1 **Stable isotope analyses of feather amino acids identify penguin migration**

2 **strategies at ocean basin scales.**

3

4 Michael J. Polito[1-2]*, Jefferson T. Hinke[3], Tom Hart[4], Mercedes Santos[5-6], Leah A. Houghton[2],

5 Simon R. Thorrold[2]

6

7 [1]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge,

8 LA 70803,

9 [2]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, U.S.A.

10 [3]Antarctic Ecosystem Research Division, Southwest Fisheries Science Center, National Marine

11 Fisheries Service, National Oceanic and Atmospheric Administration, La Jolla, California 92037,

12 U.S.A. 1.

13 [4]Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK

14 [5]Departamento Biología de Predadores Tope, Instituto Antártico Argentino, 25 de Mayo 1143,

15 B1650CSP, San Martín, Buenos Aires, Argentina

16 [6]Laboratorios Anexos, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La

17 Plata, Calle 64 N° 3, B1904AMA, La Plata, Buenos Aires, Argentina

18

19 *Author for correspondence: Michael J. Polito mpolito@lsu.edu

20   **Abstract:** Identifying the at-sea distribution of wide ranging marine predators is critical to

21   understanding their ecology. Advances in electronic tracking devices and intrinsic

22   biogeochemical markers have greatly improved our ability to track animal movements on ocean-

23   wide scales. Here we show that, in combination with direct tracking, stable carbon isotope

24   analysis of essential amino acids in tail feathers provides the ability to track the movement

25   patterns of two, wide-ranging penguin species over ocean basin scales. In addition, we use this

26   isotopic approach across multiple breeding colonies in the Scotia Arc to evaluate migration

27   trends at a regional scale that would be logistically challenging using direct tracking alone.

28

29   Keywords: migration, geolocation (GLS), seabird, stable isotopes

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 due 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 geographic 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 geographic differences as some individual amino acids (AAs) 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 (δ^{13}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 composition at the base of geographically distinct food webs [7-9]. For example, one recent study found geographic variation in penguin chick AA δ^{13}C values with latitude, thought at the time they cautioned that using AA δ^{13}C to track forging locations may not be possible [9].

52   The goal of this research is to test the ability of δ^{13}C CSIA-AA to discriminate among

53   three migrations strategies identified by archival geolocation tags (GLS) [10] in two wide-

54   ranging species, the Adélie (*Pygoscelis adeliae*) and Chinstrap (*P. antarctica*) penguin. We then

55   use this technique to assign migration strategies to untracked individual Chinstrap penguins from

56   multiple breeding colonies to evaluate regional migration trends at population-level scales.

57

58   **2. Material and methods**

59   Breeding adult Chinstrap and Adélie penguins from Cape Shirreff, Livingston Island and

60   Admiralty Bay, King George Island (Table 1 and 2) were tagged during 2011/12 breeding

61   season with Lotek Nano-Lat 2900-series GLS (Lotek Wireless, Inc.) and recaptured the

62   following year (2012/13). Tags provided daily estimates of latitude and longitude over the

63   austral winter. At recapture a central tail feather was collected, a proximal section of which

64   reflected a late-March to early-June growth period when penguins were migrating to or

65   inhabiting their winter foraging areas [10]. We restricted our spatial analyses to penguins that

66   had GLS data within the window of tail-feather synthesis and isotopic incorporation (i.e. 40-

67   100 days following the onset of molt; Adélie penguins: 25 March - 24 May; chinstrap

68   penguins: 10 April - 9 June). Details on GLS data processing, feather growth rates, and bulk

69   δ^{13}C values are provided in Hinke et al. [10]. In 2012/13 we collected tail feathers from

70   additional, untracked breeding adult Chinstrap penguins from five breeding sites (Table 2).

71   Tail feather sections (20 mg each) were acid hydrolyzed, derivatized and analyzed for

72   CSIA-AA following the methods outlined McMahon et al. [11]. Samples were analyzed in

73   duplicate with AA and fish muscle standards of known isotopic composition (mean

74   reproducibility: AA standard: ±0.2 ‰; internal fish standard: ±0.6 ‰). We focused on bulk

75   feather $\delta^{13}C$ and five essential AAs (threonine, isoleucine, valine, phenylalanine, and

76   leucine) and used linear discriminant analyses (LDA) in program R (ver. 2.15.3) [12] with

77   leave-one-out cross-validation to differentiate among the three migration strategies observed

78   by Hinke et al. [10]: Adélie penguins migrating eastward from their breeding sites into the

79   Weddell Sea, Chinstrap penguins migrating eastward into the Scotia Sea, and Chinstrap

80   penguins migrating westward to the Pacific sector of the Southern Ocean (Table 1, Fig. 1).

81   We then used LDA to discriminate between the two Chinstrap penguin migration strategies

82   in isolation and assign untracked individuals to specific migration strategies. We evaluated

83   regional migration trends using only Chinstrap penguins with known migration patterns

84   (GLS) and those that were assigned based on CSIA-AA with $\geq$ 80% probability of group

85   membership [4, 5].

86   We also applied a Bayesian mixing-model approach [13] in program R [12] to obtain a

87   probability distribution of migration strategies at the five Chinstrap penguins breeding sites

88   examined. We used essential AAs $\delta^{13}C$ values of GLS tracked Chinstrap penguin as source

89   end-members (eastward vs. westward), and values of all penguins by breeding site regardless

90   of if their migration status was known. We used a small non-zero trophic discrimination

91   factor in the model (0.1 $\pm$ 0.1‰) [7] and ran 1 million iterations, thinned by 15, with an

92   initial discard of 40,000 resulting in 64,000 posterior draws.

93

94   **3. Results**

95   LDA classification using AA $\delta^{13}C$ out-performed bulk $\delta^{13}C$ and provided clear separation

96   in canonical multivariate space (Wilk's lambda = 0.16, P < 0.001; Table 1, Fig. 1).

97   Individuals misclassified by AA $\delta^{13}C$ were assigned as Chinstrap penguins migrating

98   eastward. AA δ^{13}C LDA accuracy was ≥ 89.3% for Chinstrap penguins only (Wilk's lambda

99   = 0.34, P < 0.001) and out-performed bulk δ^{13}C (Table 1).

100   Migration strategies for 59 of the 66 untracked Chinstrap penguins were assigned with ≥

101   80% probability. When combined with individuals of known migration status, a majority of

102   Chinstrap penguins exhibited "Pacific" isotopic signatures, consistent with a westward

103   migration (81.7%). However, we also observed a relatively higher number of individuals

104   exhibiting a "Scotia Sea" signature at sites located farther north and east (Table 2; Fig. 2).

105   This was confirmed by our mixing-model approach, with 95% credibility intervals around

106   the contribution of eastward vs. westward migrants overlapping only at the most northeastern

107   breeding site (Table 2; Fig. 2).

108

109   **4. Discussion**

110   Essential AA δ^{13}C values in tail feathers successfully discriminated between the winter

111   migrations strategies observed in Adélie and Chinstrap penguins. This approach provided more

112   accurate classifications than bulk δ^{13}C and successfully differentiated species-specific habitat

113   niches between eastward moving Adélie and Chinstrap penguins (into the ice-covered Weddell

114   Sea vs. ice free Scotia Sea, respectively) [10]. In addition, our results were unaffected by trophic

115   biases [5, 8] as essential AA in penguin tail feathers most likely reflect only the baseline δ^{13}C

116   values in their specific wintering area [8]. Differences in baseline δ^{13}C values across wintering

117   areas in this study may be driven by differences in the phytoplankton and/or sea-ice algae

118   community composition and sources of inorganic carbon [14, 15].

119   Differences in essential AA δ^{13}C values among eastward vs. westward migrating

120   Chinstrap penguins also provided a basis for assignment of untracked individuals. This allowed

6

121   us to expand the overall sample sizes (i.e. number of individuals) and spatial scope (i.e. number

122   and range of breeding sites) of our study to confirm that the dominant migration strategy of

123   chinstrap penguins from the Antarctic Peninsula region and southern Scotia Sea is westward.

124   One possible hypothesis for this trend is competitive avoidance as the Scotia Sea is home to large

125   wintering populations of Macaroni (*Eudyptes chrysolophus*) and southern rockhopper (*E.*

126   *chrysocome chrysocome*) penguins [16]. In addition, we identified a spatial trend with a

127   relatively higher number of eastward migrating individuals at sites located farther northwards

128   and eastwards (Fig. 2). This may suggest that the location of breeding sites influences migration

129   patterns. Following this trend, one might expect individuals breeding in the South Sandwich

130   Islands to remain in the Scotia Sea during winter, as this archipelago is the farthest northeast and

131   contains the largest Chinstrap penguin breeding population [17]. If so, this might serve as a

132   source of intra-specific competition and further explain dominance of westward migration

133   strategies of Chinstrap penguins from our study sites. An alternate explanation is some

134   individuals from northeastern colonies may obtain a "Scotia Sea" isotopic signature while

135   migrating westward towards the Pacific.

136   In summary, to our knowledge this research represents the first use of essential AA δ^{13}C

137   values to track the migration routes and at-sea distribution of a wide-ranging marine predator.

138   While the spatial resolution of essential AA δ^{13}C is coarse compared to direct tracking, this

139   approach can significantly expand the scope of studies and help facilitate inference about

140   individual and population processes in far-ranging marine species. Future studies that elucidate

141   spatial gradients in oceanic isotopic baselines will further refine our ability to track marine

142   animal movements over ocean basin scales.

143

161   **References**

162   [1] Costa, D.P., Breed, G.A. & Robinson, P.W. 2012 New insights into pelagic migrations: implications for
163   ecology and conservation. *Annual review of ecology, evolution, and systematics* **43**, 73-96.
164   [2] Block, B.A., Jonsen, I., Jorgensen, S., Winship, A., Shaffer, S.A., Bograd, S., Hazen, E., Foley, D., Breed,
165   G. & Harrison, A.-L. 2011 Tracking apex marine predator movements in a dynamic ocean. *Nature* **475**,
166   86-90.
167   [3] Ramos, R. & González-Solís, J. 2012 Trace me if you can: the use of intrinsic biogeochemical markers
168   in marine top predators. *Frontiers in Ecology and the Environment* **10**, 258-266.
169   [4] Pajuelo, M., Bjorndal, K.A., Reich, K.J., Vander Zanden, H.B., Hawkes, L.A. & Bolten, A.B. 2012
170   Assignment of nesting loggerhead turtles to their foraging areas in the Northwest Atlantic using stable
171   isotopes. *Ecosphere* **3**, 1-18.
172   [5] Seminoff, J.A., Benson, S.R., Arthur, K.E., Eguchi, T., Dutton, P.H., Tapilatu, R.F. & Popp, B.N. 2012
173   Stable isotope tracking of endangered sea turtles: validation with satellite telemetry and δ 15 N analysis
174   of amino acids. *PLoS One* **7**, e37403.
175   [6] Madigan, D.J., Baumann, Z., Carlisle, A.B., Hoen, D.K., Popp, B.N., Dewar, H., Snodgrass, O.E., Block,
176   B.A. & Fisher, N.S. 2014 Reconstructing transoceanic migration patterns of Pacific bluefin tuna using a
177   chemical tracer toolbox. *Ecology* **95**, 1674-1683.
178   [7] McMahon, K.W., Fogel, M.L., Elsdon, T.S. & Thorrold, S.R. 2010 Carbon isotope fractionation of
179   amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. *Journal of
180   Animal Ecology* **79**, 1132-1141.
181   [8] McMahon, K.W., Polito, M.J., Abel, S., McCarthy, M.D. & Thorrold, S.R. 2015 Carbon and nitrogen
182   isotope fractionation of amino acids in an avian marine predator, the gentoo penguin (Pygoscelis
183   papua). *Ecology and evolution* **5**, 1278-1290.
184   [9] Lorrain, A., Graham, B., Ménard, F., Popp, B., Bouillon, S., Van Breugel, P. & Cherel, Y. 2009 Nitrogen
185   and carbon isotope values of individual amino acids: a tool to study foraging ecology of penguins in the
186   Southern Ocean. *Marine Ecology Progress Series* **391**, 293-306.
187   [10] Hinke, J.T., Polito, M.J., Goebel, M.E., Jarvis, S., Reiss, C.S., Thorrold, S.R., Trivelpiece, W.Z. &
188   Watters, G.M. 2015 Spatial and isotopic niche partitioning during winter in chinstrap and Adélie
189   penguins from the South Shetland Islands. *Ecosphere* **6**, 1-32.
190   [11] McMahon, K.W., Thorrold, S.R., Houghton, L.A. & Berumen, M.L. 2016 Tracing carbon flow through
191   coral reef food webs using a compound-specific stable isotope approach. *Oecologia* **180**, 809-821.
192   [12] R Core Team. 2013 R: A language and environment for statistical computing. (Vienna, Austria, R
193   Foundation for Statistical Computing.
194   [13] Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L. 2010 Source partitioning using stable isotopes:
195   coping with too much variation. *PloS one* **5**, e9672.
196   [14] McMahon, K.W., Hamady, L.L. & Thorrold, S.R. 2013 A review of ecogeochemistry approaches to
197   estimating movements of marine animals. *Limnology and Oceanography* **58**, 697-714.
198   [15] Graham, B.S., Koch, P.L., Newsome, S.D., McMahon, K.W. & Aurioles, D. 2010 Using isoscapes to
199   trace the movements and foraging behavior of top predators in oceanic ecosystems. In *Isoscapes* (pp.
200   299-318, Springer Netherlands.
201   [16] Ratcliffe, N., Crofts, S., Brown, R., Baylis, A.M., Adlard, S., Horswill, C., Venables, H., Taylor, P.,
202   Trathan, P.N. & Staniland, I.J. 2014 Love thy neighbour or opposites attract? Patterns of spatial
203   segregation and association among crested penguin populations during winter. *Journal of biogeography*
204   **41**, 1183-1192.
205   [17] Lynch, H.J., White, R., Naveen, R., Black, A., Meixler, M.S. & Fagan, W.F. 2016 In stark contrast to
206   widespread declines along the Scotia Arc, a survey of the South Sandwich Islands finds a robust seabird
207   community. *Polar Biology* **39**, 1615-1625.

209 **Table 1.** Mean±SD δ^{13}C values and classification accuracies from GLS tracked penguins

210 exhibiting three differing winter migration strategies. Parentheses identify individuals from

211 either Admiralty Bay or Cape Shirreff, and LDA classifications excluding Adélie penguins.

212

213 **Table 2.** Mean±SD essential AA δ^{13}C values and assigned winter migration strategies of

214 Chinstrap penguins from five breeding locations. Parentheses identify GLS tracked individuals at

215 each site.

216

217 **Figure 1.** Indices of A) geographic habitat utilization and B) multivariate discrimination based

218 on essential AA δ^{13}C values of C) Adélie and Chinstrap penguins. Habitat utilization data

219 modified from Hinke et al. [10]. Dotted lines represent 50% probability of assignment.
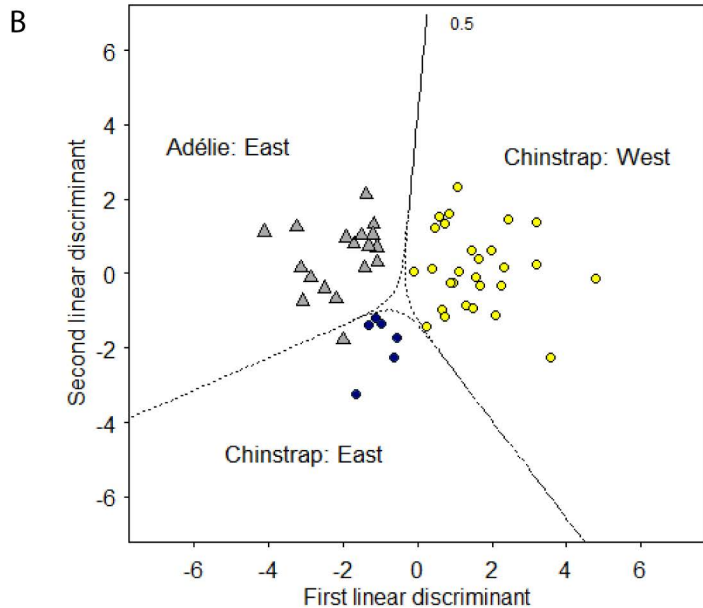
220

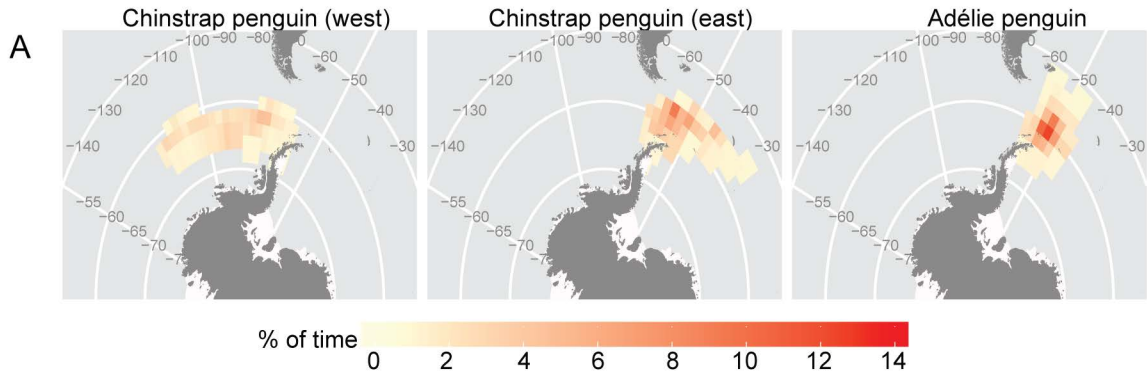221 **Figure 2.** A) Multivariate discrimination of tracked (colored points) and untracked (white points)

222 Chinstrap penguins based on essential AA δ^{13}C values and B) assigned winter migration

223 strategies (eastward or westward) in Chinstrap penguins from five breeding locations using LDA

224 (pie charts) and stable isotope mixing-models (histograms). Dotted line represents 50%
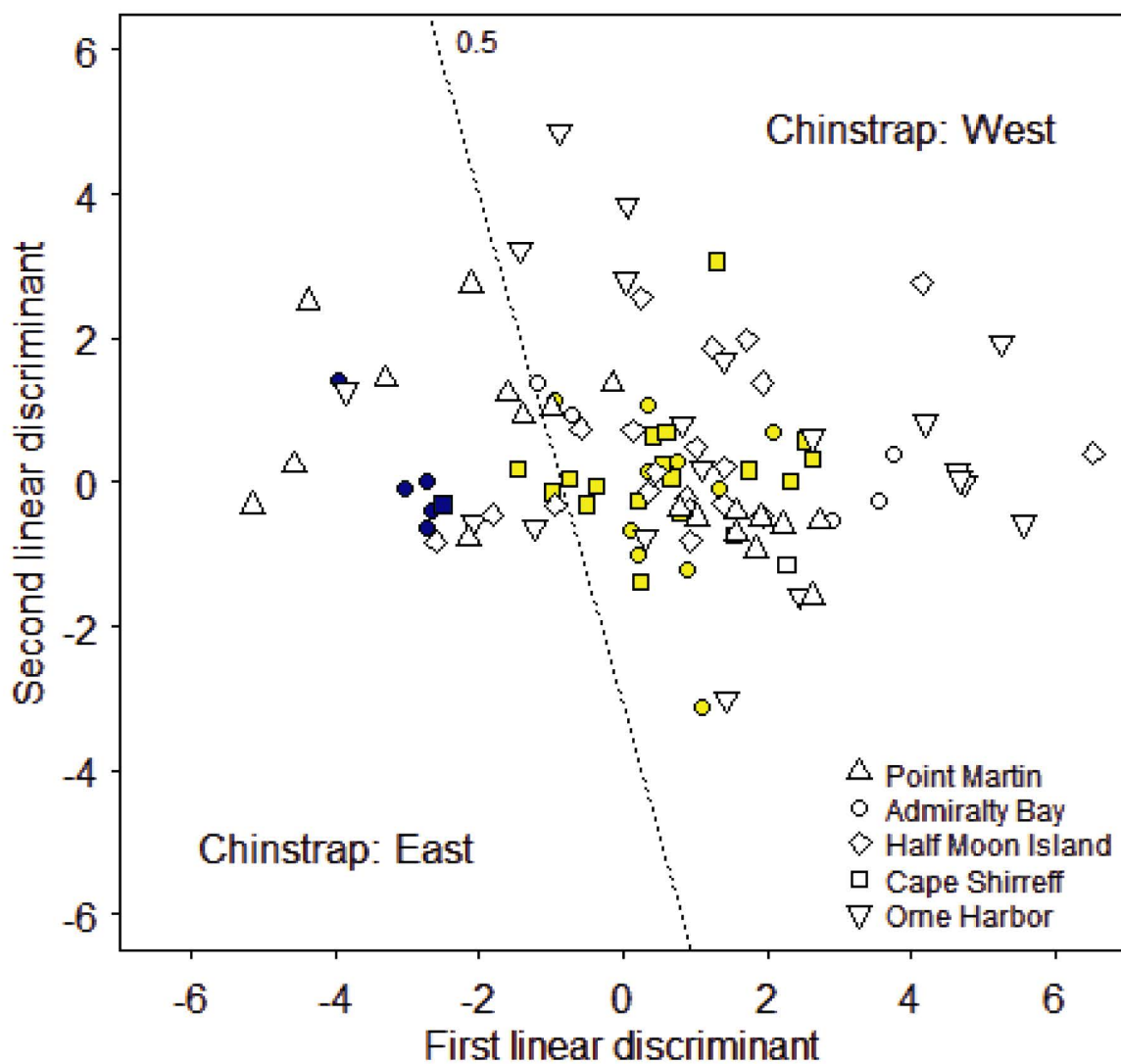
225 probability of assignment.

Table 1.

GLS tracked penguins	Adélie penguin	Chinstrap penguin	
	East, Weddell Sea	East, Scotia Sea	West, Pacific sector
n	18 *(18,0)*	6 *(5,1)*	28 *(10,18)*
$\delta^{13}C$ (‰)			
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 $\delta^{13}C$	66.7	33.3 (83.3)	82.1 (82.1)
Essential AA $\delta^{13}C$	94.4	100.0 (100.0)	96.4 (89.3)

Table 2.

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
n	20 (0)	20 (15)	20 (19)	20 (0)	20 (0)
$\delta^{13}C$ (‰)					
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)

A

Chinstrap penguin (west) | Chinstrap penguin (east) | Adélie penguin

% of time

0 2 4 6 8 10 12 14

B

Second linear discriminant

Adélie: East

Chinstrap: West

Chinstrap: East

First linear discriminant

0.5

C

Adélie penguin

Chinstrap penguin

A

Chinstrap: West

Chinstrap: East

Point Martin
Admiralty Bay
Half Moon Island
Cape Shirreff
Orne Harbor

B

Polar Front

N

Point Martin

Cape Shirreff

Admiralty Bay

Half Moon Island

Orne Harbour

East
West