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Research

Multidimensional stable isotope analysis illuminates resource partitioning in a sub-Antarctic island bird community

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A central theme in community ecology is understanding how similar species co-exist and how their interactions may evolve in the context of climate change. Most studies of resource partitioning among central place foragers, particularly birds, focus on the offspring-rearing period, when they are accessible, but breeding success may be determined earlier and little is known about how such species partition resources at the onset of breeding. We used a non-invasive approach to evaluate resource partitioning in co-existing females at a sub-Antarctic island during their pre-laying periods. Three hypotheses were tested using carbon, nitrogen and oxygen stable isotope ratios measured in shells and membranes of hatched eggs as ecological tracers: 1) resource partitioning by geographic location and trophic level will exist among the 12 bird species and will be enhanced within taxonomic groups; 2) given the absence of strong oxygen gradients in the Southern Ocean we will not detect spatial structuring based on oxygen isotopes, but differences will exist between resident and oceanic species as the former may use meteoric water; 3) capital and income breeder strategies can be differentiated using stable isotopes of egg remains.

Two and three dimensional isotopic data showed resource partitioning among species. As predicted, segregation was evident within the four main taxonomic groups: penguins, albatrosses, burrowing petrels and giant petrels. Unexpectedly, oxygen isotopes revealed widespread use of meteoric water among a suite of sub-Antarctic birds. Stable isotopes allowed us to identify females of most species as income breeders at the onset of breeding, with the exception of the females of the two crested penguin exhibiting a mix of income and capital resources use. Multidimensional isotopic analyses revealed that resource partitioning exists at multiple stages of the annual cycle in ways likely to be important under global change, exhibiting wide potential for ecosystem analysis.

Keywords: carbon, nitrogen and oxygen stable isotopes, egg membranes, egg shells, Prince Edward Islands, Southern Ocean

Introduction

The ability of multiple species to co-exist and the limits of possible niche overlap are a long-standing concern in ecology (Chesson 2000). Oceanic islands are ideal ecosystems to investigate such co-existence (Kueffer et al. 2014), particularly the sub-Antarctic islands that support diverse seabird communities often numbering millions of individuals (Chown et al. 1998). When breeding, seabirds are central place foragers, which increases potential niche overlap among species (Connan et al. 2014) and their co-existence is made possible by differences in the timing of breeding (Cooper et al. 2001), diet (Ridoux 1994) and foraging and nesting locations (Weimerskirch et al. 1986). Despite our understanding of the incubation and chick-rearing stages, it is less clear how resource partitioning is achieved in the energetically critical pre-laying period of egg formation (Moreno et al. 2008, Sorensen et al. 2009) or how this partitioning may be disrupted under what have been observed to be rapidly changing environmental conditions (Le Roux and McGeoch 2008, Constable et al. 2014, Turney et al. 2017, McQuaid 2018).

Seabird species can be ordered along a capital-to-income continuum, depending on how they allocate nutrients for reproduction (Meijer and Drent 1999). Capital breeders, such as the emperor penguin *Aptenodytes forsteri*, which fasts throughout oogenesis, use stored reserves during egg formation (Speake et al. 1999). At the other send of the spectrum, the Procellariiformes (albatrosses and petrels) are mainly income breeders that use nutrients ingested by females during the pre-laying period for egg formation (Warham 1990). After copulation on land, female Procellariiformes and some Sphenisciformes (penguins) undertake a pre-laying exodus at sea (Warham 1990, Chiaradia and Kerry 1999). Information on egg formation duration of seabirds is sparse but it appears to take 18 (little penguin *Eudyptula minor*) to 40 days (northern royal albatross *Diomedea sanfordi*; Grau 1984). During this period, females of at least some species preferentially target prey that fulfil their macro- and micro-nutrients requirements (Boersma et al. 2004).

Our understanding of trophic and foraging ecology of pelagic seabirds has been revolutionized over the last 30 years by the use of natural markers such as stable isotopes (Hobson 2011). These allow information to be collected on seabird habits away from their breeding colonies when many species are dispersed over large areas and largely inaccessible for observation or sampling. Because many seabirds are threatened (Croxall et al. 2012), this has the advantage that such sampling is mainly non-destructive, focusing on blood and feathers. However, hatched eggs potentially provide information about female foraging ecology at the start of the breeding season. Egg shell membranes are largely proteinaceous and are formed in the isthmus region of the oviduct at the end of egg formation (Burley and Vadehra 1989). Egg shells are biomineralized structures comprising 95% calcite (calcium carbonate, $CaCO_3$) embedded in an organic matrix (Nys et al. 2004). Calcification occurs in the egg shell gland (Burley and Vadehra 1989). Importantly, incubation does not change the stable isotope ratios of egg membranes (Oppel et al. 2009), and egg shells are isotopically homogeneous (Maurer et al. 2011). Shells from hatched eggs can be collected and their stable isotope compositions analysed with minimal disturbance to breeding birds. Recent isotopic studies of egg shells and egg membranes have provided insights into the foraging areas of pre-laying gentoo penguins *Pygoscelis papua* (Polito et al. 2009, Emslie et al. 2013) and slender-billed prions *Pachyptila belcheri* (Quillfeldt et al. 2009).

We used stable isotopes of three chemical elements (carbon, nitrogen and oxygen) in egg shells and membranes as ecological tracers to investigate resource partitioning and reproduction strategies in terms of nutrient allocation for reproduction among a suite of bird species that breed on a sub-Antarctic island. Carbon ($^{13}C/^{12}C$; $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$; $\delta^{15}N$) stable isotopes are frequently used to infer foraging areas and trophic levels of marine predators (Graham et al. 2010). Nitrogen stable isotopes mainly provide information on trophic position (Caut et al. 2009), whereas $\delta^{13}C$ is useful to infer foraging areas. Broad-scale latitudinal differences in carbon isoscapes of particulate organic matter in the water column (Magozzi et al. 2017) are reflected in marine top predators (Jaeger et al. 2010), while inshore–offshore $\delta^{13}C$ gradients allow the inference of foraging areas at finer spatial scales (Hill et al. 2006, Cherel and Hobson 2007). In aquatic ecosystems, oxygen stable isotopes ($^{18}O/^{16}O$; $\delta^{18}O$) have been widely used to reconstruct paleotemperatures from fossilised shells (Burgess et al. 2010) and investigate the thermal environments of aquatic animals (Torniainen et al. 2017) due to the influence of ambient water temperature on oxygen isotopic fractionation (Kim and O'Neil 1997). However, for endotherms, such as seabirds, that synthesise carbonates from body tissues at constant body temperatures (Bryant and Froelich 1995), oxygen isotopes provide information on diet, which is useful when oxygen isoscapes exist across feeding areas (Zentero et al. 2013). Variability of oxygen isotopes in water is largely driven by evaporation and condensation, and is often used to distinguish between oceanic and meteoric water sources (Gat 1996).

We used a combination of carbon, nitrogen and oxygen stable isotope compositions to investigate partitioning within seabird communities in the Southern Ocean. We report resource partitioning, drinking habits and nutrient allocation strategies among 12 co-occurring species by testing the following hypotheses: 1) resource partitioning in terms of geographic location and the trophic level of targeted prey will be observed among the 12 bird species mirroring the partitioning during chick rearing (Brown et al. 1990, Reisinger et al. 2018), and partitioning will be enhanced within taxonomic groups as interspecific competition is likely to be more intense within groups of species with related phylogenies and broadly similar nutrient requirements; 2) due to the absence of clear oxygen isoscapes in the Southern Ocean south of the Subtropical Front (McMahon et al. 2013) we did not expect any structuring based on the oxygen stable isotope ratios in the

oceanic species, but differences will be highlighted between more land-based/residential species and oceanic species based on the possibility of using meteoric water (Schaffner and Swart 1991); 3) capital and income breeding strategies can be differentiated by assessing the nutrient assimilation patterns through the comparison of carbon stable isotope ratios of egg shells and egg membranes (Schaffner and Swart 1991).

Material and methods

Study site and sampling

Sub-Antarctic Marion Island (46°55′S, 037°54′E), the larger of the two Prince Edward Islands, lies between the Sub-Antarctic Front to the north and the Antarctic Polar Front to the south (Ansorge et al. 2012). It supports huge numbers of marine predators, including 29 species of breeding seabirds and three pinniped species (Ryan and Bester 2008, Dept of Environmental Affairs unpubl.). The only land bird is the lesser sheathbill *Chionis minor*, which relies on stealing food from seabirds to raise its chicks (Burger 1981).

Egg shells and associated membranes were collected between 2011 and 2013 from 12 bird species: two species of albatrosses (wandering *Diomedea exulans* and grey-headed *Thalassarche chrysostoma*), three of penguins (king *Aptenodytes patagonicus*, macaroni *Eudyptes chrysolophus* and eastern rockhopper *E. [chrysocome] filholi*), both northern *Macronectes halli* and southern giant petrels *M. giganteus*, three species of burrowing petrels (blue *Halobaena caerulea*, great-winged *Pterodroma macroptera* and white-chinned *Procellaria aequinoctialis*), brown skuas *Catharacta antarctica* and lesser sheathbills. Collection dates varied depending on the breeding cycle of the species (Supplementary material Appendix 1 Table A1). Egg shells were collected after successful hatching and stored at −20°C as soon as possible after collection. When two hatched eggs were present in a nest, only one egg shell was collected.

Stable isotope analyses

Carbon stable isotope ratios were determined for both egg shells and egg membranes, nitrogen stable isotope ratios for egg membranes, and oxygen stable isotope ratios for egg shells. Egg shells and membranes were scrubbed in distilled water and dried at 50°C for 24–48 h. Once dried, egg membranes were cut into small pieces, and 0.4–0.5 mg weighed out in a tin capsule. Relative isotope abundances of carbon and nitrogen were determined by combusting samples in a Flash 2000 organic elemental analyzer and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (all from Thermo Scientific, Bremen, Germany).

Dried egg shells were crushed into a fine powder and the organic matrix removed using bleach rinses (Maurer et al. 2011). Relative isotope abundances of carbon and oxygen from carbonates were determined by inserting egg shell powder into 12 mL borosilicate tubes with screw-top lids containing a septum. The tubes were placed in a temperature-controlled sampler tray at 72°C. Atmospheric air was removed from the tubes by flushing with helium using a CTC Analytics A200S autosampler. Depending on sample size, 5–7 drops of warm (72°C) 85% orthophosphoric acid and phosphorus pentoxide acid (specific gravity of solution = 1.92) were then added to each sample tube through the septum using a 1 mL syringe. The samples were left to react for three hours before starting the run. The gas evolved from each reaction was sampled by the autosampler and passed to a Thermo Finnigan (Germany) model II gasbench, where the sample gas was passed through a Nafion water removal unit. It then passed through the 'Poraplot Q' GC column to separate the gas compounds released by the reaction, and then through a second Nafion water trap. The gas was then passed from the gasbench to a Delta Plus XP IRMS (Thermo electron, Bremen) computer controlled by Isodat software. The gas flow was controlled to give eight sample peaks and five reference peaks. The CO_2 reference gas (99.995% purity) was also introduced into the mass spectrometer via the gasbench and controlled by the Isodat software.

Carbon, nitrogen and oxygen results are presented in the usual δ notation relative to Vienna Pee Dee Belemnite (V-PDB), atmospheric N_2 (N_2) and Vienna Standard Mean Ocean Water (V-SMOW), respectively:

$$\delta^{13}C, \delta^{15}N \text{ or } \delta^{18}O\,(0\ \ ) = \left(R_{sample}/R_{standard}\right) - 1$$

where R_{sample} and $R_{standard}$ are the ratios of $^{13}C/^{12}C$ (for $\delta^{13}C$), $^{15}N/^{14}N$ (for $\delta^{15}N$) or $^{18}O/^{16}O$ (for $\delta^{18}O$) for the samples and the standards (V-PDB, N_2 and V-SMOW), respectively. Internal laboratory standards for $\delta^{13}C/\delta^{15}N$ analyses of egg membrane analyses were seal bone, valine and Merck gel, which were calibrated against reference materials from the International Atomic Energy Agency (IAEA, Vienna, Austria) and run throughout all runs, typically two standards for every 10–12 samples. The precision of analyses was < 0.21‰ for $\delta^{13}C$ and < 0.11‰ for $\delta^{15}N$. For $\delta^{13}C/\delta^{18}O$ analyses of carbonates, NBS18, NBS19, NBS20, Carrara marble and Lincoln Limestone were used as internal laboratory standards typically inserting three standards every five samples. The precision of analyses was < 0.29‰ for $\delta^{13}C$ and < 0.24‰ for $\delta^{18}O$. All stable isotope analyses were conducted in the Stable Light Isotope Unit, Univ. of Cape Town, South Africa.

Analysis of stable isotope data

Carbon, nitrogen and oxygen stable isotope ratios were compared among species using univariate and multivariate analyses. Multivariate normality (Mardia tests) and absence of multicollinearity were checked before running multivariate analyses of variance (MANOVA). When these assumptions were not met, one-way permutational analyses of variance (PERMANOVA; Anderson 2001) was used. When MANOVA or PERMANOVA revealed significant effects,

Table 1. Carbon, nitrogen and oxygen stable isotope ratios of egg membranes and shells of 12 bird species collected on Marion Island (mean ± SD, min/max; N: number of samples). Abbreviations and symbols identify species in Fig. 1, 2.

	Abbreviation	Symbol	Egg membrane			Egg shell		
			N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	N	$\delta^{13}C$ (‰)	$\delta^{18}N$ (‰)
King penguin	KP	■	5	−20.2 ± 0.3 (−20.5/−19.7)	12.0 ± 0.4 (11.7/12.7)	5	−15.0 ± 0.5 (−15.7/−14.2)	−3.9 ± 0.3 (−4.3/−3.5)
Macaroni penguin	MP	■	10	−18.9 ± 0.2 (−19.1/−18.5)	9.4 ± 0.2 (9.1/9.7)	10	−15.1 ± 0.6 (−16.3/−14.3)	−4.9 ± 0.3 (−5.2/−4.3)
Eastern rockhopper penguin	ERP	□	10	−19.5 ± 0.3 (−20.1/−19.1)	9.8 ± 0.2 (9.5/10.1)	10	−16.5 ± 0.6 (−17.5/−15.5)	−5.2 ± 0.3 (−5.7/−4.8)
Grey-headed albatross	GHA	◀	10	−19.5 ± 0.3 (−20.2/−19.0)	12.2 ± 0.3 (11.8/12.8)	10	−13.7 ± 1.2 (−15.3/−11.8)	−2.9 ± 0.3 (−3.4/−2.4)
Wandering albatross	WA	△	20	−18.1 ± 0.5 (−19.6/−16.7)	15.0 ± 0.3 (14.4/15.5)	24	−12.5 ± 0.9 (−13.8/−11.0)	−2.9 ± 0.2 (−3.3/−2.5)
Northern giant petrel	NGP	●	19	−19.0 ± 0.9 (−20.7/−17.4)	12.1 ± 0.6 (11.1/13.7)	21	−13.4 ± 1.5 (−17.2/−11.7)	−3.4 ± 0.3 (−4.0/−2.8)
Southern giant petrel	SGP	○	10	−20.5 ± 0.4 (−21.1/−19.5)	11.3 ± 0.2 (10.8/11.6)	10	−15.4 ± 1.2 (−18.0/−13.4)	−3.4 ± 0.2 (−3.8/−3.1)
Blue petrel	BP	◇	13	−21.9 ± 0.6 (−22.6/−20.9)	9.1 ± 0.4 (8.4/9.8)	13	−14.8 ± 0.8 (−16.2/−13.3)	−3.2 ± 0.4 (−4.0/−2.7)
Great-winged petrel	GWP	◆	10	−17.0 ± 0.5 (−17.8/−16.3)	12.0 ± 0.5 (11.2/12.6)	10	−10.8 ± 0.8 (−12.4/−9.5)	−2.4 ± 0.3 (−3.0/−2.1)
White-chinned petrel	WCP	◆	10	−15.6 ± 1.0 (−17.6/−13.8)	12.8 ± 0.7 (11.8/13.8)	10	−8.9 ± 1.4 (−11.7/−6.5)	−2.5 ± 0.5 (−3.3/−1.7)
Brown skua	BS	+	15	−18.3 ± 0.9 (−20.5/−17.3)	11.1 ± 0.8 (10.0/12.8)	15	−12.4 ± 1.3 (−14.8/−10.4)	−6.1 ± 0.9 (−8.2/−5.0)
Lesser sheathbill	LS	×	7	−19.1 ± 0.2 (−19.5/−18.8)	10.8 ± 0.6 (10.0/11.7)	7	−13.5 ± 0.9 (−14.8/−12.2)	−4.4 ± 0.8 (−5.1/−3.0)

post-hoc Hotelling's tests (MANOVA) or pairwise comparisons (PERMANOVA) were run using Bonferroni correction (Hammer et al. 2001). Each factor ($\delta^{13}C$ and $\delta^{15}N$ for egg membranes, $\delta^{13}C$ and $\delta^{18}O$ for egg shells) was then considered separately, and comparisons among species were conducted using either analyses of variance (ANOVA) or Kruskal–Wallis if the assumptions of normality and homogeneity of variances were not met. Post-hoc pairwise comparisons based on Tukey's HSD test (ANOVAs) and pairwise Mann–Whitney (Kruskal–Wallis) were then run with Bonferroni correction (Hammer et al. 2001).

Variability within and among species into a 2D projection was examined separately for egg membranes and egg shells using total convex hull areas and several isotopic metrics. Carbon and nitrogen ratios of egg membranes will inform on resource partitioning among the 12 species in terms of spatial use ($\delta^{13}C$) and trophic level ($\delta^{15}N$) during membrane formation, while carbon and oxygen ratios of egg shells will inform mostly on spatial use ($\delta^{13}C$) and water origin ($\delta^{18}O$) during shell formation. Convex hulls and isotopic metrics were estimated using a bootstrapping approach as sample numbers differed among species (Cucherousset and Villéger 2015, Table 1); a threshold of seven samples per species was applied. Isotopic metrics included isotopic divergence (IDiv), dispersion (IDis), evenness (IEve) and uniqueness (IUni) and were calculated in R ver. 3.5.1 (R Core Team) by adapting the R code supplied by Cucherousset and Villéger (2015). These four indices range between 0 and 1, and provide information on the relationships among individuals within species. Briefly, IDiv is minimal when most points are close to the center of gravity of the convex hull. IDis equals 0 when all organisms have the same stable isotope ratios. IEve tends to 0 when most organisms are packed within a small region of the stable isotope space while a few others are far from this cluster. IUni tends to 0 when each organism has at least one other organism with the same position in the stable isotope space. A principal component analysis was then run considering all these metrics to test whether any taxonomic groupings could be identified.

Three-dimensional isotopic niche space was analysed following Rossman et al. (2016) to investigate whether the isotopic niches of the 12 species were better discriminated in a three dimensional space rather than using a standard two-dimensional approach. This uses a Bayesian framework to estimate a relative centroid location, a niche volume (referred to as standard ellipsoid volume [‰^{3}]), and a volume of overlap between multiple ellipsoids among various parameters. Carbon and nitrogen stable isotope ratios from egg membranes were combined with the oxygen stable isotope ratios of the corresponding egg shells for the analysis. The R code supplied by Rossman et al. (2016) was used throughout. The priors for the model were left uninformative, and the model was run using the JAGS software in R (''jagsUI' package; Kellner 2015) for the Markov Chain Monte Carlo sampling. The model ran three chains of 5000 interations each, discarding the first 1000 as a burn-in and thinning = 1, which

produced a posterior distribution of 12 000 samples. It needs to be noted that the standard ellipsoid volume is sensitive to low sample size ($n < 6$; Rossman et al. 2016).

Because each egg component results from a different metabolic pathway and period of synthesis, the existence of a strong linear relationship between $\delta^{13}C$ measured in both egg membranes and shells most likely suggests a common origin (Hobson and Jehl 2010). Differences between $\delta^{13}C$ measured in egg membranes and $\delta^{13}C$ measured in egg shells were thus tested using paired t-tests or Wilcoxon signed-rank tests. The existence of a relationship between $\delta^{13}C$ measured in the two tissues was then tested using a linear regression among species. For the latter, species' means were used and outliers were identified using Cook's distance, with a threshold value fixed at four times the average distance (Cook 1977). Finally, we estimated the proportion of individuals within each species that followed the linear relationship obtained among species by plotting standardized residuals against the predicted $\delta^{13}C_{shells}$. A standardized residual of 0 (zero) suggests that the carbon used for the formation of egg shells and egg membranes likely originates from the same pool of carbon, whether diet or endogenous reserves. For the former, this would further suggest consistency in the foraging niche, which in case of carbon suggests consistency in the habitat use (Ceia et al. 2012).

All statistical analyses were conducted using R v3.5.1 (R Core Team) or PAST 3.21 (Hammer et al. 2001). Significance level was set at 0.05, unless a Bonferroni correction was applied (see above).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.2m0sr43> (Connan et al. 2019).

Results

Egg membranes

Membranes from 139 eggs were analysed. Blue petrels had the lowest $\delta^{13}C$ values (−22‰) and white-chinned petrels the highest (−16‰; Table 1). Blue petrels also had the lowest $\delta^{15}N$ values (9‰) and wandering albatrosses the highest (15‰; Table 1). Overall, each species' isotopic niche differed from all others (PERMANOVA $F = 103.6$, $p < 0.001$; Fig. 1). Of the 66 pairwise comparisons, all but eight were significant ($p < 0.027$; Supplementary material Appendix 1 Table A2a). These eight involved king penguins, northern and southern giant petrels, grey-headed albatrosses, lesser sheathbills and great-winged petrels.

Separately, $\delta^{13}C$ and $\delta^{15}N$ values were significantly different among the 12 bird species (Kruskal–Wallis $\delta^{13}C$ $H = 113.6$, $p < 0.001$; $\delta^{15}N$ $H = 121.3$, $p < 0.001$). Blue petrel $\delta^{13}C$ values were significantly lower than all other species (all pairwise comparisons $p < 0.038$) except king penguins ($p = 0.106$; Supplementary material Appendix 1 Table A2b).

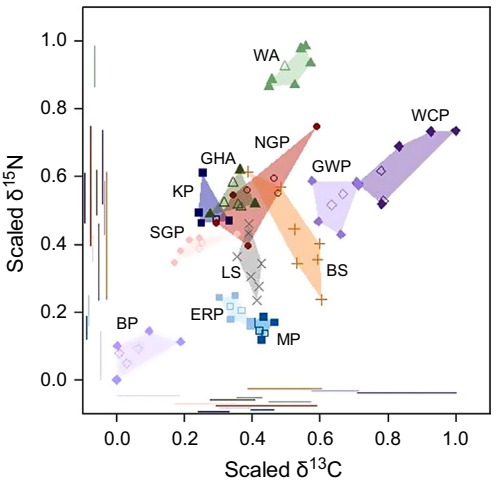

Figure 1. Scaled carbon and nitrogen stable isotope ratios for the egg membranes of the 12 bird species breeding on Marion Island. Colored areas correspond to convex hulls with bootstrapping when number of samples were > 7 (Cucherousset and Villéger 2015). See Table 1 for species abbreviations.

Wandering albatross samples were significantly enriched in ^{15}N compared to all other species (all comparisons $p < 0.024$) except king penguins ($p = 0.051$; Supplementary material Appendix 1 Table A2b).

There were marked differences among the three penguin species for both $\delta^{13}C$ (ANOVA $F = 38.4$, $p < 0.001$; king penguin < rockhopper penguin < macaroni penguin) and $\delta^{15}N$ (Kruskal–Wallis $H = 18.5$, $p < 0.001$; macaroni penguin < rockhopper penguin < king penguin). The $\delta^{13}C$ values of the three burrowing petrel species differed (Kruskal–Wallis $H = 26.4$, $p < 0.001$, blue petrel < great-winged petrel < white-chinned petrel), but their $\delta^{15}N$ mostly overlapped, with only blue petrels having lower $\delta^{15}N$ values (Kruskal–Wallis $H = 24.7$, $p < 0.001$, blue petrel < great-winged petrel = white-chinned petrel). Southern giant petrels had lower values of both $\delta^{13}C$ and $\delta^{15}N$ than northern giant petrels (Mann–Whitney $\delta^{13}C$: $U = 17.0$, $p < 0.001$; $\delta^{15}N$: $U = 22.5$, $p < 0.001$), and grey-headed albatrosses had lower $\delta^{13}C$ and $\delta^{15}N$ than wandering albatrosses (Mann–Whitney $\delta^{13}C$: $U = 8.0$, $p < 0.001$; $\delta^{15}N$: $U = 0.0$, $p < 0.001$). Lesser sheathbills and brown skuas exhibited intermediate $\delta^{13}C$ and $\delta^{15}N$ values to these species (Fig. 1).

Isotopic metrics calculated after bootstrapping showed that northern giant petrels, brown skuas and white-chinned petrels had the largest total convex hull areas, i.e. the largest isotopic niches, for the egg membranes (Table 2a). All species exhibited similar isotopic divergence, dispersion and evenness with the isotopic uniqueness being more variable among species (Table 2a). Considering all these metrics together in a principal component analysis did not highlight any structuring of the 12 species into taxonomic groups (penguins, albatrosses, burrowing petrels and giant petrels; Supplementary material Appendix 1 Fig. A1a).

Table 2. Isotopic indices (after Cucherousset and Villéger 2015) for egg membranes and egg shells for the 12 species of birds from Marion Island.

	Total convex hull area	Isotopic divergence	Isotopic dispersion	Isotopic evenness	Isotopic uniqueness
(a) Egg membrane					
King penguin	0.007	0.727	0.527	0.662	0.439
Macaroni penguin	0.002	0.809	0.706	0.756	0.556
Eastern rockhopper penguin	0.004	0.724	0.641	0.881	0.738
Grey-headed albatross	0.007	0.721	0.607	0.837	0.634
Wandering albatross	0.007	0.797	0.704	0.766	0.564
Northern giant petrel	0.028	0.653	0.440	0.756	0.421
Southern giant petrel	0.005	0.603	0.396	0.694	0.357
Blue petrel	0.013	0.729	0.490	0.814	0.516
Great-winged petrel	0.015	0.725	0.682	0.790	0.543
White-chinned petrel	0.022	0.763	0.582	0.829	0.658
Brown skua	0.024	0.678	0.510	0.822	0.691
Lesser sheathbill	0.008	0.692	0.620	0.813	0.582
(b) Egg shell					
King penguin	0.005	0.795	0.632	0.742	0.597
Macaroni penguin	0.007	0.682	0.557	0.820	0.468
Eastern rockhopper penguin	0.013	0.685	0.596	0.766	0.564
Grey-headed albatross	0.016	0.702	0.588	0.782	0.560
Wandering albatross	0.011	0.653	0.522	0.755	0.437
Northern giant petrel	0.026	0.777	0.517	0.763	0.630
Southern giant petrel	0.007	0.618	0.456	0.769	0.542
Blue petrel	0.023	0.685	0.523	0.858	0.584
Great-winged petrel	0.008	0.655	0.410	0.529	0.314
White-chinned petrel	0.022	0.628	0.430	0.700	0.544
Brown skua	0.091	0.762	0.599	0.910	0.735
Lesser sheathbill	0.035	0.757	0.530	0.794	0.552

Egg shells

The $\delta^{13}C$ and $\delta^{18}O$ values of 145 egg shells were measured. The lowest $\delta^{13}C$ values were observed in rockhopper penguins (−16‰) and the highest in white-chinned petrels (−9‰; Table 1). $\delta^{18}O$ values ranged from −6‰ in brown skuas to −2.4‰ in great-winged petrels (Table 1). Overall, all species segregated (PERMANOVA $F = 45.0$, $p < 0.001$; Fig. 2). Seventeen of the 66 pairwise comparisons were non-significant and these mainly involved king penguins, grey-headed albatrosses, blue petrels and both giant petrels (all other pairwise comparisons $p < 0.046$; Supplementary material Appendix 1 Table A3a). Considered alone, $\delta^{13}C$ and $\delta^{18}O$ values differed significantly among the 12 bird species (Kruskal–Wallis $\delta^{13}C$ $H = 106.0$, $p < 0.001$; $\delta^{18}O$ $H = 120.8$, $p < 0.001$). Only 27 pair-wise comparisons with $\delta^{13}C$ values were significant. Rockhopper penguins exhibited the lowest $\delta^{13}C$ values and white-chinned petrels the highest (all pairwise significant comparisons $p < 0.039$; Supplementary material Appendix 1 Table A3b). Similarly, only 33 pairwise comparisons of $\delta^{18}O$ were significant. Brown skua egg shells had the lowest $\delta^{18}O$ values (all pairwise significant comparisons $p < 0.039$; Supplementary material Appendix 1 Table A3b).

Comparisons within groups showed that the three penguin species were segregated in terms of $\delta^{18}O$ (Kruskal–Wallis $H = 14.5$, $p < 0.001$; rockhopper penguin < macaroni penguin < king penguin) but $\delta^{13}C$ did not differ between two species (ANOVA $F_{2,22} = 17.3$, $p < 0.001$; rockhopper penguin < macaroni penguin = king penguin). The three burrowing petrel species exhibited different $\delta^{13}C$ values (ANOVA $F_{2,30} = 102.7$, $p < 0.001$; blue petrel < great-winged petrel < white-chinned petrel) but $\delta^{18}O$ only segregated blue

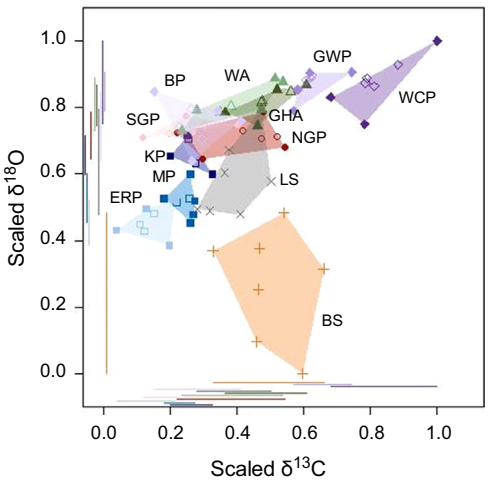

Figure 2. Scaled carbon and oxygen stable isotope ratios for the egg shells of the 12 bird species breeding on Marion Island. Colored areas correspond to convex hulls with bootstrapping when number of samples were > 7 (Cucherousset and Villéger 2015). See Table 1 for species abbreviations.

petrels (ANOVA $F_{2,30} = 12.4$, $p < 0.001$, blue petrel < white-chinned petrel = great-winged petrel). Northern and southern giant petrels segregated in $\delta^{13}C$ (t-test $t = 3.6$, $p = 0.001$; southern giant petrel < northern giant petrel) but not $\delta^{18}O$ ($t = 0.1$, $p = 0.958$). A similar pattern was observed in the two albatrosses; the two species differed in their $\delta^{13}C$ values ($t = 3.4$, $p = 0.002$; grey-headed albatross < wandering albatross) but not $\delta^{18}O$ ($t = 0.5$, $p = 0.621$).

Brown skuas had the largest total convex hull area for egg shells followed by lesser sheathbills (Table 2b). Analysing the isotopic metrics data in a principal component analysis did not highlight any structuring of the 12 species into taxonomic groups (Supplementary material Appendix 1 Fig. A1b). Overall, most species exhibited a greater area for egg shell data than for egg membrane data, and all other metrics calculated for shells were similar to those calculated for membranes (Table 2).

Isotopic niche volume and location centroids

The standard ellipsoid volumes calculated from $\delta^{13}C_{memb}$, $\delta^{15}N$ and $\delta^{18}O$ were highest for brown skuas followed by lesser sheathbills, white-chinned petrels and surprisingly king penguins (Fig. 3, Supplementary material Appendix 1 Fig. A2), suggesting that these four species had the widest isotopic niches. The broad king penguin standard ellipsoid volume is likely due to a low number of samples entered into the model ($n = 5$ and thus < 6 samples as recommended). The smallest estimate was observed for wandering albatrosses. Pairwise comparisons indicated that most species were different from each other (Supplementary material Appendix 1 Table A4). The centroid locations for all 12 species had high probabilities of being different in pairwise comparisons, indicating that most species occupy distinct 3D isotopic niches (Supplementary material Appendix 1 Table A5).

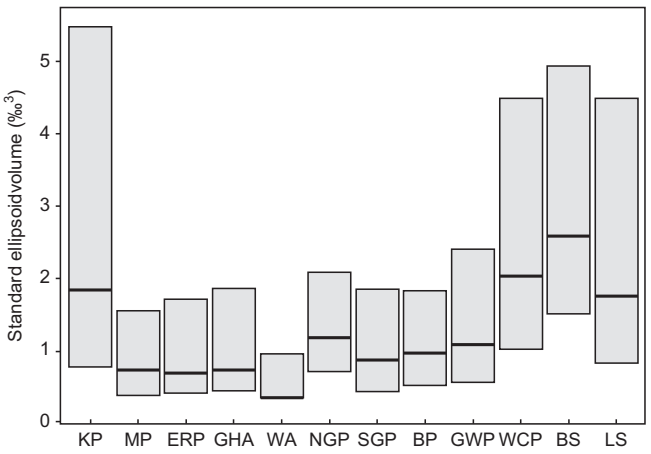

Figure 3. Standard ellipse volume (2.5th, 50.0th and 97.5th percentiles) estimated with a Bayesian framework (Rossman et al. 2016) using $carbon_{membrane}$, nitrogen and oxygen stable isotope data from 12 bird species breeding on Marion Island. See Table 1 for species abbreviations.

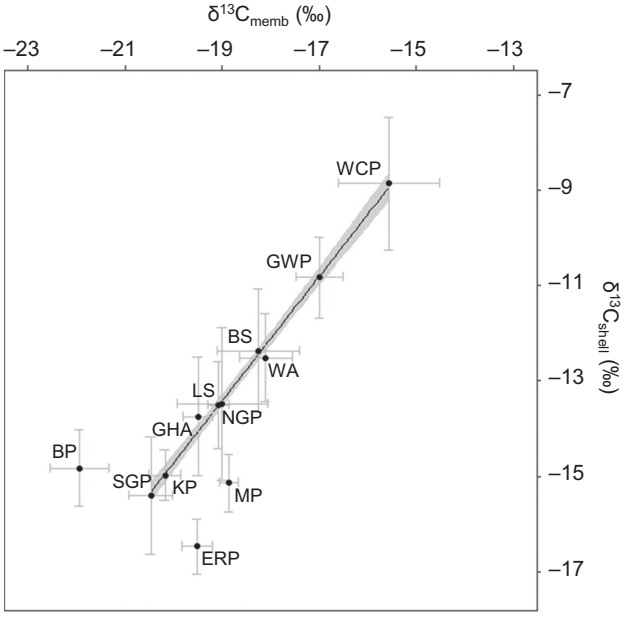

Figure 4. Linear model relationship between $\delta^{13}C$ in egg shells ($\delta^{13}C_{shell}$) and egg membranes ($\delta^{13}C_{memb}$). Solid line represents the best fit linear model with shaded area depicting the 95% confidence bands, excluding the three outliers: blue petrel (BP), macaroni penguin (MP) and eastern rockhopper penguin (ERP). See Table 1 for abbreviations.

Carbon stable isotope ratios: egg shells versus egg membranes

$\delta^{13}C$ values in egg shells were significantly higher than in membranes for all species, with no overlap for values within species, and mean differences ranging from 3.0‰ in rockhopper penguins to 7.1‰ in blue petrels (all $p < 0.002$). When considering the overall trend on the mean data among species, the Cook's Distance detected three outliers to the linear regression; macaroni penguin, rockhopper penguin and blue petrel. When the outliers were removed, $\delta^{13}C$ values in egg-shells and -membranes were highly correlated and the differences between $\delta^{13}C$ values of the two tissues remarkably consistent (F-statistic = 1149, $R_{adj}^2 = 0.993$, $p < 0.001$; Fig. 4). Blue petrels, rockhopper and macaroni penguins exhibited standardized residuals that differed markedly from 0, with all individuals exhibiting standardized residuals consistently >1 (both penguin species) or <−1 (blue petrel; Supplementary material Appendix 1 Fig. A3). None of the other species exhibited a consistent pattern (Supplementary material Appendix 1 Fig. A3).

Discussion

The development of miniaturized devices and indirect biomarkers has considerably enhanced our knowledge of seabird foraging ecology, but most studies have been performed during chick-rearing, with few studies focused on the energetically important pre-laying period. Although captive and field

studies indicate that the egg membrane is a good indicator of female foraging ecology and behaviour prior to egg laying (Schaffner and Swart 1991, Oppel et al. 2009, Maurer et al. 2011), no studies have used eggs to investigate niche overlap in bird communities such as those conducted using blood or feathers (Cherel et al. 2008, Connan et al. 2014). By using carbon, nitrogen and oxygen stable isotopes of egg membranes and shells as ecological markers, we provide new insights into the foraging ecology and behaviour of female birds prior to laying, elucidating resource partitioning within a bird community. Given the wide range of body size in the study species (from the 200 g blue petrel to the 15 000 g king penguin), and the influence of physiology on stable isotope ratios (Boecklen et al. 2011), we focus on resource partitioning within taxonomic groups where there is likely to be the greatest potential for inter-specific competition.

Isotopic partitioning by feeding

The $\delta^{13}C_{memb}$ data used to examine spatial segregation among our 12 species confirmed our view of the foraging habitats of species for which we have tracking data (breeding, overwintering; Reisinger et al. 2018) and provided new information for species lacking tracking data. Among the three small petrel species, female white-chinned and great-winged petrels both forage north of the Subtropical Front (the former slightly farther north as indicated by slightly higher $\delta^{13}C_{memb}$) during the pre-laying period, whereas blue petrels remain south of the Antarctic Polar Front. This conclusion is supported by shell oxygen data (see below) and by tracking data and at-sea observations (Hockey et al. 2005, Reisinger et al. 2018, Rollinson et al. 2018). Although diet studies suggest that great-winged petrels feed mostly on squids (Schramm 1986, Ridoux 1994, Cooper and Klages 2009), while white-chinned petrels prey more on fish (Ridoux 1994, Connan et al. 2007), we found no difference in their $\delta^{15}N$ values, likely reflecting high inter-individual variability combined with the effects of the Southern Ocean $\delta^{15}N$ isoscapes (Jaeger et al. 2010). The southerly foraging areas of blue petrels identified in our study supports previous findings from other breeding islands based on winter tracking (Navarro et al. 2015, Quillfeldt et al. 2015), feather stable isotopes (Cherel et al. 2006) and prey biogeography (Ridoux 1994, Cherel et al. 2002, Connan et al. 2008). This, together with the egg membrane data, indicates that blue petrels from Marion Island use nutrients from Antarctic waters during egg formation (this study) as well as chick-rearing (Steele and Klages 1986).

The other nine species exhibited intermediate $\delta^{13}C_{memb}$, suggesting that during egg formation they gathered nutrients from intermediate waters between the Subtropical Front and Antarctic Polar Front as suggested by tracking data for some of them (Reisinger et al. 2018). There was clear trophic segregation among species in the same taxonomic groups as shown by their $\delta^{15}N_{memb}$. We did not explicitly calculate trophic levels in our study but captive studies have estimated trophic discrimination factors between food and egg membrane to be between 3 and 4‰ in seabirds (Polito et al. 2009). Wandering albatross females obtained their nutrients at one trophic level above grey-headed albatrosses, and king penguins at a higher trophic level than the two crested penguins, showing similar trophic segregation to the chick-rearing period (Adams and Brown 1989, Hunter and Klages 1989, Cooper et al. 1992, Whitehead et al. 2017); although fasting by penguins during the last stage of oogenesis (Ancel et al. 2013) may influence the isotopic ratios of egg components. Interestingly, lesser sheathbills are often found around the colonies of crested penguins either stealing food from the penguins or scavenging from carcasses. This behaviour should place them higher than crested penguins on the $\delta^{15}N$ scale as was indeed observed with $\delta^{15}N_{memb}$ (Fig. 1).

The $\delta^{13}C_{memb}$ values suggest that female southern giant petrels forage farther south than northern giant petrels, while the $\delta^{15}N_{memb}$ values suggest that southern giant petrels feed at a similar trophic level to grey-headed albatrosses and are unlikely to rely heavily on carrion during oogenesis. In addition, the convex hull area of northern giant petrels was six times bigger than that of southern giant petrels, indicating they use a much broader range of environments and resources. Such foraging plasticity of females at the onset of breeding suggests that the northern giant petrels have a more diverse foraging repertoire than the southern giant petrels and thus may be better buffered against climate change. This is not, however, reflected in their population trends. After decreasing in the 1990s, numbers of southern giant petrels at Marion Island were stable in the 2000s, while numbers of northern giant petrels have been stable over the long term but with high inter-annual variability (Ryan et al. 2009).

Overall, both spatial and trophic niche partitioning were observed among females prior to egg laying. For species for which the information is available, this partitioning mostly mirroring that observed during chick-rearing.

Isotopic partitioning by drinking

Our hypothesis that we would not observe partitioning among oceanic species using the oxygen stable isotopes was not supported as slight structuring was present in the oceanic seabird community. Ocean $\delta^{18}O$ values mainly depend on evaporation, precipitation, advection, mixing processes and river run-off (McMahon et al. 2013). Latitudinal oxygen isoscapes have not been detected in the Southern Ocean, but waters either side of the Subtropical Front differ, with values of ~−0.5‰ from south of the front to the Antarctic continent and subtle increases up to ~+0.25‰ to the north, near the southern African coast (Schmidt et al. 1999, McMahon et al. 2013). The difference between those two bioregions may therefore explain the higher values of white-chinned and great-winged petrels that foraged in northern waters compared to the other Procellariiformes (see above). As expected, limited structuring was observed in Procellariiformes for which $\delta^{18}O$ were about −3/−3.5‰, suggesting that most water is obtained at sea (Table 1).

When the crested penguins and the more residential species, the brown skua and the lesser sheathbill, were included,

the wider range of $\delta^{18}O$ values revealed more structuring. The lowest $\delta^{18}O$ values were observed in brown skuas, followed by lesser sheathbills and the two crested penguins (Fig. 2). Fresh water on Marion Island is depleted by ~5‰ compared to seawater (~−6‰ versus ~−1‰; McMahon et al. 2013, Stowe et al. 2018), so drinking fresh water explains the much lower $\delta^{18}O$ values in skuas. They often bathe in rivers and lakes (Carneiro et al. 2014, unpubl.), so it seems likely that female skuas synthesize egg shells from chemical elements originating in part from the island itself, indicating that they spend at least the last stages of oogenesis at the island. It is not known whether brown skua females undertake a pre-laying exodus at Marion Island, but at other islands there is high inter-individual variability in this behaviour (Phillips et al. 2007, Carneiro et al. 2016). Lesser sheathbills are sedentary, mainly foraging in coastal habitats, especially in penguin colonies (Huyser et al. 2000). Their $\delta^{18}O$ values were between those for brown skuas and the Procellariiformes, suggesting intermediate use of fresh water; like skuas, they frequently bathe and probably drink from freshwater pools.

Both crested penguins have been observed drinking at freshwater waterfalls and pools (Williams et al. 1977, unpubl.) and, while the sex of the individuals was unknown, consumption of freshwater would contribute to the lower shell $\delta^{18}O$ values in these species. Surprisingly, the crested penguins exhibited $\delta^{18}O$ values ~2‰ higher than Antarctic Adélie penguins *Pygoscelis adeliae*, the only other seabird species for which egg shell oxygen isotopes have been investigated apart from our study (Emslie and Patterson 2007). This is surprising as such low $\delta^{18}O$ values have not been recorded around Antarctica (McMahon et al. 2013). This may indicate either a difference at the species level (e.g. difference in utilizing oxygen, degree of fasting during oogenesis, etc.) or that fine-scale sampling is necessary to gain a better understanding of coastal patterns in $\delta^{18}O$ values around Antarctica. King penguins showed minimal effects of freshwater, with $\delta^{18}O$ values similar to the procellariiform species. The differences among the three penguin species may also relate to their diets, as the crested penguins prey mainly on crustaceans (Adams and Brown 1989), which have body fluids that are two to three times more concentrated than the body fluids of vertebrates such as fish (Withers 1992). Ingestion of fresh water by crested penguins is contrary to the assumption that their only source of water is from their diet (Goldstein 2002), and suggests potential vulnerability to long-term change as rainfall on Marion Island has decreased dramatically in recent decades (Le Roux and McGeoch 2008).

Influence of physiology: income versus capital breeders

There was a strong linear relationship between the $\delta^{13}C$ values of egg membranes and the corresponding shell, with shells being relatively enriched by an average of 5.8‰ (Fig. 4). This likely reflects different discrimination factors during carbon incorporation into the two tissues as these may vary greatly as shown in gentoo penguins (Polito et al. 2009). Three species deviated from this trend. All blue petrels exhibited higher $\delta^{13}C_{shell}$ values than expected, whereas all individuals of the two crested penguins had lower than expected $\delta^{13}C_{shell}$ values, presumably reflecting different energy intake strategies during egg formation. Those three species were the only ones to show a consistent pattern among individuals within species.

Blue petrels perform a pre-laying exodus of several weeks, with laying well synchronized shortly after they return to their nests (Fugler et al. 1987). Chastel et al. (1995) classified blue petrels as capital breeders based on their low body condition at the start of breeding, but our results suggest that they are income breeders with regards to egg formation. The discrepancy between $\delta^{13}C_{shells}$ and $\delta^{13}C_{memb}$ may be attributed to the use of different foraging areas during formation of the two tissues. As discussed, blue petrels breeding in the sub-Antarctic forage in Antarctic waters. The high $\delta^{13}C$ in their egg shells may indicate that the egg membrane was formed with nutrients originating mainly from southern waters, whereas the egg shell contains nutrients ingested during the females' trip back to the colony. Timing in egg formation has not been described in small Procellariiformes, but dye marker studies on albatrosses indicate a lag of 9–12 days between the end of yolk deposition (during which albumen, membrane and shell are formed) and laying (Astheimer et al. 1985). The use of different foraging areas is less plausible for the discrepancy between the two crested penguins; it is more likely that these species use a different nutrient allocation strategies during egg formation. For example, congeneric Fiordland penguins, *E. pachyrhynchus*, fast during oogenesis, relying on stored nutrients (Grau 1982).

The combination of egg membrane and shell isotopic data therefore supported our hypothesis that these could differentiate between capital and income breeding strategies, and the analysis suggests that most of the 12 species are income breeders with regards to egg formation, with the exception of the two crested penguins that may use a mix of capital and income nutrient sources.

Conclusions

The onset of breeding is a period crucial to breeding success in many birds. We used a multidimensional stable isotope approach to examine resource partitioning within a seabird community at this stage of the breeding season. We found marked segregation in isotope ratios among taxa, even in species that breed at different times of the year. Most study species show some spatial and trophic segregation during the breeding season; our analyses showed similar partitioning during the earlier period of egg formation. Resource partitioning may arise from nutrient requirements of each species and was well defined within taxonomic groups where the potential for niche overlap is high. Our analyses also bring new insights into a reliance on freshwater by sub-Antarctic seabirds that needs further research in the context of long term reductions in rainfall and marked drying of these islands (Le Roux and McGeoch 2008). Our findings highlight the efficacy of using the remains of hatched eggs as a non-invasive

means of investigating resource partitioning prior to egg-laying that can reveal behaviours not easily inferred using other means. Our multidimensional isotopic approach applied to a sub-Antarctic island bird community as a case study revealed its potential for other ecosystems by identifying previously undetected behaviours that may be crucial to monitor in the context of global change.

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Author contributions – MC conceived the idea and design methodology; CMQ and PGR obtained funding; MC, BJD, TOW, DD collected the samples; MC analysed the data and led the writing of the manuscript. All authors contributed to interpretation of the data and writing of the manuscript. All authors gave final approval for publication.

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Supplementary material (available online as Appendix ecog-04560 at <www.ecography.org/appendix/ecog-04560>). Appendix 1.