Northern rockhopper penguins prioritise future reproduction over chick provisioning

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ABSTRACT: As iteroparous species, seabirds must balance present against future reproduction. We used stable-isotope (SIA) and stomach content analysis (SCA) to examine the effects of sex, breeding stage, pre-moulting period, ontogeny and chick age on the diet of northern rockhopper penguins (NRP; *Eudyptes moseleyi*) breeding at the Tristan da Cunha archipelago during 2010. Stomach contents were obtained from birds during the guard and crèche stages. $\delta^{15}N$ and $\delta^{13}C$ signatures of whole blood of adults were measured at Tristan Island (during the incubation, guard and crèche stages), and of adult feathers at Tristan and nearby (~38 km) Nightingale Islands. $\delta^{15}N$ and $\delta^{13}C$ signatures of the whole blood of chicks were also measured during both stages. Adult pre-moult diets were significantly enriched in both elements at Nightingale compared to Tristan Islands. Adult female diet was dominated by zooplankton during the guard stage, and in both sexes by fish (predominantly Photichthyidae) in the crèche stage. No effects of chick size within the guard stage, or of sex within the crèche stage were observed on diet composition of adult birds. $\delta^{13}C$ values of adult blood were higher in females relative to chicks, and in crèche relative to guard stage individuals. $\delta^{15}N$ values of blood showed a significant breeding stage × chick age interaction. Sex affected blood $\delta^{15}N$ and $\delta^{13}C$ only in the guard and incubation stages. Stage and sex interacted significantly on $\delta^{13}C$ and $\delta^{15}N$ signatures of adult feathers. NRP were opportunistic foragers, hunting in different areas during breeding and non-breeding periods, with different pre-moult foraging patterns observed in adults from colonies separated by only 38 km. Adults seemed to favour future reproduction, through brood reduction and by feeding chicks lower trophic level prey than they consumed themselves, although provisioning shifted from zooplankton to fish later in the breeding season.

KEY WORDS: Sex-specific foraging · Selective chick provisioning · Breeding stage · Stable isotope analysis · Stomach content analysis · Northern rockhopper penguins

INTRODUCTION

Energy requirements of predators are increased during breeding; the ability of parents to procure resources affects their fitness in terms of provisioning current offspring (offspring growth and condition), and the chance of future reproduction (parental condition) (Hughes 1993). The fitness of parents is determined by a combination of the number and fitness of their offspring (Lalonde 1991). The evolution of different life history strategies is driven by the habitat, which acts as the 'template' against which these strategies evolve (Southwood 1988). However, in dimorphic species the sexes may differ in the constraints under which they operate—including their nutritional requirements (Morehouse et al. 2010). Long-lived species are believed to have evolved fixed levels of reproductive investment (Saether et al.

1993) and generally the potential for future re-production (i.e. adult survival) is assumed to take precedence over present reproductive effort (Mills et al. 2005).

Seabirds forage within a spatially and temporally heterogeneous habitat (Hunt et al. 1999) and can exhibit considerable intra-specific variation in diet to ensure their own survival and successful breeding. This flexibility can manifest itself through changes in foraging areas and in the trophic positions or size-classes of prey (Morrison & Hobson 2004, Polito et al. 2011). On an evolutionary scale, specific life-history traits such as low fecundity, delayed sexual maturity and high adult survivorship (Pianka 1970) allow them to cope with the variability in food availability (Hunt et al. 1999). Nevertheless, their population dynamics are strongly regulated by food availability (Hunt & Schneider 1987). Breeding seabirds are central place foragers (Orians & Pearson 1979). During this period, they are challenged to meet the nutritional require-ments of rapidly growing chicks as well as their own energetic requirements, while foraging is restricted in time and space due to the necessity of returning frequently to the colony to provision the young (Ydenberg et al. 1994). Seabirds are known to alter reproductive behaviour to ensure their own survival over that of their chicks (the 'individual optimisation hypothesis'; Nur 1986) in periods of altered prey availability (Erikstad et al. 1997). For example, Adélie penguins *Pygoscelis adeliae* regulate their own body mass at the cost of reduced chick provi-sioning (Ballard et al. 2010), conforming to a life-history strategy of 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 providing their young with different food than that which is used for self-feeding (Swihart & Johnson 1986). Some species have demonstrated selective provi-sioning of chicks with higher quality prey items (for example, prey that are more enriched in protein or lipid) than those which they themselves digest (Davoren & Burger 1999), and this strongly affects chick growth (Dahdul & Horn 2003). Provisioning of young with higher trophic level prey has been observed in Antarctic petrels *Pterodroma incerta* (Hodum & Hobson 2000), Magellanic penguins *Spheniscus magellanicus* (Forero et al. 2002) and grey-headed albatrosses *Thalassarche chrysostoma* (Richoux et al. 2010).

Flightless seabirds, such as penguins, face an even greater challenge, as they are unable to increase their foraging range substantially when prey (which are typically highly mobile and patchily distributed) are not abundant close to the colony (Davis & Cuth-bert 2001). Within the breeding season, opportunistic foragers such as northern rockhopper penguins *Eudyptes moseleyi* (NRP) can exhibit a high degree of foraging plasticity and dietary shifts (Tremblay & Cherel 2003). While the high energetic requirements of breeding are likely to play an important role in dietary changes of penguins (Gales & Green 1990), the influence of changes in local prey abundance and availability are undoubtedly important (Boersma et al. 2009).

We would expect changes in diet to be most prominent between the breeding season, when for-aging range is constrained, and the non-breeding season, when birds are not tied to breeding sites. For example, there can be a switch between trophic levels, or between a specialist and generalist diet (Hobson et al. 1994). Cherel et al. (2007) docu-mented a widening of the trophic niches of sub-Antarctic penguins, including southern rockhopper penguins *Eudyptes chrysocome*, at the Crozet archi-pelago during winter. Within species, dietary segre-gation may also be pronounced between sexes, not only in sexually size-dimorphic birds (reviewed in Thaxter et al. 2009), but also in size-monomorphic birds (Hamer et al. 2005). Penguins show a slight degree of sexual dimorphism, with males generally having larger beaks. In species such as Magellanic penguins, males are known to prey more on fish than females; a behaviour attributed to their larger bill (Forero et al. 2001, 2002). Sexual dietary differ-ences, through trophic or spatial segregation, are thought to be linked to a reduction in intersexual competition (Forero et al. 2002), with benefits to overall fitness (Schoener 1970).

Stable isotope analysis (SIA) is particularly useful in identifying seasonal and trophic segrega-tion in the diet of seabirds, which are difficult to observe in their natural habitat (Cherel et al. 2007). Organisms are enriched in carbon (C^{13}) and nitrogen (N^{15}) compared to the organisms on which they feed (N^{15} by 3.0–4.0‰, C^{13} by ~1‰; DeNiro & Epstein 1978, 1981, Peterson & Fry 1987). $\delta^{15}N$ can indicate the trophic level at which animals are feeding, while $\delta^{13}C$ can be used to de-termine the carbon sources in a food web (Cherel & Hobson 2005). Diet assimilated over different time scales can be revealed through SIA of differ-ent tissues, which have different metabolic rates, and consequently different isotopic turnover rates (Tieszen et al. 1983). Blood has a rapid turnover

rate of 3–4 wk, while feathers are inert and carry the isotopic signature of the diet during the moulting period. Penguins, however, fast ashore during moult and feather growth, and amino acids for keratin synthesis are derived from protein catabolism from skeletal muscles (Cherel et al. 1994). Being metabolically inert and fixed in their isotopic composition (Hobson & Clark 1992), feathers thus reflect the penguins' pre-moult diet (Mizutani et al. 1992, Hobson & Clark 1992). Together, these are the preferred tissues for avian SIA studies, as they can be sampled without harm to the bird (Bearhop et al. 2002). The combination of SIA and stomach content analyses (SCA), from which dietary information of the last stages of the most recent foraging trip can be obtained, provides considerable time-integrated and taxonomic dietary information (e.g. Karnovsky et al. 2008).

NRP are considered endangered, and more than 80% of the world's population breeds at the Tristan da Cunha archipelago (Cuthbert et al. 2009). Here, we use SIA and SCA to examine how the diet of this marine predator is influenced by the stage of breeding cycle (incubation, guard and crèche), the pre-moult period, the age of offspring and sex of parents. We also take advantage of the existence of distinct breeding colonies to explore colony-level differences in adult foraging patterns.

MATERIALS AND METHODS

Study area and species

Fieldwork was carried out at the Stony Beach colony on Tristan Island (37° 09′ 46″ S, 12° 16′ 09″ W) and on Nightingale Island (37° 09′ 46″ S, 12° 16′ 09″ W) in the Tristan da Cunha Archipelago, South Atlantic Ocean during the breeding season (September to December) 2010. The marine macroplankton faunal composition around the islands reflects a warm subtropical influence (Miller & Tromp 1982).

Egg incubation is carried out by both parents and typically commences at the beginning of September, lasting for 32–34 d. Both birds remain at the nest for the first 12 d; males then depart to sea to feed for the next 12 d whilst females incubate eggs. When males return, they swap roles until hatching. *Eudyptes* penguins display brood reduction (Lack 1966). Typically 2 eggs are laid, exhibiting reversed egg size dimorphism and a unique reversed hatching synchrony (St Clair 1996). Rarely are 2 chicks reared per clutch, and if both eggs hatch, the A-chick usually dies

within days from starvation (Lamey 1990). Of 262 nests monitored at the study colonies, only 4 had 2 chicks, and none supported both chicks beyond the guard stage (J. M. Booth pers. obs.). During the guard stage (24–26 d), males fast ashore and brood the chicks, whilst females forage, returning daily to provision chicks. After this brooding period, when thermally emancipated, chicks form loose crèches and are provisioned by both parents (Williams 1995).

Diet and tissue sampling

Stomach contents were obtained from birds returning from sea, using the water-offloading technique (Gales 1987) on different days during the guard stage (n = 31 females, 4 d) and crèche stage (n = 20 males and 22 females, 5 d). Birds were only flushed twice to minimise stress, so it is possible that complete stomach contents were not obtained (Neves et al. 2006) and for this reason total meal mass was not analysed. Samples were drained through a 0.5 mm sieve (Hull 1999) and frozen at −20°C.

Blood samples were taken from the birds during the 3 breeding stages: incubation (n = 39 females), guard (n = 27 females and 27 males) and crèche (n = 18 females and 19 males). In addition, 8 incubating males from Nightingale Island were opportunistically sampled for blood. A small amount (0.5 ml) of blood was collected from the tarsal vein of each bird using a 25-gauge needle and 3 ml syringe containing no additives (Cherel et al. 2007). Whole blood was frozen at −30°C. 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. 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 (1.1–1.7 kg) were sampled for blood. Fasting behaviour of birds during the incubation stage, and of males during the guard stage, may result in $\delta^{15}N$ enrichment of blood (Cherel et al. 2005a); to minimise this effect, whole blood, which is less affected by fasting compared to plasma, was used for the analysis (Cherel et al. 2005a).

Five back feathers per individual were taken from the same adults as those sampled for blood during the incubation and guard stages at Stony Beach (n = 107), and from birds on Nightingale Island (n = 64) to determine if birds from the 2 islands have different diets outside the breeding season. Feathers were stored in a sealed plastic bag until analysed (Bearhop et al. 2006).

Dietary analysis

In the laboratory, stomach samples were thawed, drained through a 0.5 mm sieve, and total wet weight was obtained in order to obtain percentage contribution of different prey categories. Samples were sorted into the categories macrozooplankton, cephalopods and fish, and then weighed. Samples were examined and sorted under a dissecting microscope to ensure all otoliths, squid beaks and crustacean exoskeletons were removed. Whole prey items of the crayfish *Jasus tristani*, the amphipod *Themisto gaudichaudii*, the fish *Phosichthys argenteus*, an ommastrephid squid and the octopus *Ocythoe tuberculata* were retained for SIA.

Numbers of individual crustaceans consumed were estimated from whole bodies (when present), and from pairs of eyes in digested material (Raya Rey & Schiavini 2005). Cephalopods were identified from lower beaks and enumerated by counting the lower beaks, either free or in buccal masses (Raya Rey & Schiavini 2005). Lower rostral lengths (LRL) of squid beaks were measured under a microscope with an eyepiece graticule, and used in a regression equation to estimate mantle length (ML = −11.3 + 41.36 LRL; Clarke 1986). Fish were identified from otoliths (separated into left and right), and the number of fish consumed was taken to be equal to the greater of left or right otoliths (Silva 1999). Total length (TL) of *Phosichthys argenteus* specimens were calculated from a regression of otolith diameter (OD) (lnTL = ln3.9374 + 0.9549 lnOD; Smale et al. 1995). Highly eroded cephalopod beaks and otoliths were excluded from analysis as they are highly resistant to mechanical and chemical digestion and their contribution to diet can be overestimated (Heezik & Seddon 1989).

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); and beaks and otoliths were compared with material in the reference collection at the Port Elizabeth Museum, South Africa and specimens were confirmed by an expert (M. Smale pers.comm). Otoliths identified as *Vinciguerria* sp., *Phosichthys argenteus* and *Maurolicus muelleri* are closely related and are morphologically similar; therefore key features described in Smale et al. (1995) were used to differentiate among them. The percentage contribution of prey types to diet was described in terms of both number and mass (Duffy & Jackson 1986), and percentage frequency of occurrence of prey types in stomach samples was calculated.

Stable isotope analysis

Surface contaminants were removed from feathers by rinsing them in 2:1 chloroform:methanol at room temperature in a sonicating water bath, followed by 2 methanol rinses, and a final rinse in distilled water (Cherel et al. 2005b). Feathers were dried at 50°C in an oven, and cut into small fragments with stainless steel scissors (Hilton et al. 2006); all feathers from an individual bird were analysed together. Blood and prey items retrieved from stomach contents were dried in an oven at 60°C (Cherel et al. 2007), and then ground to a fine powder with a pestle and mortar. Removal of lipids from whole blood is not necessary for SIA due to the low lipid content (Cherel et al. 2005b). Crayfish were treated to remove carbonates with 1 mol HCL 1^{-1} and rinsed with distilled water (Cherel 2008). Lipids were removed from all powdered prey items using a 2:1 chloroform:methanol soak and rinse and air dried in a fume hood to control for differences in lipid content (Hobson & Cherel 2006).

Blood and feathers have different protein turnover rates, requiring correction before comparison (Hobson & Clark 1992). We used mean blood and feather isotopic discrimination rates for marine birds (obtained from Caut et al. 2009 and Bearhop et al. 2002, respectively) following Dehnhard et al. (2011), where the discrimination factor (diet–blood $\delta^{13}C$ = 0.6‰, $\delta^{15}N$ = 2‰; diet–feather $\delta^{13}C$ = 2‰, $\delta^{15}N$ = 4‰) was subtracted from the values of each tissue. 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, which has a precision of 0.9‰ for $\delta^{15}N$ and 0.13‰ for $\delta^{13}C$. These analyses were performed in the Light Stable Isotope Laboratory in the Archaeology Department, University of Cape Town. Results of isotopic analyses are reported using standard δ notation as ratios relative to international standards (Vienna Pee Dee Belemnite for carbon, Bond & Hobson (2012), atmospheric air for nitrogen) according to the following equation:

$$\delta X = \{(R_{\text{sample}} - R_{\text{standard}})/ R_{\text{standard}}\} \times 1000 \quad (1)$$

where X = ^{13}C or ^{15}N, and R = the ratio of $^{13}C/^{12}C$ or $^5N/^{14}N$. All isotope ratios are reported as means ± SE.

Statistical analysis

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 after transformation). Independent sample t-tests were used to compare percentage mass of cephalopods, fish and zooplankton in: (1) stomach contents of guard females that were provisioning chicks, (2) stomach contents of males and females during the crèche stage, and (3) stomach contents of guard and crèche stage birds. Non-parametric Mann Whitney U-tests were used to compare lengths of ommastrephid squid A (see Table 1), and the fish *Phosichthys argenteus* found in stomach contents of (1) guard and crèche stage females, and (2) male and female birds. One-way ANOVA was used to compare the mass of cephalopods, fish and macrozooplankton in stomach contents of birds foraging on different dates (n = 5), firstly within the guard stage, and secondly within the crèche stage. One-way ANOVA was used to test the effects on $\delta^{15}N$ and $\delta^{13}C$ values of blood and feathers (corrected for tissue discrimination), firstly of breeding stage (fixed factor, 4 levels: moult, incubation, guard and crèche) and sex (fixed factor, 2 levels: male and female), and secondly of breeding stage and age (fixed factor, 2 levels: adult and chick). Independent sample t-tests were used to compare $\delta^{15}N$ and $\delta^{13}C$ values of feathers between Tristan and Nightingale and between male and female feathers from Tristan Island. In addition, the effect of squid size was tested by comparing 3 size classes of ommastrephid squid A: small (mantle length <30 mm); medium (mantle length 60–80 mm); and large (mantle length >120 mm). Stable isotope values from crayfish, photichthyid fish and the 3 size classes of squid were compared using 1-way ANOVA. Student-Newman-Keuls (SNK) tests were used to compare means when post-hoc comparisons were required with an α value of 0.05 in all cases.

RESULTS

Temporal and sex variation in diet composition

In total, 3340 prey items were identified from 73 NRP stomach contents (31 guard females, 22 crèche females, and 20 crèche males), comprising 1373 individual fish, 348 individual cephalopods and 1619 crustaceans (Table 1). Analysing diet by mass, no difference in composition of prey categories was observed between crèche stage adult males and

Table 1. *Eudyptes moseleyi.* Fish, cephalopod and crustacean species (listed alphabetically) found in northern rockhopper penguin stomach contents (n = 73) during the breeding season 2010 at Tristan da Cunha. No. = number of individuals, FO = frequency of occurrence in samples, and %FO = percentage frequency of occurrence

Species	No.	FO	% FO
FISHES			
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
CEPHALOPODS			
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			
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 spp., *Thysanoessa* sp.	1484	57	78.1
Copepoda			
Unknown species	13	9	12.3

females (fish t_{32} = −0.72, p = 0.48; cephalopods t_{32} = −0.26, p = 0.80; zooplankton t_{32} = 0.68, p = 0.50), so these data were pooled.

Guard stage adult female diet was dominated by zooplankton (t_{63} = 9.6865, p < 0.0001). Within this stage, no difference in the proportions of different prey types was found in adult female stomach con-

tents which were provisioning small (<600 g) and large (>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).

Diet of both adult males and females in the crèche stage was dominated by fish ($t_{63} = -15.0929$, p < 0.0001; Fig. 1a). Diet composition was generally stable within stages. Within the guard stage period, date had no effect on the contribution by mass to diet by fish ($t_{14} = -1.73$, p = 0.11), cephalopods ($t_{14} = -0.94$, p = 0.36) or zooplankton ($t_{14} = -1.66$, p = 0.12). The same was true for fish and zooplankton during the crèche stage, while in contrast, foraging date had a significant effect on the percentage contribution by mass of cephalopods to diet ($F_{4, 34} = 3.7645$, p = 0.0122), though the dates did not form separate homogenous groups (SNK p > 0.05), and there was no pattern of overall increase or decrease over this stage.

Zooplankton

The composition of the zooplankton component of diet differed between stages, consisting of copepods, euphausiids, amphipods and fish larvae in the guard stage, but euphausiids, amphipods and copepods in the crèche stage. Data on numbers were not analysed statistically as samples were highly digested so that macrozooplankton were likely to have been under-represented (Neves et al. 2006).

Fish

Fish representing 5 families and at least 11 species were identified. Myctophids were the best represented family, whilst photichthyids were the numerically dominant family (Table 1). *Vinciguerria* sp. and *Phosichthys argenteus* were the most important fish items by number and frequency of occurrence (Table 1). Photichthyid fish were the most important prey group for crèche stage birds and were found in 74% of stomach samples. Fish were not an important prey item for guard stage birds, but formed a greater proportion of prey numbers for crèche stage birds (Fig. 1b). The fish *Phosichthys argenteus* was not eaten by guard stage birds. During the crèche stage, it was taken at body lengths ranging from 35.6 to 57.9 mm, with male birds feeding on larger size classes than females (p = 0.007; Fig. 2a).
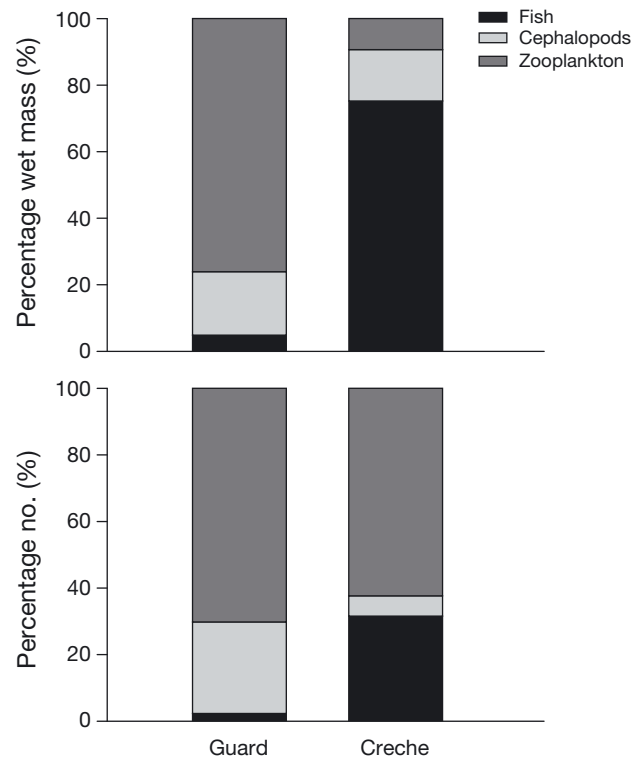

Fig. 1. *Eudyptes moseleyi.* Composition of northern rockhopper penguin stomach contents by (a) percentage of total wet mass and (b) percentage of total number of prey items, at Tristan da Cunha during the guard (females n = 31) and crèche (females n = 22, male = 20) stages in 2010. Sexes pooled for crèche stage

Crustaceans

Euphausiids were important prey in both breeding stages (Fig. 3), and were the most abundant crustaceans in stomach contents (Table 1), comprising *Euphausia spinifera*, *E. lucens* and *Thysanoessa* sp.. The crayfish *Jasus tristani* was more abundant and more frequent in crèche birds than guard birds (Fig. 3). Crustaceans in guard stage samples may have been underestimated as they were in an advanced state of digestion.

Cephalopods

Birds in both stages consumed a similar proportion of cephalopods ($t_{63} = 1.4426$, p = 0.1541; Fig. 1a). Cephalopods were found to contribute more than 50% of stomach content mass in only 3 birds; however, they were an important prey item, contributing to the diet of guard and crèche birds throughout the whole study period (Fig. 1). Guard stage birds con-
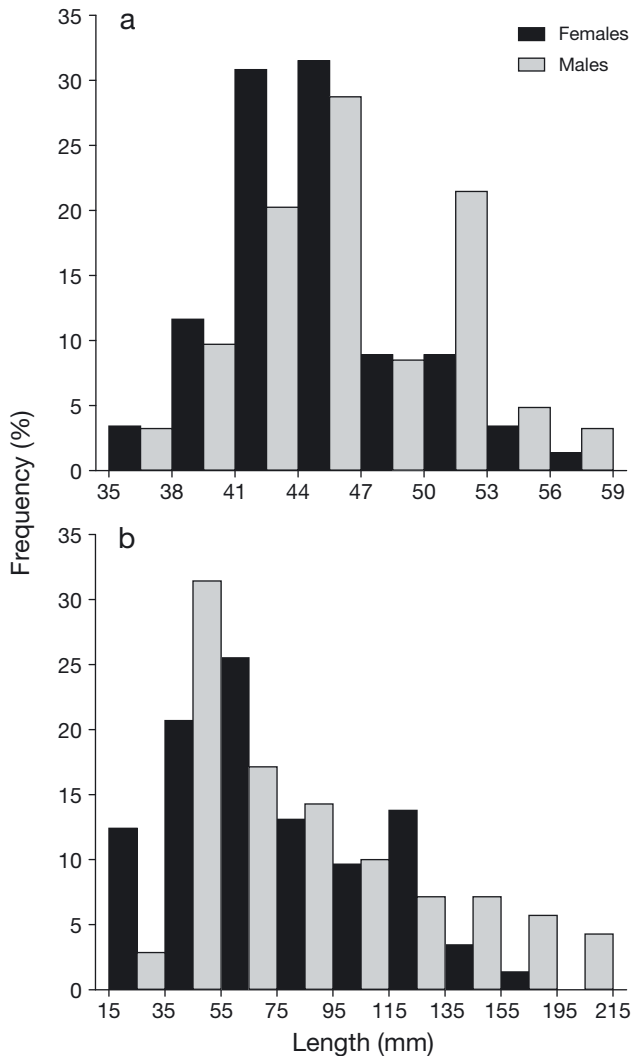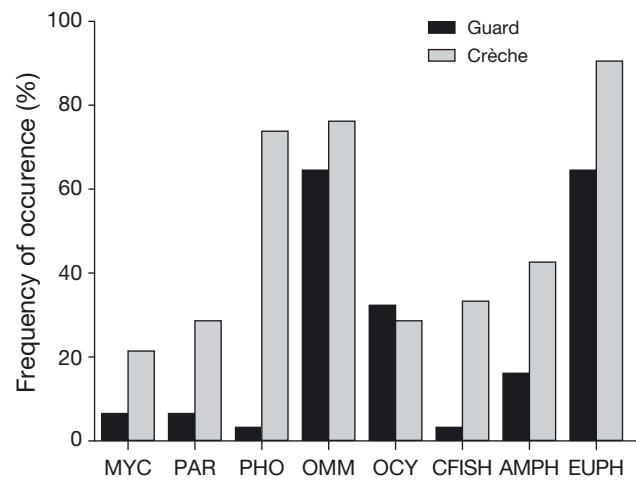
Fig. 3. *Eudyptes moseleyi.* Frequency of occurrence of the main prey types found in stomach contents of guard (n = 31) and crèche (n = 42) stage adult northern rockhopper penguins during the breeding season (fish: MYC = myctophids, PAR = paralepidids, PHO = photichthyids; cephalopods: OMM = ommastrephids, OCY = *Ocythoe tuberculata*; crustaceans: CFISH = *Jasus tristani*, AMPH = amphipods, EUPH = euphausiids)

Fig. 2. *Eudyptes moseleyi.* Size-class distribution of (a) the total length of *Phosichthys argenteus* (n = 393) and (b) the mantle length of ommastrephid squid species A (n = 215) found in stomach contents of crèche stage male (n = 22) and female (n = 20) northern rockhopper penguins during the breeding season

sumed a greater proportion of cephalopods by number than crèche stage birds (Fig. 1b), the most important by both number and frequency of occurrence being a squid in the family Ommastrephidae. This was found in similar proportions in guard and crèche stage birds (Table 1, Fig. 3), as was the octopus *Ocythoe tuberculata* (Fig. 3). The unidentified squid, ommastrephid A, was taken at mantle lengths of 17.6–195.5 mm (Fig. 2b). Guard and crèche stage females consumed ommastrephid squids of similar lengths (17.6–166 mm, U_{72} = 543.50, p = 0.1765), while males consumed larger individuals (U_{140} = 1964.0, p = 0.0428; Fig. 2b).

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

Mean $\delta^{13}C$ and $\delta^{15}N$ signatures of adult and chick blood and feathers are given in Table 2. Significant effects of both age ($F_{1,68}$ = 193.2) and stage ($F_{1,68}$ = 17.7) were observed in $\delta^{13}C$ values of blood (p < 0.0001 in both cases), with no significant interaction ($F_{1,68}$ = 0.0; p > 0.05). Both chicks and females were significantly enriched in $\delta^{13}C$ during the crèche stage compared to their guard counterparts (Fig. 4; SNK p < 0.01). In both stages, females were enriched in $\delta^{13}C$ compared to their chicks (Fig. 4, SNK p < 0.01).

A significant interaction of stage and age was observed for $\delta^{15}N$ values of blood ($F_{1,68}$ = 17.69, p < 0.0001). Guard females were enriched in $\delta^{15}N$ compared to crèche females (SNK p < 0.01), and in both the guard and crèche stage, adult females were enriched in nitrogen compared to chicks (SNK p < 0.01). In the guard stage, this enrichment was approximately 1.9‰ between adults and their chicks (Fig. 4). $\delta^{15}N$ signatures of chicks in the crèche and guard stages did not differ significantly (SNK p > 0.05). Thus, as with $\delta^{13}C$, females were enriched in $\delta^{15}N$ relative to chicks in both breeding stages, but stage had no effect on chicks; and its effect for females was the opposite to $\delta^{13}C$, as guard individuals were relatively enriched.

Table 2. δ^{15}N and δ^{13}C signatures (means ± SE and range; ‰) of adult and chick northern rockhopper penguins *Eudyptes moseleyi*. Samples were taken from blood and feathers during each stage of the breeding and the moulting period of 2010, from Tristan and Nightingale islands (n = number of birds)

| Colony Age	Stage	Sex	Tissue	n	δ^{15}N	Range δ^{15}N	δ^{13}C	Range δ^{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
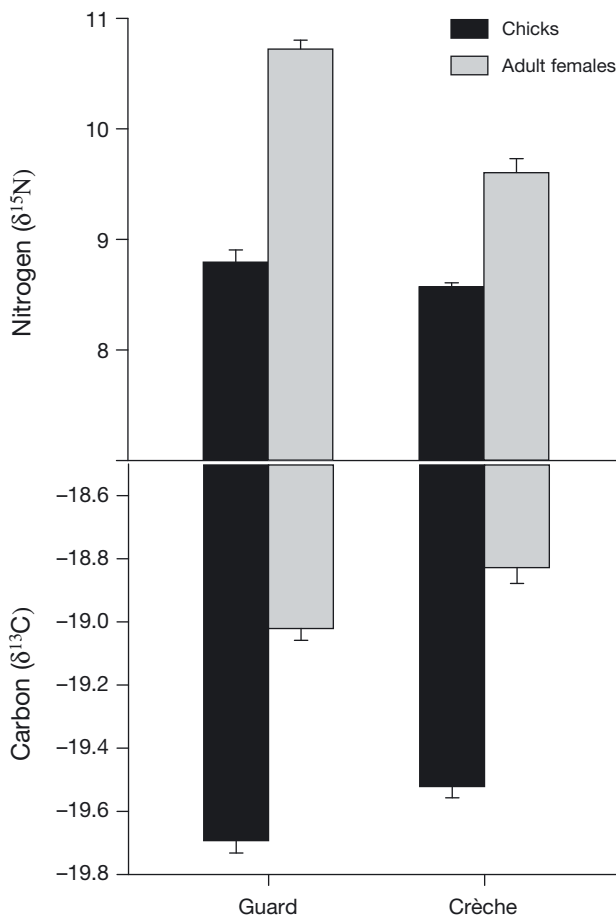
Fig. 4. *Eudyptes moseleyi*. δ^{15}N and δ^{13}C signatures (mean ±SE) of blood of adult and chick northern rockhopper penguins during the guard and crèche stages. Guard females n = 27, crèche females n = 18, guard chicks n = 27, crèche chicks n = 32

Temporal variation in δ^{13}C and δ^{15}N signatures of adult blood and feathers

A significant interaction of stage and sex was observed in carbon ($F_{3,56}$ = 6.7, p = 0.0006) and nitrogen ($F_{3,56}$ = 14.97, p = < 0.0001) blood isotope signatures of adult penguins. Moulting male birds were more enriched in δ^{13}C than males in all other stages (SNK p < 0.01). Moulting females were significantly enriched in δ^{13}C (SNK p < 0.01), and guard females significantly depleted in carbon compared to all other female birds (SNK p < 0.05). All adult blood signatures (representing diet during the breeding season) fell within a narrow δ^{13}C range of −19.02‰ (guard females) to −18.67‰ (crèche males) (Table 2, Fig. 5). Guard males were more enriched and crèche males more depleted in δ^{15}N than males in all other stages (SNK p < 0.01, Fig. 6), and incubating females were enriched in δ^{15}N compared to all other females (SNK p < 0.01, Fig. 6).

Male/female differences differed among stages. In the guard stage, males were significantly enriched in both isotopes compared to females (SNK p < 0.01; Fig. 6), while during incubation the reverse was true (SNK p < 0.01; Fig. 6). There were no sex-based differences in δ^{15}N or δ^{13}C during the crèche or moult stages (SNK p > 0.05).

As there was no significant effect of sex on δ^{13}C (t_{104} = −0.1612) or δ^{15}N (t_{104} = −0.3510; p > 0.05 in both cases) of feathers from Tristan Island birds, these data were pooled and compared with data from Nightingale Island. Nightingale feathers were enriched in both carbon (t_{168} = −35.7891) and nitrogen (t_{168} = −31.6620; p < 0.0001 in both cases) relative
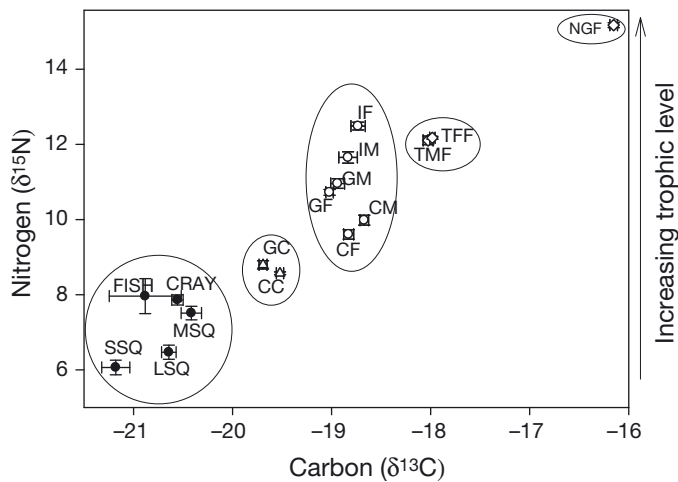
Fig. 5. *Eudyptes moseleyi.* δ¹⁵N and δ¹³C values (mean ± SE) of northern rockhopper penguins from samples of adult blood and feathers, and from chick blood (original values, uncorrected for trophic discrimination). Abbreviations (with sample sizes in brackets): GC = guard chick blood (28); CC = crèche 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)

to feathers from Tristan birds. For both islands, feathers were enriched in δ¹³C relative to blood from incubating birds. Enrichment was by an average of 0.8‰ for Tristan and 0.9‰ for Nightingale (Table 2, Fig. 5). Nightingale feathers had the highest δ¹⁵N signatures of all tissues sampled (15.2‰; Table 2, Fig. 5).

δ¹³C and δ¹⁵N signatures of prey items

A significant effect of body size (measured as mantle length) was found for δ¹⁵N of ommastrephid squid A tissue ($F_{2,29}$ = 16.21, p < 0.0001), with medium-sized individuals being significantly enriched in δ¹⁵N (SNK p < 0.05) compared to small and large individuals (Fig. 5). Small ommastrephid squid A were significantly depleted in δ¹³C compared to medium and large individuals ($F_{2,29}$ = 12.30, p < 0.001; Fig. 5).

Whilst there was a significant effect of prey item on δ¹⁵N ($F_{4,10}$ = 2.27, p < 0.05) — with small ommastrephid squid A individuals being depleted and photichthyid fish enriched in δ¹⁵N compared to other prey items (Fig. 5) — the SNK did not separate prey items into distinct homogenous groups. A significant effect

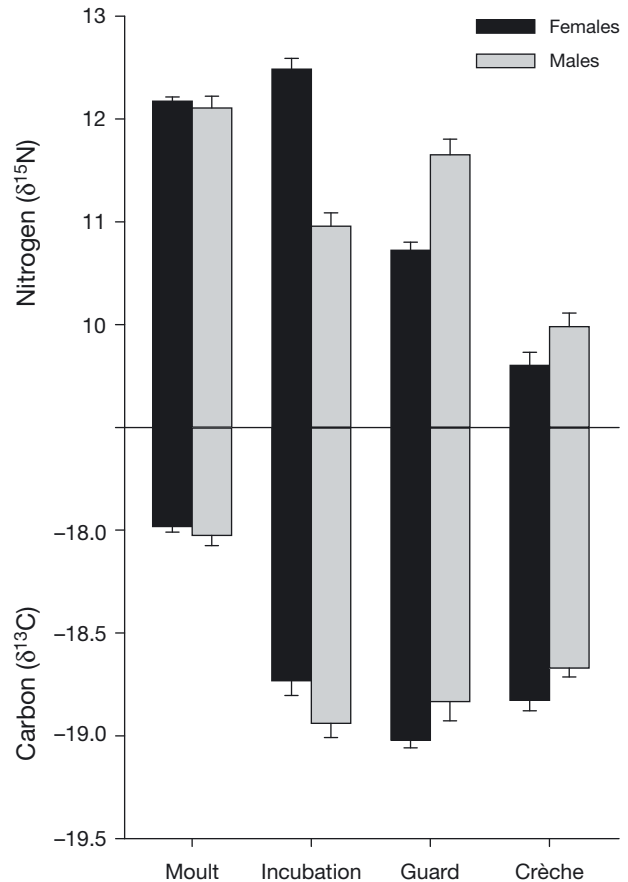Fig. 6. *Eudyptes moseleyi.* δ¹⁵N and δ¹³C signatures (mean ±SE) from adult northern rockhopper penguin blood samples (incubation, guard and crèche stages) and feathers (moult) during the breeding and non-breeding (moult) seasons (original values, uncorrected for trophic discrimination). Incubation: 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

of prey item on δ¹³C was found ($F_{4,10}$ = 3.70, p < 0.05), with small ommastrephid squid A individuals being depleted and medium ommastrephid squid A individuals more enriched in δ¹³C than other prey items (Fig. 5), but again the SNK did not separate these prey items into homogenous groups.

DISCUSSION

Rockhopper penguin diet composition

Breeding NRP feeding around islands in the Atlantic and Indian Oceans are obligatory oceanic foragers, as these islands are volcanic and lack a peri-insular shelf (Tremblay et al. 1997). Unrestricted in feeding on coastal species, birds can forage in

water over 1000 m deep within approximately 5 km of the island. Rockhopper penguins are generally opportunistic feeders (Table 3), and birds at Tristan accord with this behaviour, feeding on a mixture of fish, cephalopods and macrozooplankton. Euphausiids dominate Rockhopper diet by mass at several locations (Table 3), including Gough Island (Klages et al. 1988). *Thysanoessa gregaria*, which has a circumpolar distribution (Mauchline & Fisher 1969), contributed largely to the crustacean component of diet in this study and at Amsterdam (Tremblay et al. 1997, Cherel et al. 1999) and Gough Islands (Klages et al. 1988). Fish typically contribute only a small amount to rockhopper diet at most of their breeding locations (Table 3), although they contribute more to the diet of NRP breeding at Amsterdam Island (both coastal and epi-pelagic photichthyids; Tremblay et al. 1997, Cherel et al. 1999). The predominant fish group preyed upon by penguins at Tristan Island were epi-pelagic photichthyids, namely *Phosichthys argenteus* and *Vinciguerria* sp. Rockhopper penguins at the Tristan archipelago are diurnal feeders (J. M. Booth unpubl. data). It seems unusual that fish that normally migrate into shallower waters at night and reside beyond the diving depth of penguins during the day form a large part of the diet of these birds; however, some invididuals of such species, including *Vinciguerria lucetia* and *V. nimbaria*, remain in surface waters during the daytime (Armstrong & Prosch 1991, Marchal & Lebourges 1996, Cornejo & Koppelmann 2006). Cephalopods, particularly ommastrephid squids, were an important diet component in both breeding stages (guard and crèche), and contribute

similarly to the diet of NRP at Amsterdam Island (Duroselle & Tollu 1977, Tremblay & Cherel 2003) and of southern rockhopper penguins in the Falkland Islands (Croxall et al. 1985). Ommastrephid squids are reported in the diet of other seabirds, including grey-headed albatrosses (Cherel et al. 2002) and Atlantic petrels (Klages & Cooper 1997). The pelagic octopus *Ocythoe tuberculata* was the only octopod species found in stomach contents, and has been reported in the diet of several species of procellariiformes (Imber 1992). Interestingly, the larval Tristan rock-lobster *Jasus tristani*, which is highly abundant around Tristan and fished commercially, was found to occur almost exclusively in the stomachs of crèche stage birds. Rock-lobsters in the area spawn from September to November (T. Glass pers. comm.), so their presence in crèche-stage diet is probably due to opportunistic consumption by birds at times when this prey item is readily available.

Spatial and temporal dietary segregation

Trophic segregation was apparent between the guard and incubation stages of breeding, and spatial segregation was apparent between the breeding and pre-moult period in both colonies. The absence of significant inter-individual variation in isotope signatures within the colonies suggests that birds from the same colony tend to feed on similar prey in similar oceanic areas. This lack of variation has also been observed in the Falkland Islands during the pre-moult period in gentoo penguins *Pygoscelis papua*, whereas Magel-

Table 3. Diet data (percentage by wet mass) collected from southern *Eudyptes chrysocome* and northern rockhopper penguins *E. moseleyi* at various geographic locations. Replicate data for each prey category indicate data collected in different years. ne: not estimated

Species	Location	Cephalopods	Crustaceans	Fish	Source
		Diet composition (%)			
Northern rockhopper	Gough Island	4, 1, 1	94, 92, 90	2, 6, 9	Klages et al. (1988)
Northern rockhopper	Amsterdam Island	50	40	10	Duroselle & Tollu (1977)
		44, 15	31, 21	25, 64	Tremblay et al. (1997)
		44	44	12	Tremblay & Cherel (2003)
Southern rockhopper	Falkland Islands	53	45	2	Croxall et al. (1985)
		50	49	1	Cooper et al. (1990)
		29	53	18	Pütz et al. (2001)
Southern rockhopper	Heard Island	1	60	29	Hull (1999)
Southern rockhopper	Staten Island	74, 87, 67	26, 14, 33	ne	Raya Rey & Schiavini (2005)
Southern rockhopper	Kerguelen Archipelago	1	95	4	Tremblay & Cherel (2003)
Southern rockhopper	Marion Island	1, 3	100, 91	0, 6	Brown & Klages (1987)

lanic and southern rockhopper penguins displayed a large degree of individual variability in both carbon and nitrogen isotope signatures, reflecting different foraging areas and prey, respectively (Weiss et al. 2009). The elevated isotope values in feathers from Nightingale birds compared to birds from ca. 38 km away on Tristan Island indicate spatial segregation or different foraging behaviours during the pre-moult period. The difference in $\delta^{13}C$ values suggests that birds from the 2 islands fed in different areas during the pre-moult period, although differences in $\delta^{13}C$ values may also reflect a difference in prey species targeted, while Nightingale birds also occupied a higher trophic niche. Inter-colony differences in $\delta^{13}C$ and $\delta^{15}N$ have also been observed in Magellanic penguin colonies separated by less than 100 km (Forero et al. 2002). In our study, some birds fed on larger fish (Paralepididae and Myctophidae) for which isotope signatures could not be obtained. 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.

Sexual segregation

The sexual-size dimorphism hypothesis (Clutton-Brock et al. 1987) suggests that males will feed at a higher trophic level, on higher energy content prey, as they have greater energy requirements. In fact, male seabirds do commonly forage at a higher trophic level, and this is linked to their enhanced physiological capabilities including the ability to dive deeper and target higher trophic level prey (e.g. gentoo penguins, macaroni penguins *Eudyptes chrysolophus*, and South Georgian shags *Phalacrocorax georgianus*; Volkman et al. 1980, Bearhop et al. 2006), though trophic segregation in monomorphic species (e.g. northern gannets *Morus basanus;* Lewis et al. 2002) suggests that body size is not the sole factor. We expected such sex-based differences in diet to be most pronounced during the pre-moult stage, as birds are not restricted to foraging close to the colony and are presumably able to take advantage of a wider variety of prey; but this was not observed. In fact our results accord with those of Dehnhard et al. (2011), who found no differences between male and female southern rockhopper penguins (based on SIA analysis of feathers), and Thiebot et al. (2012) who found no effect of sex on whole blood isotopic signatures of eastern rockhopper penguins *Eudyptes filholi* and NRP.

Sexes also differ in their nutritional requirements and, although there is little literature on the energetic costs of egg formation in penguins, there is evidence that it demands a high protein requirement (e.g. Adélie penguins; Astheimer & Grau 1985). Nitrogen isotope signatures of incubating females were higher than for all other groups of breeding birds, indicating that they occupy a higher trophic niche — and this could reflect the assimilation of protein-rich food prior to egg-laying. During the guard stage, males fast and metabolise the endogenous fat and skeletal protein reserves built up during incubation feeding trips so that during this stage, their isotopic signatures reflect food consumed during the incubation stage (Cherel et al. 2005a). Thus, our results suggest that incubating males do not feed on such high trophic level prey as incubating females.

Although no sex-based differences were observed in stomach contents, males tended to prey on larger size classes of squid and fish, presumably because they have larger beaks than females (J. M. Booth unpubl. data). 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). Given the severe reductions in NRP numbers (Cuthbert et al. 2009), present-day competition for food is unlikely. While males did take some larger prey than females, they fed predominantly on the same size classes. Consequently, we suggest that the difference between the sexes reflects opportunistic capture by males of prey beyond the sizes available to females, rather than niche partitioning. Although no large differences in blood carbon signatures were revealed amongst breeding penguins, the possibility that they forage in different areas cannot be excluded as there is no longitudinal $\delta^{13}C$ isoscape within the Southern Ocean (Cherel & Hobson 2007).

Chick provisioning

In this study, chicks in both the guard and crèche stages were significantly depleted in nitrogen compared to their parents (~1.9 and ~1.2‰, respectively). In comparison, Magellanic penguin chicks have been found to have higher nitrogen isotope signatures than their parents, and adults are known to feed chicks a diet containing a larger percentage of fish than they themselves consume (Forero et al. 2002). Conversely, no effect of age on nitrogen isotope signatures has been observed in Adélie, king (*Aptenodytes patagonicus*), emperor (*A. forsteri*),

southern rockhopper or macaroni penguins (Cherel et al. 2007, Cherel 2008, Tierney et al. 2008). The majority of seabird studies have found that adults provision chicks with food of similar or higher trophic levels than the prey used for self-provisioning (e.g. Antarctic fulmarine petrels *Fulmarus glacialoides*, Magellanic and Adélie penguins; Hodum & Hobson 2000, Forero et al. 2002, Cherel 2008). The provisioning of chicks with lower trophic level prey in this study indicates that precedence is given to adult survival, in accord with the individual-optimisation theory (Nur 1986).

Isotopic discrimination factors between seabird adults and their chicks have been linked to the concentration of blood uric acid, a waste product of protein catabolism that is depleted in nitrogen compared to body protein (Bearhop et al. 2000). As chicks have increased catabolism, and should have lower blood urea concentrations than adults, dietary interpretation may be confounded by a reduction of δ^{15}N in chick blood. Earlier studies assumed that age does not affect isotopic fractionation (e.g. Hodum & Hobson 2000), but Sears et al. (2009) found blood nitrogen isotope signatures of rhinoceros auklet *Cerorhinca monocerata* chicks to be depleted in response to rapid growth and moderate nutritional stress (a decrease of ~0.92‰ in δ^{15}N). We found no relationship between the body mass and nitrogen signatures of chicks (r = −0.2208, p = 0.0988, n = 57), suggesting that chick age was not a confounding factor.

The fact that carbon isotope signatures of chicks were depleted compared to their parents suggests that adults foraged in different oceanic areas for self-provisioning purposes in both breeding stages. This has also been observed in Magellanic (Forero et al. 2002) and Adélie penguins (Tierney et al. 2008), with adults and chicks displaying significantly different carbon isotope signatures. This is probably only possible through temporal segregation, i.e. feeding for self-maintenance at the beginning of a foraging trip, and for chicks towards the end. Foraging trips of NRP at Tristan last on average 15 to 18 h (J. M. Booth unpubl. data), 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 the crèche stage, it would appear that these are the items on which adults were feeding. Less digested material brought ashore was predominantly squid in the guard stage and squid and crustaceans in the crèche stage, suggesting these were the food items selected towards the end of the foraging trips for chick provisioning.

The shift from a zooplankton-dominated diet in the guard stage to a euphausiid-fish- (predominantly small photichthyids) dominated diet in the crèche stage was distinct. 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 southern rockhopper penguins at Marion Island (Brown & Klages 1987) and Adélie penguins in Antarctica (Lyver et al. 2011). Chick growth rate is influenced by calorific content of prey (e.g. 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). Cephalopods have a lower nutritional value than crustaceans and fish (Heath & Randall 1985), and this is possibly why birds do not feed solely on cephalopods despite their high frequency of occurrence in stomach contents throughout the breeding season. Daily energy requirements of rockhopper chicks increase from ca. 211 kJ d^{-1} during the first week after hatching to ca. 1170 kJ d^{-1} halfway through the growth period (Brown 1987). Despite this, there was no evidence of a switch to higher energy content prey between small and large chicks during the guard stage. Thus, the energy requirements of chick-rearing seem unlikely to be responsible for the dietary shift observed between breeding stages; rather, changes in local prey abundance may have been the cause. This may partially reflect the life history of the fish. The abundance of demersal and pelagic fish differs between nearshore and offshore waters (Barrera-Oro 2002), but many fish show seasonal or ontogenetic changes in diet (e.g. Schafer et al. 2002, Llopiz & Cowen 2009) and this could affect their availability to predators. For example, Southern Ocean nototheniid fish can show seasonal shifts between benthophagy and vertical migrations for pelagic feeding (Casaux et al. 1990). Ainley et al. (1996) observed a similar dietary shift in the foraging behaviour of the common murre *Uria aalge*, with birds targeting euphausiids and juvenile rockfish *Sebastes* spp. early in the year and predominantly targeting rockfish later in the year once these prey had attained a larger size.

We did not analyse the stable isotopes of zooplankton prey as they were always highly digested. Two more intact specimens of *Themisto gaudichaudii* obtained from stomach contents had mean carbon and nitrogen isotope values of −21.7 and 5.1‰, respectively, confirming the lower trophic level of crustaceans. Cherel (2008) found that carbon and nitrogen values were reduced in digested *Euphausia*

superba, attributing this to an increase in the relative importance of pure chitin, since exoskeleton material is more resistant to digestion than soft tissues and has a higher C/N ratio than protein and whole crustaceans from which lipids have been removed (Smyntek et al. 2007). Treatment of samples to remove lipids and carbonates may also have altered isotope signatures as lipid extraction can result in higher carbon isotope values, and acidification in lower carbon and nitrogen isotope values (Stenroth et al. 2006). Given the problems of specimen treatment and their partially digested state, nitrogen values measured for these crustaceans may have been artificially low.

Both analyses of stomach contents and of stable isotope signatures have limitations; but the combination of both techniques has allowed us to identify the importance (or at least the possibility) of dietary segregation at different temporal and spatial scales. As marine predators foraging in a highly variable environment, *Eudyptes* penguins have evolved as generalists, yet among the most remarkable of our findings was the absence of strong sex-linked differences or inter-individual variability within colonies. This was in striking contrast to the differences observed in feather isotope signatures for birds from 2 colonies only 38 km apart. A second key finding, in keeping with the concept of unpredictable food sources, was that adult NRP appeared to favour future over present reproductive effort by provisioning chicks with lower trophic level food than prey used for self-provisioning. We assume this was achieved by foraging for themselves at the start of a foraging bout and for chicks towards the end. This was tempered by a switch from a low-calorific content zooplankton-dominated diet for chicks in the guard stage to a higher quality euphausiid/fish (predominantly small photichthyids) diet in the crèche stage when the chicks were older. As long-lived species living in an unpredictable environment, NRP have evolved a reproductive approach that emphasises future reproduction, through brood reduction and favouring self over chick provisioning.

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