PNAS
www.pnas.org

Supplemental Information Appendix for:

Divergent Trophic Responses of Sympatric Penguin Species to Historic Anthropogenic Exploitation and Recent Climate Change

Kelton W. McMahon[1], Chantel I. Michelson[2], Tom Hart[3], Matthew D. McCarthy[4], William P. Patterson[5], Michael J. Polito[2#]

[1]Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island, 02882, USA. ORCID: 000-0002-9648-4614

[2]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, Louisiana, 70803, USA. Michelson ORCID:0000-0001-6117-1346, Polito ORCID: 0000-0001-8639-443

[3]Department of Zoology, University of Oxford, 11a Mansfield Road, South Parks Road, OX1 3SZ, UK. ORCID: 0000-0002-4527-5046

[4]Ocean Sciences Department, University of California, Santa Cruz, California, 95064, USA. ORCID: 0000-0002-5426-7005

[5]Department of Geological Sciences, Saskatchewan Isotope Laboratory, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5E2, Canada. ORCID: 0000-0003-3387-708X

#Author for correspondence: Michael J. Polito, 1239 Energy, Coast and Environment Building, Louisiana State University, Baton Rouge, Louisiana, 70803, USA, ph: +1-225-578-9403 email: mpolito@lsu.edu

This PDF file includes:

Supplementary Information Text

1. Methods for compound-specific stable isotope analysis of amino acids:

Compound-specific stable isotope analyses of penguin feathers followed protocols modified from McMahon *et al.* (1). Three breast feathers per specimen (~3 mg) were homogenized and acid hydrolyzed in 1 ml of 6 N HCl at 110°C for 20 hrs to isolate the total free amino acids (AAs). Samples were evaporated to dryness under a gentle stream of N_2. The total free AAs were derivatized by esterification with acidified iso-propanol followed by acylation with trifluoroacetic anhydride (2) and brought up in ethyl acetate for stable isotope analysis. The derivatized AAs were injected on column in splitless mode at 250°C and separated on a BPX5 column (60 m x 0.32 mm inner diameter, 1.0 mm film thickness; SGE Analytical Science, Austin, Texas, USA) in a Thermo Trace Ultra gas chromatograph (GC) at the University of California - Santa Cruz, Santa Cruz, California, USA. The separated AA peaks were analyzed on a Finnegan MAT Delta^{Plus} XL isotope ratio monitoring mass spectrometer (irm-MS) interfaced to the GC through a GC-C III combustion furnace (980°C), reduction furnace (650°C), and a liquid nitrogen trap.

We analyzed five individuals per species from four discrete time periods (i.e. 1930s, 1960s, 1980s, and 2010s) for a total of 40 specimens. Feather samples were analyzed in triplicate along with a homogeneous laboratory algal standard (>100 repeat injections) and a mixed AA standard of known isotopic composition (Sigma-Aldrich Co., St. Louis, MO, USA). Standardization of runs was achieved using intermittent pulses of an N_2 reference gas of known isotopic value. Mean reproducibility of the laboratory algal standard and the mixed AA standard across all individual AAs was ±0.3‰.

We analyzed the $\delta^{15}N$ values of twelve individual AAs, accounting for approximately 77% of the total hydrolysable AAs in feathers. We assigned glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), leucine (Leu), isoleucine (Ile), proline (Pro), and valine (Val) as trophic AAs, and phenylalanine (Phe) and lysine (Lys) as source AAs. Note that glycine (Gly), serine (Ser), and threonine (Thr) do not behave similarly to either of these main groups (3-6) and have not been included in the trophic dynamic calculations. Acid hydrolysis converts glutamine (Gln) and asparagine (Asn) into Glu and Asp, respectively, due to cleavage of the terminal amine group, resulting in the measurement of combined Gln + Glu (referred to hereby as Glu), and Asn +Asp (referred to hereby as Asp).

The mean and variance of $\delta^{15}N_{Glu}$, $\delta^{15}N_{Phe}$, and $TP_{TDF\text{-}multi}$ across each time period were estimated separately using Bayesian Analysis of Variances (BANOVA) for gentoo and chinstrap penguins. Parameter estimates were unbiased for small sample sizes. Equal variance among time periods was assumed for each analysis. Parameter estimates were obtained with Markov chain Monte Carlo (MCMC) and JAGS. Vague priors were used for the mean (normal distributions with a mean of 0 and variance of 1000) and the variance (uniform distribution between 0 and 100). Each analysis was run with three chains, each for 100,000 iterations, a burn in of 10,000 iterations, and thinning of three, producing posterior distributions of 90,000 values. Pairwise comparison between time periods and between species are expressed as Bayesian posterior probabilities (PP) that the posterior distributions of $\delta^{15}N_{Glu}$, $\delta^{15}N_{Phe}$, and $TP_{TDF\text{-}multi}$ from one group (time period or species) are different in a one-way comparison with another group. We consider $PP > 0.99$ as a conservative indication of significant differences in $\delta^{15}N_{Glu}$, $\delta^{15}N_{Phe}$, and $TP_{TDF\text{-}multi}$. All statistics were performed in R version 3.5.0 (7).

2. Overview of CSIA: Disentangling baseline and trophic nitrogen isotope variability

Differential isotopic discrimination of amino acid (AA) nitrogen between diet and consumer allows for the disentanglement of trophic and baseline variability contributing to consumer $\delta^{15}N$ values (reviewed in McMahon and McCarthy (8)). Protein AAs are commonly divided into two groups, termed the "trophic" and the "source" AAs, based upon their nitrogen isotope fractionation during trophic transfer (after Popp, *et al.* (9)). The "source" amino acids (e.g. phenylalanine [Phe] and lysine [Lys]) undergo minimal isotopic discrimination between diet and metazoan consumers (10, 11), because N bonds are neither formed nor cleaved during typical metabolic reactions (10, 12, 13). Source AAs (e.g., Phe $\delta^{15}N$ [$\delta^{15}N_{Phe}$]) provide proxies for the isotopic signature of nitrogen cycling at the base of the food web, without the confounding factor of trophic modification (14-16). Conversely, "trophic" amino acids (e.g. glutamic acid [Glu], aspartic acid [Asp], alanine [Ala], leucine [Leu], isoleucine [Ile], proline [Pro], valine [Val]) exhibit strong isotopic discrimination during metazoan trophic transfer (10, 11) as a result of N-bond cleavage during transamination/deamination reactions (17). Together, these differentially fractionating AAs can be used to assess the trophic position of a consumer while simultaneously controlling for the nitrogen isotope value of the base of the food web (10). This approach is particularly valuable when working in complex or dynamic systems, where multiple different baseline end-members are present (18-20), when working on highly mobile or high trophic level consumers that may integrate across multiple food webs (21-23), and in paleoreconstructive context when it is challenging, if not impossible, to *a priori* characterize the isotopic baseline of past ecosystems (14, 24, 25).

Trophic dynamics of consumers can be assessed using a compound-specific trophic position (TP_{CSIA}) equation that quantifies trophic transfers and baseline nitrogen isotope

variability from a single sample. Successful application of the TP_{CSIA} equation requires proper parameterization. A recent meta-analysis of controlled feeding experiments examining AA isotopic discrimination across 70 species (88 distinct species-diet combinations) clearly showed that both diet quality and mode of nitrogen excretion significantly affect the trophic discrimination factor (TDF) parameter, the degree of fractionation of individual AAs between diet and consumer (8). In the gentoo and chinstrap penguin food webs, where consumers differ in their mode of nitrogen excretion (e.g., ammonia for krill and fish vs uric acid for penguins) and diet quality (e.g, krill feeding on phytoplankton vs penguins feeding on high protein fish), McMahon, Polito, Abel, McCarthy and Thorrold (1) proposed using a multi-TDF equation ($TP_{TDF\text{-}multi}$) with trophic AA Glu and source AA Phe:

$$TP_{TDF-multi} = 2 + \left[\frac{\delta^{15}N_{(Glu)} - \delta^{15}N_{(Phe)} - TDF_{(Glu-Phe)\,penguin} - \beta}{TDF_{(Glu-Phe)average}}\right]$$

where $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ represent the stable nitrogen isotope values of penguin Glu and Phe, respectively, β represents the difference in $\delta^{15}N$ between Glu and Phe of primary producers (3.4‰ for aquatic microalgae) (4, 26, 27), $TDF_{(Glu\text{-}Phe)\ average}$ represents an average trophic discrimination factor (TDF) of 6.3‰ ($\Delta^{15}N_{Glu} - \Delta^{15}N_{Phe}$) characteristic of planktonic marine food webs (see meta-analysis by McMahon and McCarthy (8)), and $TDF_{(Glu\text{-}Phe)\ penguin}$ represents the *Pygoscelis* penguin-specific $TDF_{Glu\text{-}Phe}$ value (3.5‰) derived from a controlled feeding experiment on *Pygoscelis* penguins (1).

Very few studies have used multi-TDF equations to examine consumer trophic dynamics. In fact, of the 60 environmental application studies identified by the meta-analysis of McMahon and McCarthy (8), 92% used a single TDF approach with trophic AA Glu and source AA Phe of either 7 or 7.6‰ $TP_{7.6\text{-}TDF}$ *sensu* Chikaraishi, *et al.* (10):

$$TP_{7.6-TDF} = 1 + \left[\frac{\delta^{15}N_{(Glu)} - \delta^{15}N_{(Phe)} - \beta_{Glu-Phe}}{TDF_{7.6}}\right]$$

where $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ represent the stable nitrogen isotope values of penguin Glu and Phe, respectively, $\beta_{Glu\text{-}Phe}$ represents the difference in $\delta^{15}N$ between Glu and Phe of primary producers (3.4‰ for aquatic microalgae) (4, 26, 27), and $TDF_{7.6}$ represents the commonly used trophic discrimination factor (TDF) of 7.6‰ ($\Delta^{15}N_{Glu} - \Delta^{15}N_{Phe}$) from the seminal paper on TP_{CSIA} by Chikaraishi (10). However, a number of field studies calculating TP_{CSIA} in upper trophic level consumers (including cephalopods, teleost fishes, elasmobranchs, marine mammals, and seabirds) have noted that assuming a constant $TDF_{Glu\text{-}Phe}$ value of 7.6‰ often led to substantially underestimated TP_{CSIA} (20-22, 28-30).

Alternatively, several studies have proposed using a single TDF approach but with averaged trophic and source AAs to improve the accuracy and precision of TP_{CSIA} (31-33):

$$TP_{Avg-TDF} = 1 + \left[\frac{\delta^{15}N_{(Avg-TrAA)} - \delta^{15}N_{(Avg-SrAA)} - \beta_{Avg-TrAA\,-\,Avg-SrAA}}{TDF_{Avg-TrAA\,-\,Avg-SrAA}}\right]$$

where $\delta^{15}N_{Avg\text{-}TrAA}$ and $\delta^{15}N_{Avg\text{-}SrAA}$ represent the average stable nitrogen isotope values of penguin trophic AAs (Glu, Ala, Leu, Ile, Pro, Val) and source AAs (Phe, Lys), respectively, $\beta_{Avg\text{-}TrAA\,-\,Avg\text{-}SrAA}$ represents the difference in average $\delta^{15}N$ between trophic AAs (Glu, Ala, Leu, Ile, Pro, Val) and source AAs (Phe, Lys) of primary producers (2.8‰ for aquatic microalgae) (4, 27, 32), and $TDF_{Avg\text{-}TrAA\,-\,Avg\text{-}SrAA}$ represents the average trophic discrimination factor (TDF) of 5.4‰ ($\Delta^{15}N_{TrAA} - \Delta^{15}N_{SrAA}$ for pairwise combinations of trophic AAs [Glu, Ala, Leu, Ile, Pro, Val] and source AAs [Phe, Lys]) for aquatic consumers (meta-analysis by McMahon and McCarthy (8). Nielsen, Popp and Winder (33) found that modeled uncertainties in TP_{CSIA} estimates significantly decreased when increasing the number of trophic and source AAs in the calculation.

We found that all three TP_{CSIA} approaches produced similar trends in penguin trophic dynamics (Fig. S1). Chinstrap penguin TP_{CSIA} did not change significantly through time while gentoo penguin TP_{CSIA} increased significantly between the 1960s and 1980s. On average, $TP_{7.6\text{-}TDF}$ produced the lowest TP_{CSIA} of any approach (chinstrap 2.4±0.1, gentoo 2.8±0.3), which was not ecologically realistic for consumers foraging on krill (chinstrap penguins) or krill, fish, and squid (gentoo penguins). $TP_{TDF\text{-}multi}$ produced the highest and most realistic TP_{CSIA} of any approach (chinstrap 3.2±0.1, gentoo 3.6±0.4). $TP_{Avg\text{-}TDF}$ was intermediate between the two other approaches but similar to $TP_{7.6\text{-}TDF}$, was often unrealistically low given the known diet patterns of these penguin species (34, 35).

3. Individual amino acid isotope value statistics

Penguin feather $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Glu}$ values differed between species in three of four decades examined (Fig. 2). Bayesian analysis indicated gentoo penguins had higher $\delta^{15}N$ values than chinstrap penguins in the 1960s ($\delta^{15}N_{Phe}$ PP=1.0; $\delta^{15}N_{Glu}$ PP>0.99), 1980s ($\delta^{15}N_{Phe}$ PP>0.99; $\delta^{15}N_{Glu}$ PP=1.0), and 2010s ($\delta^{15}N_{Phe}$ PP=1.0; $\delta^{15}N_{Glu}$ PP=1.0), but not in the 1930s ($\delta^{15}N_{Phe}$ PP=0.09; $\delta^{15}N_{Glu}$ PP=0.68). For each species considered individually over time, we found that chinstrap penguin $\delta^{15}N_{Phe}$ values were lower in the 1960s relative to the 1980s (PP>0.99) and $\delta^{15}N_{Glu}$ values were higher in the 1930s relative to the1960s (PP>0.99; Fig 3.). Gentoo penguin $\delta^{15}N_{Phe}$ values from the 1930s were lower than all other time periods (PP=1.0), and $\delta^{15}N_{Phe}$ values from the 1980s were lower than the 2010s (PP>0.99; Fig. 3). Gentoo penguin $\delta^{15}N_{Glu}$ values increased over time and differed (all PP<0.99) among all comparisons except those between the 1930s and 1960s (PP=0.84; Fig. 3).

Penguin TPs differed between species in two of four decades examined (Fig. 2). TP did not differ between species in the 1930s (PP=0.91) and 1960s (PP=0.42), while gentoo penguins

had higher TP relative to chinstrap penguins in the 1980s (PP=1.0) and 2010s (P=1.0). In addition, Bayesian analysis indicated no difference in chinstrap penguin TP across decades (all PP<0.96; Fig. 3). In contrast, gentoo penguin TP from the 1930s and 1960s were lower than those from the 1980s and 2010s (all PP>0.99; Fig. 3). These shifts in TP resulted in gentoo and chinstrap penguins having similar TPs in the 1930s and 1960s that diverged during the 1980s and 2010s (Fig. 2; Fig. 3).

4. Alternative hypothesis for variations in $\delta^{15}N_{Phe}$

The observed shifts in penguin trophic dynamics in the northern Antarctic Peninsula over the last 100 years were overlain on apparent shifts in biogeochemical cycling at the base of the food web. The $\delta^{15}N$ values of the source AA phenylalanine ($\delta^{15}N_{Phe}$), which serve as a proxy for the sources and cycling of nitrogen at the base of the food web (reviewed in McMahon and McCarthy (8), varied over the ~100-year record for both penguin species, though trend and magnitude of their values were not in parallel. In the 1930s, both gentoo penguins (median (2.5%-97.5% credible intervals): 4.0‰ (3.5-4.5‰) and chinstrap penguins (4.4‰ (3.9-5.0‰) had similarly low $\delta^{15}N_{Phe}$ values (PP=0.09). Over the next century, chinstrap penguin $\delta^{15}N_{Phe}$ values oscillated but remained relatively low (between 3.8 and 4.7‰), while gentoo penguin $\delta^{15}N_{Phe}$ values increased significantly between the 1930s and the 1960s (median (2.5%-97.5% credible intervals): 6.7‰ (6.2-7.2‰)) and 1980s (6.2‰ (5.7-6.7‰)), and then again by the 2010s (7.4‰ (6.9-7.9‰)) (All PP>0.99).

The divergent shifts in penguin $\delta^{15}N_{Phe}$ values over the last 100 years could reflect temporal shifts in diet among prey items that occupy food webs with different isotopic baselines. In the 1930s, TP data suggest both penguin species were foraging almost exclusively on krill and at this time both gentoo penguins and chinstrap penguins had similar $\delta^{15}N_{Phe}$ values. While

chinstrap penguins continued to forage almost exclusively on krill throughout the rest of the century, maintaining relatively low $\delta^{15}N_{Phe}$ values, gentoo penguins shifted their diet to higher trophic level consumers and their $\delta^{15}N_{Phe}$ values also increased. A recent study of food web dynamics in the Scotia Sea found that *Euphausiid* krill, which forage under sea ice, had lower bulk $\delta^{15}N$ values (4.3±1.0‰) than copepods foraging in the water column (5.6±0.9‰) and the myctophid fish (9.2±0.7‰) and *Galiteuthis* squid (8.7±0.1‰) that feed on them (36). However, this dietary shift is unlikely to be the sole driver of the shift in isotopic baseline recorded in the penguin $\delta^{15}N_{Phe}$ values, as gentoo penguin $\delta^{15}N_{Phe}$ values increased significantly between the 1930s and 1960s, yet their TP did not significantly change until after the 1960s, indicating that the baseline shift began before the trophic shift. This indicates that the underlying driver of changes in penguin $\delta^{15}N_{Phe}$ values between these species is likely a function of shifts in baseline nitrate $\delta^{15}N$ values in their foraging locations as discussed earlier.

SI References

1. K. W. McMahon, M. J. Polito, S. Abel, M. D. McCarthy, S. R. Thorrold, Carbon and nitrogen isotope fractionation of amino acids in an avian marine predator, the gentoo penguin (*Pygoscelis papua*). *Ecology and Evolution* **5**, 1278-1290 (2015).
2. J. Silfer, M. Engel, S. Macko, E. Jumeau, Stable carbon isotope analysis of amino acid enantiomers by conventional isotope ratio mass spectrometry and combined gas chromatography/isotope ratio mass spectrometry. *Analytical Chemistry* **63**, 370-374 (1991).
3. P. E. Hare, M. L. Fogel, T. W. Stafford, A. D. Mitchell, T. C. Hoering, The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. *Journal of Archaeological Science* **18**, 277-292 (1991).
4. J. W. McClelland, J. P. Montoya, Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology* **83**, 2173-2180 (2002).
5. A. K. Styring, J. C. Sealy, R. P. Evershed, Resolving the bulk $\delta^{15}N$ values of ancient human and animal bone collagen via compound-specific nitrogen isotope analysis of constituent amino acids. *Geochimica et Cosmochimica Acta* **74**, 241-251 (2010).
6. L. R. Germain, P. L. Koch, J. Harvey, M. D. McCarthy, Nitrogen isotope fractionation in amino acids from harbor seals: implications for compound-specific trophic position calculations. *Marine Ecology Progress Series* **482**, 265-277 (2013).
7. R Core Team (2018) R: A language and environment for statistical computing. (R Foundation for Statistical Computing, Vienna, Austria).

8. K. W. McMahon, M. D. McCarthy, Embracing variability in amino acid $\delta^{15}N$ fractionation: mechanisms, implications, and applications for trophic ecology. *Ecosphere* **7**, e01511 (2016).

9. B. N. Popp *et al.*, Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. *Terrestrial Ecology* **1**, 173-190 (2007).

10. Y. Chikaraishi *et al.*, Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnology Oceanography: methods* **7**, 740-750 (2009).

11. K. W. McMahon, S. R. Thorrold, T. S. Elsdon, M. D. McCarthy, Trophic discrimination of nitrogen stable isotopes in amino acids varies with diet quality in a marine fish. *Limnology and Oceanography* **60**, 1076-1087 (2015).

12. N. Patel, D. L. Pierson, R. A. Jensen, Dual enzymatic routes to L-tyrosine and L-phenylalanine via pretyrosine in *Pseudomonas aeruginosa*. *Journal of Biological Chemistry* **252**, 5839-5846 (1977).

13. D. Tomé, C. Bos, Lysine requirement through the human life cycle. *The Journal of Nutrition* **137**, 1642S-1645S (2007).

14. O. A. Sherwood, T. P. Guilderson, F. C. Batista, J. T. Schiff, M. D. McCarthy, Increasing subtropical North Pacific Ocean nitrogen fixation since the Little Ice Age. *Nature* **505**, 78 (2014).

15. A. Lorrain *et al.*, Nitrogen isotopic baselines and implications for estimating foraging habitat and trophic position of yellowfin tuna in the Indian and Pacific Oceans. *Deep Sea Research Part II: Topical Studies in Oceanography* **113**, 188-198 (2015).

16. E. D. Hetherington *et al.*, Spatial food-web structure in the eastern tropical Pacific Ocean based on compound-specific nitrogen isotope analysis of amino acids. *Limnology and Oceanography* **62**, 541-560 (2017).

17. A. Braun, A. Vikari, W. Windisch, K. Auerswald, Transamination governs nitrogen isotope heterogeneity of amino acids in rats. *Journal of Agricultural Food Chemistry* **62**, 8008-8013 (2014).

18. N. F. Ishikawa *et al.*, Stable nitrogen isotopic composition of amino acids reveals food web structure in stream ecosystems. *Oecologia* **175**, 911-922 (2014).

19. K. Maki *et al.*, Influence of nitrogen substrates and substrate C:N ratios on the nitrogen isotopic composition of amino acids from the marine bacterium *Vibrio harveyi*. *Geochimica et Cosmochimica Acta* **140**, 521-530 (2014).

20. R. I. Ruiz-Cooley, P. L. Koch, P. C. Fiedler, M. D. McCarthy, Carbon and nitrogen isotopes from top predator amino acids reveal rapidly shifting ocean biochemistry in the outer California Current. *PloS ONE* **9**, e110355 (2014).

21. A. Lorrain *et al.*, Nitrogen and carbon isotope values of individual amino acids: a tool to study foraging ecology of penguins in the Southern Ocean. *Marine Ecology Progress Series* **391**, 293-306 (2009).

22. J. J. Dale, N. J. Wallsgrove, B. N. Popp, K. N. Holland, Nursery habitat use and foraging ecology of the brown stingray *Dasyatis lata* determined from stomach contents, bulk and amino acid stable isotopes. *Marine Ecology Progress Series* **433**, 221-236 (2011).

23. Y. P. Papastamatiou, C. G. Meyer, R. K. Kosaki, N. J. Wallsgrove, B. N. Popp, Movements and foraging of predators associated with mesophotic coral reefs and their

potential for linking ecological habitats. *Marine Ecology Progress Series* **521**, 155-170 (2015).

24. Y. Itahashi, Y. Chikaraishi, N. Ohkouchi, M. Yoneda, Refinement of reconstructed ancient food webs based on the nitrogen isotopic compositions of amino acids from bone collagen: a case study of archaeological herbivores from Tell Ain el-Kerkh, Syria. *Geochemical Journal* **48**, e15-e19 (2014).

25. R. Schwartz-Narbonne, F. J. Longstaffe, J. Z. Metcalfe, G. Zazula, Solving the woolly mammoth conundrum: amino acid ^{15}N-enrichment suggests a distinct forage or habitat. *Scientific Reports* **5**, 9791 (2015).

26. Y. Chikaraishi, N. O. Ogawa, N. Ohkouchi, "Further evaluation of the trophic level estimation based on nitrogen isotopic composition of amino acids" in Earth, life, isotopes, N.Ohkouchi, I. Tayasu, K. Koba, Eds. (Kyoto University Press), pp. 37-51. (2010).

27. M. D. McCarthy, J. Lehman, R. Kudela, Compound-specific amino acid δ^{15}N patterns in marine algae: tracer potential for cyanobacterial vs. eukaryotic organic nitrogen sources in the ocean. *Geochimica et Cosmochimica Acta* **103**, 104-120 (2013).

28. C. A. Choy *et al.*, Global trophic position comparison of two dominant mesopelagic fish families (Myctophidae, Stomiidae) using amino acid nitrogen isotopic analyses. *PLoS ONE* **7**, e50133 (2012).

29. R. I. Ruiz-Cooley, L. T. Ballance, M. D. McCarthy, Range expansion of the jumbo squid in the NE Pacific: δ^{15}N decrypts multiple origins, migration and habitat use. *PLoS ONE* **8**, e59651 (2013).

30. C. J. Matthews, S. H. Ferguson, Spatial segregation and similar trophic-level diet among eastern Canadian Arctic/north-west Atlantic killer whales inferred from bulk and

compound specific isotopic analysis. *Journal of the Marine Biological Association of the United Kingdom* **94**, 1343-1355 (2014).

31. M. D. McCarthy, R. Benner, C. Lee, M. L. Fogel, Amino acid nitrogen isotopic fractionation patterns as indicators of heterotrophy in plankton, particulate, and dissolved organic matter. *Geochimica et Cosmochimica Acta* **71**, 4727-4744 (2007).
32. C. J. Bradley *et al.*, Trophic position estimates of marine teleosts using amino acid compound specific isotopic analysis. *Limnology and Oceanography-Methods* **13**, 476-493 (2015).
33. J. M. Nielsen, B. N. Popp, M. Winder, Meta-analysis of amino acid stable nitrogen isotope ratios for estimating trophic position in marine organisms. *Oecologia* **178**, 631-642 (2015).
34. A. K. Miller, M. A. Kappes, S. G. Trivelpiece, W. Z. Trivelpiece, Foraging-niche separation of breeding gentoo and chinstrap penguins, South Shetland Islands, Antarctica. *The Condor* **112**, 683-695 (2010).
35. M. J. Polito *et al.*, Contrasting specialist and generalist patterns facilitate foraging niche partitioning in sympatric populations of *Pygoscelis* penguins. *Marine Ecology Progress Series* **519**, 221-237 (2015).
36. G. Stowasser *et al.*, Food web dynamics in the Scotia Sea in summer: a stable isotope study. *Deep Sea Research Part II: Topical Studies in Oceanography* **59**, 208-221 (2012).

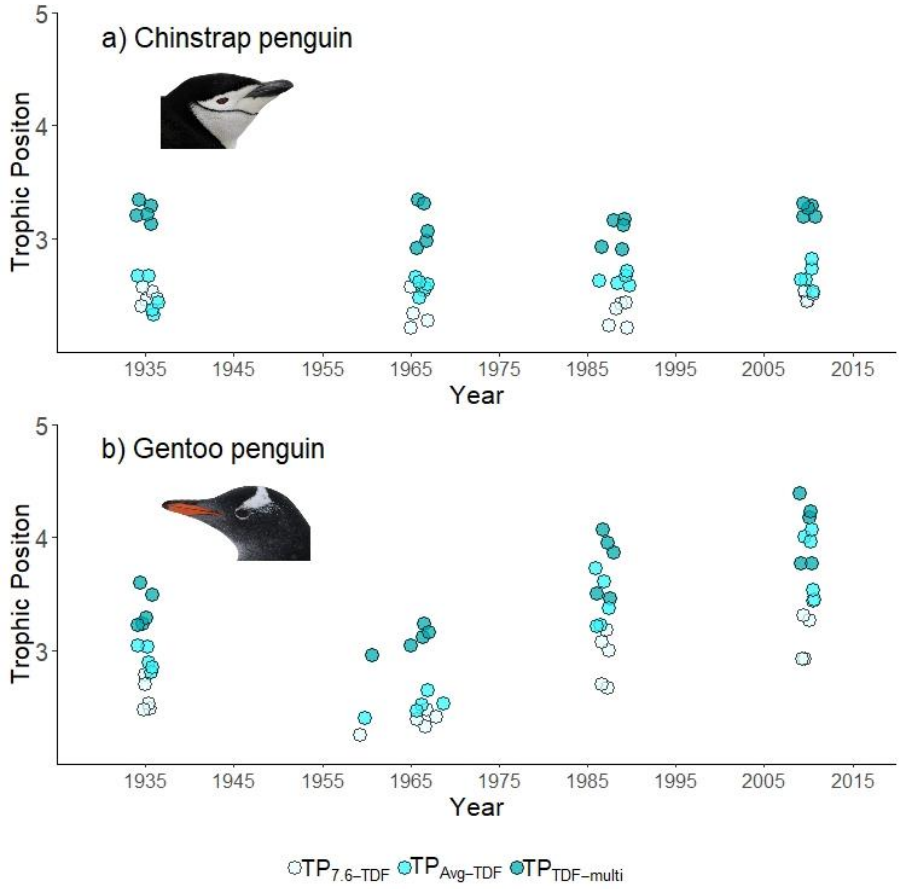

Figure S1. A comparison of the calculated trophic positions of archived historic museum specimens of chinstrap and gentoo penguins collected in the Antarctic Peninsula region in the 1930s, 1960s, 1980s, and 2010s using three compound-specific amino acid nitrogen isotope methods. Each symbol represents one individual penguin (five individuals per species per time period). $TP_{7.6\text{-}TDF}$ used a single TDF approach with trophic AA Glu and source AA Phe, *sensu* Chikaraishi et al. (2007), $TP_{Avg\text{-}TDF}$ used a single TDF approach but with averaged trophic and source AAs, *sensu* McCarthy, Benner, Lee and Fogel (31), and $TP_{TDF\text{-}multi}$ used a multi-TDF approach that accounted for key transitions in diet quality and consumer mode of nitrogen excretion within the food web supporting penguins *sensu* McMahon, Polito, Abel, McCarthy and Thorrold (1).

Table S1. Collection dates, locations, and $\delta^{15}N$ values of individual amino acids from historic, archival museum specimens of adult chinstrap penguins (*Pygoscelis antarctica*) collected in the Antarctic Peninsula region. 'Trophic' amino acids include glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), leucine (Leu), isoleucine (Ile), proline (Pro), valine (Val), glycine (Gly), and serine (Ser). Phenylalanine (Phe) and lysine (Lys) are classified as a 'source' amino acids and threonine (Thr) as a 'metabolic' amino acid following McMahon and McCarthy (8). Institution codes: American Museum of Natural History (AMNH), National Museum of Natural History (USNM), Swedish Museum of Natural History (NRM), Royal Belgian Institute of Natural Sciences (RBINS), Natural History Museum at Tring (NHMUK), and University of North Carolina Wilmington (UNCW).

Institution	Catalog no.	Sex	Locality	Year	Glu	Asp	Ala	Leu	Ile	Pro	Val	Gly	Ser	Phe	Lys	Thr	$TP_{7.6\text{-}TDF}$	$TP_{Avg\text{-}TDF}$	$TP_{TDF\text{-}muli}$
AMNH	Skin-442419	Unknown	Deception Island	1935	18.9	12.4	17.2	18.3	18.2	19.3	19.2	6.7	7.9	4.4	4.1	-19.5	2.5	2.6	3.2
AMNH	Skin-442420	Unknown	Deception Island	1935	19.9	13.7	18.6	20.0	18.8	21.3	21.2	7.6	8.2	4.8	5.1	-18.1	2.5	2.7	3.3
AMNH	Skin-442421	Unknown	Deception Island	1935	19.2	13.1	17.8	18.8	18.3	20.8	20.2	8.4	8.2	3.9	3.7	-20.5	2.6	2.8	3.3
AMNH	Skin-442419bis	Unknown	Deception Island	1935	18.2	12.8	17.9	18.0	17.3	20.4	19.1	8.1	8.4	4.2	4.3	-18.2	2.4	2.6	3.1
NHMUK	1938.12.19.137	Male	Deception Island	1936	19.5	12.2	19.1	18.0	17.5	18.8	19.4	8.1	8.6	4.9	5.0	-19.1	2.5	2.5	3.2
USNM	548079	Female	Tower Island	1966	19.1	12.0	16.7	17.6	18.0	17.9	18.2	8.6	9.1	3.7	3.4	-18.3	2.6	2.7	3.3
USNM	548080	Female	Tower Island	1966	16.8	10.7	15.9	15.6	16.9	16.6	17.1	7.7	8.0	4.2	4.1	-17.5	2.2	2.3	2.9
USNM	548078	Male	Tower Island	1966	18.2	12.3	16.5	17.4	17.9	17.7	18.1	9.9	10.1	3.1	3.5	-20.1	2.5	2.7	3.3
USNM	548039	Male	Gaston Island	1966	17.2	10.4	16.1	15.9	16.3	16.8	17.3	8.1	8.4	4.1	3.9	-17.9	2.3	2.4	3.0
USNM	548131	Female	Penguin Island	1966	17.6	11.5	16.1	16.5	16.8	16.5	17.2	8.5	9.1	4.0	3.7	-17.3	2.3	2.4	3.1
RBINS	2	Unknown	King George Island	1987	17.6	13.4	17.9	18.6	18.4	20.7	19.1	9.6	10.5	4.8	4.7	-21.5	2.2	2.6	2.9
NRM	896280	Female	Deception Island	1989	18.7	12.3	16.8	17.6	17.5	20.3	18.1	10.0	10.3	4.5	3.8	-22.2	2.4	2.6	3.2
NRM	896290	Female	Deception Island	1989	18.6	12.6	17.1	18.3	18.9	20.0	18.6	9.0	10.2	4.2	4.0	-23.0	2.4	2.7	3.2
NRM	896291	Female	Deception Island	1989	18.0	13.7	18.1	18.2	18.7	20.6	19.6	9.8	11.0	5.4	5.4	-21.2	2.2	2.5	2.9
NRM	896292	Male	Deception Island	1989	18.8	12.6	17.1	18.3	18.5	21.0	18.9	10.4	10.8	4.8	4.3	-21.4	2.4	2.6	3.1
UNCW	66	Female	King George Island	2010	19.2	12.0	16.2	17.5	17.0	19.8	18.9	9.2	10.0	4.1	3.6	-19.2	2.5	2.6	3.3
UNCW	67	Male	King George Island	2010	18.6	11.9	15.9	17.3	16.8	18.7	19.1	8.1	8.9	4.2	3.3	-18.1	2.5	2.6	3.2
UNCW	68	Female	King George Island	2010	19.0	12.4	16.5	17.6	16.8	20.6	18.7	9.6	10.1	4.6	3.9	-19.5	2.4	2.6	3.2
UNCW	72	Male	King George Island	2010	18.5	11.8	15.4	17.4	17.1	19.2	18.8	8.3	9.1	3.6	3.2	-19.4	2.5	2.7	3.3
UNCW	73	Female	King George Island	2010	18.8	12.6	16.3	18.2	17.3	20.5	19.2	10.2	10.6	3.7	3.7	-19.9	2.5	2.7	3.3

Table S2. Collection dates, locations, and $\delta^{15}N$ values of individual amino acids from historic, archival museum specimens of adult gentoo penguins (*Pygoscelis papua*) collected in the Antarctic Peninsula region. 'Trophic' amino acids include glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), leucine (Leu), isoleucine (Ile), proline (Pro), valine (Val), glycine (Gly), and serine (Ser). Phenylalanine (Phe) and lysine (Lys) are classified as a 'source' amino acids and threonine (Thr) as a 'metabolic' amino acid following McMahon and McCarthy (8). Institution codes: American Museum of Natural History (AMNH), National Museum of Natural History (USNM), Royal Belgian Institute of Natural Sciences (RBINS), Texas A&M University (TCWC), and University of North Carolina Wilmington (UNCW).

Institution	Catalog no.	Sex	Locality	Year	Glu	Asp	Ala	Leu	Ile	Pro	Val	Gly	Ser	Phe	Lys	Thr	$TP_{7.6\text{-}TDF}$	$TP_{Avg\text{-}TDF}$	$TP_{TDF\text{-}multi}$
AMNH	Skin-442413	Unknown	Deception Island	1935	18.2	12.7	19.1	18.8	19.4	18.8	20.1	8.6	9.7	3.5	3.2	-19.4	2.5	2.9	3.2
AMNH	Skin-442414	Unknown	Deception Island	1935	19.3	14.0	20.4	19.2	19.3	20.5	20.1	9.7	10.6	4.3	4.5	-20.0	2.5	2.8	3.3
AMNH	Skin-442415	Unknown	Deception Island	1935	21.6	15.1	21.5	21.7	21.9	21.9	20.9	10.2	11.7	4.6	4.8	-19.9	2.8	3.0	3.6
AMNH	Skin-442416	Unknown	Deception Island	1935	18.1	12.5	18.5	18.5	18.9	18.9	20.3	8.5	10.2	3.5	3.2	-23.1	2.5	2.9	3.2
AMNH	Skin-442418	Unknown	Deception Island	1935	20.4	14.9	20.0	21.2	21.7	20.8	20.6	9.1	10.1	4.0	3.9	-18.3	2.7	3.0	3.5
AMNH	SKIN-775712	Female	Brabant Island	1960	19.0	13.2	18.5	18.4	19.0	20.4	20.5	14.3	16.8	6.1	6.3	-17.2	2.3	2.4	3.0
USNM	548097	Male	Laurie Island	1966	20.4	14.9	19.8	20.2	20.6	21.5	22.3	16.0	18.0	6.9	6.7	-17.6	2.3	2.5	3.0
USNM	548098	Female	Laurie Island	1966	22.1	16.9	21.6	21.5	22.2	23.2	23.5	17.3	19.5	7.4	7.5	-18.3	2.5	2.7	3.2
USNM	547952	Female	Anvers Island	1966	20.9	15.7	19.7	20.3	20.6	21.4	22.0	16.2	17.9	6.9	7.3	-17.8	2.4	2.5	3.1
TCWC	13447	Unknown	Hope Bay, Palmer Peninsula	1968	20.3	14.7	19.3	19.3	20.1	20.8	22.2	15.2	17.1	6.1	6.6	-16.7	2.4	2.5	3.2
RBINS	69799	Female	King George Island	1987	25.8	21.9	25.6	26.7	27.2	28.6	28.9	13.1	14.5	6.5	6.2	-22.1	3.1	3.6	4.0
RBINS	3	Unknown	King George Island	1987	22.9	18.6	23.7	24.4	24.9	25.9	26.0	12.5	12.9	6.8	5.8	-20.9	2.7	3.2	3.5
RBINS	4	Unknown	King George Island	1987	26.1	21.6	26.8	27.1	27.5	27.7	29.7	10.7	12.5	6.1	5.9	-23.1	3.2	3.7	4.1
RBINS	7	Unknown	King George Island	1987	24.6	19.8	24.8	25.0	25.5	26.6	26.7	11.6	11.9	6.0	6.5	-21.4	3.0	3.4	3.9
RBINS	9	Unknown	King George Island	1987	22.1	17.8	24.3	23.6	24.4	25.0	25.3	11.1	13.0	5.8	6.1	-23.3	2.7	3.2	3.5
UNCW	43	Female	King George Island	2010	25.6	21.4	27.0	26.8	27.7	28.7	28.9	12.5	13.1	7.5	7.4	-22.1	2.9	3.5	3.8
UNCW	48	Male	King George Island	2010	29.7	26.3	28.7	31.3	32.0	32.3	33.4	11.7	12.2	7.8	7.6	-24.9	3.4	4.0	4.4
UNCW	50	Male	King George Island	2010	28.1	24.5	29.3	30.1	30.7	31.6	31.6	11.0	12.6	7.5	6.8	-22.3	3.3	4.0	4.2
UNCW	51	Male	King George Island	2010	25.7	22.9	26.7	27.7	28.7	29.0	30.0	11.2	13.2	7.7	7.3	-22.2	2.9	3.5	3.8
UNCW	58	Female	King George Island	2010	27.3	24.3	29.1	30.0	30.3	30.6	31.2	11.3	12.6	6.4	5.8	-23.2	3.3	4.1	4.2