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Ecological forensics: using single point stable isotope values to infer seasonal schedules of animals after two diet switches

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Summary

1. Animals adjust to seasonal challenges in physical, behavioural and spatial ways. Such adjustments are commonly associated with diet changes that often can be characterised isotopically.

2. We introduce the 'double diet switch model', with which the occurrence and timing of two subsequent diet switches of an individual animal can be traced with a single sample assayed for stable isotopes. We demonstrate the model for Sanderling, *Calidris alba,* a small shorebird that migrates from the Nearctic tundra breeding grounds to the intertidal flats of the Wadden Sea; during this migration some birds may stage in the North Atlantic areas.

3. The 'double diet switch model' successfully predicted the occurrence and timing of two diet switches in 59 Sanderlings captured in the Wadden Sea in July–September. Excluding birds that likely had over-summered at North Atlantic staging areas, the model predicted that Sanderlings departed from the Arctic on 13 July (range: 9–17 July), had a staging duration of 18·6 days in the North Atlantic, and arrived in the Wadden Sea on 1 August (31 July–1 August).The estimated mean Arctic departure dates coincided with the mean hatching date, suggesting that many individuals failed to produce young or left the care to a partner. Estimated mean arrival date matched the main arrival period in the Wadden Sea obtained from observation data. In this study we did not use lipid-free tissues, which may bias model predictions. After correcting for lipid components, the estimated departure date was 11 days later and the staging duration 8·5 days shorter, while arrival date was similar.

4. The 'double diet switch model' successfully identified the occurrence and timing of two subsequent diet switches. The 'double diet switch model' will not only apply to switches between three isotopic levels (as in the case study on Sanderling) but also to scenarios where the second switch reverses to the initial isotopic level. Due to this general applicability, the model can be adapted to a wide range of taxa and situations. Foreseeable applications include changes in habitat and food type, ontogenetic development or drastic phenotypic changes such as the metamorphosis in insects and amphibians.

Key-words: *Calidris alba*, dietary change, migration, seasonal patterns, shorebirds, stable isotopes, staging duration, timing, trophic change, Wadden Sea

Introduction

Animals adjust to seasonal challenges by movements and by physical and behavioural changes (Piersma & van Gils 2011). Quite commonly, these adjustments are associated with diet changes that can be isotopically characterised (Hobson 1999; Caut, Angulo & Courchamp 2009). The accompanying shifts in isotopic value enables researchers to illuminate seasonal phenomena such as migration, metamorphosis or (temporary) increasing or declining food availability (Phillips & Eldridge 2006; Karasov & Martínez del Rio 2007; Schwemmer *et al.* 2016). No surprise that 'ecological forensics' is thriving (Dawson & Siegwolf 2007).

Stable isotope analyses can track the occurrence and timing of diet switches based on differences in (i) isotopic values generated by foraging on isotopically distinct food sources and (ii) incorporation times of an isotope in distinct consumer tissues (e.g. plasma and red blood cells: Hobson 1999; Klaassen *et al.* 2010). After a diet switch, the isotopic incorporation of the new diet in a consumer's tissues follows a first order kinetics model, mostly described by an exponential decay function. This model can estimate the time since a single diet switch using stable isotope values of one, or preferably two, tissue

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types (Phillips & Eldridge 2006; Klaassen *et al.* 2010; Oppel & Powell 2010). For animals that change their foraging location or diet more than once over relatively short time spans, we here describe a 'double diet switch model'. This model can deal with three successive isotopically distinct diets based on a single assessment of isotopic values in two tissues with distinct turnover rates in one individual and gives estimates of the timing of the two consecutive diet switches.

To demonstrate the functionality of the model, we estimate the timing of post-breeding migration of Sanderlings *Calidris alba* upon their arrival in the Dutch Wadden Sea. After a breeding season in the High Arctic, these long-distance migratory shorebirds depart from the tundra where they fed on terrestrial arthropods (Wirta *et al.* 2015). Before arrival in the Wadden Sea, where they mainly feed on Brown Shrimp *Crangon crangon* (JR pers. comm.), Sanderlings may or may not make refuelling stops in coastal habitats in the North Atlantic where soft-bodied marine invertebrates comprise the diet (Reneerkens *et al.* 2009).

Materials and methods

THE DOUBLE DIET SWITCH MODEL

The isotopic change in body tissues after a diet switch typically follows a first-order kinetic response which is generally well described by a negative exponential function (Tieszen *et al.* 1983; Phillips & Eldridge 2006; Klaassen *et al.* 2010). Specifically, consider a focal animal on a diet A, with a corresponding isotope ratio δ_{A1} in tissue 1. If at time $t = 0$ the animal switches from diet A to diet B, then after t_B days on the new diet, its tissue-specific isotope ratio is given by the formula:

$$\delta(t_{B1}) = \delta_{B1} + (\delta_{B1} - \delta_{B1})e^{-\lambda_1 t_B}, \qquad \text{eqn 1}$$

where δ_{B1} is the characteristic isotope ratio of diet B in issue 1, and λ_1 is the tissue-specific turnover rate (1 per day) of the isotope. Given estimates of δ_{A1}, δ_{B1} and λ_1, this 'single diet switch model' allows estimation of t_B, the amount of time since the diet switch occurred (Phillips & Eldridge 2006; Klaassen *et al.* 2010).

Here we expand this 'single diet switch model' to one which describes two diet switches: the 'double diet switch model'. Suppose that at time $t = t_B$, our focal animal switches once again, from diet B to diet C, the latter having characteristic isotopic ratio δ_{C1} in tissue 1. After t_C days on diet C, at time $t = t_B + t_C$, the animal's isotope ratio is now given by

$$\begin{aligned}\delta(t) &= \delta_{C1} + [\delta(t_B) - \delta_{C1}]e^{-\lambda_1 t_C} \\ &= \delta_{C1} + [\delta_{B1} + (\delta_{A1} - \delta_{B1})e^{-\lambda_1 t_B} - \delta_{C1}]e^{-\lambda_1 t_C},\end{aligned} \qquad \text{eqn 2}$$

where we substituted the right-hand side of formula (1) for $\delta(t_B)$ in the first line. Note that this formula is not very useful by itself, since any observed value of $\delta(t)$ within the range spanned by δ_{A1}, δ_{B1} and δ_{C1} is typically consistent with infinitely many combinations of t_B and t_C. However, if a sample is taken simultaneously from a second tissue with a different turnover rate λ_2, then we have a system of two equations for the two unknowns t_B and t_C:

$$\begin{aligned}\delta_1(t) &= \delta_{C1} + [\delta_{B1} - \delta_{C1} + (\delta_{A1} - \delta_{B1})e^{-\lambda_1 t_B}]e^{-\lambda_1 t_C} \\ \delta_2(t) &= \delta_{C2} + [\delta_{B2} - \delta_{C2} + (\delta_{A2} - \delta_{B2})e^{-\lambda_2 t_B}]e^{-\lambda_2 t_C}\end{aligned} \qquad \text{eqn 3}$$

Geometrically, the two equations correspond to two curves in the t_B–t_C plane, and solutions to the two equations occur if and where the curves intersect. As we shall see below, these solutions are precisely the maximum likelihood (ML) estimates of t_B and t_C, provided that $\delta_1(t)$ and $\delta_2(t)$ are normally distributed around their predicted values. Solving both equations for t_C gives explicit formulas for the two curves:

$$\begin{aligned}t_C &= \frac{1}{\lambda_1}\ln\left(\frac{\delta_{C1} - \delta_{B1} + (\delta_{B1} - \delta_{A1})e^{-\lambda_1 t_B}}{\delta_{C1} - \delta_1(t)}\right) \\ t_C &= \frac{1}{\lambda_2}\ln\left(\frac{\delta_{C2} - \delta_{B2} + (\delta_{B2} - \delta_{A2})e^{-\lambda_2 t_B}}{\delta_{C2} - \delta_2(t)}\right)\end{aligned} \qquad \text{eqn 4}$$

Equating both right-hand-sides yields an equation in t_B, which does not have closed-form solutions, but which may be solved by standard numerical routines. If a solution is found, it can be put back into either of the right-hand sides of (4) to give a corresponding solution for t_C.

Thus, the 'double diets switch model' allows estimation of seasonal scheduling of animals with three subsequent diets, such as migrant birds consuming isotopically distinct diets before the start of migration, during a staging episode and after arrival to final destination, respectively, or grizzly bears (*Ursus arctos*) switching temporarily from a diet with mainly whitebark pine (*Pinus albicaulis*) to a diet with mainly elk (*Cervus elaphus*) (Schwartz *et al.* 2014). The conditions for the use of the 'double diet switch model' are presented in Table 1. In the next section we describe a statistical method to estimate t_B and t_C.

THE LIKELIHOOD MODEL

We use a ML approach to estimate the parameters t_B and t_C in the nonlinear model (3), given estimates of all other parameters and the

Table 1. An overview of the required conditions for the 'double diet switch model' to estimate the timing of two consecutive diet switches

Conditions for using the 'double diet switch model':
a) Stable isotope analysis (eg. $\delta^{13}C$) of individuals of the study species should be measured while on the third diet. This should be done for two tissue types: one with a relatively high turnover rate such as plasma and one with a relatively low turnover rate such as RBC). Both tissues should be sampled at the same moment. Tissue sampling needs to be performed before the individual has reached isotopic adaptation of the new equilibrium of the third diet.
b) At all three stages (or locations), the stable isotope values of the study species itself or that of its food are known (plus a discrimination factor; but see Data S1) and sufficiently distinct from each other Ideally, stable isotope values are known for both separate tissue types at all three stages.
c) Turnover rates of the two tissues are known for the study species (or can be estimated sufficiently accurately)
d) Preferably, sampling dates of the tissue types are known With this information durations of the use of a diet can be transferred to dates instead of number of days.
e) Diet uniformity among individuals
f) The sampling moment is important, since there should not have been enough time to approach equilibration to diet C Besides, the animal's staging duration should be shorter than the time to approach the equilibration to diet B.

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measured values of δ_1 and δ_2. We assume that measurement errors have a normal density:

$$p(\delta_1, \delta_2 | t_B, t_C) = \frac{1}{2\pi\sigma_\delta^2} \exp\left(-\frac{1}{2\sigma_\delta^2} ((\delta_1 - \mu_1)^2 + (\delta_2 - \mu_2)^2) \right) \quad \text{eqn 5}$$

Here σ_δ^2 is the variance, assumed known and identical for both tissues, whereas μ_1 and μ_2 are the expected values of δ_1 and δ_2 according to model (3):

$$\begin{aligned} \mu_1(t_B, t_C) &= \delta_{C1} + [\delta_{B1} - \delta_{C1} + (\delta_{A1} - \delta_{B1}) e^{-\lambda_1 t_B}] e^{-\lambda_1 t_C} \\ \mu_2(t_B, t_C) &= \delta_{C2} + [\delta_{B2} - \delta_{C2} + (\delta_{A2} - \delta_{B2}) e^{-\lambda_2 t_B}] e^{-\lambda_2 t_C} \end{aligned} \quad \text{eqn 6}$$

The log-likelihood is then, up to a constant term:

$$l(t_B, t_C) = -\frac{1}{2\sigma_\delta^2} \left((\delta_1 - \mu_1)^2 + (\delta_2 - \mu_2)^2 \right) \quad \text{eqn 7}$$

The score, the partial derivatives of the log-likelihood with respect to both parameters is then given by

$$\begin{aligned} \frac{\partial l}{\partial t_B} &= \frac{1}{\sigma_\delta^2} \left((\delta_1 - \mu_1) \frac{\partial \mu_1}{\partial t_B} + (\delta_2 - \mu_2) \frac{\partial \mu_2}{\partial t_B} \right) \\ \frac{\partial l}{\partial t_C} &= \frac{1}{\sigma_\delta^2} \left((\delta_1 - \mu_1) \frac{\partial \mu_1}{\partial t_C} + (\delta_2 - \mu_2) \frac{\partial \mu_2}{\partial t_C} \right) \end{aligned} \quad \text{eqn 8}$$

Clearly the score vanishes if $\mu_1 = \delta_1$ and $\mu_2 = \delta_2$, which shows that the ML estimates of t_B and t_C are indeed the solutions to the system of equations (4). We used the function uniroot in R version 3.3.0 (R Core Team 2016) to find numerical solutions. All R scripts are available as online appendices to this paper.

The Hessian matrix of second order derivatives, evaluated at the candidate ML estimates, is

$$H = \begin{bmatrix} \frac{\partial^2 l}{\partial t_B^2} & \frac{\partial^2 l}{\partial t_B \partial t_C} \\ \frac{\partial^2 l}{\partial t_B \partial t_C} & \frac{\partial^2 l}{\partial t_C^2} \end{bmatrix} = \frac{1}{\sigma_\delta^2} \begin{bmatrix} -\left(\frac{\partial \mu_1}{\partial t_B}\right)^2 - \left(\frac{\partial \mu_2}{\partial t_B}\right)^2 & -\frac{\partial \mu_1}{\partial t_B}\frac{\partial \mu_1}{\partial t_C} - \frac{\partial \mu_2}{\partial t_B}\frac{\partial \mu_2}{\partial t_C} \\ -\frac{\partial \mu_1}{\partial t_B}\frac{\partial \mu_1}{\partial t_C} - \frac{\partial \mu_2}{\partial t_B}\frac{\partial \mu_2}{\partial t_C} & -\left(\frac{\partial \mu_1}{\partial t_C}\right)^2 - \left(\frac{\partial \mu_2}{\partial t_C}\right)^2 \end{bmatrix} \quad \text{eqn 9}$$

The Hessian has two uses here: first, to verify that candidate ML solutions are indeed maxima of the likelihood, and secondly, to provide approximate standard errors for the ML estimates. A local maximum is verified if $\mathrm{tr}(H) = H_{11} + H_{22} < 0$, which is easily seen to be true, and if $\det(H) = H_{11}\,H_{22} - H_{12}\,H_{21} > 0$, which is also true since $\det(H) = \left(\frac{\partial \mu_1}{\partial t_B}\frac{\partial \mu_2}{\partial t_C} - \frac{\partial \mu_1}{\partial t_C}\frac{\partial \mu_2}{\partial t_B}\right)^2 > 0$. Approximate standard errors and covariances for the ML estimates $\hat{t}_B$ and $\hat{t}_C$ follow from

$$-H^{-1} \approx \begin{bmatrix} \sigma^2_{\hat{t}_B} & \sigma_{\hat{t}_B \hat{t}_C} \\ \sigma_{\hat{t}_B \hat{t}_C} & \sigma^2_{\hat{t}_C} \end{bmatrix} \quad \text{eqn 10}$$

The matrix $-H$ is called the information matrix, since the inverse of information is uncertainty, as quantified by standard errors. To evaluate H we need to evaluate the partial derivatives for tissues $i = 1, 2$:

$$\begin{aligned} \left.\frac{\partial \mu_i}{\partial t_B}\right|_{t_B = \hat{t}_B, t_C = \hat{t}_C} &= -\lambda_i (\delta_{Ai} - \delta_{Bi}) e^{-\lambda_i (\hat{t}_B + \hat{t}_C)} \\ \left.\frac{\partial \mu_i}{\partial t_C}\right|_{t_B = \hat{t}_B, t_C = \hat{t}_C} &= -\lambda_i (\delta_{Bi} - \delta_{Ci} + (\delta_{Ai} - \delta_{Bi}) e^{-\lambda_i \hat{t}_B}) e^{-\lambda_i \hat{t}_C} \end{aligned} \quad \text{eqn 11}$$

Plugging these into (9) clearly shows that the uncertainty about $\hat{t}_B$ and $\hat{t}_C$ increases exponentially with their estimated mean values. Specifically, according to the first equation in (11), information regarding t_B decays exponentially if either t_B or t_C grows large, whereas according to the second equation information regarding t_C is especially sensitive to large t_C but not t_B values. Thus, unless turnover rates are very low, it is clearly preferable to sample not too long after the second diet switch, nor should the time between diet switches be too long.

We have attempted to take a full Bayesian approach to estimate t_B and t_C, but the ML approach was superior. Simulations indicated (results not shown) that even weakly informative priors produced considerable bias in estimates. The use of flat priors is ruled out for our model since the likelihood does not converge to zero as t_B and t_C go to infinity, rendering the corresponding posterior distribution non-integrable.

SENSITIVITY ANALYSIS

The model has eight parameters: for each tissue $i = 1, 2$ and diet $j = A, B, C$ the equilibrium isotope ratios are denoted by δ_{ij} and turnover rates by λ_i. For the Sanderling data, the diet-and tissue-specific isotope ratios and associated standard deviations were estimated directly from blood and indirectly from prey items (Table 2, Data S1, Supporting Information). No direct information about turnover rates was available for the Sanderling. Instead values for λ_i were predicted on the basis of interspecific allometric regressions, whereas standard deviations were obtained as averages of intraspecific standard deviations (Table S1).

To assess the sensitivity of model predictions to uncertainty in the 8 parameters, for each bird in our data set we drew 10 000 random normal deviates for each of the 6 isotope ratios and for the logarithms of the turnover rates (which must be positive), based on our estimates of mean values and standard deviations. For the isotope ratios we used independent draws, whereas for turnover rates we allowed for a positive correlation between tissues since it seems plausible that variation in metabolic rate affects turnover rates in the same direction. For each of the draws we attempted to obtain ML estimates for t_B and t_C by solving system (4). When we obtained a candidate solution, we calculated the Hessian to verify it corresponded to a maximum and to estimate standard errors for the parameter estimates. Thus, for each bird we obtained 1000 distributions, one for each successful random draw, which we approximated as a mixture of 10 000 gamma distributions to avoid negative values in the tails of the distributions. The mixture was stored as a 'posterior distribution' from which we calculated mean values and 89% highest posterior density intervals.

As an alternative to our simulation approach to sensitivity analysis, parameter likelihoods may also be incorporated into an overall likelihood for all model parameters, in addition to t_B and t_C, and corresponding confidence levels calculated. Such an extended likelihood-approach would have to be tailored to the study-specific way the additional parameters were estimated.

THE CASE: TIMING OF SOUTHWARD MIGRATION IN SANDERLING

Using the 'double diet switch model', we reconstructed the timing of southward migration by Sanderlings from the tundra breeding grounds (where they ate diet A) and subsequently flew, with or without staging in the North Atlantic (diet B), to the Wadden Sea (diet C). In July–September 2011 and 2012, 65 adult Sanderlings were captured with mist-nets during new moon nights near high-tide roosts in the western Dutch Wadden Sea (53°N, 4–5°E). In addition, 10 adult Sanderlings were caught on their nests in Greenland (Zackenberg, 74°30′N, 21°00′W) in the second half of June 2009. Blood samples of these latter birds were used to determine the $\delta^{13}C$ value of red blood cells (RBC) and plasma of birds on the initial diet in the Arctic (diet A; see Data S1). Immediately after capture, all 75 Sanderlings were (colour)ringed, weighed and aged based on plumage criteria (Prater, Marchant & Vuorinen 1977), and a blood sample (~300 μL) for stable isotope

Table 2. Summary of all general input variables of the 'double switch model' to estimates individual schedules in migrating Sanderling

Diet tissue type		Calc. (prey + DiF)*	*N*	True (bird blood)	*N*	*t*-test
Arctic[†]	Plasma			**−25·99 ± 0·29‰**	10	
	RBC			**−25·33 ± 0·29‰**		
Staging area[‡]	Plasma	**−18·29 ± 0·24‰**	25	−18·28 ± 0·14[§]‰	4	$t(27) = 0{\cdot}02$, $P = 0{\cdot}99$
	RBC	**−17·62 ± 0·24‰**		−17·94 ± 0·30[§]‰		$t(27) = 0{\cdot}53$, $P = 0{\cdot}60$
Wadden Sea	Plasma	**−14·56 ± 0·09‰**	20	−14·54 ± 0·16[¶]‰	6	$t(24) = 0{\cdot}16$, $P = 0{\cdot}91$
	RBC	**−13·90 ± 0·09‰**		−13·94 ± 0·13[¶]‰		$t(24) = 0{\cdot}23$, $P = 0{\cdot}82$
Turnover rate[‖]	Tissue type	Mean	SD			
	Plasma	**0·303**	**0·033**			
	RBC	**0·056**	**0·012**			

Presented are the $\delta^{13}C$ values of Sanderling in equilibrium with the diets on the three locations along southward migration (mean ± SE). The $\delta^{13}C$ values were calculated in two ways and shown in two columns: obtained from Sanderling blood (True) and a calculated value with help of $\delta^{13}C$ values of prey and a discrimination factor (Calc.). The results of the two methods did not differ significantly (see *t*-test in last column and Data S1). Bold values were used in the model.

*See Data S1 for details about indirect calculations of the $\delta^{13}C$ signal of Sanderlings. DiF = discrimination factor.

[†]Based on blood of Sanderlings caught in northeast Greenland.

[‡]North Atlantic staging area.

[§]Blood of Sanderlings caught in Wadden Sea in summer with $\delta^{13}C$ values of plasma and RBC that both represented the staging location ($\delta^{13}C_{plasma}$ minus $\delta^{13}C_{RBC}$ <0·23‰). These birds were suspected to have just arrived in the Wadden Sea after using a staging area somewhere in the North Atlantic.

[¶]Blood of Sanderlings caught in September in the Wadden Sea with $\delta^{13}C$ values of plasma and RBC that both represented the Wadden Sea ($\delta^{13}C_{plasma}$ minus $\delta^{13}C_{RBC}$ <0·23‰).

[‖]See Data S2 for calculation of the turnover rate of $\delta^{13}C$ in plasma and RBC of Sanderlings.

analysis was drawn from the brachial vein into heparinised capillaries. Note that second calendar year Sanderlings cannot be distinguished from older Sanderling based on their plumage after their first basic moult in spring (Prater, Marchant & Vuorinen 1977; Lemke, Bowler & Reneerkens 2012). Immediately after sampling, the blood was centrifuged in Eppendorf cups in a haematocrit centrifuge (microfuge Sigma 1–13, 6 min on 5000 rpm). Plasma and RBC were pipetted in separate glass vials and stored in a freezer (−20 °C) until analysis.

The Sanderling data set serves all conditions for the 'double diet switch model', as described in Table 1: (i) Stable carbon isotope analysis were performed on plasma and RBC of Sanderlings caught in the Wadden Sea. (ii) The $\delta^{13}C$ values of plasma and RBC of Sanderlings differed between all three locations along the migration route (Table 2). North Atlantic staging areas were assigned based on eight re-sightings of colour-ringed Sanderlings (2007–2014) recorded within the same season of southward migration at both a North Atlantic staging area and the Wadden Sea (Fig. 1). The isotope values of Sanderling's RBC and plasma at locations A and C were obtained from Sanderling blood samples, whereas the isotope values of RBC and plasma at the North Atlantic staging location (location B) were estimated via prey tissues and a discrimination factor (see Data S1). (iii) The turnover rates for plasma ($\lambda_{plasma} = 0{\cdot}303 \pm 0{\cdot}033$ SD) and RBC ($\lambda_{RBC} = 0{\cdot}056 \pm 0{\cdot}012$ SD) were estimated for an average adult Sanderling (see Data S2). (iv) The tissue sampling dates of all Sanderlings captured in the Wadden Sea were known. (v) There is no indication for non-uniformity in diet between individual Sanderlings under any of the three diets. Besides, it is unlikely that individual diet specialisation alters the average stable isotope signature of the diet, because we took all important prey species into account, intra-diet variation was within the limits of inter-diet variation, and the consumed prey species differed between the three sites. (vi) Samples were collected in the period shortly after the mean arrival period in the Wadden Sea. The ten samples that were collected in late summer, some weeks after the arrival period, indeed showed that the majority of these birds were already adapted to the Wadden Sea diet (Fig. 2).

Figure 2 shows the predictions of the 'double diet switch model' for Sanderlings with different staging durations. The steepness of the slopes of the model predictions increases with turnover rate of the tissue, showing that plasma $\delta^{13}C$ values (dashed lines) adapt more quickly to the new diet than RBC $\delta^{13}C$ values (solid lines). The model is based on the combined differences in values for $\delta^{13}C_{plasma,}$ $\delta^{13}C_{RBC}$ and the difference between plasma and RBC isotope values ($\delta^{13}C_{plasma}$ minus $\delta^{13}C_{RBC}$) over time (t_B and t_C). Therefore, the seasonal schedule of an individual Sanderling can be predicted using a single time point measurement of the stable isotopic value of two tissues. Birds with an Arctic isotopic value in both RBC and plasma are still in equilibrium with the Arctic diet and must have flown directly to the Wadden Sea. Birds with a very short staging period in the North Atlantic staging area and recently arrived in the Wadden Sea will also show a predominantly Arctic signature. Birds with Wadden Sea isotopic values in both RBC and plasma are birds that have been long enough in the Wadden Sea for both tissues to achieve equilibrium with the Wadden Sea diet. We expect that the 'double diet switch model' cannot assign a staging duration to Sanderlings that are already isotopically resident in the Wadden Sea (cf. Hobson 1999). Birds with intermediate values might have been in the Wadden Sea for some time, but not long enough to be in equilibrium with the Wadden Sea diet, and/or may have staged in the North Atlantic region.

Note that migratory flights from the Arctic breeding area in Greenland to the Wadden Sea, which we expect to last approximately 2 days (65 km h^{-1} ground speed for the whole flight of approximately 2850 km; Zwarts *et al.* 1990), are not taken into account in the model. Although this could potentially affect the biological interpretation of departure dates from the Arctic, the time in flight is short in comparison with the mean error term of t_B (9·1 days, $N = 52$). We assumed (i) that a diet switch started upon arrival at a new location and (ii) uniform isotopic diets in the three reference areas are representative for the different regions (Arctic,

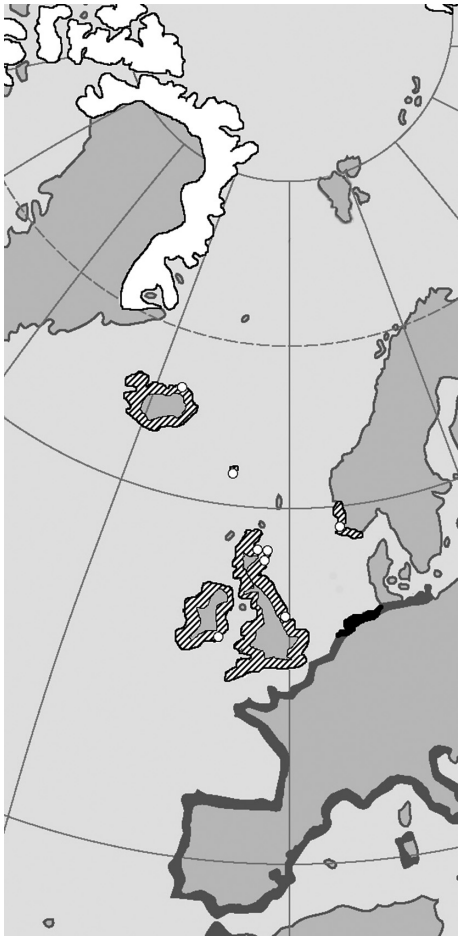

Fig. 1. Arctic breeding areas (white), North Atlantic staging areas (striped) and the Wadden Sea (black) used by Sanderlings visiting the Wadden Sea in late summer. Known wintering areas are shown in dark grey, but the Wadden Sea area (black) is a wintering area too. The coastal North Atlantic staging areas were determined based on observations of eight colour-ringed Sanderlings (white dots in striped area) that were observed in the Dutch Wadden Sea a few days later.

North Atlantic staging areas and Wadden Sea) used by Sanderlings during southward migration to the Wadden Sea. Note also that output dates were reconstructed from termination of 'day of the year' of 2011, since most birds were caught in that year, while the day of the year differs 1 day between 2011 and 2012.

To evaluate the seasonal schedules of Sanderlings estimated by our 'double diet switch model', we compared our model data with observation data of seasonal schedules of Greenlandic breeding Sanderlings migrating southwards. In 2007–2014, Sanderling nests were annually searched for in northeast Greenland (Reneerkens *et al.* 2014). Dates of hatch were often exactly known or, in case of clutch predation, estimated based on egg flotation (Hansen *et al.* 2011). For families found post-hatch, a body mass growth curve based on local data was used to estimate the hatching date. In total we determined hatching dates of 417 clutches and broods (annual range 25–77). The timing of southward migration of Sanderlings was determined based on sightings of individually colour-ringed birds. More than 5600 Sanderlings were individually marked in 12 countries produced over 58 000 unique observations along the East Atlantic flyway collected by us and many volunteers. This data set was used to extract information of birds sighted in the North Atlantic region and the Wadden Sea within the same season of southward migration.

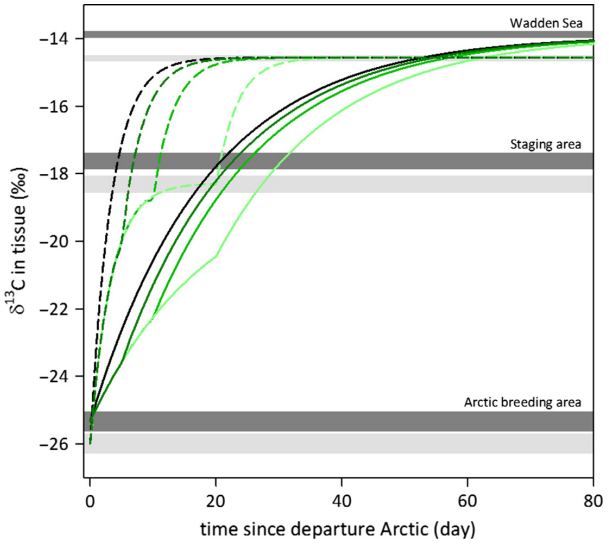

Fig. 2. Predicted changes in $\delta^{13}C$ values in plasma and RBC of Sanderlings with different staging durations during southward migration. The horizontal bars for plasma (light grey) and RBC (dark grey) represent $\delta^{13}C$ values in equilibrium with diets used in the Arctic breeding area, the North Atlantic staging area and in the Wadden Sea. The isotopic changes of $\delta^{13}C_{plasma}$ (dashed lines, turnover rate of 0·303) and $\delta^{13}C_{RBC}$ (solid lines, turnover rate of 0·056) are given for staging durations of 0, 5, 10 and 20 days. Black lines show a migration without a stopover. Green lines show migrations with a stopover in the North Atlantic staging area, with colour-darkness corresponding with ascending staging durations.

STABLE ISOTOPE ANALYSIS

All bird plasma, RBC and prey items were stored at −20 °C before analysis. The samples were freeze-dried before grinding them with a mortar and pestle. We used a microbalance (Sartorius CP2P) to weigh 0·4–0·8 mg of the sample material in 5 × 8 mm tin capsules. The $\delta^{13}C$ values were determined with a Thermo Flash 2000 elemental analyser coupled to a Thermo Delta V isotope ratio mass spectrometer. Isotope values were calibrated to a laboratory acetanilide standard ($\delta^{13}C$ −26·1‰ calibrated on NBS-22) and corrected for blank contribution. 72% of the plasma and RBC samples were analysed in duplicate. The results are reported on the per mill scale with respect to Vienna Pee Dee Belemnite [VPDB]. The replicate error on the standard, acetalinide, ranged between 0·03 and 0·08, using one standard every 4·3 to 7 bird samples.

ELIMINATION OF BIRDS OVERSUMMERING IN THE NORTH ATLANTIC REGION

Out data set on stable isotope profiles appeared to contain Sanderlings that probably over-summered in the North Atlantic 'staging area' and did not migrate to the Arctic tundra. The estimated staging duration of these individuals was so exceptionally long that if they would have arrived from the Arctic they would have had to depart unrealistically early (as early as 14 May, when Sanderlings are still on northward migration to the Arctic). The 'double diet switch model' cannot eliminate birds that over-summered in the North Atlantic, but simply predicts that these birds have exceptionally long staging durations. To eliminate the birds that may have over-summered in the North Atlantic, we excluded birds with a $\delta^{13}C_{RBC}$ that fell within or was higher than the $\delta^{13}C$ of the North Atlantic staging area and also had a $\delta^{13}C_{plasma}$

that was still not yet adapted to the Wadden Sea diet (7 birds; see Fig. 3a).

Results

The $\delta^{13}C$ values of RBC and plasma of Sanderlings caught in the Wadden Sea varied from −24·32‰, which is close to a signature of bird's blood in equilibrium with a diet on the Arctic terrestrial arthropods, to −13·5‰, which is a signature for bird's blood in equilibrium with the Wadden Sea diet (Fig. 3a). Whereas most birds captured in late summer showed Wadden Sea diet type isotopic values in both RBC and plasma, birds captured in the main arrival period (23 July to 2 August) showed a variety of patterns ranging from almost purely Arctic signatures, North Atlantic isotopic signatures, intermediate isotopic values, to Wadden Sea diet signatures (Fig. 3a).

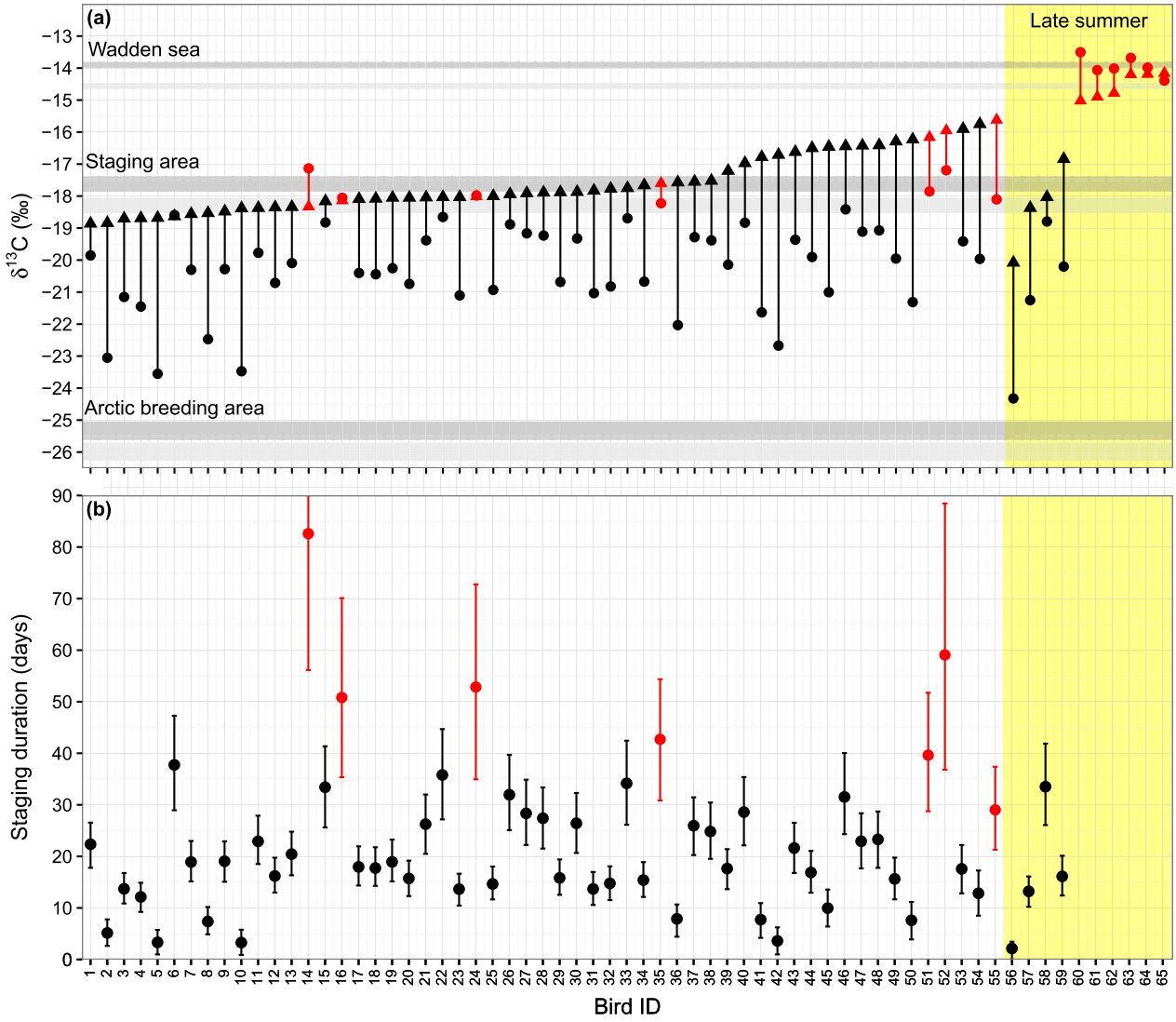

Fig. 3. $\delta^{13}C$ values of Sanderlings caught in the Wadden Sea after southward migration and their corresponding estimated staging duration along North Atlantic coasts. For clarity, individuals are sorted along the *X*-axis according to raw $\delta^{13}C$ values. Depicting individuals in chronological order of arrival caused many overlaying points because multiple birds were mist-netted per day. Birds in the yellow bar were caught in late summer and represented separately to show the high number of birds that are adapted to the Wadden Sea diet in late summer. (a) Measured values of $\delta^{13}C_{plasma}$ (triangles) and $\delta^{13}C_{RBC}$ (dots) of all 65 individual Sanderlings. Although the model was able to fit a t_B and t_C for all birds, only birds that had been in the Arctic breeding area, indicated with black symbols, were taken into account for further interpretations (N = 52). Birds with a $\delta^{13}C_{RBC}$ within or above the $\delta^{13}C$ of the diet of the North Atlantic staging area and a $\delta^{13}C_{plasma}$ that was not already adapted to the Wadden Sea (red symbols) were considered to have oversummered and not used for further interpretations of the migration schedule of Sanderlings. For individuals that were already resident to the Wadden Sea (Late summer, N = 6, Table 1), the model could (and should) not fit t_B and t_C. (b) The staging duration of all individuals not yet adjusted to the Wadden Sea diet (N = 59) as calculated by the 'double diet switch model'. The confidence limits of the staging duration (t_B) for each individual bird are expressed with standard deviation bars. Again, red symbols indicate birds that likely oversummered in the North Atlantic staging area and therefore were left out for further interpretations of the migration schedule (N = 7). For visualisation, we distinguished between birds caught in the main arrival period in summer (23 July–2 August) and birds caught after the main arrival period in late summer (20 August–1 September; yellow bar) in the graphs.

Based on the 'double diet switch model' we assessed the individual seasonal schedules of the Sanderlings (Fig. 3). Sanderlings had a wide range of migration strategies with staging periods along North Atlantic coasts ranging from 2·2 to 37·6 days (Fig. 3b). Sanderlings departed from the Arctic on average on 13 July (range: 9–17 July, $N = 52$, Fig. 4), to arrive in the Wadden Sea on 1 August (31 July–1 August, $N = 52$, Fig. 4). When we include the seven birds that over-summered in the North Atlantic staging areas, the mean arrival date remained 1 August (range: 31 July–1 August, $N = 59$, Fig. 4). Departure dates from the Arctic and arrival dates in the Wadden Sea for all individual birds are presented in Fig. 4b.

Discussion

Here we developed a new inferential statistical tool to estimate the timing of movements between distinct habitats on the basis of chemical markers in animal tissues. Ecological forensic problems by their nature are particular and specific, and for this reason we will discuss the Sanderling case before zooming out to the wider range of situations to which our new tool can be applied.

Interestingly, with the help of the 'double diet switch model', we are the first to describe the timing of southward migration of Sanderlings. Our results shows that Sanderlings that spend the summer in the Arctic, as well as those which over-summered in the North Atlantic, arrive simultaneously in the Wadden Sea, matching the main arrival date obtained by observations (Loonstra, Piersma & Reneerkens 2016). As surmised by Reneerkens *et al.* (2009), the 'double diet switch model' revealed that Sanderlings show large temporal variation in the autumn migration schedules. Contrary to the work of Dietz *et al.* (2010) who, with the help of a 'single diet switch model' found that Red Knots *Calidris canutus* do not stage in the North Atlantic during southward migration, we show that Sanderlings stage for variable lengths of time in the North Atlantic before moving on the Wadden Sea. The mean staging duration in coastal areas between Greenland and the Netherlands of southward migrating Sanderlings was estimated to last 18·6 days. The mean departure date from the Arctic was estimated as 13 July. This coincides with the mean hatching date in northeast Greenland (13 July). The majority of clutches fails due to depredation (Reneerkens *et al.* 2014) and Sanderlings often leave their partner with the care of eggs (Reneerkens

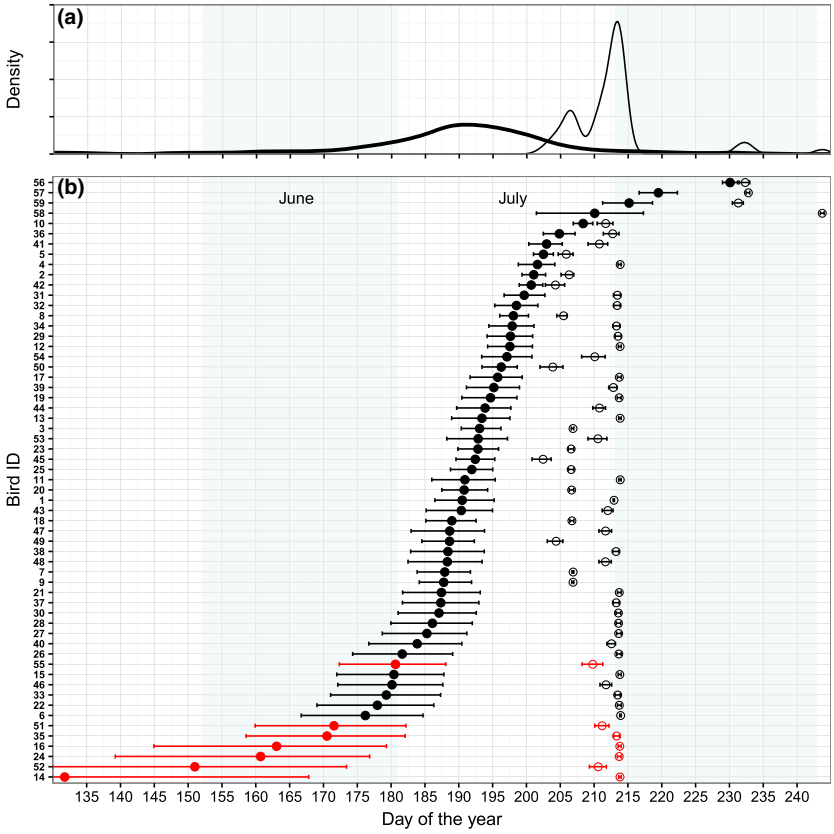

Fig. 4. Migration schedule of Sanderlings, shown as departure dates from the Arctic and arrival dates in the Wadden Sea. (a) The distribution of the departure date from the Arctic (thick line) and arrival date in the Wadden Sea (thin line), for birds that likely arrived from the Arctic breeding area and thus completed the entire migration ($N = 52$). The mean departure date from the Arctic is 13 July, the mean arrival date in the Wadden Sea is 1 August. (b) Individual migrating schedules of all 59 Sanderlings with the estimated departure date from the Arctic (filled dots) and the arrival date in the Wadden Sea (open circles), both given as mean ± SD. Black symbols represent birds that likely arrived from the Arctic ($N = 52$), whereas red symbols represent birds that likely over-summered in the North Atlantic ($N = 7$). Grey and white alternating zones refer to months. Bird ID shown on the *Y*-axis of this figure, correspond with Bird ID of Fig. 3.

et al. 2011). When clutches are incubated by two adults, one of the partners always leaves the other parent with the chicks, as soon as they hatch (Reneerkens *et al.* 2014). This would explain the early departures from the Arctic tundra by the majority of assayed birds. The seven individuals that seemed to have over-summered in the North Atlantic were most likely second calendar year birds (Summers, Underhill & Prŷs-Jones 1995). The proportion over-summering Sanderlings in the North Atlantic (12%) is comparable to an earlier study by Lemke, Bowler & Reneerkens (2012) who estimated the percentage of juveniles in a wintering population in Scotland to be 6–9%.

At time of our isotope analyses, it was not common practice to use lipid-free tissues. It is clear now that lipids may influence isotopic values substantially, also in blood tissue (e.g. Rode *et al.* 2016). Specifically, high lipid contents in tissue biases $\delta^{13}C$ values downwards, whereas lipid contents may vary between individual and tissue type. Although our case study with Sanderlings clearly demonstrates the applicability of the double diet switch model, the estimated migration schedule may be biased for not using lipid-free tissues. To explore this possible bias, we corrected for lipid contents following the method of Post *et al.* (2007), who suggested to use *C*:*N* ratios of the sampled tissue to correct for lipid contents by adding a correction term to the estimated $\delta^{13}C$ values, and we reran the model with the 'lipid-free' approximate $\delta^{13}C$ values (of all tissues, from Sanderlings and prey). Using the 'lipid-free' data, the model did not converge for 14 birds (while all 59 birds converged when using incorrected values), indicating that corrections were inconsistent with the model. Using the approximated 'lipid-free' data of the remaining birds, resulted in an estimated departure date from the Arctic that was later than when using uncorrected data (24 July [CI 20–26 July], rather than 13 July), a shorter estimated staging duration (10·1 days [CI 7·6–14·9], rather than 18·6 days), but a similar arrival date in the Wadden Sea (31 July [29 July–2 August] compared with 1 August) ($N = 45$). The model estimates using the 'lipid-free' data matched better with our expectations on the timing of southward Sanderling migration.

As it is likely that Sanderlings show moderate intraspecific variation, we used distributions of the input parameters rather than the mean values, for two reasons. First, individual dietary preferences cause stable isotopic values to vary slightly among individuals. Moreover, the discrimination factor that may be used to distinguish between diet and consumer may vary between individuals as well (Data S1; Caut, Angulo & Courchamp 2009). Second, intraspecific variation in turnover rates is rather large and poorly understood (Martínez del Rio *et al.* 2009; Hahn *et al.* 2012). More accurate information about intraspecific variation in turnover rates is needed for more accurate estimations of individual seasonal scheduling. As the conditions for using the 'double diet switch model' can be met rather easily on the basis of a single time point stable isotope measurement of the target species (Table 1), the 'double diet switch model' allows a relatively simple way to assess seasonal schedules.

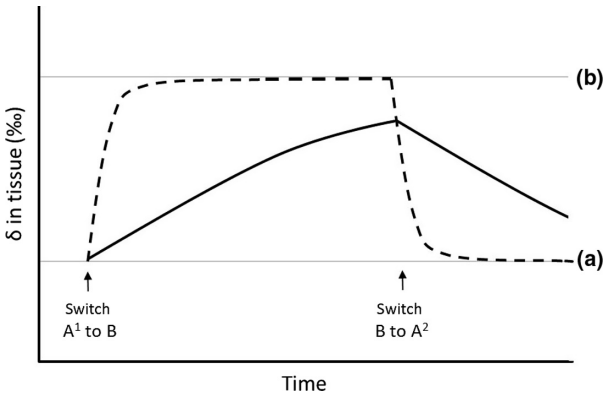

Fig. 5. A special case of the double diet switch model, the ABBA-switch. This is a simplified representation of a 'switch-switching back' situation, from diet A^1 to B and from B back to A^2 describing how the isotopic values of two tissues, one with a fast turnover rate (striped black line) and a slow turnover rate (solid black line), adapt from diet A to(wards) diet B back to(wards) diet A. The two grey lines (line A and B) represent the isotopic signature of the tissue in equilibrium with the two diets. The two arrows indicate the time of the two diet switches.

We encourage future use of our model for estimation of seasonal schedules of animals and emphasise that other isotopes than carbon can also be used (e.g. nitrogen or sulphur). The 'double diet switch model' might be particularly interesting in deciphering the timing and occurrence of migration in other migratory animals, animals with changes in food availability during a season (e.g. an animal that follows the food peak of different prey species), or in the timing of ontogenetic development of animals (e.g. from egg to juvenile to adult). Although not tested here, the 'double diet switch model' might not be limited to studies with switches between three isotopic levels, i.e. with diet switches from diet A to B to C, but might also be applicable to scenarios where the second switch reverses to the initial isotopic level, so a double diet switches from diet A^1 to B and from B back to A^2. We call this an 'ABBA switch' (see Fig. 5). An ABBA switch may occur under temporary changing conditions such as, e.g. breeding, drought, frozen foraging surfaces (no access to regular food) or injuries of the animal that restricts regular prey consumption. The ABBA switch could, theoretically, be studied with the regular formula of the 'double diet switch model' (see eqn. 2), where diet A^2 can be interpreted in the model as diet C. The model is thus generally applicable, and can be adapted to a wide range of taxa and situations in which animals use two or three distinct diets within a short period of time.

Authors' contributions

J.J., I.P., M.D. and E.R. conceived the ideas and designed methodology; J.J., J.R, G.H. and M.D. collected the data; J.J., M.D., I.P., J.R. and T.P. analysed the data; J.J. (and MR and IP) led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data are deposited in the Dryad Repository http://dx.doi.org/10.5061/dryad.t72b0 (Jouta *et al*. 2016).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Data S1. Estimating diet-specific $\delta^{13}C$ values.

Data S2. Estimating $\delta^{13}C$ turnover rates in Sanderlings.

Data S3. R-scripts.