

BIOLOGY LETTERS

rsbl.royalsocietypublishing.org

Research



Cite this article: Polito MJ, Hinke JT, Hart T, Santos M, Houghton LA, Thorrold SR. 2017 Stable isotope analyses of feather amino acids identify penguin migration strategies at ocean basin scales. *Biol. Lett.* **13**: 20170241. <http://dx.doi.org/10.1098/rsbl.2017.0241>

Received: 19 April 2017

Accepted: 18 July 2017

Subject Areas:

behaviour, ecology

Keywords:

migration, geolocation, seabird, stable isotopes

Author for correspondence:

Michael J. Polito

e-mail: mpolito@lsu.edu

Marine biology

Stable isotope analyses of feather amino acids identify penguin migration strategies at ocean basin scales

Michael J. Polito^{1,2}, Jefferson T. Hinke³, Tom Hart⁴, Mercedes Santos^{5,6}, Leah A. Houghton² and Simon R. Thorrold²

¹Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

²Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

³Antarctic Ecosystem Research Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, La Jolla, CA 92037, USA

⁴Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK

⁵Departamento Biología de Predadores Tope, Instituto Antártico Argentino, 25 de Mayo 1143, San Martín, Buenos Aires B1650CSP, Argentina

⁶Laboratorios Anexos, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Calle 64 N° 3, La Plata, Buenos Aires B1904AMA, Argentina

 MJP, 0000-0001-8639-4431; JTH, 0000-0002-3600-1414; TH, 0000-0002-4527-5046

Identifying the at-sea distribution of wide-ranging marine predators is critical to understanding their ecology. Advances in electronic tracking devices and intrinsic biogeochemical markers have greatly improved our ability to track animal movements on ocean-wide scales. Here, we show that, in combination with direct tracking, stable carbon isotope analysis of essential amino acids in tail feathers provides the ability to track the movement patterns of two, wide-ranging penguin species over ocean basin scales. In addition, we use this isotopic approach across multiple breeding colonies in the Scotia Arc to evaluate migration trends at a regional scale that would be logistically challenging using direct tracking alone.

1. Introduction

Identifying the at-sea distribution of wide-ranging marine animals is critical to aid in their conservation [1] and advances in electronic tracking devices have revolutionized our ability to track animal movements on ocean-wide scales [2]. However, tracking studies can be limited in scale owing to logistical, financial and ethical constraints. Intrinsic biogeochemical markers that retain spatial information, including stable isotope analysis (SIA), have therefore been used to complement direct tracking [3]. SIA can increase the scale of tracking studies by examining a greater number of individuals and/or locations to better generalize population-level movements [4]. However, interpreting bulk tissue SIA can be challenging, because it is often difficult to distinguish the influence of a consumer's diet (i.e. what it eats) from geographical differences in isotopic values (i.e. where it is eating) [3,5].

Compound-specific SIA of amino acids (CSIA-AA) may offer a solution to the bulk SIA problem of distinguishing between diet and geographical differences as some individual amino acids faithfully reflect ecosystem baseline isotopic values that can be used to independently evaluate animal movement [5]. However, few studies have applied CSIA-AA at ocean basin scales and most have focused on nitrogen isotopes [5,6]. Carbon isotope values ($\delta^{13}\text{C}$) of essential AA are also likely to be useful for estimating movement patterns of wide-ranging marine species. This is because essential AAs transfer from diet without alteration and reflect primary producer community

Table 1. Mean \pm s.d. $\delta^{13}\text{C}$ values and classification accuracies from GLS tracked penguins exhibiting three differing winter migration strategies. (Parentheses identify sample sizes of individuals from either Admiralty Bay or Cape Shirreff, and LDA classifications excluding Adélie penguins.)

GLS tracked penguins	Adélie penguin	chinstrap penguin	
	east, Weddell Sea	east, Scotia Sea	west, Pacific sector
<i>n</i>	18 (18,0)	6 (5,1)	28 (10,18)
$\delta^{13}\text{C}$ (‰)			
bulk feather	-24.3 ± 0.3	-24.5 ± 0.5	-22.8 ± 0.6
valine	-30.7 ± 0.7	-29.7 ± 0.4	-27.9 ± 0.9
isoleucine	-20.4 ± 2.1	-17.7 ± 0.9	-19.4 ± 1.5
leucine	-34.9 ± 0.7	-33.4 ± 1.7	-33.4 ± 1.7
threonine	-14.1 ± 1.7	-11.4 ± 1.5	-11.7 ± 2.6
phenylalanine	-30.2 ± 0.7	-30.1 ± 0.4	-28.7 ± 1.5
LDA (%)			
bulk $\delta^{13}\text{C}$	66.7	33.3 (83.3)	82.1 (82.1)
essential AA $\delta^{13}\text{C}$	94.4	100.0 (100.0)	96.4 (89.3)

Table 2. Mean \pm s.d. essential AA $\delta^{13}\text{C}$ values and assigned winter migration strategies of chinstrap penguins from five breeding locations. (Parentheses identify sample size of GLS tracked individuals at each site and 95% credibility intervals for mixing model analyses.)

breeding site	South Orkney Islands	South Shetland Islands			Western Antarctic Peninsula
	Point Martin, Laurie Is.	Admiralty Bay, King George Is.	Cape Shirreff, Livingston Is.	Half Moon Is., Livingston Is.	Orne Harbour, Arctowski Peninsula
lat., long.	60.76°S, 44.68°W	62.17°S, 58.45°W	62.47°S, 60.78°W	62.58°S, 62.58°W	64.62°S, 62.53°W
<i>n</i>	20 (0)	20 (15)	20 (19)	20 (0)	20 (0)
$\delta^{13}\text{C}$ (‰)					
valine	-27.9 ± 1.8	-28.5 ± 1.1	-27.8 ± 1.0	-27.2 ± 1.3	-27.2 ± 2.1
isoleucine	-18.8 ± 1.9	-19.5 ± 2.0	-19.1 ± 1.5	-19.5 ± 2.6	-21.0 ± 1.6
leucine	-32.7 ± 2.1	-33.3 ± 1.7	-33.5 ± 1.6	-32.4 ± 1.8	-33.9 ± 1.6
threonine	-12.1 ± 3.4	-11.6 ± 2.7	-11.5 ± 2.2	-12.1 ± 2.7	-10.5 ± 4.6
phenylalanine	-30.2 ± 1.9	-29.1 ± 1.2	-28.6 ± 1.6	-30.7 ± 1.6	-30.5 ± 1.6
LDA (%)					
east	38.9	26.3	5.0	10.5	11.8
west	61.1	73.7	95.0	89.5	88.2
mixing-model (%)					
east	32.6 (2.1–58.7)	23.8 (5.9–41.3)	9.0 (0.0–19.9)	10.0 (0.0–28.5)	11.5 (0.0–32.7)
west	67.4 (41.3–97.9)	76.2 (58.7–94.1)	91.0 (80.1–100)	90.0 (71.5–100)	88.5 (67.3–100)

composition at the base of geographically distinct food webs [7–9]. For example, one recent study found geographical variation in penguin chick AA $\delta^{13}\text{C}$ values with latitude, though at the time they cautioned that using AA $\delta^{13}\text{C}$ to track foraging locations may not be possible [9].

The goal of this research is to test the ability of $\delta^{13}\text{C}$ CSIA-AA to discriminate among three migration strategies identified by archival geolocation tags (GLS) [10] in two wide-ranging species, the Adélie (*Pygoscelis adeliae*) and chinstrap (*P. antarctica*) penguin. We then use this technique to assign migration strategies to untracked individual

chinstrap penguins from multiple breeding colonies to evaluate regional migration trends at population-level scales.

2. Material and methods

Breeding adult Chinstrap and Adélie penguins from Cape Shirreff, Livingston Island and Admiralty Bay, King George Island (tables 1 and 2) were tagged during the 2011/2012 breeding season with Lotek Nano-Lat 2900-series GLS (Lotek Wireless, Inc.) and recaptured the following year (2012/2013). Tags provided daily estimates of latitude and longitude over the austral

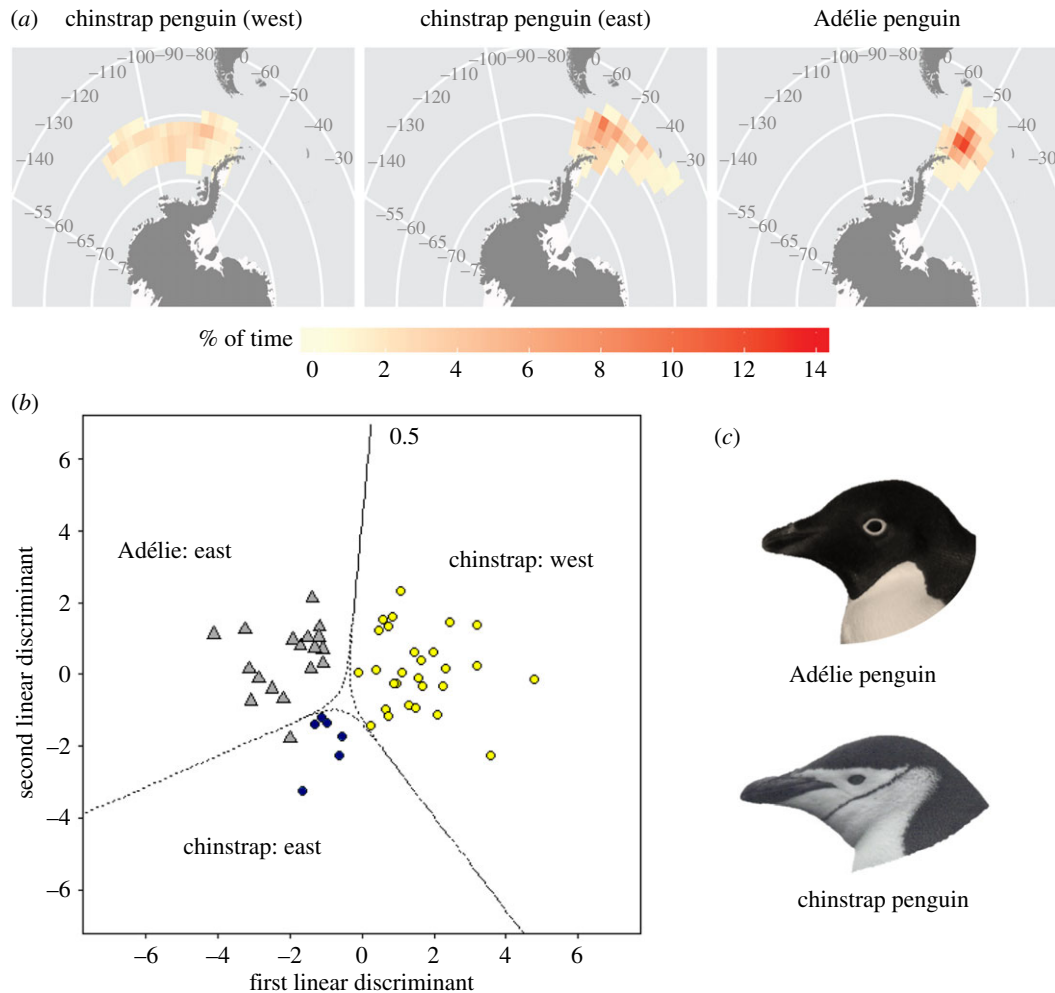


Figure 1. Indices of (a) geographical habitat use and (b) multivariate discrimination based on essential AA $\delta^{13}\text{C}$ values of (c) Adélie and chinstrap penguins. Habitat use data modified from Hinke *et al.* [10]. Dotted lines represent 50% probability of assignment. (Online version in colour.)

winter. At recapture, a central tail feather was collected, a proximal section of which reflected a late-March to early June growth period when penguins were migrating to or inhabiting their winter foraging areas [10]. We restricted our spatial analyses to penguins that had GLS data within the window of tail-feather synthesis and isotopic incorporation (i.e. 40–100 days following the onset of molt; Adélie penguins: 25 March–24 May; chinstrap penguins: 10 April–9 June). Details on GLS data processing, feather growth rates and bulk $\delta^{13}\text{C}$ values are provided in Hinke *et al.* [10]. In 2012/2013, we collected tail feathers from additional, untracked breeding adult chinstrap penguins from five breeding sites (table 2).

Tail feather sections (20 mg each) were acid hydrolysed, derivatized and analysed for CSIA-AA following the methods outlined McMahon *et al.* [11]. Samples were analysed in duplicate with AA and fish muscle standards of known isotopic composition (mean reproducibility: AA standard: $\pm 0.2\%$; internal fish standard: $\pm 0.6\%$). We focused on bulk feather $\delta^{13}\text{C}$ and five essential AAs (threonine, isoleucine, valine, phenylalanine and leucine) and used linear discriminant analyses (LDA) in program R (v. 2.15.3) [12] with leave-one-out cross-validation to differentiate among the three migration strategies observed by Hinke *et al.* [10]: Adélie penguins migrating eastward from their breeding sites into the Weddell Sea, chinstrap penguins migrating eastward into the Scotia Sea, and chinstrap penguins migrating westward to the Pacific sector of the Southern Ocean (table 1 and figure 1). We then used LDA to discriminate between the two chinstrap penguin migration strategies in isolation and assign untracked individuals to specific migration strategies. We evaluated regional migration

trends using only chinstrap penguins with known migration patterns (GLS) and those that were assigned based on CSIA-AA with greater than or equal to 80% probability of group membership [4,5].

We also applied a Bayesian mixing-model approach [13] in program R [12] to obtain a probability distribution of migration strategies at the five chinstrap penguins breeding sites examined. We used essential AAs $\delta^{13}\text{C}$ values of GLS tracked chinstrap penguins as source end-members (eastward versus westward), and values of all penguins by breeding site regardless of if their migration status was known. We used a small non-zero trophic discrimination factor in the model ($0.1 \pm 0.1\%$) [7] and ran 1 million iterations, thinned by 15, with an initial discard of 40 000 resulting in 64 000 posterior draws.

3. Results

LDA classification using AA $\delta^{13}\text{C}$ out-performed bulk $\delta^{13}\text{C}$ and provided clear separation in canonical multivariate space (Wilk's $\lambda = 0.16$, $p < 0.001$; table 1 and figure 1). Individuals misclassified by AA $\delta^{13}\text{C}$ were assigned as chinstrap penguins migrating eastward. AA $\delta^{13}\text{C}$ LDA accuracy was greater than or equal to 89.3% for chinstrap penguins only (Wilk's $\lambda = 0.34$, $p < 0.001$) and out-performed bulk $\delta^{13}\text{C}$ (table 1).

Migration strategies for 59 of the 66 untracked chinstrap penguins were assigned with greater than or equal to 80% probability. When combined with individuals of known

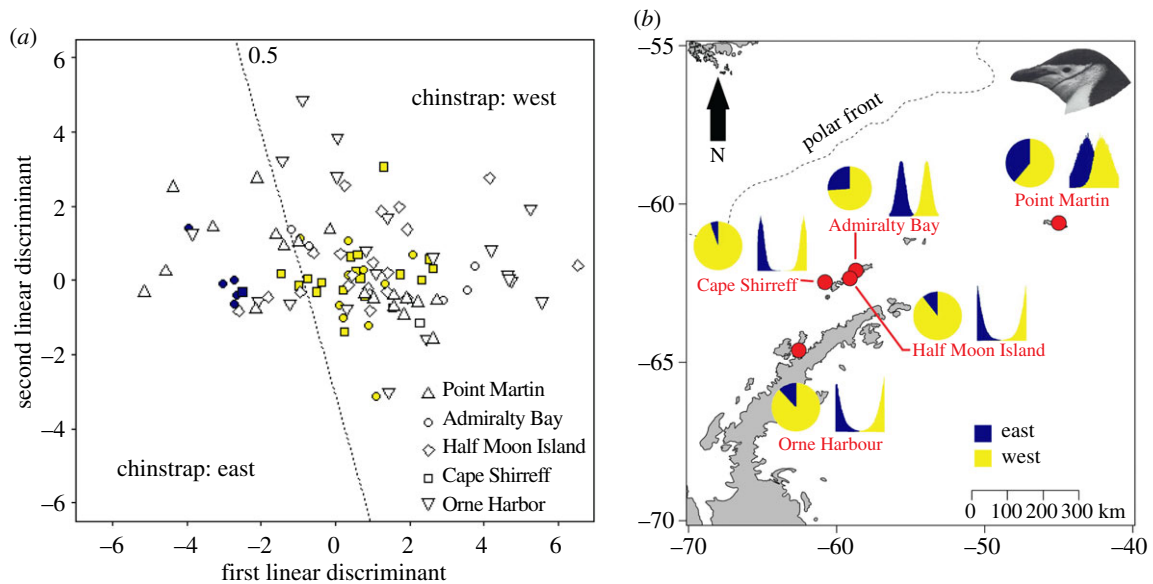


Figure 2. (a) Multivariate discrimination of tracked (coloured points) and untracked (white points) chinstrap penguins based on essential AA $\delta^{13}\text{C}$ values and (b) assigned winter migration strategies (eastward or westward) in chinstrap penguins from five breeding locations using LDA (pie charts) and stable isotope mixing-models (histograms). Dotted line represents 50% probability of assignment. (Online version in colour.)

migration status, a majority of chinstrap penguins exhibited 'Pacific' isotopic signatures, consistent with a westward migration (81.7%). However, we also observed a relatively higher number of individuals exhibiting a 'Scotia Sea' signature at sites located farther north and east (table 2 and figure 2). This was confirmed by our mixing-model approach, with 95% credibility intervals around the contribution of eastward versus westward migrants overlapping only at the most northeastern breeding site (table 2 and figure 2).

4. Discussion

Essential AA $\delta^{13}\text{C}$ values in tail feathers successfully discriminated between the winter migration strategies observed in Adélie and chinstrap penguins. This approach provided more accurate classifications than bulk $\delta^{13}\text{C}$ and successfully differentiated species-specific habitat niches between eastward moving Adélie and chinstrap penguins (into the ice-covered Weddell Sea versus the ice free Scotia Sea, respectively) [10]. In addition, our results were unaffected by trophic biases [5,8] as essential AA in penguin tail feathers most probably reflect only the baseline $\delta^{13}\text{C}$ values in their specific wintering area [8]. Differences in baseline $\delta^{13}\text{C}$ values across wintering areas in this study may be driven by differences in the phytoplankton and/or sea-ice algae community composition and sources of inorganic carbon [14,15].

Differences in essential AA $\delta^{13}\text{C}$ values among eastward versus westward migrating chinstrap penguins also provided a basis for assignment of untracked individuals. This allowed us to expand the overall sample sizes (i.e. number of individuals) and spatial scope (i.e. number and range of breeding sites) of our study to confirm that the dominant migration strategy of chinstrap penguins from the Antarctic Peninsula region and southern Scotia Sea is westward. One possible hypothesis for this trend is competitive avoidance as the Scotia Sea is home to large wintering populations of Macaroni (*Eudyptes chrysolophus*) and southern rockhopper (*E. chrysolophus*) penguins [16]. In addition, we identified a spatial trend with a relatively higher number of

eastward migrating individuals at sites located farther northwards and eastwards (figure 2). This may suggest that the location of breeding sites influences migration patterns. Following this trend, one might expect individuals breeding in the South Sandwich Islands to remain in the Scotia Sea during winter, as this archipelago is the farthest northeast and contains the largest chinstrap penguin breeding population [17]. If so, this might serve as a source of intra-specific competition and further explain dominance of westward migration strategies of chinstrap penguins from our study sites. An alternate explanation is some individuals from northeastern colonies may obtain a 'Scotia Sea' isotopic signature while migrating westward towards the Pacific.

In summary, to our knowledge this research represents the first use of essential AA $\delta^{13}\text{C}$ values to track the migration routes and at-sea distribution of a wide-ranging marine predator. While the spatial resolution of essential AA $\delta^{13}\text{C}$ is coarse compared to direct tracking, this approach can significantly expand the scope of studies and help facilitate inference about individual and population processes in far-ranging marine species. Future studies that elucidate spatial gradients in oceanic isotopic baselines will further refine our ability to track marine animal movements over ocean basin scales.

Ethics. Fieldwork was conducted via an Antarctic Conservation Act permit (ACA 2013-007) and animal use approved by WHOI (27071382) and UCSD (S05480) IACUC.

Data accessibility. GLS and isotope data are available online at <https://swfsc.noaa.gov/AERD-Data/>.

Authors' contributions. Study design: M.J.P., J.T.H. and S.R.T.; fieldwork: M.J.P., J.T.H., T.H. and M.S.; data analysis: M.J.P., J.T.H., T.H. and L.H.; manuscript: M.J.P.; all authors revised and gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. We have no competing interests.

Funding. Funded by the National Marine Sanctuary Foundation (GLS tags), Ocean Life Institute (M.J.P., L.H. and S.R.T.), Darwin Initiative (T.H.) and SeaWorld Bush Gardens Conservation Fund (M.J.P. and S.R.T.).

Acknowledgements. We thank the US AMLR program, Instituto Antártico Argentino, and Quark Expeditions, K. McMahon and P. McDowell for field and analysis assistance.

- Costa DP, Breed GA, Robinson PW. 2012 New insights into pelagic migrations: implications for ecology and conservation. *Annu. Rev. Ecol. Evol. Syst.* **43**, 73–96. (doi:10.1146/annurev-ecolsys-102710-145045)
- Block BA *et al.* 2011 Tracking apex marine predator movements in a dynamic ocean. *Nature* **475**, 86–90. (doi:10.1038/nature10082)
- Ramos R, González-Solís J. 2012 Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. *Front. Ecol. Environ.* **10**, 258–266. (doi:10.1890/110140)
- Pajuelo M, Bjørndal KA, Reich KJ, Vander Zanden HB, Hawkes LA, Bolten AB. 2012 Assignment of nesting loggerhead turtles to their foraging areas in the Northwest Atlantic using stable isotopes. *Ecosphere* **3**, 1–18. (doi:10.1890/ES12-00220.1)
- Seminoff JA, Benson SR, Arthur KE, Eguchi T, Dutton PH, Tapilatu RF, Popp BN. 2012 Stable isotope tracking of endangered sea turtles: validation with satellite telemetry and $\delta^{15}\text{N}$ analysis of amino acids. *PLoS ONE* **7**, e37403. (doi:10.1371/journal.pone.0037403)
- Madigan DJ, Baumann Z, Carlisle AB, Hoen DK, Popp BN, Dewar H, Snodgrass OE, Block BA, Fisher NS. 2014 Reconstructing transoceanic migration patterns of Pacific bluefin tuna using a chemical tracer toolbox. *Ecology* **95**, 1674–1683. (doi:10.1890/13-1467.1)
- McMahon KW, Fogel ML, Elsdon TS, Thorrold SR. 2010 Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. *J. Anim. Ecol.* **79**, 1132–1141. (doi:10.1111/j.1365-2656.2010.01722.x)
- McMahon KW, Polito MJ, Abel S, McCarthy MD, Thorrold SR. 2015 Carbon and nitrogen isotope fractionation of amino acids in an avian marine predator, the gentoo penguin (*Pygoscelis papua*). *Ecol. Evol.* **5**, 1278–1290. (doi:10.1002/ece3.1437)
- Lorrain A, Graham B, Ménard F, Popp B, Bouillon S, Van Breugel P, Cherel Y. 2009 Nitrogen and carbon isotope values of individual amino acids: a tool to study foraging ecology of penguins in the Southern Ocean. *Mar. Ecol. Prog. Ser.* **391**, 293–306. (doi:10.3354/meps08215)
- Hinke JT, Polito MJ, Goebel ME, Jarvis S, Reiss CS, Thorrold SR, Trivelpiece WZ, Watters GM. 2015 Spatial and isotopic niche partitioning during winter in chinstrap and Adélie penguins from the South Shetland Islands. *Ecosphere* **6**, 1–32. (doi:10.1890/ES14-00287.1)
- McMahon KW, Thorrold SR, Houghton LA, Berumen ML. 2016 Tracing carbon flow through coral reef food webs using a compound-specific stable isotope approach. *Oecologia* **180**, 809–821. (doi:10.1007/s00442-015-3475-3)
- R Core Team. 2013 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Parnell AC, Inger R, Bearhop S, Jackson AL. 2010 Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* **5**, e9672. (doi:10.1371/journal.pone.0009672)
- McMahon KW, Hamady LL, Thorrold SR. 2013 A review of ecogeochemistry approaches to estimating movements of marine animals. *Limnol. Oceanogr.* **58**, 697–714. (doi:10.4319/lo.2013.58.2.0697)
- Graham BS, Koch PL, Newsome SD, McMahon KW, Aurioles D. 2010 Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In *Isoscapes*, (eds JB West, GJ Bowen, TE Dawson, KP TU pp. 299–318. Dordrecht, The Netherlands: Springer.
- Ratcliffe N *et al.* 2014 Love thy neighbour or opposites attract? Patterns of spatial segregation and association among crested penguin populations during winter. *J. Biogeogr.* **41**, 1183–1192. (doi:10.1111/jbi.12279)
- Lynch HJ, White R, Naveen R, Black A, Meixler MS, Fagan WF. 2016 In stark contrast to widespread declines along the Scotia Arc, a survey of the South Sandwich Islands finds a robust seabird community. *Polar Biol.* **39**, 1615–1625. (doi:10.1007/s00300-015-1886-6)