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DO RED KNOTS (*CALIDRIS CANUTUS ISLANDICA*) ROUTINELY SKIP ICELAND DURING SOUTHWARD MIGRATION?

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Abstract. Subspecies *Calidris canutus islandica* of the Red Knot breeds on the arctic tundra of northeastern Canada and northern Greenland and winters along the coasts of northwestern Europe. During northward migration, it stops over in either Iceland or northern Norway. It has been assumed that it does the same during southward migration. Using ratios of stable carbon isotopes ($\delta^{13}\text{C}$) in whole blood, blood cells, and plasma, we investigated evidence for a stopover in Iceland en route from the breeding grounds to the Dutch Wadden Sea. With the expected diet (shellfish) and stopover duration at Iceland (12–15 days, maximum 17 days) and the turnover rates of blood cells (15.1 days) and plasma (6.0 days), Red Knots that stopped in Iceland should arrive with a blood (cell) $\delta^{13}\text{C}$ midway between a tundra (-24.7‰) and a marine value (-14.0‰) and a plasma $\delta^{13}\text{C}$ approaching the marine value (-15.3‰). However, many adults arriving at the Wadden Sea had $\delta^{13}\text{C}$ ratios in blood (cells) and plasma below these levels, and some arrived with clear tundra signals in blood cells, suggesting that they skipped Iceland during southward migration. Surprisingly, available data suggest this also to be true for juveniles during their first southward migration. The $\delta^{13}\text{C}$ signature of second-year birds confirmed that they overwintered in the Wadden Sea. Our findings contradict the largely untested idea that juvenile shorebirds make more stopovers than adults as well as the idea that the migration between the Nearctic and Europe is necessarily a two-leg process.

Key words: age effect, stable carbon isotope, migration, sex, shorebird, stopover ecology, Red Knot, *Calidris canutus*.

¿Es Islandia Evitada Consistentemente durante la Migración hacia el Sur por Parte de *Calidris canutus islandica*?

Resumen. *Calidris canutus islandica* se reproduce en la tundra ártica en el noreste de Canadá y norte de Groenlandia y pasa el invierno a lo largo de la costa del noroeste de Europa. Durante la migración hacia el norte, los individuos de esta subespecie hacen paradas migratorias en Islandia o en el norte de Noruega. Para la migración hacia el sur, se ha asumido el mismo patrón de paradas migratorias. Utilizando cocientes de isótopos estables de carbono ($\delta^{13}\text{C}$) en la sangre, células sanguíneas y plasma sanguíneo investigamos si existe evidencia a favor de paradas en Islandia durante la ruta desde las áreas de cría hacia el Mar de Wadden en Holanda. Con base en la dieta esperada (mariscos) y en la duración esperada de la parada en Islandia (12–15 días, máximo 17 días) y en las tasas de recambio de las células sanguíneas (15.1 días) y plasmáticas (6.0 días), los individuos de *C. c. islandica* que habrían parado en Islandia deberían llegar con un $\delta^{13}\text{C}$ en la sangre (células) con un valor medio entre tundra (-24.7‰) y marino (-14.0‰) y con $\delta^{13}\text{C}$ plasmático cercano al valor marino (-15.3‰). Sin embargo, muchos adultos que llegaron al Mar de Wadden tuvieron un cociente de $\delta^{13}\text{C}$ en la sangre (células) y en el plasma menor al de los niveles esperados, y algunos llegaron con señales claras de tundra en las células sanguíneas, lo que sugiere que estos individuos se saltaron la parada en Islandia durante la ruta migratoria hacia el sur. Sorpresivamente, los datos disponibles también surgieron este mismo patrón para los juveniles durante su primera migración hacia el sur. La señal del $\delta^{13}\text{C}$ de los individuos en su segundo año confirmó que estos prolongaron su estadía durante el verano en el Mar de Wadden. Nuestros resultados contradicen la idea no corroborada de que los juveniles de aves costeras hacen más paradas que los adultos, como también la idea de que la migración entre el Neártico y Europa es un proceso que necesita dos etapas.

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INTRODUCTION

Calidris canutus islandica has the shortest migration route of all six subspecies of the Red Knot, ca. 4800 km (Piersma et al. 2005, Piersma 2007). *Calidris c. islandica* breeds on the arctic tundra of northeastern Canada and northern Greenland and winters along the coasts of northwestern Europe. During northward migration it stops over along the coasts of Iceland or northern Norway and has been assumed to make a similar stopover during southward migration (Dick et al. 1976, Wilson 1981, Davidson and Wilson 1992, Morrison and Wilson 1992, Wilson and Morrison 1992, Gudmundsson and Gardarsson 1993, Piersma et al. 2005, Gunnarsson 2006, Wilson et al. 2008). In Iceland, higher numbers of *C. c. islandica* have been counted in May, en route to the breeding grounds, than in July and August, during the return migration (Wilson 1981, Morrison and Wilson 1992). The lower numbers in July and August might be due to less observation effort, to the asynchrony of southward migration, and/or possibly to a briefer stay (Wilson 1981, Wilson and Morrison 1992). In addition, arrivals in early July in the United Kingdom (Morecambe Bay and the Ribble) and the Dutch Wadden Sea suggest that these birds may have bypassed Iceland on their southward migration (Wilson 1981, Davidson and Wilson 1992, Morrison and Wilson 1992). Considering the length of nonstop migration flights by other subspecies of the Red Knot, up to 6900 km (Piersma et al. 2005), it should be physically possible for *C. c. islandica* to cover the distance between wintering and breeding areas in a single flight.

In this study, using ratios of stable carbon isotopes in whole blood, blood cells, and plasma, we investigate if not only the early-arriving birds but also later-arriving ones captured in the Dutch Wadden Sea skip stopovers in intertidal areas in Iceland. Ratios of stable isotopes in body tissues reflect the isotopic composition of the organism's diet (Gannes et al. 1997, Evans Ogden et al. 2004, Karasov and Martínez del Río 2007:433–478). When the organism shifts from one diet to another of a different isotopic signature, after the switch, body tissues will adjust to the isotopic ratio of the new diet at a tissue-specific rate. The tundra diet in the Red Knot's breeding range and the marine diet at its stopover sites and in its winter range differ considerably in ratios of stable carbon isotopes (−26.1‰ vs. −16.8‰, respectively; Morrison and Hobson 2004). Therefore, after a stopover in Iceland, knots arriving in the Dutch Wadden Sea are expected to have ratios of stable carbon isotopes enriched (higher) in comparison with those in the arctic breeding range. In contrast, knots arriving directly from the arctic tundra are expected to have ratios similar to those of knots in the breeding range.

In the Red Knot, as in most shorebirds, the timing of departure from the breeding range varies with sex and age. Adult females leave the breeding grounds when the eggs have hatched, adult males when the young are independent, and the juveniles leave latest (Helseth et al. 2005, Piersma et al. 2005).

In addition, there are indications that juvenile shorebirds use more, and different, stopovers than adults (Davidson and Wilson 1992, Lindström et al. 2002, Meissner 2007). Because sex and age affect the timing of migration and may affect the migration route, we also investigated effects of age and sex on the southward migration strategy of *C. c. islandica*. A special case are the second-year birds (hatched in the previous year), most of which overwinter in the winter range (Boere 1976, Wilson et al. 1998; Piersma et al., unpubl. data), though some may overwinter in Iceland (Morrison and Wilson 1992).

METHODS

ANIMALS AND EXPERIMENTAL PROCEDURE

In July, August, and September in 2001 and 2002, we mist-netted Red Knots near high-tide roosts in the Dutch Wadden Sea on nights of the new moon. The birds were measured, weighed (± 1 g), aged (by plumage; Prater et al. 1977), and banded (a metal band from the Dutch banding scheme and an individual combination of four color bands and one leg flag), and their molt was scored. We took a small blood sample (~ 120 μ L) for DNA analysis (sex determination) and stable-isotope analysis by puncturing the wing vein and drawing blood into heparinized capillaries. Blood for DNA analysis was stored in ethanol (95%) and kept in a freezer (-20 °C aboard a research vessel, later at -80 °C) until analysis at the Royal Netherlands Institute for Sea Research (NIOZ, Texel, the Netherlands). Sexing followed a verified standard molecular technique (Baker et al. 1999). Blood samples destined for stable-isotope analyses were centrifuged (12 min at 6900 g) as soon as possible after being taken for separation of plasma from blood cells. Blood cells and plasma samples were stored in a freezer (-20 °C) until transport to the Netherlands Institute of Ecology (NIOO-KNAW), Centre for Limnology, for analysis. In addition, leftovers of blood samples from DNA analyses of knots caught in July, August or September in 1998 and 1999 (caught by a protocol similar to that of 2001 and 2002) were used for stable-isotope analyses.

From mid July onward, two subspecies of the Red Knot occur in the Dutch Wadden Sea, *C. c. islandica* and *C. c. canutus*. Subspecies *islandica* remains in the Dutch Wadden Sea to overwinter, while *canutus* fuels up to continue migrating to west Africa (Nebel et al. 2000, Piersma et al. 2005). We distinguished *islandica* as follows: adults and second-year birds in primary molt were identified as *islandica* because, with the exception of juveniles, all knots molt their flight feathers in the winter range (Boere 1976, Davidson and Wilson 1992); knots of all ages were identified as *islandica* when resighted at locations where that subspecies is known to winter. Note that in the field, assigning recently arrived birds and juveniles to subspecies is possible only on the basis of resightings, as these birds have not started primary molt. This complication explains the rather small sample sizes of recently arrived and juveniles of *islandica*. Subspecies determination was kept conservative,

and in total we identified 46 adult males, 69 adult females, 21 second-year males, 20 second-year females, 6 juvenile males, and 4 juvenile females as subspecies *islandica*.

PREY SAMPLING

Each year in July and August, samples of shorebird prey are taken in our research area in the Dutch Wadden Sea (e.g., van Gils et al. 2006, Kraan et al. 2009). Samples were stored in a freezer until analysis (-20°C). Of specimens collected in 1998, 1999, 2001 and 2002 at depths accessible to knots, not used in other analyses, and of a size eaten by knots, we took random samples of two favorite species of hard-shelled prey (Baltic tellin, *Macoma balthica*, and edible cockle, *Cerastoderma edule*; $n = 104$) and two of soft prey (juvenile shore crab, *Carcinus maenas*, and common shrimp, *Crangon crangon*; $n = 49$) (Zwarts and Blomert 1992, van Gils et al. 2005). Prey samples were transported to the Netherlands Institute of Ecology (NIOO-KNAW), Centre for Limnology, for stable-isotope analysis.

STABLE-ISOTOPE ANALYSIS

Whole blood, blood cells, and plasma samples of *Calidris c. islandica* and prey samples were freeze-dried to constant mass prior to the stable-isotope analysis. Prey samples were ground whole. Ratios of stable carbon isotopes (parts per thousand, ‰, difference from the $^{13}\text{C}:^{12}\text{C}$ ratio in Vienna PeeDee limestone; further referred to as $\delta^{13}\text{C}$) were determined in a Eurovector EuroAE3000 elemental analyzer coupled online to a Finnigan Delta S isotope-ratio mass spectrometer via a Finnigan continuous-flow interface. Average reproducibility based on replicate measurements was $<0.2\text{‰}$. We assume that $\delta^{13}\text{C}$ ratios and turnover rates of whole blood and blood cells are similar, given the overwhelming effect of isotope ratios in blood cells on the isotope ratios in the blood as a whole (Evans Ogden et al. 2004; M. Klaassen, pers. obs.), which results from the fact that by far the most carbon in a sample of whole blood stems from the blood cells and not from the plasma.

STATISTICS

The change in isotopic ratios in body tissue after a diet switch is usually described by an exponential decay curve (Karasov and Martínez del Río 2007:433–478): $\delta(t) = \delta(\infty) + [\delta(0) - \delta(\infty)] \times e^{-\lambda t}$, where $\delta(t)$ is the isotopic signature of the tissue at time t after the diet switch (t in days), $\delta(\infty)$ is the isotopic signature of the tissue in equilibrium with the new diet, $\delta(0)$ is the isotopic signature of the tissue in equilibrium with the old diet, and λ is the turnover rate of the isotope in the tissue due to metabolism and growth. Fitting this decay curve to our field data was not possible for several reasons. For juveniles, sample sizes were too small, and the data were not evenly distributed over time. Second-year birds had most likely overwintered in the area and thus not switched diet. For adults, the sample size was sufficient, but at our study site adult knots arrive very asynchronously. The variation in

arrival dates also precluded the fitting of sex-specific exponential decay curves. Therefore, instead we fitted quadratic curves through the data of adults (NONLIN 2.5; Sherrod 1994, based on the nonlinear least-squares algorithm described in Dennis et al. 1981), because this relatively simple model best described the data. To test for differences by sex, we also fitted through the data an extended quadratic model that fitted separate quadratic curves for males and females in one model:

$$\delta^{13}\text{C} = \text{dummy}_1 \times (a_1 + b_{11} \times \text{day} + b_{12} \times \text{day}^2) + \text{dummy}_2 \times (a_2 + b_{21} \times \text{day} + b_{22} \times \text{day}^2),$$

where dummy_1 was set at 1 for males and 0 for females and dummy_2 was set at 0 for males and 1 for females, a_i is the constant for males ($i = 1$) or females ($i = 2$), and b_{ij} is the first ($j = 1$) or second ($j = 2$) coefficient for males ($i = 1$) or females ($i = 2$). Results of the extended quadratic model and a normal quadratic model fitted through all data were compared via an F -test.

In the transition from diet to tissue, $\delta^{13}\text{C}$ increases as a result of diet–tissue fractionation (Gannes et al. 1997, Evans Ogden et al. 2004, Karasov and Martínez del Río 2007:433–478). To allow for comparison of the blood (cells) and plasma ratios with $\delta^{13}\text{C}$ in the various alternative tundra and estuarine diets we compared our data with the expected signals for blood (cells) and plasma. The expected signals were obtained as follows: (1) for the tundra signal, we used for blood (cells) the $\delta^{13}\text{C}$ of blood of *C. c. islandica* captured in July and August on the tundra around Alert, Canada ($-24.7 \pm 0.6\text{‰}$ SD; Morrison and Hobson 2004). For plasma, we calculated the expected isotopic signature at Alert by using the difference in isotopic signature between blood cells and plasma of captive knots ($-24.7 - 1.6 = -26.3\text{‰}$; Morrison and Hobson 2004; Klaassen et al. in press). (2) For the Wadden Sea signal, we used diet values corrected for diet–tissue discrimination based on the discrimination factors found in *C. c. islandica* in captivity, namely, 0.8‰ for blood plasma and 2.2‰ for blood (cells). These values are in range with the discrimination factors found in captive Dunlins (*C. alpina pacifica*; Evans Ogden et al. 2004).

Means are presented \pm SE, unless stated otherwise, and compared with ANOVA or independent Student t -tests. The significance level α was set at 0.05 for all analyses.

RESULTS

PREY SAMPLES

Hard-shelled prey (bivalves) had $\delta^{13}\text{C}$ ratios lower than those of soft crustacean prey ($-16.1 \pm 0.2\text{‰}$ vs. $-12.9 \pm 0.2\text{‰}$, $t_{151} = -11.9$, $P < 0.001$). Within each type of prey, $\delta^{13}\text{C}$ differed by year (ANOVA $F_{3,100} = 10.6$ and $F_{3,45} = 7.2$, for hard and soft prey, respectively; both $P < 0.001$), but this was due solely to lower ratios in 2001 (Bonferroni analyses, for hard prey $P < 0.01$ for all comparisons with 2001; for soft prey $P < 0.05$ for comparisons of 2001 with 1998 and 1999, while 2001 and

2002 did not differ from each other, $P = 0.09$). Nevertheless, we used mean $\delta^{13}\text{C}$ ratios in prey to calculate the expected Wadden Sea signature (see above).

ADULT KNOTS

Adults of *C. c. islandica* expected to have arrived recently in the Wadden Sea area (captured in late July, Julian date range 205–210), had lowest $\delta^{13}\text{C}$ ratios (Fig. 1a, b). In these knots, $\delta^{13}\text{C}$ ratios of males and females did not differ ($t_{17} = -1.3$ and $t_{14} = -1.1$, $P = 0.21$ and $P = 0.30$ for blood (cells) and plasma, respectively). The isotopic signature of blood (cells) of some knots was within the range of the expected tundra isotopic signature. The mean $\delta^{13}\text{C}$ ratio of blood (cells) in late July ($-21.4 \pm 0.7\text{‰}$), however, was higher than the mean isotopic signature of whole blood of knots on the Canadian tundra ($t_{17} = 4.7$, $P < 0.001$; Fig. 1b). The mean $\delta^{13}\text{C}$ ratio of plasma in late July was also higher than the expected tundra signature ($t_{14} = 13.6$, $P < 0.001$; Fig. 1a), but note that the $\delta^{13}\text{C}$ ratio of plasma of some knots was slightly above the expected tundra signature.

With time, ratios of $\delta^{13}\text{C}$ became more enriched in both blood (cells) and plasma, until ratios approached the expected Wadden Sea signature. The enrichment of $\delta^{13}\text{C}$ of blood (cells) with time differed by sex ($F_{3,110} = 3.5$, $P < 0.05$); $\delta^{13}\text{C}$ of blood (cells) of females was slightly more enriched than that of males early in the season, which is consistent with females leaving the breeding range earlier than males. The increase in $\delta^{13}\text{C}$ of plasma did not differ by sex ($F_{3,57} = 1.2$, $P > 0.05$). Adults' body mass did not vary with time (Fig. 1c). In late September, the $\delta^{13}\text{C}$ of blood (cells) and plasma were close to the expected mean Wadden Sea signature for hard prey, indicating that the birds had been eating mainly hard prey.

For comparison, we calculated the expected pattern of enrichment of blood (cells) and plasma of an individual Red Knot that flew nonstop from the arctic tundra to the Dutch Wadden Sea, where it arrived on Julian day 210 (29 July). For these exponential decay curves, we used the turnover rates (λ) of blood cells and plasma determined in a diet-switch experiment in captive knots (0.046 and 0.144, respectively, Klaassen et al. in press). As isotopic signature under the old diet [$\delta(0)$], we used the $\delta^{13}\text{C}$ ratio of whole blood and plasma expected on the tundra. As isotopic signature under the new diet [$\delta(\infty)$], we used the mean expected Wadden Sea signature of blood (cells) and plasma, assuming the birds ate a diet of hard prey (-14.0‰ and -15.3‰ , respectively). The resulting exponential decay curves indicate how fast plasma is expected to change, while the enrichment of blood (cells) is much slower (Fig. 1a, b). The effect of variation in arrival time on $\delta^{13}\text{C}$ ratios will thus be larger for plasma than for blood (cells).

JUVENILE KNOTS

Juvenile knots were caught in two periods: mid August 1999 and mid September 2002 (Fig. 2). Ratios of $\delta^{13}\text{C}$ in blood (cells) and plasma of the August birds were lower than those of the

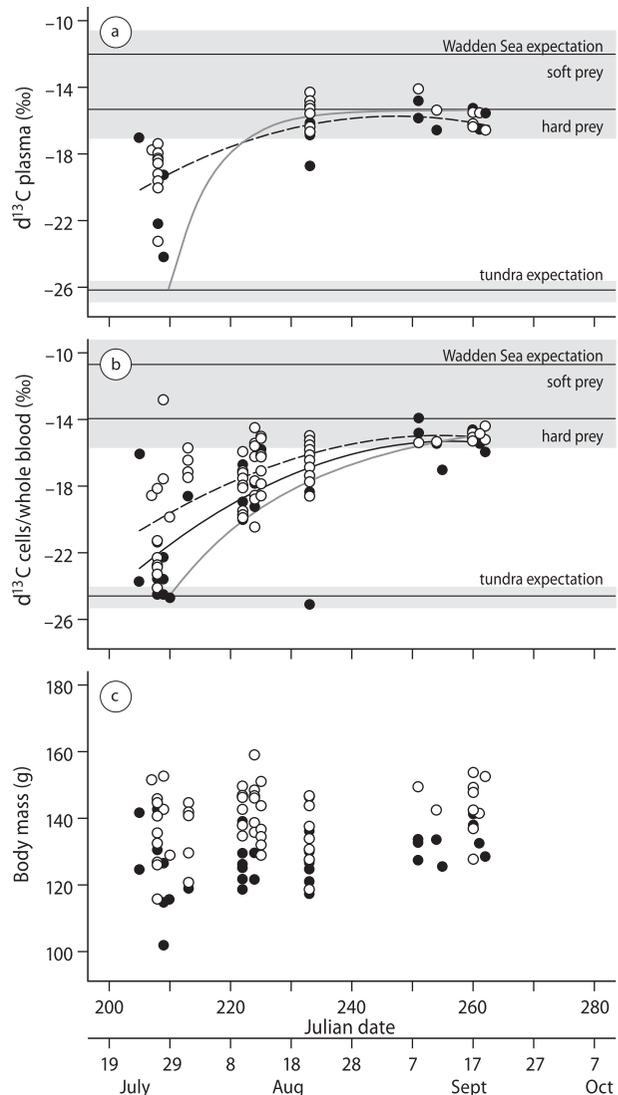


FIGURE 1. Variation with time in (a) $\delta^{13}\text{C}$ ratios in blood plasma, (b) $\delta^{13}\text{C}$ ratios in blood (cells), and (c) body mass in adult males (black dots) and adult females (white circles) of *Calidris canutus islandica*. For comparison of the sexes, quadratic relationships were fitted through the data. These relationships are presented by the solid and dashed lines [male and female in (b), and all knots in (a), respectively]. To indicate the expected patterns of turnover of blood (cells) and plasma, the expected turnover rates for an individual arriving at the Julian date 210 are given (solid gray lines, see also text). Quadratic equations: for plasma, $\delta^{13}\text{C} = -163.56 (\pm 27.63) + 1.19 (\pm 0.24) \text{ Julian date} - 0.002 (\pm 0.001) \text{ Julian date}^2$, $n = 60$, $R^2 = 0.60$, $P < 0.001$; for cells in males, $\delta^{13}\text{C} = -210.56 (\pm 56.39) + 1.53 (\pm 0.48) \text{ Julian date} - 0.003 (\pm 0.001) \text{ Julian date}^2$; for cells in females, $\delta^{13}\text{C} = -162.68 (\pm 46.00) + 1.16 (\pm 0.39) \text{ Julian date} - 0.002 (\pm 0.001) \text{ Julian date}^2$. For males and females combined, $n = 113$, $R^2 = 0.52$, $P < 0.001$. The mean expected $\delta^{13}\text{C}$ ratios of blood (cells) and plasma (see text) for the Wadden Sea and for tundra are indicated with horizontal solid lines. The light gray areas around these lines represent the range ± 1 SD around the means.

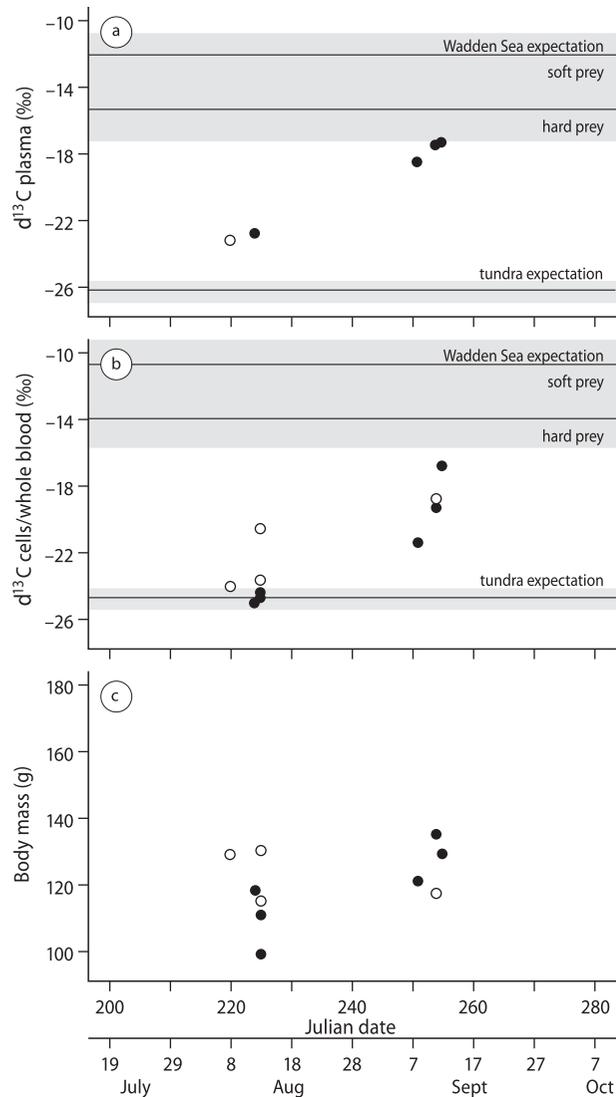


FIGURE 2. Variation with time in (a) $\delta^{13}\text{C}$ ratios in blood plasma, (b) $\delta^{13}\text{C}$ ratios in blood (cells), and (c) body mass in first-year males (black dots) and females (white circles) of *Calidris canutus islandica*. Horizontal lines and gray areas as in Fig. 1.

September birds ($t_8 = -4.2$, $P < 0.01$, and $t_3 = -10.6$, $P < 0.01$, for blood (cells) and plasma, respectively). Low sample sizes did not allow a comparison by sex. Values of $\delta^{13}\text{C}$ of blood (cells) in most August juveniles were within or close to the range of expected tundra $\delta^{13}\text{C}$ ratios and did not differ significantly from those of knots on the Canadian tundra ($t_4 = 1.5$, $P > 0.05$; Fig. 2b). In August, ratios of $\delta^{13}\text{C}$ in plasma were slightly above the expected tundra signature; sample size was too small to allow a statistical comparison. Body mass increased slightly but non-significantly with time ($t_8 = -1.3$, $P = 0.24$; Fig. 2c).

SECOND-YEAR KNOTS

In contrast to adults and juveniles, in which $\delta^{13}\text{C}$ ratios of blood (cells) and plasma were enriched with time, $\delta^{13}\text{C}$ ratios of blood

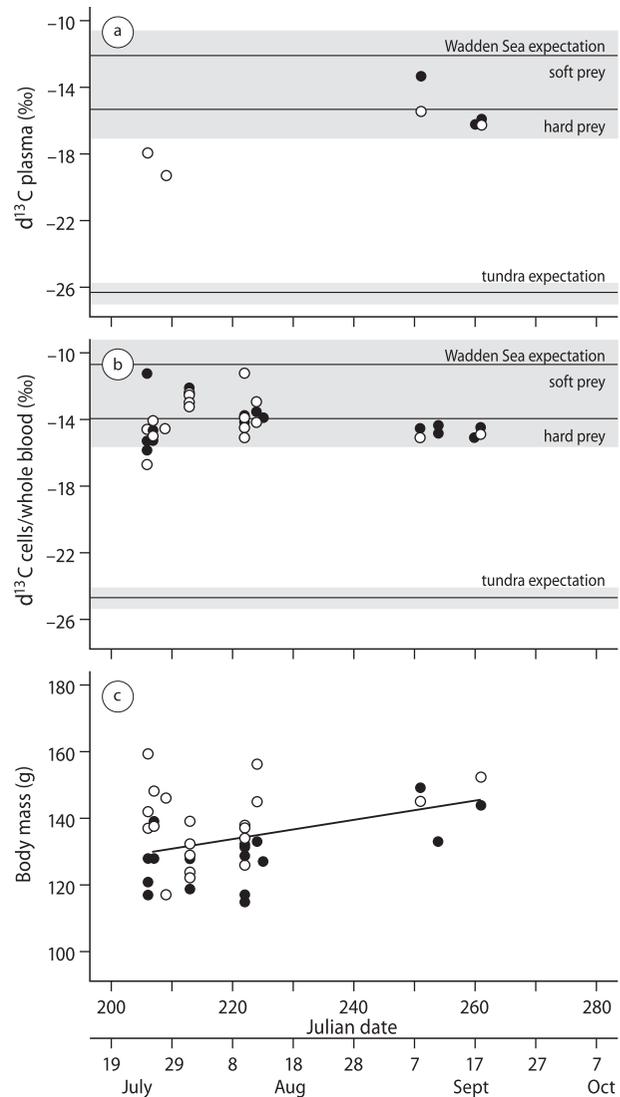


FIGURE 3. Variation with time in (a) $\delta^{13}\text{C}$ ratios in blood plasma, (b) $\delta^{13}\text{C}$ ratios in blood (cells), and (c) body mass in second-year males (black dots) and females (white circles) of *Calidris canutus islandica*. Horizontal lines and gray areas as in Fig. 1. The solid line in (c) gives the linear regression: body mass = $70.22 + 0.29$ Julian date, $n = 42$, $R^2 = 0.165$, $P < 0.01$.

(cells) and plasma of second-year knots did not vary with time (Fig. 3a, b). Most second-year birds had ratios around the expected Wadden Sea signature. Body mass increased with time (linear regression, $n = 42$, $R^2 = 0.17$, $P < 0.01$).

DISCUSSION

The results confirm that in *Calidris canutus islandica* most one-year-old birds overwinter in the winter range. Many adults and juveniles, however, do not seem to make the expected stopover in Iceland. To show this more clearly, we ascertained the expected duration of stopover in Iceland via the known constraining effect of gizzard mass on fueling rate and the available literature.

At stopover sites and in the winter range, Red Knots occur mainly on soft sediments in intertidal areas, where they forage chiefly on hard prey such as shellfish (Alerstam et al. 1992, Zwarts and Blomert 1992, van Gils et al. 2005, Piersma et al. 2005, Quaintenne et al. 2010). Red Knots ingest their prey whole and crack them with their large muscular gizzard (Piersma et al. 1993). Gizzard size is therefore an important determinant of the maximum prey size and the maximum amount of indigestible matter that a knot can process. On the tundra, knots forage mainly on much softer prey such as spiders and arthropods (Tulp et al. 1998, Klaassen et al. 2001, Tulp and Schekkerman 2008) for which they do not require a large gizzard. Indeed, in the breeding range *C. c. islandica* has a gizzard smaller than at stopover sites or in the winter range (Piersma et al. 1993, 1999, Morrison et al. 2005). Therefore, during southward migration, Red Knots can be expected to arrive at their first stopover site with relatively small gizzards that must increase before they can process larger, more profitable hard prey and the large amounts of prey required to fuel up (Piersma et al. 1993). A knot's gizzard size can change rapidly, but the increase from small on arrival to large for processing shellfish still requires ~6 days (Dekinga et al. 2001). Since the required fueling rates can be achieved only after the gizzard's size has increased sufficiently, the minimum stopover time is likely to exceed 1 week. Stopover time is therefore expected to exceed the half-life of $\delta^{13}\text{C}$ in plasma (6.0 days, Klaassen et al. in press).

In the 1970s, Red Knots were caught in Iceland during southward migration (Wilson 1981, Morrison and Wilson 1992, Wilson and Morrison 1992). Wilson and Morrison (1992) used the data on body mass and the few recaptures to estimate fueling rate and duration of the stopover. They estimated that during southward migration Red Knots stayed in Iceland on average 12–15 days (maximum 17 days), confirming the expectation based on gizzard mass. Estimated time for staging is similar to the half-life of $\delta^{13}\text{C}$ in blood cells (15.1 days, Klaassen et al. in press) and two to three times the half-life of $\delta^{13}\text{C}$ in plasma.

In Iceland, Red Knots eat mainly gastropods (*Littorina* sp.) and bivalves (edible mussel, *Mytilus edulis*; Alerstam et al. 1992) whose $\delta^{13}\text{C}$ signature is similar to that of Wadden Sea shellfish (–15.9 and –17.1‰, respectively; Sarà et al. 2007, Steinarsdóttir et al. 2009). Combining this with the estimated stopover time from Wilson and Morrison (1992) and half-life times of blood cells and plasma, knots stopping over in Iceland are expected to arrive in the Wadden Sea with blood (cells) $\delta^{13}\text{C}$ ratios that are halfway between tundra and marine signatures, while plasma $\delta^{13}\text{C}$ ratios should approach the marine signature. Many adults, however, have $\delta^{13}\text{C}$ ratios in blood (cells) and plasma much below these expectations. In fact, a fair number of adults had $\delta^{13}\text{C}$ blood (cells) ratios at or near the tundra signature. These low ratios can be explained only if (1) *C. c. islandica* skips Iceland during southward migration or (2) the stopover is confined to fresh water. For the latter explanation

we have no supporting field observations (Wilson 1981, Morrison and Wilson 1992, Wilson and Morrison 1992).

However, there are also many adults that fall within the range of values expected for knots that stopped over in Iceland. To determine if these birds skipped Iceland or not, we made use of the fact that Red Knots molt their primaries only in the wintering areas (Boere 1976, Davidson and Wilson 1992) and plotted a score of primary molt versus $\delta^{13}\text{C}$ ratios of blood (cells) (Fig. 4). Because the signature of stable carbon isotopes in the shellfish diet in Iceland and the Wadden Sea are similar, $\delta^{13}\text{C}$ ratios of blood (cells) can be used for all knots, whether they stopped over or not, to estimate time since the switch from a tundra to a marine diet as follows (second x axis in Fig. 4):

$$t = -\ln\left(\frac{\delta(0) - \delta(\infty)}{\delta(t) - \delta(\infty)}\right) \times \lambda^{-1},$$

with t in days and $\delta(0)$ and $\delta(\infty)$ set equal to the values used to calculate the expected change in $\delta^{13}\text{C}$ in blood (cells) with time (Fig. 1). Next, using 11 years of field data, we calculated the average rate of change in primary-molt score in adults of *C. c. islandica* during the period of linear increase in this score (no difference between males and females, $n = 516$, $P = 0.627$; common rate of change was $0.637 \pm 0.016 \text{ day}^{-1}$). From the common rate of change in primary-molt score and the time conversion, we calculated the expected primary-molt score versus $\delta^{13}\text{C}$ ratio, assuming that, on basis of our data, the average knot reached a primary-molt score of 40 (which is near the end of the period of linear change and in range of our data set) at a blood (cell) $\delta^{13}\text{C}$ of –14.5‰, i.e., at 66.6 days after the diet switch (solid black line in Fig. 4). This line of expected change in primary-molt score is not linear because the time axis is not linear. The expected change in primary-molt score indicates that the average knot must have started primary molt well before the $\delta^{13}\text{C}$ ratio of its blood (cells) was halfway between the tundra and marine signature, thus below the range expected for knots that stopped over at Iceland. In other words, all knots with primary-molt scores around or above the line of expected change, which represent the majority of data points, especially for males (Fig. 4), must have skipped Iceland because knots do not molt their primaries at a stopover site (Boere 1976, Davidson and Wilson 1992). We therefore conclude that most adults of *C. c. islandica* wintering in the Dutch Wadden Sea skip Iceland during southward migration and fly directly from the nearctic breeding range to the Wadden Sea and possibly other European molting and wintering areas.

Since males leave the breeding range later than do females (Helseth et al. 2005, Piersma et al. 2005), they are more time-stressed to arrive in the Wadden Sea before the abundance of profitable prey decreases (Zwarts et al. 1992) and more likely to skip Iceland (Piersma et al. 1999). In the Wadden Sea, males of *C. c. islandica* finish flight-feather molt by the end of October (Boere 1976; Dietz et al., unpubl. data), after a molt

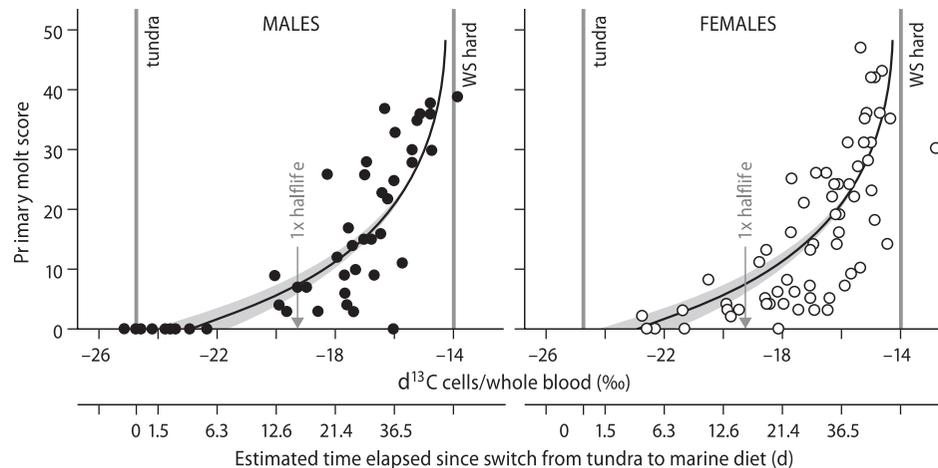


FIGURE 4. The variation in score of primary molt in adults of *Calidris canutus islandica* in relation to the $\delta^{13}\text{C}$ ratio of their blood (cells) for males (left panel, black dots) and females (right panel, white circles). The time elapsed since a Red Knot switched from the tundra diet to a marine diet is indicated on the second x axis, a time calculated from the $\delta^{13}\text{C}$ ratio of blood (cells) (see text). The vertical gray lines represent the expected tundra (left) and Wadden Sea (right) signature for blood (cells), under the assumption that the knots in the Wadden Sea had been eating their preferred prey, shellfish. The arrow indicates the point of half-life of $\delta^{13}\text{C}$ ratio in blood (cells) (-19.3‰ or 15 days). All data left of the arrow are therefore from birds that skipped Iceland. The solid black line represents the expected change in primary-molt score in relation to time since the switch from the tundra to the marine diet. The expected change was calculated from 11 years of field data on the average rate of change in primary-molt score during this variable's period of linear increase in *C. c. islandica* in the Wadden Sea ($0.637 \pm 0.016 \text{ day}^{-1}$) and by assuming that a score of 40, which is near the end of the period of linear increase and in range of our data set, was obtained at a blood (cells) $\delta^{13}\text{C}$ ratio of -14.5‰ , i.e., 66.6 days after the diet switch (see text). The gray area around the solid black line indicates the 95% confidence interval around the average rate of change in primary-molt score.

of ~ 100 days (Boere 1976; Dietz et al., unpubl. data), so they must have arrived in the very first days of August. Female Red Knots are less time-stressed than males, which may allow a stopover in Iceland during southward migration. Nevertheless, $\delta^{13}\text{C}$ ratios showed that females en route to the winter range also bypass Iceland, which may indicate that time stress is not the predominant reason for *C. c. islandica* skipping Iceland.

Our most remarkable finding is the evidence that juveniles of *C. c. islandica* may also fly nonstop from their natal tundra to their wintering grounds in the Dutch Wadden Sea (Fig. 2), as it contradicts the expectation that during southward migration young shorebirds make more stopovers than adults (Davidson and Wilson 1992, Lindström et al. 2002, Meissner 2007). That juveniles of *C. c. islandica* wintering in the Netherlands fly nonstop from the natal site to the winter range implies that juveniles may be able to fuel up to a level similar to that of adults. To examine whether on the tundra juveniles fuel themselves at a rate comparable to that of adults, we compared the body sizes and masses of recently arrived birds of the two age classes. We selected individuals conservatively by using only Red Knots with $\delta^{13}\text{C}$ ratios in blood (cells) below -24.1‰ (i.e., mean $\delta^{13}\text{C}$ signature on the tundra around Alert in July and August + 1 SD; Morrison and Hobson 2004). Body size (bill, tarsus, and wing length) of recently arrived juveniles ($n = 3$) and adults ($n = 5$; ANOVA, all $P > 0.05$) did not differ. However, recently arrived juveniles weighed less than recently arrived adults ($109 \pm 6 \text{ g}$ vs. $128 \pm 5 \text{ g}$, respectively; ANOVA,

$F_{1,6} = 5.8, P = 0.05$). This difference suggests that juveniles leave the breeding areas with body stores smaller than the adults' or do not fly the migration route as efficiently as adults.

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