

Subtle but significant segregation in the feeding ecology of sympatric penguins during the critical pre-moult period

T. Otto Whitehead^{1,*}, Maëlle Connan², Yan Ropert-Coudert³, Peter G. Ryan¹

¹Percy FitzPatrick Institute of African Ornithology, DST-NRF Centre of Excellence, University of Cape Town, Rondebosch 7701, South Africa

²Department of Zoology, Nelson Mandela Metropolitan University, Port Elizabeth 6031, South Africa

³Station d'Écologie de Chizé-La Rochelle, UMR 7372, CNRS, Centre d'Etudes Biologiques de Chizé, 79360 Villiers-en-Bois, France

ABSTRACT: Niche partitioning plays an important role in minimising interspecific competition for resources. Using carbon and nitrogen stable isotopic analysis of feathers, we investigated how macaroni penguins *Eudyptes chrysolophus* and eastern rockhopper penguins *E. chrysocome filholi* breeding at the Prince Edward Islands partition the marine environment during the critical pre-moult period over 5 consecutive years (2011 to 2015). Both species consistently foraged immediately south of the Antarctic Polar Front, with macaroni penguins foraging farther south in years of reduced primary productivity, minimising spatiotemporal overlap between species. Macaroni penguins consistently foraged at a higher trophic level than rockhopper penguins, indicating trophic niche differentiation. Male rockhopper penguins fed at a higher trophic level than females, but macaroni penguins showed no differences between sexes. The observed dietary differences, partial allochrony and species-specific spatial responses to reduced primary productivity (i.e. prey availability) indicate that macaroni and eastern rockhopper penguins exhibit contrasting foraging strategies that limit interspecific competition during the pre-moult period.

KEY WORDS: Niche partitioning · Crested penguins · Stable isotopes · Pre-moult

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The ecological niche is defined as the role or position of a species in its environment, particularly with respect to the utilisation of resources needed to survive and reproduce. In cases where sympatric species compete for a limited resource, ecological theory predicts that each species should evolve to exploit different parts of the resource (Hardin 1960). Niche partitioning may occur along several ecological axes (Hutchinson 1957), including spatial (e.g. horizontal or vertical segregation), temporal (e.g. asynchrony in peak resource use) and/or trophic (e.g. dietary differences) (Trivelpiece et al. 1987, Nakano et al. 1999, Field et al. 2005).

Penguins (Spheniscidae) are one of the most numerous mesopelagic predators in the Southern Ocean, consuming more than 23 million tonnes of marine resources every year (Brooke 2004). The most diverse and abundant group is the crested penguins (genus *Eudyptes*), ranging from temperate to Antarctic climates (Williams 1995). Eudyptids breed sympatrically at many localities, usually involving the smaller rockhopper *E. chrysocome* and the larger royal *E. schlegeli*, erect-crested *E. sclateri* or macaroni penguins *E. chrysolophus* (Warham 1975). Most eudyptids occupy broadly similar ecological niches, feeding primarily on swarming crustaceans and myctophid fish in offshore waters (Cooper et al. 1990). Owing to the high potential for interspecific competi-

tion for resources, sympatric eudyptids provide a good model by which to explore niche partitioning.

Approximately 302 000 and 80 000 pairs of macaroni and eastern rockhopper penguins *E. chrysochrome filholi*, respectively, breed at the Prince Edward Islands in the Indian Ocean sector of the Southern Ocean (Crawford et al. 2009). During the breeding season, diet and foraging areas overlap considerably (Brown 1987, Brown & Klages 1987, Adams & Brown 1989), with the only major difference being that macaroni penguins commence breeding 2 to 3 wk earlier (Brown 1987). This difference extends into the pre-moult period, when penguins must head to sea to replenish body condition and fatten up for their annual moult ashore. As penguins lose 40 to 50% of their body mass during the moult (Brown 1986), finding sufficient resources during the pre-moult foraging trip is critical, as failure to do so may impact survival. In such circumstances it is expected that each species should maximise foraging gain by adopting strategies that minimise energetic costs, such as avoiding interspecific competition for resources. Although macaroni and rockhopper penguins exhibit similar habitat preferences during their month-long foraging trip to waters 400 to 1000 km south of the islands, temporal segregation exists for the initial 2 to 3 wk (Whitehead et al. 2016). However, potential interspecific competition may still occur while both species are concurrently at sea, particularly in years when reduced productivity prolongs temporal overlap (Whitehead et al. 2016). It is possible that trophic niche differentiation may further reduce interspecific competition for resources; however, few studies have focused on dietary comparisons during this period (Jaeger & Cherel 2011).

The use of stable isotopes as natural tracers allows us to reconstruct the diet and quantify the isotopic niche of penguins during the pre-moult foraging trip (Cherel & Hobson 2007, Newsome et al. 2007). In marine food webs, the 2 most commonly used isotopic markers are carbon and nitrogen, which provide information on prey origin and trophic level, respectively (Cherel & Hobson 2007). The ratio of carbon-13 to carbon-12 ($\delta^{13}\text{C}$) varies according to inshore versus offshore, benthic versus pelagic and, in the Southern Ocean, low-latitude versus high-latitude food webs, thus providing information on the broad-scale foraging areas of predators (Cherel & Hobson 2007). $\delta^{13}\text{C}$ is also an indicator of ecosystem productivity due to phytoplankton-driven changes in baseline $\delta^{13}\text{C}$ of the food web (Hilton et al. 2006, Jaeger & Cherel 2011). The ratio of nitrogen-15 to

nitrogen-14 ($\delta^{15}\text{N}$) varies according to trophic level, with higher-order consumers having higher $\delta^{15}\text{N}$ than lower-order consumers within a given geographic area (Cherel & Hobson 2007). The turnover rate of an isotopic marker in consumer tissues is tissue-dependent, but as feathers remain chemically inert once grown and start developing while penguins are at sea (Cherel et al. 2005), they provide useful information on the diet during the pre-moult foraging trip.

In this study, we used stable isotope ratios in the feathers of macaroni and rockhopper penguins at the Prince Edward Islands to investigate niche partitioning during the pre-moult period over 5 consecutive years (2011 to 2015). As knowledge of how penguins respond to fluctuations in the marine environment is vital to better understand their susceptibility to ecosystem changes, we also investigated inter-annual variation of stable isotopic ratios and niche overlap in relation to at-sea conditions. We predicted that in years of reduced prey availability, penguins would adopt strategies that minimise competition for resources.

MATERIALS AND METHODS

Data collection

Marion Island (47.0° S, 37.9° E), the larger of the 2 Prince Edward Islands, supports 96 and 53% of the breeding population of macaroni and rockhopper penguins, respectively (Crawford et al. 2009). Penguins depart for the pre-moult foraging trip in mid-February and early March, respectively, returning in late March or in April (Whitehead et al. 2016). Feathers were collected in April from moulting penguins at 2 colonies, Funk Bay and Ship's Cove, in April 2012, 2013 and 2015. As melanin is known to influence the isotopic values of feathers (Michalik et al. 2010), only white feathers were used in this study. Old and new feathers were plucked from the breast of each individual and stored in sealed bags at room temperature until laboratory analysis. As feathers remain chemically inert after synthesis, old and new feathers represent the previous and current year's pre-moult foraging trip, respectively (Jaeger & Cherel 2011). Thus, we could investigate the diet of the eudyptids in 2011, 2012, 2013, 2014 and 2015. Each bird was sexed in the field according to bill morphometrics (Williams & Croxall 1991, Poisbleau et al. 2010), except in 2013, when the sex of macaroni penguins was not recorded.

Stable isotope analysis

All preparations and analyses were conducted at the Stable Light Isotope Unit, University of Cape Town, South Africa. Prior to isotopic analysis, each feather was washed in a glass tube containing a 2:1 solution of chloroform:methanol in an ultrasonic bath for 5 min to remove any surface lipids and impurities. The feather was further rinsed in methanol and distilled water, then placed in the oven to dry (40°C for 48 h). As isotopic values in the distal half of each feather represent the diet of each penguin while at sea (Bearhop et al. 2002), only the distal half of each feather was cut and homogenised using scissors. Sub-samples (~0.4 mg) were weighed in tin cups on a microbalance to the nearest microgram. The relative abundance of stable carbon and nitrogen isotopes were determined by combusting samples in a Flash 2000 organic elemental analyzer and passing gasses through a Delta V Plus isotope ratio mass spectrometer via a ConFlo IV gas control unit (Thermo Scientific). Replicate measurements of internal laboratory standards indicated minimal standard deviations (Merck gel: $\delta^{13}\text{C} = 0.2\text{‰}$, $\delta^{15}\text{N} < 0.1\text{‰}$; valine: $\delta^{13}\text{C} = 0.1\text{‰}$, $\delta^{15}\text{N} = 0.1\text{‰}$; seal bone: $\delta^{13}\text{C} = 0.2\text{‰}$, $\delta^{15}\text{N} < 0.1\text{‰}$). All internal standards were calibrated against International Atomic Energy Agency standards. Carbon is expressed in terms of its value relative to Vienna PeeDee Belemnite, while nitrogen is expressed in terms of its value relative to atmospheric nitrogen.

Environmental data

Surface chl *a* concentrations (mg m^{-3}) derived from Aqua Moderate Resolution Imaging Spectroradiometer (MODIS) data and sea surface temperature (SST) anomalies (°C) derived from NASA Jet Propulsion Laboratory multi-scale ultra-high resolution (JPL MUR) data were downloaded as monthly composites from the NOAA CoastWatch website (<http://coastwatch.pfeg.noaa.gov>). As conditions leading up to the pre-moult period may influence prey availability, mean chl *a* concentrations (proxy for primary productivity) and mean SST anomalies within a region corresponding to the core pre-moult foraging area of penguins (49° to 54° S, 33° to 40° E; Whitehead et al. 2016) were calculated for January, February and March each year. Means were calculated using the 'calc' function in the R package 'raster'. Sea surface height data were downloaded from AVISO (<http://www.avis0.altimetry.fr>) and the southern extent of the

Antarctic Polar Front (APF) was characterised using the -0.63 m contour following Swart et al. (2010).

Statistical analysis

Data were analysed using R (version 3.1.0; R Development Core Team 2016). To validate our assumption that old and new feathers represented the previous and current pre-moult periods, respectively, linear models (LMs) were used to test for the influence of feather age on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in feathers representative of the 2012 pre-moult foraging trip. Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between species and sexes were determined using linear mixed-effects models (LMEs) (R package 'nlme'; Pinheiro et al. 2013). To account for repeated observations from the same individual (old and new feathers) and the potential influence of sampling year, individual identity nested within year was included as a random effect. Within-year comparisons of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between species and sexes were made using LMs. All model residuals were checked to ensure normality. Bivariate ellipses were used to plot the isotopic niche of each species using the stable isotopes Bayesian ellipses in R (SIBER) package (Jackson et al. 2011). The total and core niche space and overlap between species were calculated within each year and overall, with a correction for small sample sizes. To investigate potential drivers of inter-annual variation, mean annual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of each species were modeled in relation to mean primary productivity and SST anomalies during January, February and March using LMs. Models were weighted by the standard deviation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ each year to account for differences in variation between years. Model selection was performed according to Akaike's information criterion (AIC), with the most parsimonious of the top models ($\Delta\text{AIC} < 4$) selected. To reconstruct the diet of penguins during the pre-moult foraging trip, a 2-source isotopic mixing model was used (Phillips 2001, Forero et al. 2002). This simplistic approach uses a single isotopic axis to estimate the relative contribution of 2 sources to consumer diet. This method was preferred over the 2-axis approach ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) as the $\delta^{13}\text{C}$ values of available isotopic data of potential prey species were unlikely to be representative of prey at known foraging areas south of the APF (Whitehead et al. 2016). Mean $\delta^{15}\text{N}$ values of the 2 dominant prey groups, crustaceans and fish (Adams & Brown 1989), were calculated based on available isotopic data (Table S1 in the Supplement at www.int-res.com/articles/suppl/m565p227_suppl.pdf).

Owing to the potential variation of isotopic signatures within prey groups (i.e. according to species, life history stage, year, locality), $\delta^{15}\text{N}$ values used are not definite but approximate. The following equation was used to estimate relative contribution of fish in the diet from isotopic data:

$$P_A (\%) = 100 \times (D_T - D_B) / (D_A - D_B) \quad (1)$$

where P_A is the proportion of source A in consumer diet, D_T is the isotopic value of consumer tissue, D_A and D_B are the isotopic values of sources A and B (fish and crustaceans), respectively, after the addition of discrimination factors. We used the mean of known discrimination factors for feathers derived from captive penguin studies: $\Delta^{15}\text{N}_{\text{diet-feathers}} = 4.1 \pm 0.7\%$ (Table S2; Connan et al. 2016).

RESULTS

Feathers were collected from 28 macaroni and 40 rockhopper penguins, representing 53 and 80 pre-moult foraging trips, respectively. Feather age (old

and new) had no influence on isotopic signatures of macaroni ($\delta^{13}\text{C}$: $t = -2.0$, $p = 0.07$; $\delta^{15}\text{N}$: $t = 1.1$, $p = 0.29$) and rockhopper penguins ($\delta^{13}\text{C}$: $t = 1.4$, $p = 0.19$; $\delta^{15}\text{N}$: $t = 0.2$, $p = 0.86$). No correlations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were present in macaroni ($t = -0.3$, $p = 0.75$) or rockhopper penguins ($t = 0.01$, $p = 0.99$).

Interspecific differences

Overall, the core isotopic niche space of both species was $0.39\% ^2$ with an overlap of $0.01\% ^2$ (Fig. 1). Within-year comparisons also revealed minimal overlap (Fig. 1; Table S3 in the Supplement). Feathers of rockhopper penguins were $\delta^{13}\text{C}$ enriched by $0.2 \pm 0.0\%$ (mean \pm SD) compared to those of macaroni penguins ($t = 6.2$, $p < 0.001$; Table 1). This difference varied between years, being similar in 2012, 2013 and 2015, but significantly higher in rockhopper penguins in 2011 and 2014 (Fig. 1). Overall, feathers of macaroni penguins were $\delta^{15}\text{N}$ enriched by $0.8 \pm 0.1\%$ compared to rockhopper penguins ($t = 9.7$, $p < 0.001$; Table 1). This difference varied between

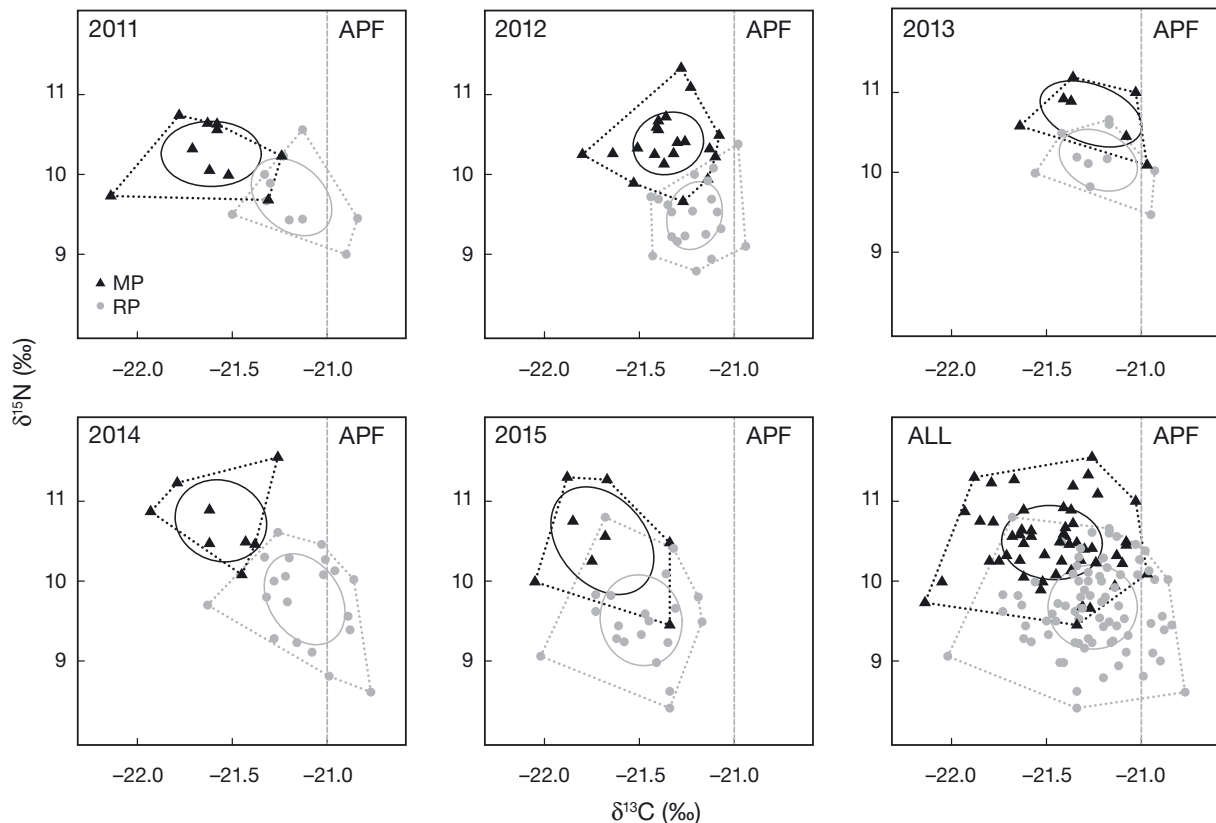


Fig. 1. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios in feathers of macaroni (MP; ▲) and eastern rockhopper (RP; ●) penguins. Total isotopic niche space (dotted convex hulls), core isotopic niche space corrected for small samples size (solid ellipses) and the estimated position of the Atlantic Polar Front (APF) (dashed vertical grey line) are shown

Table 1. Summary of carbon and nitrogen stable isotope ratios in feathers of macaroni and eastern rockhopper penguins in 2011 to 2015 and the estimated percentage of fish in the diet (mean \pm SD (range)) (see 'Materials and methods')

Species	Sex	N individuals (n feathers)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N ratio	Percentage of fish in diet
Macaroni penguin	Male	10 (20)	-21.5 ± 0.2	10.5 ± 0.5	3.12 ± 0.03	51 ± 11 (30–77)
	Female	8 (16)	-21.5 ± 0.3	10.3 ± 0.5	3.12 ± 0.03	46 ± 12 (25–70)
	Both	28 (53)	-21.5 ± 0.3	10.5 ± 0.5	3.12 ± 0.03	50 ± 11 (25–77)
Eastern rockhopper penguin	Male	21 (42)	-21.3 ± 0.2	9.9 ± 0.4	3.11 ± 0.02	37 ± 11 (19–58)
	Female	19 (38)	-21.2 ± 0.2	9.4 ± 0.5	3.11 ± 0.02	23 ± 11 (0–43)
	Both	40 (80)	-21.3 ± 0.2	9.7 ± 0.5	3.11 ± 0.02	30 ± 13 (0–58)

years, being similar in 2011 and 2013, but significantly higher in macaroni penguins in 2012, 2014 and 2015 (Fig. 1). $\delta^{15}\text{N}$ of both macaroni and rockhopper penguins reflected a mixed diet of crustaceans and myctophid fish, with macaroni penguins consuming ~40% more fish (Table 1).

Sexual differences

Macaroni penguins exhibited no sexual differences in $\delta^{13}\text{C}$ ($t = 0.8$, $p = 0.45$) or $\delta^{15}\text{N}$ ($t = 0.5$, $p = 0.65$) overall, or in any within-year comparison when data were available (Fig. 2; Table S4). Rockhopper penguins also lacked sexual differences in $\delta^{13}\text{C}$ overall ($t = 0.5$, $p = 0.62$) and in each year (Fig. 2; Table S4), but $\delta^{15}\text{N}$ of male rockhopper penguins was 0.6 ± 0.1 ‰ higher than females ($t = 5.8$, $p < 0.001$). This was consistent for all within-year comparisons except in 2011, when both sexes had similar $\delta^{15}\text{N}$ values (Table S4).

Inter-annual variation

Primary productivity was higher in 2012 and 2013 compared to other years (Fig. 2). Mean annual $\delta^{13}\text{C}$ of macaroni penguins was significantly related to primary productivity in January and February (Table S5), with February explaining the most variation. When years were categorised into high and low productivity in February ($>0.30 \text{ mg m}^{-3}$ versus $<0.22 \text{ mg m}^{-3}$), feathers of macaroni penguins were $\delta^{13}\text{C}$ enriched by 0.3 ± 0.1 ‰ in more productive years ($t = 5.2$, $p < 0.05$). There were no significant trends in $\delta^{15}\text{N}$ of macaroni penguins or $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of rockhopper penguins in relation to primary productivity (Table S5). Similarly, there were no trends in mean annual $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ in relation to SST anomalies (Table S5). When years were categorised into cooler ($<0^\circ\text{C}$) and warmer ($>0.4^\circ\text{C}$) SST anomalies in Feb-

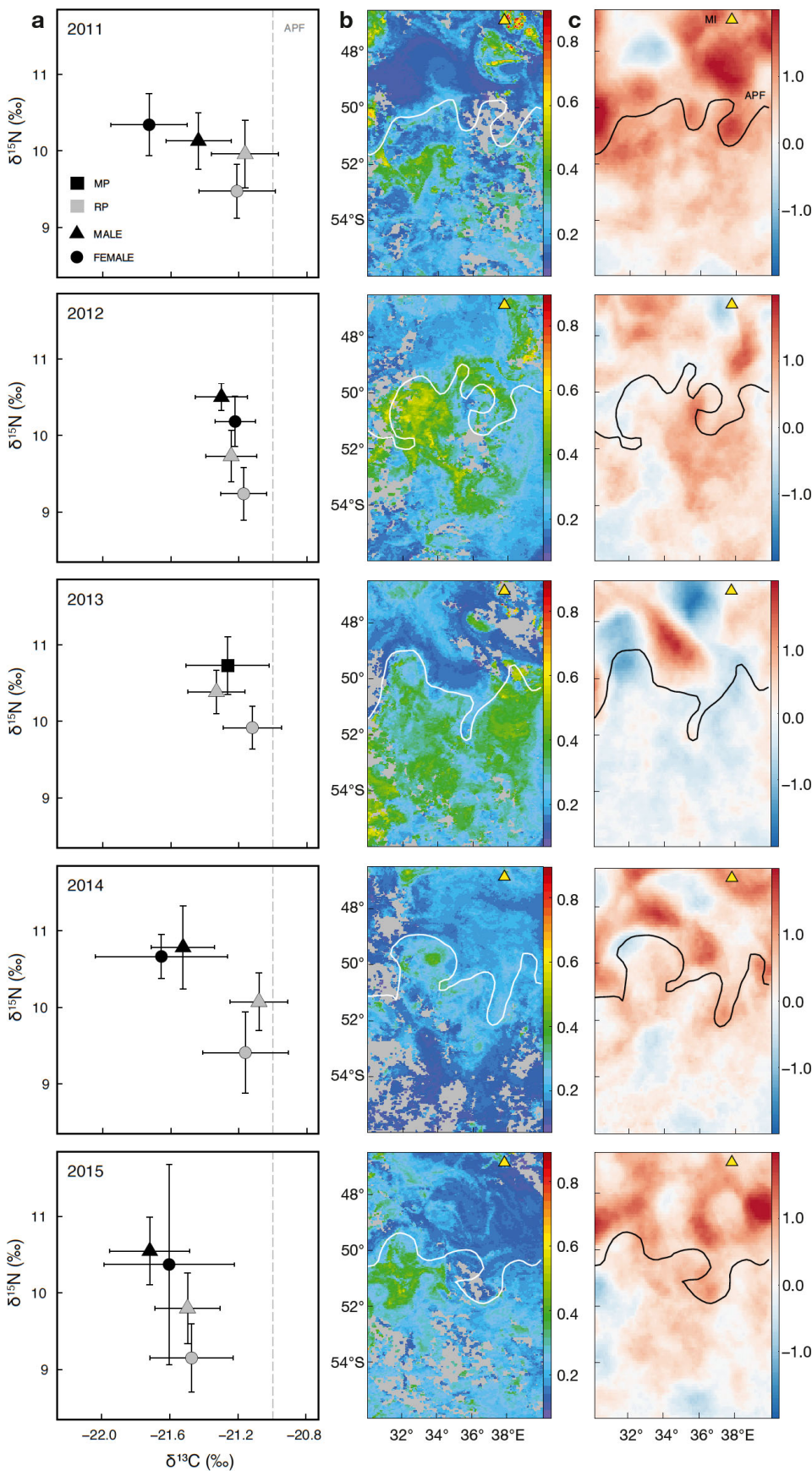
ruary, feathers of rockhopper penguins were $\delta^{15}\text{N}$ enriched by 0.5 ± 0.2 ‰ in cooler years, i.e. 2013 ($t = 3.2$, $p < 0.05$). $\delta^{13}\text{C}$ of macaroni penguins was significantly higher in 2012 and 2013 than 2011 and 2015, with 2014 intermediate between these 2 sets of years (Table 2). There were no inter-annual differences in $\delta^{15}\text{N}$ of macaroni penguins (Table 2). $\delta^{13}\text{C}$ of rockhopper penguins was significantly lower in 2015 than other years, and $\delta^{15}\text{N}$ was significantly higher in 2013 compared to 2012 and 2015, but similar for other comparisons (Table 2).

DISCUSSION

Our study provides evidence for trophic niche differentiation between macaroni and rockhopper penguins during the pre-moult period. By monitoring resource use over 5 successive years, we were able to compare trophic niches across a range of environmental conditions, revealing species-specific responses to changes in primary productivity. However, niche overlap was very limited, irrespective of inter-annual variation in productivity in their preferred foraging areas. Ours is also the first study to reveal consistent sexual differences in the diet of rockhopper penguins during the pre-moult foraging trip, which contrasts with the lack of sexual differences among macaroni penguins. Potential reasons for these inter- and intraspecific niche differences are discussed below.

Trophic differences

The consistently higher trophic level at which macaroni penguins foraged during this study is indicative of a greater proportion of myctophid fish in the diet and/or potentially larger prey, as larger individuals of some fish and crustaceans may be $\delta^{15}\text{N}$ enriched



(Pakhomov et al. 1994, Schmidt et al. 2003). This concurs with dietary comparisons during the breeding season at Marion Island and at other localities, where macaroni penguins may consume nearly twice as much fish as rockhopper penguins (Cooper et al. 1990). The size class of myctophids consumed may also differ; for example, at the Crozet Islands, macaroni penguins feed mainly on adult myctophids whereas rockhopper penguins mainly target post-larval myctophids (Cherel et al. 2010). Dietary differences may be linked to vertical distribution of prey, as macaroni penguins spend more time foraging at >80 m (22%) than rockhopper penguins (4%) during the pre-moult foraging trip (Whitehead et al. 2016). Diving deeper may increase encounters with myctophid fish, as macaroni penguins at Heard Island consumed more fish when performing deeper dives (Deagle et al. 2008), and specialist myctophid-feeding king penguins *Aptenodytes patagonicus* dive to considerably deeper depths (Adams & Brown 1989; 100 to 200 m, Scheffer et al. 2012). Bill size might also influence prey selection; macaroni penguins have larger bills (Agnew & Kerry 1995) that may better

Fig. 2. (a) Carbon and nitrogen stable isotopes in feathers of male (triangle) and female (circle) macaroni (MP; black) and eastern rockhopper (RP; grey) penguins representative of diet during the pre-moult foraging trip across 5 consecutive years. Mean (b) chl a concentrations (mg m^{-3}) and (c) SST anomalies ($^{\circ}\text{C}$) during February each year, showing positions of the APF and Marion Island (MI; \blacktriangle)

Table 2. Carbon and nitrogen stable isotope ratios in feathers of macaroni and eastern rockhopper penguins (mean \pm SD). ^{a,b}Different letters indicate significant within-species inter-annual differences ($p < 0.05$)

Species	Year	N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N ratio
Macaroni penguin	2011	10	-21.6 ± 0.3^a	10.3 ± 0.4	3.12 ± 0.02
	2012	20	-21.4 ± 0.2^b	10.4 ± 0.4	3.12 ± 0.03
	2013	7	-21.3 ± 0.2^b	10.7 ± 0.4	3.10 ± 0.01
	2014	8	-21.6 ± 0.2^{ab}	10.8 ± 0.5	3.15 ± 0.03
	2015	8	-21.7 ± 0.3^a	10.5 ± 0.6	3.13 ± 0.03
Eastern rockhopper penguin	2011	10	-21.2 ± 0.2^b	9.7 ± 0.5^{ab}	3.10 ± 0.01
	2012	20	-21.2 ± 0.1^b	9.5 ± 0.4^a	3.12 ± 0.02
	2013	10	-21.2 ± 0.2^b	10.2 ± 0.4^b	3.12 ± 0.03
	2014	20	-21.1 ± 0.2^b	9.8 ± 0.6^{ab}	3.11 ± 0.02
	2015	20	-21.5 ± 0.2^a	9.5 ± 0.6^a	3.13 ± 0.02

equip them to handle larger prey. Ultimately, dietary differences between macaroni and rockhopper penguins appear to be a function of body size, as larger-bodied animals are able to load greater O_2 stores and have lower mass-specific metabolic rates (Schreer & Kovacs 1997), presumably resulting in species-specific optimal foraging depths, such as with pygoscelids (Wilson 2010). Being larger, macaroni penguins also have higher energetic requirements (Brown 1989) and thus it may be more energy-efficient to feed on energy-dense, lipid-rich myctophids (Cherel & Ridoux 1992, Meynier et al. 2008) to acquire sufficient energy stores for their upcoming moult ashore. Compared to

sympatric macaroni and rockhopper penguins at the Crozet Islands, conspecifics in this study fed at a higher trophic level (Fig. 3), which either indicates a greater availability of higher trophic level prey or a higher baseline $\delta^{15}\text{N}$ in waters south of the Prince Edward Islands. Macaroni penguins at South Georgia fed at a similar trophic level to macaroni penguins from Marion Island. The higher $\delta^{15}\text{N}$ of southern rockhopper penguins *E. c. chrysocome* at the Falklands is likely due to differences in stable isotopic values at the base of the food web influenced by productivity over the Patagonian Shelf (Dehnhard et al. 2011).

Spatial differences

Previous studies estimate the isotopic position of the APF as 21.2‰ $\delta^{13}\text{C}$ based on data from king penguins (Cherel & Hobson 2007) and wandering albatrosses *Diomedea exulans* (Jaeger et al. 2010). Given that macaroni and rockhopper penguins forage almost exclusively in Antarctic zone waters south of the APF during the pre-moult trip (Whitehead et al. 2016), and that most $\delta^{13}\text{C}$ values are -21.8 to -21.0‰ $\delta^{13}\text{C}$ (Fig. 1), these values are likely representative of Antarctic zone waters. Conspecifics at Crozet and South Georgia also feed in Antarctic waters (see Whitehead et al. 2016 for review) and feather $\delta^{13}\text{C}$ has a similar range to those in the present study (Fig. 3). It is possible that species-specific tissue characteristics (e.g. metabolic routing, turn-over rates and discrimination factors) may result in different estimates of $\delta^{13}\text{C}$ at the APF, and thus we redefined the isotopic position of the APF for macaroni and rockhopper penguins for this study as 21‰ $\delta^{13}\text{C}$. This indicates that rockhopper penguins consistently foraged in Antarctic zone waters south of the APF (with the exception of 2015), whereas macaroni penguins foraged in similar areas in productive years but farther south in less productive years (Fig. 2). Therefore, although spatial overlap between species was high in productive years, the shorter foraging trips associated with such conditions (Whitehead et al. 2016) means that spatiotemporal overlap was negligible. Similarly, in years of reduced productivity when both species spend more time concurrently at sea (Whitehead et al. 2016), spatial overlap would have been reduced because macaroni penguins forage farther south. It is important to note that although variation in $\delta^{13}\text{C}$ may be attributed to phytoplankton-driven shifts in baseline $\delta^{13}\text{C}$ of the food web (Jaeger &

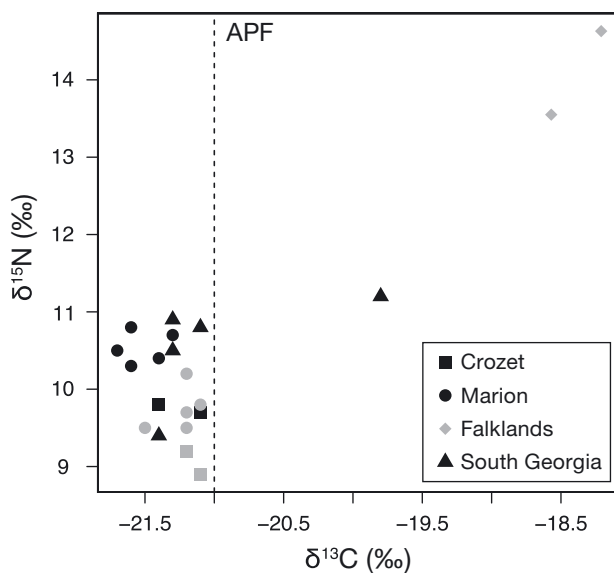


Fig. 3. Carbon and nitrogen stable isotopes in feathers of macaroni (black) and eastern/southern rockhopper penguins (grey) at Marion Island, Crozet (Jaeger & Cherel 2011), Falklands (Dehnhard et al. 2011) and South Georgia (Horswill et al. 2016)

Cherel 2011), the contrasting variation of $\delta^{13}\text{C}$ in macaroni and rockhopper penguin feathers (Fig. 2) indicates that fluctuations in $\delta^{13}\text{C}$ are primarily driven by species-specific behavioral responses to productivity rather than productivity itself. These contrasting responses may be related to species-specific energetic requirements and dietary preferences that force the larger macaroni penguins to travel farther south in search of more energy-rich myctophids.

Sexual differences

Although macaroni penguins did not exhibit sex-specific feeding differences, male rockhopper penguins fed at a higher trophic level compared to females, indicating a larger contribution of fish and/or larger-sized prey in the diet. As both macaroni and rockhopper penguins exhibit similar degrees of sexual dimorphism (Agnew & Kerry 1995), it is surprising that sex has a contrasting influence on diet. Macaroni penguins at other localities also lack sexual differences in diet during the pre-moult period (Thiebot et al. 2014, Horswill et al. 2016), as well as during the breeding period (Bearhop et al. 2006, Horswill et al. 2016). Although male southern rockhopper penguins in the southwest Atlantic Ocean forage at a higher trophic level than females during the late winter and incubation periods (Dehnhard et al. 2011, Ludynia et al. 2013), no sexual differences in diet were recorded during the pre-moult period (Dehnhard et al. 2011). Sexual differences in diet exist in Adélie *Pygoscelis adeliae*, gentoo *P. papua* and Magellanic penguins *Spheniscus magellanicus* (Ainley & Emison 1972, Volkman et al. 1980, Forero et al. 2002), as well as other sexually dimorphic seabirds (Kato et al. 1996, Forero et al. 2005, Quillfeldt et al. 2011), with larger males generally feeding on larger and/or higher trophic level prey.

Environmental drivers

The fact that macaroni penguins foraged farther south during years of reduced primary productivity suggests that such conditions are likely associated with reduced availability of myctophid fish immediately south of the APF. With the exception of 2012, poor productivity coincided with strong positive SST anomalies near the APF (Fig. 2). Warmer surface waters may force ectothermic myctophids to migrate farther south or deeper in the water column, making them less accessible to penguins (Péron et al. 2012).

Conversely, when cooler waters were present in 2013, rockhopper penguins foraged at a higher trophic level (Fig. 2), possibly due to increased accessibility to fish. Warmer SSTs have been linked to a global shift of rockhopper penguins to a lower trophic level diet over the last century (Hilton et al. 2006), which is suggested to have caused the decline of rockhopper penguin populations in the sub-Antarctic (Cunningham & Moors 1994). However, extremely cool SST anomalies are also known to negatively affect the survival of rockhopper penguins at the Falklands (Dehnhard et al. 2013), and local warming has been shown to have a positive effect on the survival of adult macaroni penguins at South Georgia (Horswill et al. 2014), suggesting that the effects of SST on the distribution and abundance of preferred prey may be regional. It is also important to consider that the foraging strategies that penguins have evolved to buffer the consequences of environmental variability may be regional. For instance, macaroni penguins at South Georgia exhibited high intrapopulation variation and low sensitivity to fluctuations in krill density (Horswill et al. 2016), which contrasts with the low intrapopulation variation and high sensitivity to primary productivity (i.e. prey availability) observed in this study.

CONCLUSIONS

The trophic niche differentiation observed between macaroni and rockhopper penguins during the relatively unconstrained pre-moult period indicates that subtle, but inherently different dietary preferences exist between the 2 species. The combination of such differences with partial allochrony and species-specific spatial responses to reduced primary productivity/prey availability provides a better perspective of the overall niche differentiation in space, time and diet, limiting interspecific competition during the pre-moult period. As it is vital that penguins accumulate enough resources in order to endure the moult ashore and retain sufficient energy stores for their return to winter foraging areas, any changes in the availability of preferred prey during the pre-moult period may have severe consequences for adult survival. With the predicted southward shift of major fronts and increasing SST in the Southern Ocean (Hunt et al. 2001, Mélice et al. 2003), it is possible that penguins may have to travel farther and/or dive deeper to find sufficient energy-rich myctophids (Péron et al. 2012), exacerbating energetic constraints during the most critical stage of their annual

cycle. Continued monitoring of penguin foraging behavior during the pre-moult period coupled with annual survival estimates is needed to determine whether changes in at-sea conditions during the pre-moult period are key drivers of population dynamics in macaroni and rockhopper penguins at the Prince Edward Islands.

Acknowledgements. We thank Ben Dilley, Delia Davies, Stefan Schoombie and Kim Stevens for assistance with fieldwork. The South African National Antarctic Programme provided funding and logistical support; additional funding was provided by the South African Department of Science and Technology, National Research Foundation and the University of Cape Town.

LITERATURE CITED

- Adams NJ, Brown CR (1989) Dietary differentiation and trophic relationships in the sub-Antarctic penguin community at Marion Island. *Mar Ecol Prog Ser* 57:249–258
- Agnew DJ, Kerry KR (1995) Sexual dimorphism in penguins. In: Dann P, Norman I, Reilly P (eds) *The penguins*. Surrey Beatty & Sons, Sydney, p 299–318
- Ainley DG, Emison WB (1972) Sexual size dimorphism in Adélie penguins. *Ibis* 114:267–271
- Bearhop S, Waldron S, Votier SC, Furness RW (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiol Biochem Zool* 75:451–458
- Bearhop S, Phillips RA, McGill R, Chérel Y, Dawson DA, Croxall JP (2006) Stable isotopes indicate sex-specific and long-term individual foraging specialisation in diving seabirds. *Mar Ecol Prog Ser* 311:157–164
- Brooke ML (2004) The food consumption of the world's seabirds. *Proc R Soc B* 271:S246–S248
- Brown CR (1986) Feather growth, mass loss and duration of moult in macaroni and rockhopper penguins. *Ostrich* 57:180–184
- Brown CR (1987) Traveling speed and foraging range of macaroni and rockhopper penguins at Marion Island. *J Field Ornithol* 58:118–125
- Brown CR (1989) Energy requirements and food consumption of *Eudyptes* penguins at the Prince Edward Islands. *Antarct Sci* 1:15–21
- Brown CR, Klages NT (1987) Seasonal and annual variation in the diets of macaroni (*Eudyptes chrysolophus chrysolophus*) and southern rockhopper (*E. chrysocome chrysocome*) penguins at sub-Antarctic Marion Island. *J Zool (Lond)* 212:7–28
- Chérel Y, Hobson K (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Mar Ecol Prog Ser* 329:281–287
- Chérel Y, Ridoux V (1992) Prey species and nutritive-value of food fed during summer to king penguin *Aptenodytes patagonicus* chicks at Possession Island, Crozet archipelago. *Ibis* 134:118–127
- Chérel Y, Hobson KA, Bailleul FR, Groscolas R (2005) Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. *Ecology* 86:2881–2888
- Chérel Y, Fontaine C, Richard P, Labat JP (2010) Isotopic niches and trophic levels of myctophid fishes and their predators in the Southern Ocean. *Limnol Oceanogr* 55:324–332
- Connan M, Hofmeyr GJG, Pistorius P (2016) Reappraisal of the trophic ecology of one of the world's most threatened spheniscids, the African penguin. *PLOS ONE* 11:e0159402
- Cooper J, Brown CR, Gales RP, Hindell MA and others (1990) Diets and dietary segregation of crested penguins (*Eudyptes*). In: Davis LS, Darby JT (eds) *Penguin biology*. Academic Press, San Diego, CA, p 131–156
- Crawford RJM, Whittington PA, Upfold L, Ryan PG, Petersen SL, Dyer BM, Cooper J (2009) Recent trends in numbers of four species of penguins at the Prince Edward Islands. *Afr J Mar Sci* 31:419–426
- Cunningham DM, Moors PJ (1994) The decline of rockhopper penguins *Eudyptes chrysocome* at Campbell Island, Southern Ocean and the influence of rising sea temperatures. *Emu* 94:27–36
- Deagle BE, Gales NJ, Hindell MA (2008) Variability in foraging behaviour of chick-rearing macaroni penguins *Eudyptes chrysolophus* and its relation to diet. *Mar Ecol Prog Ser* 359:295–309
- Dehnhard N, Voigt CC, Poisbleau M, Demongin L, Quillfeldt P (2011) Stable isotopes in southern rockhopper penguins: foraging areas and sexual differences in the non-breeding period. *Polar Biol* 34:1763–1773
- Dehnhard N, Poisbleau M, Demongin L, Ludynia K, Lecoq M, Masello JF, Quillfeldt P (2013) Survival of rockhopper penguins in times of global climate change. *Aquatic Conserv: Mar Freshw Ecosyst* 23:777–789
- Field IC, Bradshaw CJA, Burton HR, Sumner MD, Hindell MA (2005) Resource partitioning through oceanic segregation of foraging juvenile southern elephant seals (*Mirounga leonina*). *Oecologia* 142:127–135
- Forero MG, Hobson KA, Bortolotti GR, Donazar JA, Bertelotti M, Blanco G (2002) Food resources utilisation by the Magellanic penguin evaluated through stable-isotope analysis: segregation by sex and age and influence on offspring quality. *Mar Ecol Prog Ser* 234:289–299
- Forero MG, González-Solis J, Hobson KA, Donazar JA, Bertelotti M, Blanco G, Bortolotti GR (2005) Stable isotopes reveal trophic segregation by sex and age in the southern giant petrel in two different food webs. *Mar Ecol Prog Ser* 296:107–113
- Hardin G (1960) The competitive exclusion principle. *Science* 131:1292–1297
- Hilton GM, Thompson DR, Sagar PM, Cuthbert RJ, Chérel Y, Bury SJ (2006) A stable isotopic investigation into the causes of decline in a sub-Antarctic predator, the rockhopper penguin *Eudyptes chrysocome*. *Glob Change Biol* 12:611–625
- Horswill C, Matthiopoulos J, Green JA, Meredith MP and others (2014) Survival in macaroni penguins and the relative importance of different drivers: individual traits, predation pressure and environmental variability. *J Anim Ecol* 83:1057–1067
- Horswill C, Matthiopoulos J, Ratcliffe N, Green JA and others (2016) Drivers of intrapopulation variation in resource use in a generalist predator, the macaroni penguin. *Mar Ecol Prog Ser* 548:233–247
- Hunt BPV, Pakhomov EA, McQuaid CD (2001) Short-term variation and long-term changes in the oceanographic environment and zooplankton community in the vicinity

- of a sub-Antarctic archipelago. *Mar Biol* 138:369–381
- ✦ Hutchinson GE (1957) Concluding remarks. *Cold Spring Harb Symp Quant Biol* 22:415–427
- ✦ Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER—stable isotope Bayesian ellipses in R. *J Anim Ecol* 80:595–602
- ✦ Jaeger A, Cherel Y (2011) Isotopic investigation of contemporary and historic changes in penguin trophic niches and carrying capacity of the southern Indian Ocean. *PLOS ONE* 6:e16484
- ✦ Jaeger A, Lecomte VJ, Weimerskirch H, Richard P, Cherel Y (2010) Seabird satellite tracking validates the use of latitudinal isoscapes to depict predators' foraging areas in the Southern Ocean. *Rapid Commun Mass Spectrom* 24:3456–3460
- ✦ Kato A, Nishiumi I, Naito Y (1996) Sexual differences in diet of king cormorants at Macquarie Island. *Polar Biol* 16:75–77
- ✦ Ludynia K, Dehnhard N, Poisbleau M, Demongin L, Masello JF, Voigt CC, Quillfeldt P (2013) Sexual segregation in rockhopper penguins during incubation. *Anim Behav* 85:255–267
- Mélice JL, Lutjeharms JRE, Rouault M, Ansorge IJ (2003) Sea-surface temperatures at the sub-Antarctic islands Marion and Gough during the past 50 years. *S Afr J Sci* 99:363–366
- ✦ Meynier L, Morel PCH, MacKenzie DDS, MacGibbon A, Chilvers BL, Duignan PJ (2008) Proximate composition, energy content, and fatty acid composition of marine species from Campbell Plateau, New Zealand. *NZ J Mar Freshw Res* 42:425–437
- ✦ Michalik A, McGill RAR, Furness RW, Eggers T, van Noordwijk HJ, Quillfeldt P (2010) Black and white—does melanin change the bulk carbon and nitrogen isotope values of feathers? *Rapid Commun Mass Spectrom* 24:875–878
- ✦ Nakano S, Fausch KD, Kitano S (1999) Flexible niche partitioning via a foraging mode shift: a proposed mechanism for coexistence in stream-dwelling charrs. *J Anim Ecol* 68:1079–1092
- ✦ Newsome SD, Martinez del Rio C, Bearhop S, Phillips D (2007) A niche for isotopic ecology. *Front Ecol Environ* 5:429–436
- ✦ Pakhomov EA, Perissinotto R, McQuaid CD (1994) Comparative structure of the macro zooplankton / micronekton communities of the Subtropical and Antarctic Polar Fronts. *Mar Ecol Prog Ser* 111:155–169
- ✦ Péron C, Weimerskirch H, Bost CA (2012) Projected poleward shift of king penguins' (*Aptenodytes patagonicus*) foraging range at the Crozet Islands, southern Indian Ocean. *Proc R Soc B* 279:2515–2523
- ✦ Phillips DL (2001) Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia* 127:166–170
- ✦ Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2013) nlme: linear and nonlinear mixed effects models. R package version 3.1-113. <https://CRAN.R-project.org/package=nlme>
- ✦ Poisbleau M, Demongin L, van Noordwijk HJ, Strange IJ, Quillfeldt P (2010) Sexual dimorphism and use of morphological measurements to sex adults, immatures and chicks of rockhopper penguins. *Ardea* 98:217–224
- ✦ Quillfeldt P, Schroff S, van Noordwijk HJ, Michalik A, Ludynia K, Masello JF (2011) Flexible foraging behaviour of a sexually dimorphic seabird: large males do not always dive deep. *Mar Ecol Prog Ser* 428:271–287
- ✦ R Development Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.r-project.org
- ✦ Scheffer A, Bost CA, Trathan PA (2012) Frontal zones, temperature gradient and depth characterize the foraging habitat of king penguins at South Georgia. *Mar Ecol Prog Ser* 465:281–297
- ✦ Schmidt K, Atkinson A, Stübing D, McClelland JW, Montoya JP, Voss M (2003) Trophic relationships among Southern Ocean copepods and krill: some uses and limitations of a stable isotope approach. *Limnol Oceanogr* 48:277–289
- ✦ Schreer JF, Kovacs KM (1997) Allometry of diving capacity in air-breathing vertebrates. *Can J Zool* 75:339–358
- Swart S, Speich S, Ansorge IJ, Lutjeharms JRE (2010) An altimetry-based gravest empirical mode south of Africa: 1. Development and validation. *J Geophys Res* 115:C03002
- ✦ Thiebot JB, Cherel Y, Acqueberge M, Prudor A, Trathan PN, Bost CA (2014) Adjustment of pre-moult foraging strategies in macaroni penguins *Eudyptes chrysolophus* according to locality, sex and breeding status. *Ibis* 156:511–522
- ✦ Trivelpiece WZ, Trivelpiece SG, Volkman NJ (1987) Ecological segregation of Adelie, gentoo and chinstrap penguins at King George Island, Antarctica. *Ecology* 68:351–361
- ✦ Volkman NJ, Presler P, Trivelpiece WZ (1980) Diets of pygoscelid penguins at King George Island, Antarctica. *Condor* 82:373–378
- Warham J (1975) The crested penguins. In: Stonehouse B (ed) *The biology of penguins*. Macmillan, London, p 189–269
- ✦ Whitehead TO, Kato A, Ropert-Coudert Y, Ryan P (2016) Habitat use and diving behaviour of macaroni *Eudyptes chrysolophus* and eastern rockhopper *E. chrysolophus filholi* penguins during the critical pre-moult period. *Mar Biol* 163:19–37
- Williams TD (1995) *The penguins*. Oxford University Press, Oxford
- ✦ Williams TD, Croxall JP (1991) Annual variation in breeding biology of macaroni penguins, *Eudyptes chrysolophus*, at Bird Island, South Georgia. *J Zool (Lond)* 223:189–202
- ✦ Wilson RP (2010) Resource partitioning and niche hypervolume overlap in free-living pygoscelid penguins. *Funct Ecol* 24:646–657

Editorial responsibility: Stephen Wing,
Dunedin, New Zealand

Submitted: October 25, 2016; Accepted: December 13, 2016
Proofs received from author(s): February 8, 2017