Lochmiller & Deerenberg 2000; Norris & Evans 2000; Lee *et al.* 2006). Denhard *et al.* (2011) found a strong investment in the acquired immune system of Southern Rockhopper penguin chicks, *Eudyptes chrysocome*, with age. It is possible that Northern Rockhopper penguins also invest a large amount of energy in the acquired immune system, and this may explain why the prevalence of blood parasites was not greater.

5.4.3 Reproductive success of Northern Rockhoppers

Parental behaviour can be reduced in response to elevated corticosterone levels (Silverin 1986; Wingfield & Kitaysky 2002; Love *et al.* 2004). Contrary to the hypothesis, birds that were unsuccessful breeders, specifically those that did not successfully rear chicks

to the early-guard stage, did not show any signs of greater stress through elevated plasma corticosterone levels, lower body mass, or higher infestation by blood parasites, compared to successful breeders. This suggests that abandonment of eggs or chicks was not induced by stress, i.e. elevated corticosterone leading to birds favouring self maintenance over the current reproductive effort. However, the decision to abandon reproductive effort is likely a complex interaction of intrinsic and extrinsic factors, and levels of the hormone prolactin, which were not assessed in this study, play an important role in birds (Chastel *et al*. 2005; Criscuolo *et al.* 2005; Angelier *et al*. 2007, 2009c; Spée *et al.* 2010). Angelier *et al.* (2010b) found that male Black-browed albatrosses, *Thalassarche melanophrys*, with a history of successful breeding had lower basal corticosterone levels, although the same pattern was not observed in females, which the authors attributed to differential breeding investment between the sexes. There is likely a strong mechanistic link between corticosterone and prolactin the and suppression of parental behaviour, and prolactin probably mediates circulating corticosterone, as it has the opposite effect, stimulating incubation and brooding behaviour (Buntin 1996; Youngren *et al.* 1991). Adélie penguins abandoning their nests are known to have high corticosterone and low prolactin levels (Spée *et al.* 2010).

I suspect that the failed breeding attempts, and also the low overall breeding success, in the colonies at Stony Beach and Stony Hill are due to predation on eggs and chicks by Tristan skuas, *Catharacta antarctica hamiltoni*. Less than ten pairs of skuas breed on Tristan Island (Ryan 2007), however at least one breeding pair nests close to these colonies, and a minimum of one egg or chick was killed every day throughout the duration of fieldwork (personal observation). Using the Stony Beach sub-colony as an example, where 262 nests were recorded, over the chick rearing period, which is approximately 45 days, skua predation alone can account for breeding failure of approximately 17% of breeding pairs. Nesting habitat certainly plays an important role in the susceptibility of eggs and chicks to attack by

aerial predators, with dense vegetation, such as tussock grass, providing a safer habitat (St Clair & St Clair 1996).

In the study population of Northern Rockhopper penguins, potential stressors, such as infestation with blood parasites and periods of fasting did not induce an elevation in plasma corticosterone levels. Furthermore, there was no relationship between body mass and plasma corticosterone levels. It is recognised that the role of hormones in physiology is highly complex, however, I suggest that if indeed fasting and infection with *Babesia* were sources of stress for these birds, higher levels of plasma corticosterone would have been observed in these individuals. Reproduction is not as stressful for Northern Rockhopper penguins as anticipated, and nor is infection with the blood parasite *Babesia*, therefore, reproductive failure in this population cannot be linked to stress. Predation, however, certainly plays an important role in reducing breeding success in the Stony Beach and Stony Hill sub-colonies.

Chapter 6

General discussion

Seabirds have evolved life-history traits, such as low fecundity and delayed sexual maturity (Pianka 1970; Stearns 1992), that enable them to exploit prey and survive in the spatio-temporally heterogeneous marine environment (Hunt *et al.* 1999). On a life-time scale, foraging plasticity is an essential attribute of seabirds, increasing their chance of survival and reproductive success (Burger & Piatt 1990), and this is especially important for flightless central place foragers such as breeding penguins (Orians & Pearson 1979). The breeding period is energetically demanding (Williams 1966; Stearns 1976; Ydenberg *et al.* 1994), and life-history theory predicts that seabird parents should make decisions to maximise their own survival (Stearns 1992), and in times of low energy availability, resources should be directed towards self-maintenance and future reproductive events (Drent & Dann 1980). The fitness of a seabird reflects how well they are able to cope with a changing prey availability to provision chicks and acquire food for self-maintenance, and is dependent on the interaction of numerous behavioural and physiological factors (Ricklefs & Wikelski 2002).

Populations of Northern Rockhopper penguins are declining globally for unknown reasons; it is possible that 'at-sea' factors, such as low, or poor-quality, prey availability may be responsible, as the relationship between food availability and seabird reproductive success is strong. However, these declines could also reflect reduced juvenile and/or adult survival rather than reproductive success. Physiological factors may also be responsible, such as the natural constraints placed on parents during breeding, for example fasting, if sufficient fat stores have not been accumulated, or exposure to disease in breeding colonies, which can result in elevated corticosterone, which if prolonged can lead to abandoned breeding attempts. Thus analysis of foraging behaviour and physiological condition may contribute to

the understanding of causal factors behind population declines. In this thesis, sexual dimorphism and intra-specific variation in foraging behaviour were examined, focusing on detailed analysis of diet during the chick rearing period, in conjunction with diving behaviour, and broad-scale changes in diet between the breeding and moult periods. Additionally, the basal plasma corticosterone concentrations of breeding birds were examined to assess the stress imposed on birds by fasting, and the presence of blood parasites.

6.1 Foraging plasticity

Northern Rockhopper penguins breeding in the Tristan da Cunha archipelago, like their conspecifics in the Indian Ocean (Tremblay & Cherel 2003), can forage in deep oceanic waters within only a few kilometres of their breeding colonies, and feed opportunistically on a wide variety of prey, displaying a high degree of foraging plasticity. Opportunistic behaviour allows predators to exploit different prey (Montevecchi *et al.* 2009), and this is probably why Rockhopper penguins are able to inhabit such a diverse range of geographic habitats (Chapter 3). Considering birds foraged closer to their colonies during their pre-moult trips (based on $\delta^{13}C$), despite being less spatially restricted during this period, it is difficult to conceive that Rockhopper penguin prey is not abundant around Tristan and Nightingale Islands. Therefore the opportunistic behaviour of these birds likely reflects local small-scale changes in prey distribution.

Birds fed on fish, cephalopods and macrozooplankton throughout the breeding season, although intra-seasonal variation was observed in the proportions these prey items contributed to overall diet. By mass, diet was dominated by zooplankton in the guard stage, and small meso-pelagic fish in the crèche stage. Cephalopods were an important prey item throughout the breeding season. Northern Rockhopper penguins were able to modify their diving tactics to cope with presumed changes in prey diversity and abundance intra-

seasonally, taking advantage of locally abundant prey. Birds were generally diurnal, daily foragers with some unusual behaviour; extended trips and nocturnal diving were recorded in a few individuals. Birds dived well within their physiological limits, predominantly utilising the upper 20m of the water column, and two distinct strategies were observed. Long, deep (30 – 40 m) dives were performed when targeting fish, and a greater number of shorter, shallower (5 – 20 m) dives when targeting zooplankton. This behaviour seems to accord with optimal foraging theory (Stephen & Krebs 1986), as, although deeper dives are more energetically costly, this is balanced by capturing prey of higher energy content (fish). In comparison, a series of comparatively energetically inexpensive shallow dives will maximise net energy gain when the energetic value of prey is low (zooplankton).

No sexual segregation was observed in terms of benthic/ pelagic or inshore/ offshore foraging (carbon isotope signatures) during either the breeding season or the pre-moult period, although males were found to prey on larger individuals of squid and fish. Whilst this may suggest that Northern Rockhopper penguins conform to the niche divergence hypothesis (Schoener 1970), which would reduce inter-sexual competition, males predominantly fed on the same size classes as females, so I would suggest that this again reflects their opportunistic behaviour. As males have larger beaks, they are able to capture and handle larger prey when it is encountered. The fact that throughout the breeding season birds foraged in similar habitats suggests no intra-specific competition occurred, despite both sexes feeding on the same prey. On Tristan Island, colonies are very small, breeding pairs numbering in the hundreds, however at Nightingale and Alex Islands tens of thousands of birds breed in a very small area and intra-specific competition may play a greater role in foraging behaviour of birds in these colonies.

6.2 Chick provisioning versus self-maintenance

The value of an integrated approach to diet determination was highlighted in this study. On interpretation of stomach content data alone it would appear that Northern Rockhopper penguins at Tristan fed their chicks on energy-rich fish in the crèche stage to meet increasing energy demands (Chapter 3), however SIA revealed these prey items to be enriched in nitrogen compared to chick blood, so chicks were clearly fed on lower trophic level prey, likely zooplankton (Chapter 4). The fact that SIA revealed that guard and crèche chicks have very similar diets, and that adults fed on a higher trophic level, suggests that the fresh otoliths found in stomach contents of many crèche stage adult birds were remnants of food used for self-maintenance rather than that which was provisioned to chicks. Based on this assumption, we would expect to find similar otoliths in guard adult stomach contents, as they also fed on a much higher trophic level than the zooplankton with which they predominantly provisioned chicks. In fact, fish were notably absent in guard stage stomach contents (Chapter 3), therefore cephalopods with similar nitrogen isotope signature to fish were an important dietary component of guard stage adults.

An increase in chick growth over the breeding season warrants some form of increase in foraging effort from adults, whether this is increased meal size, an increase in frequency of provisioning, or an increase in the quality of prey. In this study, this did not come from an increase in individual foraging effort (although this may happen in the crèche stage because both adults forage to provision chicks), or an increase in energy-rich prey, but meal mass was not assessed. Adult birds may have selected higher trophic level prey for themselves, or may again simply feed opportunistically on local temporally abundant prey. SIA indicated that adults foraged in benthic/ inshore water for self-maintenance, and in pelagic/ offshore water for chick provisioning, and temporal segregation is likely the only way birds are able to segregate prey items for self-consumption and chick provisioning. It is not possible to

determine if these lower trophic level prey were actively 'selected'. If they were, this would suggest that Northern Rockhopper penguins conform to the individual optimisation hypothesis (Nur 1986), and favour self-maintenance and future reproduction over their current breeding effort as they could have fed chicks on similarly energetically valuable prey. I suggest that this is not the case as birds would exhibit this behaviour if energy availability in the environment was low (Goodman 1974; Perrins & Moss 1975; Chaurand & Weimerskirch 1994, Erikstad *et al.* 1997), and, as mentioned, it does not seem that penguin prey around Tristan Island is limiting.

6.3 Factors affecting reproductive success

In long lived seabirds, any factor that increases mortality can have detrimental effects on population growth (Furness 2003). As discussed in Chapter 5, predation by Tristan skuas, *Catharacta antarctica hamiltoni*, was observed to be a significant cause of egg and chick mortality, and could have significant impacts on the small breeding colonies on Tristan Island (Stony Beach, the smallest colony, had 262 breeding pairs in 2010, Big Gulch, the largest colony, had 2410 breeding pairs in 2010). Inaccessible, Nightingale and Alex Island present very different conditions for breeding birds, in terms of significantly larger breeding colonies (approximately 30 000, 20 000 and 80 000 breeding pairs respectively), which is more likely to result in intra-specific competition and prey depletion around these colonies, particularly Nightingale and Alex Island, as Alex Island is really an islet rather than a separate island and only a few metres separate the two (Hunt *et al.* 1986; Birt *et al.* 1987). Whilst birds nest under tussock grass on these neighbouring islands, and are less threatened by aerial predators, there are large breeding populations of Sub-Antarctic fur seals, *Arctocephalus tropicalis*, which may prey on penguins, as has been observed at Amsterdam Island (Guinard *et al.* 1998), as well as competing for prey. Northern Giant petrels, *Macronectes halli*, have also

been observed to hunt and kill Northern Rockhopper penguins offshore of penguin colonies at Nightingale Island (Ryan *et al.* 2008). Notably, birds from Nightingale Island foraged on a higher trophic level and close to the colony during the pre-moult period, and it is possible this is a way to mitigate one of the above mentioned factors. It is unlikely that inter-specific competition with other breeding seabirds at Tristan da Cunha plays any significant role in affecting prey availability, as these are predominantly pelagic-feeding Procellariiformes, and the few species that forage inshore, such as terns, skuas, and occasionally petrels, feed at the surface (Ryan 1991).

Breeding success and juvenile and adult survival of Rockhopper penguins is largely unknown, as no long-term monitoring programmes exist, and it is therefore unknown if mortality is greater during a certain life stage. During 2010 at Stony Beach, breeding success in the Stony Hill sub-colony was 0.64 chicks per pair and in the Stony Hill sub-colony 0.5 chicks per pair. This is generally higher than breeding success of Northern Rockhopper penguins recorded at Amsterdam Island (0.28, 0.35 and 0.52 chicks per pair, 1993 – 1995, Guinard *et al.* 1998). However, at Amsterdam Island chicks were counted closer to fledgling than in this study, and breeding success at Stony Beach may have been lower at the end of 2010 when chick mortality in the late crèche stage is accounted for. Breeding success at Stony Beach was closer to that of Southern Rockhopper penguins breeding in the Falkland Islands, with 0.35 to 0.61 chicks raised per pair (Clausen and Pütz 2002). Investigation into the number of juveniles returning to colonies to moult may help to clarify upon which stage of the life cycle detrimental factors are acting. In seabirds, recruitment only occurs if juveniles survive to several years of age, due to delayed sexual maturity, therefore factors increasing mortality may be significant once chicks fledge from colonies.

No signs of physiological stress, in the form of elevated basal corticosterone levels, were observed within fasting birds in the study population of Northern Rockhopper penguins,

suggesting they had built up sufficient fat reserves prior to incubating, in the case of both sexes, and, in the case of guard males when guarding chicks (Chapter 5), again supporting the idea that penguin prey around Tristan is not limiting. Birds that were failed breeders were not characteristically different from successful breeders, in terms of body mass during incubation, corticosterone levels or infection with *Babesia* (Chapter 5). More than half of the sampled study population were infected with the intra-cellular blood parasite *Babesia*, yet this appeared to have no effect on the physiological state of individuals, with no relationship to body mass, corticosterone levels or breeding success.

6.4 Conclusions

This is the first comprehensive study of Northern Rockhopper penguins at Tristan da Cunha, with the exception of a historical isotopic analysis of the carbon signature of feathers and stomach contents data from Gough Island (1984 - 1986), thus there is a paucity of baseline data with which to compare the results of this study, and this prohibits any link between foraging behaviour and changing 'at-sea' conditions. To gain a broad understanding of the foraging ecology and physiological condition of Northern Rockhopper penguins in the Tristan da Cunha archipelago, similar investigations should be carried out at neighbouring Inaccessible, Alex and Nightingale Islands. The different foraging behaviour observed in penguins from Tristan and Nightingale Islands, in addition to the contrasting breeding conditions, highlights the fact that on a small geographical scale, large differences between populations may be observed and emphasises the fact that foraging behaviour of a species should not be extrapolated from one colony to another.

Indeed population declines of this species have been dramatic and world-wide, and processes that influence the population dynamics of penguins are complex, therefore determining the mechanisms that drive changes is difficult. It is thus imperative that the

factors that may influence survival are understood. The link between seabird survival and food availability around breeding colonies is strong, and it is tempting to link population declines to the effects of climate and changing prey availability, however opportunistic feeders, which demonstrate such a high degree of plasticity in their foraging behaviour, such as Northern Rockhopper penguins, are probably less affected by this than specialist, for example piscivorous, feeders.

This thesis did not aim to address the causal factors of global Rockhopper population declines and I recognise that there are limitations to this study. For example, there could be other measures of stress affecting birds that were not assessed, foraging locations of the birds are unknown, and, importantly, past breeding success is unknown. It is highly likely that a combination of causal factors are involved in population declines of Northern Rockhopper penguins, and of course the absence of baseline data with which to compare change is lacking for this species at Tristan da Cunha. The lack of temporal replication is also problematic; all of the variables investigated show large inter-annual variation in other penguin species. However, in the present study, predation on eggs and chicks by Tristan skuas was a significant causal factor of egg and chick mortality at small, open colonies such as those on Tristan Island. Founded on the lack of sex-based differences in foraging, the change to inshore foraging habitat when free to range farther afield, and absence of signs of stress based on the physiological measures made, I conclude that there are no obvious signs of high levels of stress or food limitations during breeding at Tristan da Cunha.

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