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How large is large: estimating ecologically meaningful isotopic differences in observational studies of wild animals

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RATIONALE: In ecological studies of wildlife movements and foraging, bio-logging and isotopic data are routinely collected and increasingly analyzed in tandem. Such analyses have two shortcomings: (1) small sample size linked with the number of telemetric tags that can be deployed, and (2) the observational nature of isotopic gradients. Wildlife ecologists are thus put in a statistical conundrum known as the *small n, large p* problem.

METHODS: Using shrinkage regression, which directly addresses the issue of accurately estimating effects from sparse data, we studied what counts as a biologically meaningful isotopic difference (a prerequisite to delineate isoscapes) in the southern elephant seal (*Mirounga leonina*), a large and elusive marine predator.

RESULTS: Seals foraging in Antarctic waters had a lower carbon isotopic value (by $\approx 2\%$) than seals foraging either in the interfrontal zone or on the Kerguelen Plateau. The latter two foraging strategies were indistinguishable on the sole basis of $\delta^{13}\text{C}$ values with our data.

CONCLUSIONS: Shrinkage regression is a conservative statistical technique that has wide applicability in isotopic ecology to help separate robust biological signals from noise. Copyright © 2012 John Wiley & Sons, Ltd.

A popular application of stable isotopes in ecology is as natural 'tags' to study the movement of animals between habitats.^[1,2] The rationale for this approach follows from the premise that "in equilibrium situations, animal tissues reflect the isotopic structure of local food webs".^[3] In other words, the foraging behaviour of consumers is investigated indirectly by taking advantage of naturally occurring differences in stable isotope composition between ecosystems. However, this reliance on natural gradients also means that stable isotopic data collected from wild animals are more observational than experimental.^[4] Reviews on the use of stable isotopes in wildlife ecology have stressed some vexing problems linked to observational data, for example the accurate estimation of discrimination factors.^[5,6]

Because stable isotopes provide indirect evidence, their analysis requires both substantial biological knowledge and an adequate statistical analysis to account for relevant sources of variations. Hobson *et al.*^[3] stated three major conditions for an accurate interpretation of wildlife movements: (1) animals must move between isotopically distinct food webs, (2) bias between the stable isotopic values of prey and consumer are accounted for (that is, discrimination factors are accurately known), and (3) the isotopic turnover of the sampled tissue is known. When experiments cannot be performed under controlled conditions, requirements (2) and (3) may be quite difficult to meet for

wildlife ecologists, more so when studying rare or cryptic or endangered species; the kind of situation where the use of stable isotopes is especially attractive.

Even the first requirement of Hobson *et al.*^[3] may be challenging. While for terrestrial ecosystems geographical regions with known differences in isotopic composition, or isoscapes, have been mapped (e.g.^[7,8]), there are comparatively fewer data to compile such maps for marine ecosystems.^[2,9–11] Ecological knowledge of marine organisms has, however, greatly increased with the use of stable isotopes.^[12] In practice, marine biologists may have to estimate the isotopic signature of oceanic basins, fractionation factors or tissue turnover rates from limited data given the difficulties associated with sampling the world's oceans. Sampling preys in the marine environment may also be challenging for deep-diving predators. In general, we can expect the stable isotope values of animal tissues to be affected by many sources of variations, each of small magnitude and of different signs. The expected trend of the sum of all these variations is hard to predict, and there is no strong *a priori* reason that it should be exactly zero, although its magnitude may be small. All the aforementioned difficulties may put marine biologists into a situation where data (*n*) are scarce, yet many parameters (*p*) have to be accurately estimated: a *small n, large p* setting.

Our aim is to illustrate with an example on a large marine predator, the southern elephant seal (*Mirounga leonina*), how to conduct inferences with stable isotopes in a *small n, large p* situation. We want to bring forward to a greater audience some statistical tools that may help wildlife ecologists to meet the first and, to a lesser extent, the third requirements of Hobson *et al.*:^[3] how to estimate isoscapes

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and tissue turnover rate from observational data. To achieve this goal, we used both isotopic and bio-logging data and conducted all analyses with the free statistical software R.^[13]

Estimating tissue turnover

Bentaleb *et al.*^[14] and Trull and Armand^[15] documented a decreasing latitudinal gradient of $\delta^{13}\text{C}$ values of Particulate Organic Matter in the Southern Ocean. Studies on marine predators such as penguins or procellariiforms have taken advantage of this gradient to infer the foraging grounds of these predators.^[16] Jaeger *et al.*^[10] were the first to explicitly attempt to estimate isoscapes for a top predator, the wandering albatross (*Diomedea exulans*), without relying on isotopic data on the lowest trophic levels of the Southern Ocean food web. Jaeger *et al.*^[10] first equipped animals with ARGOS tags; then, upon tag retrieval, they took a blood sample to measure the $\delta^{13}\text{C}$ values of plasma, a tissue with a high turnover rate of proteins.^[17] Although the precise turnover rate of albatross' plasma protein was unknown to Jaeger *et al.*,^[10] the short duration of trips (on average 11 days) allowed for a reasonable match between the temporal resolution of the isotopic and bio-logging data. This, however, need not always be the case.

Southern elephant seals, for example, undertake foraging trips that may last several months. Relating a single number, a $\delta^{13}\text{C}$ value, to the complex behaviour of foraging is a challenge that ecologists have to overcome. Stable isotopes are especially appealing for this precise reason: they integrate the whole foraging trip of an animal. However, when tissue turnover is unknown, which part of the trip is reflected in the measured $\delta^{13}\text{C}$ value has to be inferred. In a heterogeneous environment, such as the Southern Ocean, where food resources are patchily distributed in space and time, an area-restricted search (ARS) may be linked to resource acquisition.^[18] Upon capturing a prey in a patch, an animal can intensify its foraging in this patch: the swimming speed decreases and the track sinuosity (that is, the turning angle between two successive localizations) increases.^[19] Thus, foraging may be inferred from ARS patterns along a track from speed and turning angle data.

The mean latitude at which an animal may exhibit ARS behaviour can be inferred with state-space models.^[20] Because tissue protein turnover is a continuous physiological process, this computation can be carried out for several time steps (chosen according the species biology) before tag retrieval and isotopic sampling. The mean ARS latitude for each time step may then be correlated with measured $\delta^{13}\text{C}$ values. Any significant relationship between latitude and $\delta^{13}\text{C}$ value is expected to gradually wear-off because of tissue turnover. The lag at which this significant relationship disappears would then indicate the temporal resolution of the sample tissue. This procedure would allow the third requirement of Hobson *et al.*^[3] to be met.

How large is large enough for an isotopic difference?

In the Southern Ocean, mesoscale features, such as fronts and eddies, structure and enhance primary productivity.^[21] They are likely to influence the spatial structure of prey fields and play a key role in the creation of preferred foraging regions for oceanic top-predators.^[22] The interfrontal zone, between the southern boundary of the Antarctic Circumpolar Front

(PF) and the Subtropical Front (STF), is very dynamic with locally productive eddies. South of the Polar Front, the vast Antarctic zone presents important seasonal variations in sea-ice extent. The interactions between sea-ice extent and the local oceanographic conditions determine the dynamics of key Antarctic species such as Antarctic krill (*Euphasia superba*),^[23] a keystone species of the Southern Ocean food web. Southern elephant seals from the Kerguelen population present several foraging strategies.^[24] Seals mainly forage in three rich and dynamic areas: in Antarctic waters, on the Kerguelen Plateau (a very productive shelf at the local scale^[25]), and in the interfrontal zone (Fig. 1).

A priori, the Antarctic $\delta^{13}\text{C}$ signature is expected to be smaller than that of interfrontal waters,^[26,27] the $\delta^{13}\text{C}$ signature of interfrontal waters itself is expected to be smaller than that of waters above the Kerguelen Plateau because of increased primary productivity.^[16,28,29] However, the large bloom of phytoplankton over the Kerguelen Plateau is carried eastward by currents, forming a long plume that extends into the oceanic waters of the interfrontal zone.^[28] Thus, large differences between the carbon isotopic signature of the interfrontal zone and water masses over the Kerguelen Plateau are not likely. The following relationship with carbon isotopes may be expected:

$$\delta^{13}\text{C}_{\text{Antarctic}} < \delta^{13}\text{C}_{\text{Interfrontal}} \leq \delta^{13}\text{C}_{\text{Plateau}}$$

The challenge is to estimate accurately these three carbon isotopic signatures using isotopic data from individuals equipped with tags. If tracks are known, the animals may be classified according to their foraging strategies. However, it is important to realize that at the time of tag equipment, the strategy of individuals is unknown, which may result in a more or less severely unbalanced design. Equipped animals may be an heterogeneous group, differing with respect to (st) age or sex. Animals of different ontogenetic stages may have different foraging grounds.^[30] Such differences will typically be accounted for in a statistical analysis at the price of a lower parameter-to-data ratio and larger standard errors. Because tag price is usually what limits the number of deployments,^[31] ecologists may have to work with relatively small sample size, which in turn may limit the ability to estimate small effects.^[32] This is a serious limitation on our inferential abilities given the costly nature of the data collected. To remedy this limitation, we propose to use shrinkage regression^[33] to estimate accurately how large is large enough for an isotopic difference under uncontrolled conditions.

EXPERIMENTAL

Field work

From 2003 to 2011, 80 southern elephant seals were captured on îles Kerguelen (49°20'S, 70°20'E) at the end of the breeding (October) or moulting (February to April) seasons. They were anaesthetized using a 1:1 combination of Tiletamine and Zolazepam (Zoletil 100) which was injected intravenously.^[34] Before logger deployment, the seals were blood sampled and weighed. Data loggers were then glued with a quick-setting epoxy resin (Araldite AW 2101) on the seals' heads after cleansing their fur with acetone. The deployed tags were satellite-relayed-data devices that collected and transmitted Argos locations

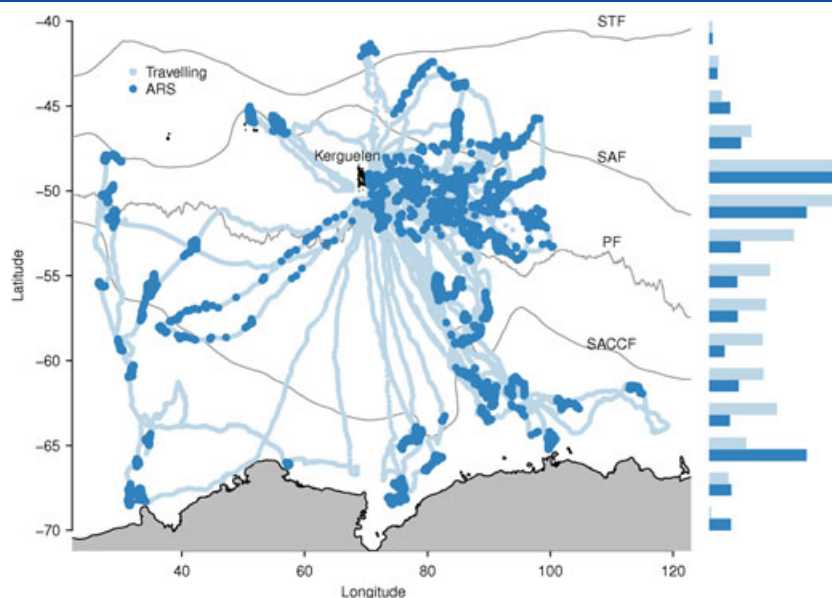


Figure 1. Satellite tracking of southern elephant seal breeding on îles Kerguelen. Twenty-six complete tracks are represented (light blue crosses) to illustrate the different strategies: seals mainly forage in the Antarctic Zone, on the Kerguelen Plateau or in the interfrontal zone (waters lying between the Sub-Tropical Front and the Southern Antarctic Circum-Polar Front). Dark blue dots symbolize Area Restricted Search (ARS) behaviour. Black lines symbolize frontal structures within the Southern Ocean: Southern Sub-Tropical Front (SSTF), Sub-Antarctic Front (SAF), Polar Front (PF) and Southern Antarctic Circum-Polar Front (SACCF). A marginal histogram of localisations' latitude is represented on the side to illustrate the different strategies.

during the seals' post-breeding or post-moulting trips at sea. Upon returning to îles Kerguelen, 26 animals were recaptured and blood sampled. Blood was collected from the dorsal venous sinuses using 90×1.2 mm needles. Ethanol (70%) was added for sample preservation before laboratory analysis.

Tracking data analysis

To take into account measurement errors, ARGOS locations from the 26 complete tracks were analyzed using a switching state-space model.^[20,35] State-space models are an efficient and robust analytic method for detecting areas of intensive foraging from ARGOS tracking data.^[35,36] A preliminary analysis of speed and turning angle distributions (not shown) suggested fitting models with two behavioural modes. Such state-space models allowed us to estimate the probability that a seal exhibited intensive foraging (that is ARS behaviour) or extensive foraging along its track (for details, see Dragon *et al.*^[36]).

Stable isotopes

Before isotopic analysis, whole blood was oven-dried for 48 h at 50–60 °C. Samples were weighed (range: 3–5 mg) into tin cups prior to combustion in an elemental analyzer (Flash EA 1112, Thermo Fisher, Waltham, MA, USA) coupled to a continuous flow mass spectrometer (Thermo Fisher, Delta V Advantage). The carbon to nitrogen (C/N) mass ratios were checked and, when they were above 3.7, lipids were extracted using cyclohexane. Lipids are depleted in ^{13}C relative to

proteins and carbohydrates,^[37] but lipid extraction is usually unnecessary due to the typically small lipid content of blood. Stable isotopic ratios are presented in the usual δ notation (in ‰) relative to Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ values. Replicate measurements of internal laboratory standards (acetanilide, $\delta^{13}\text{C} = -27.5$ ‰, 2 every 23 samples) indicated precisions of 0.15 ‰ for carbon.

To assess the turnover rate of blood ^{13}C , we used the complete track of 26 southern elephant seals which were blood sampled both upon tag deployment and upon retrieval (data set 1). The mean latitude at which a seal exhibited ARS behaviour was computed for each seal before tag retrieval. We focused on ARS as we expected this behavioural state to reflect increased foraging effort. The mean latitude ARS was estimated for each cumulative month before a seal came back on land (hereafter, haul-out), that is we computed the mean latitude of ARS for the last month, the last two months, and so forth, before haul-out. We then sequentially correlated this mean latitude with the $\delta^{13}\text{C}$ value obtained upon tag retrieval. We expected any significant relationship between latitude and $\delta^{13}\text{C}$ value to gradually wear-off because of blood protein turnover. The lag at which this significant relationship disappeared would then indicate the temporal resolution of blood $\delta^{13}\text{C}$ values. Tissue isotopic turnover scales with body mass.^[38] Because there was a strong correlation between body length and body mass ($\rho = 0.87$, $p < 0.001$), we included body length as a covariate in the models. Southern elephant seals only haul-out on land to moult at the end of the Austral Summer, or to breed at the beginning of the Austral Spring. Accordingly, we included whether a seal performed a post-moulting or post-breeding trip as a covariate.

Upon assessing the temporal resolution of the $\delta^{13}\text{C}$ values, we then included in the regression the $\delta^{13}\text{C}$ value corresponding to tag deployment, which is the value reflecting the previous foraging trip to assess foraging fidelity (denoted $\delta^{13}\text{C}_{t-1}$ value). If seals are faithful to a foraging strategy, we can expect (1) a slope of 1 between $\delta^{13}\text{C}_t$ and $\delta^{13}\text{C}_{t-1}$ values, and (2) latitude to lose its statistical significance.

Because the temporal resolution of the $\delta^{13}\text{C}$ values was at least 4 months and, because seals were faithful to a strategy (see Results section), we analyzed isotopic data collected upon tag deployment. Using track data, we were able to determine whether a seal foraged predominantly on the Kerguelen Plateau, in the interfrontal zone or in Antarctic waters for 80 equipped seals (data set 2) whose foraging track was not necessarily complete (thus assuming the observed part to be representative of the whole track). We used Linear Models to determine the isotopic signature of these three water masses (those of the Kerguelen Plateau, interfrontal and Antarctic zones), and tested the effect of sex, body length, trip type (post-moulting vs. post-breeding trips), foraging location and all interactions between sex and other covariates. Since males foraged over the Antarctic Shelf or the Kerguelen Plateau,^[24,39] an interaction between sex and foraging location is expected.

This data set is not balanced: females ($n = 54$) outnumbered males ($n = 26$) by a factor of 2, only five males were tracked over a post-breeding trip, and only one female foraged over the Kerguelen Plateau. Thus, some covariates (or combination thereof) are largely correlated. To circumvent this issue, variable selection was performed using shrinkage regression with a spike-and-slab prior.^[40] The key idea of shrinkage regression is to *a priori* penalize a regression coefficient by using a weakly-informative prior that shrinks this coefficient towards zero unless there is a strong signal in the data that it is not zero. The expression *slope-and-slab* refers to a mixture prior for regression coefficients: this prior is made up of a diffuse and vague distribution (the slab) and a degenerate distribution at 0 (the spike, Fig. 2). The spike pulls coefficients for which there is no information in the data to 0, thereby achieving variable selection, while the slab allows the other coefficients to escape this gravitational pull and to be accurately estimated. Spike-and-slab priors prevent the overestimation of effect size, or type-M (magnitude) error.^[32] Finally, spike-and-slab regression necessitates fitting only one model to achieve model selection compared with alternative procedures based on Information Criterion, which require fitting all the $2^{\text{number of parameters}}$ possible models.

The stability of a selected variable was checked by cross-validation: a value of 1.00 means that a variable was always selected during the cross-validation trials. The original dataset was randomly split into two halves, one for training and the other for prediction. This procedure was repeated 40 times. The results are summarized in Table 2.

All linear models were fitted in R version 12.0^[13] using the *arm* package. The *arm* mathematical library of functions implements Linear Models with weakly informative priors that shrink regression coefficients towards zero. The threshold for statistical significance was set to 5%. Values for parameter θ are reported as $\hat{\theta}_{se}$ where *se* is the standard error.^[41] Spike-and-slab variable selection for linear models is implemented in the R package *spikeslab*.^[42] This mathematical library of functions also implements a cross-validation procedure (function *cv.spikeslab*). For all computations, a burn-in of 10 000 iterations was run to

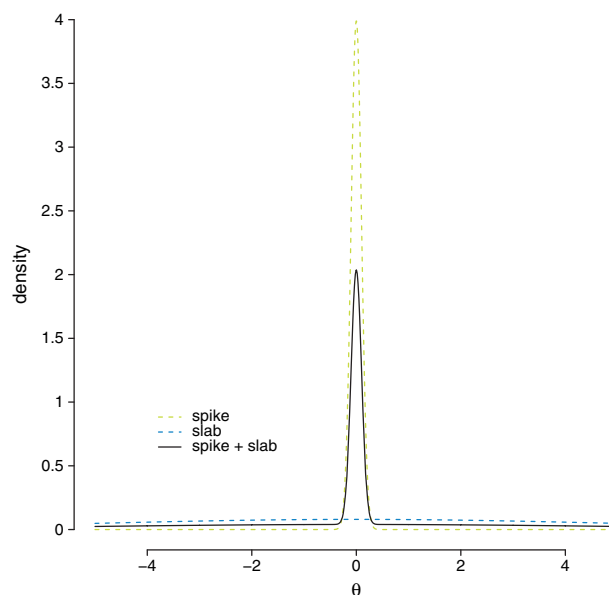


Figure 2. Graphical representation of a spike-and-slab prior. The spike corresponds to a sharp density around zero, which effectively shrinks the posterior estimate towards zero. The slab is a diffuse density over a wide interval, which gives a small, but non-zero, weight to other values than zero.

achieve model convergence. Posterior means are computed from an additional 10 000 iterations. The package *spikeslab* does not allow running multiple chains in parallel: computations were carried out several times with different seeds (random initial values) to check the stability of the results.

RESULTS

Tracking data summary

ARGOS tags emitted on average 7 ± 4 locations per day. The 26 animals equipped (data set 1) spent an average of 2.8 months at sea (85 ± 9 days) for post-breeding animals, and an average of 5.7 months at sea (171 ± 38 days) for post-moulting animals. They spent an average of $84.3 \pm 11.1\%$ of their time in oceanic waters (Fig. 1). State-space models identified two statistically distinct behavioural modes: intensive foraging, which corresponds to ARS, and extensive foraging. ARS behaviour was displayed on average during 43% of their time at sea ($43.5 \pm 13.1\%$) and similar proportions of ARS were displayed in oceanic waters ($45.1 \pm 14.9\%$) and over the Kerguelen Plateau ($45.6 \pm 21.7\%$).

Carbon turnover

There was a statistically significant relationship between $\delta^{13}\text{C}$ value and the mean latitude where a seal exhibited ARS behaviour, up to 4 months before hauling-out ($p < 0.001$, Table 1). Small sample size prevented conclusions being drawn about this relationship beyond 4 months before haul-out. In fact, there was a statistically significant relationship between $\delta^{13}\text{C}$ value and the mean ARS latitude of the *whole trip* (Fig. 3). The blood $\delta^{13}\text{C}$ value thus seemed to reflect the whole previous trip of a southern elephant seal. When the $\delta^{13}\text{C}_{t-1}$ value was

Table 1. Regression coefficients for the relationship between blood $\delta^{13}\text{C}$ value and latitude in southern elephant seals

Month before haul-out	N	$\bar{\theta}$	se	z score	p value
1	26	1.49	0.18	8.3	< 0.01
2	26	1.69	0.72	2.4	< 0.01
3	26	1.66	0.71	2.3	< 0.01
4	13	1.65	0.15	11.0	< 0.01
5	7	0.90	0.41	2.2	< 0.05
Whole trip	26	2.04	0.29	7.0	< 0.01

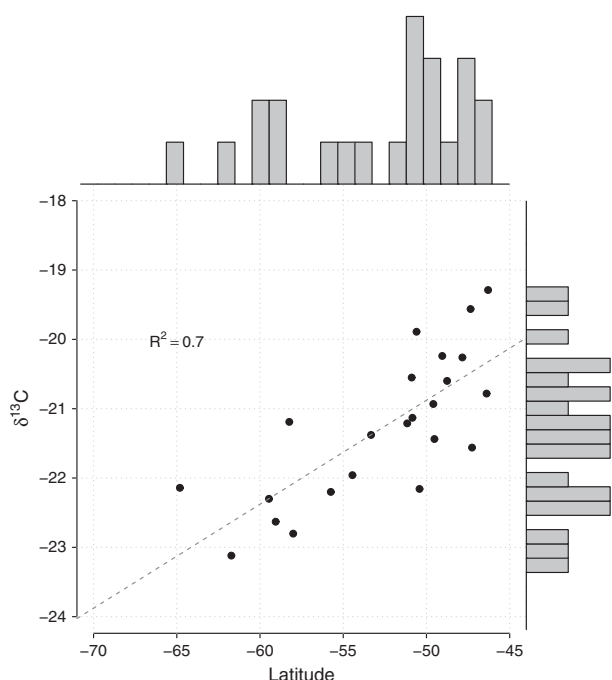


Figure 3. Relationship between the mean ARS latitude of a foraging trip and blood $\delta^{13}\text{C}$ value in southern elephant seals from îles Kerguelen. Marginal distributions are depicted on the margins of the plot.

included in the regression model, the relationship with latitude disappeared ($\bar{\theta}_{se} = 0.01_{0.26}$, $p = 0.97$, Fig. 4). The coefficient of regression for the $\delta^{13}\text{C}_{t-1}$ value was $\bar{\theta}_{se} = 0.987_{0.005}$, which was statistically different from either 0 ($p < 0.001$) or 1 ($p = 0.004$). Whether the $\delta^{13}\text{C}_{t-1}$ value was included or not, trip type (post-moulting vs. post-breeding) was a statistically significant factor: post-moulting trips were associated with smaller $\delta^{13}\text{C}$ values.

Carbon isoscapes

From a dataset of 80 seals which were blood-sampled upon tag deployment, our stability analysis suggested that the two most important variables were whether a seal foraged in Antarctic waters and the trip type (Table 2). As expected, Antarctic foraging was associated with a smaller $\delta^{13}\text{C}$ value than foraging in the interfrontal zone ($\Delta\delta^{13}\text{C}_t = -1.7\text{‰}$).

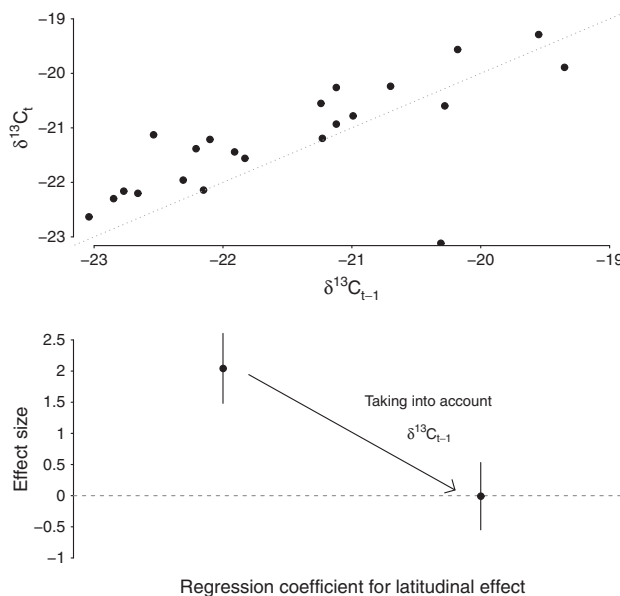


Figure 4. Relationship between blood carbon stable isotopic values at tag deployment ($\delta^{13}\text{C}_{t-1}$) and retrieval ($\delta^{13}\text{C}_t$) in southern elephant seals from îles Kerguelen, and its effect on the regression coefficient for latitude.

There were no other variables whose statistical stability was greater than 0.8, but the next most stable variables were whether seals foraged on the Kerguelen Plateau and an interaction between sex and body length. The carbon isotopic signature of the waters of the Kerguelen Plateau is very similar to that of the interfrontal zone, with the former being ^{13}C -enriched by 0.2‰ compared to the latter. This small difference is of similar magnitude to the measurement error of the $\delta^{13}\text{C}$ values. Given the residual standard deviation of 0.8‰, the water masses of the Kerguelen Plateau and the interfrontal zone had indistinguishable $\delta^{13}\text{C}$ signatures with our data.

DISCUSSION

Like many wildlife ecologists, we have endeavoured to couple stable isotopic analysis with bio-logging data to unravel the foraging ecology of wide-ranging, elusive marine predators.^[10,43–46] Our incremental contribution is to advocate the use of shrinkage regression to address the issue of small sample size currently associated with bio-logging.^[31]

We first estimated blood turnover rate from the coupling of bio-logging data analysis and stable isotopes. To achieve this, we relied on the ARS concept to infer behaviour from track data. We acknowledge that this is may be a limitation on the generality of our proposal for species which do not exhibit ARS behaviour. In southern elephant seals, ARS behaviour was associated with improved body condition,^[47] although foraging is not necessarily restricted to ARS behaviour in this species.^[48] Analyses conducted on the mean latitude of the whole trip yielded the same results as reported in Table 1. The analysis of blood protein turnover rate strongly suggested that seals were faithful to a foraging strategy as the previous $\delta^{13}\text{C}_{t-1}$

Table 2. Spike-and-slab regression summary. Regression coefficients for 10 variables/interactions are reported relative to a baseline taken as the $\delta^{13}\text{C}$ value of a female seal of average snout-to-tail length of 250 cm that foraged in interfrontal waters ($\delta^{13}\text{C} = -20.2\text{‰}$). Standardized coefficients allow the relative importance of each variable to be assessed, while the scaled ones are useful for predictions. For example, a seal that foraged into Antarctic waters has a carbon signature of $-20.2 - 1.7 = -21.9\text{‰}$. Snout-to-tail length was standardized.^[59] the scaled regression coefficient corresponds to the value for being 50 cm larger than average. OLS stands for the 'Ordinary Least Squares' regression coefficient and are reported to illustrate shrinkage

Parameter	Posterior Mean			
	Standardized	Scaled	OLS	Stability
Antarctic	-0.77	-1.7	-2.0	0.900
Trip	-0.64	-1.3	-1.6	0.800
Male \times Snout-to-tail length	-0.38	-0.9	-1.9	0.675
Plateau	0.08	0.2	0.2	0.675
Male \times Interfrontal	0.07	0.3	-0.1	0.650
Male \times Plateau	0.10	0.1	NA	0.600
Snout-to-tail length	0.20	0.4	-0.1	0.500
Male	0.03	0.1	0.1	0.475
Male \times Antarctic	-0.05	-0.2	-0.2	0.475
Male \times Trip	0.02	0.1	0.4	0.475

value, when included in the regression, cancelled the statistically significant effect of latitude on the $\delta^{13}\text{C}_t$ value. This finding is in agreement with previous work on this species.^[46,49,50]

Using shrinkage regression, we further sought to estimate the isotopic signature of three large marine zones in the Southern Ocean from the blood isotopic values of southern elephant seals. We found that we could identify Antarctic foragers, but we could not reliably distinguish between the interfrontal or a Kerguelen Plateau strategy with $\delta^{13}\text{C}$ values alone. However the estimated difference was plausible both in sign and magnitude: waters of the Kerguelen Plateau are expected to have an enriched ^{13}C signature due to their large productivity.^[28] It is also noteworthy that the estimated difference has not been shrunk toward 0 compared with the Ordinary Least Squares estimate (Table 2). The modest size of our dataset might have prevented an unambiguous assessment of the carbon isotopic difference between the waters of the Kerguelen Plateau and those of the interfrontal zone.

Shrinkage regression is a conservative analysis: it pulls many effects down to zero. Among the 10 variables that we considered, only two were unambiguously identified as important (Table 2). An interaction between sex and body length was, however, suggestive (stability close to 0.7): bigger males had a more negative blood $\delta^{13}\text{C}$ value (-0.9‰ for a 3 m long seal compared with a 2.5 m long one). The posterior mean of this coefficient is considerably shrunk toward 0 compared with a traditional Ordinary Least Squares estimate (Table 2). The latter is overly influenced by a single datum with high leverage corresponding to the largest male (3.8 m long) with a blood $\delta^{13}\text{C}$ value of -22.2‰ . This illustrates the usefulness of shrinkage regression in preventing type-M errors. This interaction between sex and body length is nevertheless plausible: these seals have an indeterminate growth,^[51] and body length can thus be used as a proxy for age. This interaction term could reflect a tendency for older males to forage at higher latitudes than other individuals (juvenile males or females). Foraging in Antarctic waters is characterized by a relative difference the

$\delta^{13}\text{C}$ value of 1.7‰ for whole blood in southern elephant seals. In contrast, Jaeger *et al.*^[10] found that a difference of 10° of latitude was associated with a relative $\delta^{13}\text{C}$ difference of 3.1‰ for wandering albatrosses, nearly twice the size of our estimate. This discrepancy may result from the different turnover rates of whole blood and blood plasma, the huge different size in the compared species, type-M errors or, more likely, extrapolation: among the 45 wandering albatrosses studied by Jaeger *et al.*,^[10] none went as south as -60°S .

Isotopic data on southern elephant seals could also be compatible with a slight non-linear relationship between latitude and $\delta^{13}\text{C}$ value: a steeper slope may be observed for lower latitudes. François *et al.*^[52] found a very steep negative relationship between particulate organic matter $\delta^{13}\text{C}$ values and latitudes between -40°S and roughly -45°S , followed by a less steep relationship below -45°S . Very few animals among the 80 tagged ones went into subtropical waters, and we deem this strategy to be minor. A linear relationship between the range of latitudes covered by southern elephant seals and $\delta^{13}\text{C}$ values thus seems reasonable. In contrast, wandering albatrosses flew north of -45°S of latitude which, in light of the results from François *et al.*,^[52] suggests that these data may overestimate the relationship between $\delta^{13}\text{C}$ values and higher latitudes. Our slope estimate is in agreement with that of Bentaleb *et al.*^[14] who measured the $\delta^{13}\text{C}$ values of particulate organic matter between -40°S and -50°S in the vicinity of îles Kerguelen and found an average decrease of $\approx 2\text{‰}$ per 10° of latitude. Finally, a consistent finding in our analysis was how post-moult trips were associated with smaller $\delta^{13}\text{C}$ values than post-breeding trips. The post-moult trips, which take place in the Austral winter, can last 7 months while the post-breeding one lasts a shorter 2 months at the beginning of the Austral summer. The observed difference may be linked to overall primary productivity, which varies greatly between the two trips,^[25] although the observational nature of our data precludes the identification of the precise causes of the observed effects.

CONCLUSIONS

Our analysis showed how the carbon isotopic signature of Antarctic waters is reflected in southern elephant seal tissues, thus allowing one to infer where a seal has been feeding. For our purposes, a $\delta^{13}\text{C}$ value difference of -2‰ is enough to conclude that there is a difference in the foraging strategy of southern elephant seals. Knowledge of this effect size is fundamental to the correct interpretation of isotopic data^[3] and to delineate relative isoscapes. Whereas an Antarctic signal was statistically detectable, it seems not possible to distinguish the $\delta^{13}\text{C}$ signature of waters above the Kerguelen Plateau from that of the interfrontal zone with our current data. This may be a consequence of (1) the important biological productivity over the Kerguelen Plateau that is carried eastward in a long plume that extends into oceanic waters,^[28] or (2) the capital breeding strategy of southern elephant seals. In addition, the large body size of seals means that they store large amount of energy reserves: any isotopic signal may then be considerably smoothed out of small variations. This can explain why the $\delta^{13}\text{C}$ signatures of waters over the Kerguelen Plateau and of the interfrontal zone were not distinguishable. Nevertheless, we found shrinkage regression very useful for estimating this difference. The accurate estimation of such biologically meaningful differences is a prerequisite in order to be able to use and interpret methods of probabilistic assignment of geographic origins.^[53]

Currently, statistical analyses of stable isotopes in wildlife ecology tend to emphasize null hypothesis testing over estimation.^[54] While testing hypotheses is certainly important, estimation is often more of interest to ecologists.^[54,55] We hope that we have illustrated how other regression techniques^[56] may be useful for this task. The modelling strategy behind shrinkage regression is implicitly one of conservative scepticism since many effects are *a priori* shrunk towards zero. Shrinkage regression contrasts with techniques such as stepwise model selection, which may generate more hypotheses. However, given (1) how little true replication of studies takes place and the absence of incentives to replicate published work^[57] and (2) the seriousness of issues about "undisclosed flexibility in data analysis",^[58] being conservative may be the more reasonable option to guarantee that reported effects are true biological signals.

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