Mar Biol (2014) 161:229–237
DOI 10.1007/s00227-013-2314-5

METHOD

Differences in $\delta^{13}C$ and $\delta^{15}N$ values between feathers and blood of seabird chicks: implications for non-invasive isotopic investigations

Yves Cherel · Sébastien Jaquemet · Alessio Maglio · Audrey Jaeger

Received: 12 June 2013 / Accepted: 8 August 2013 / Published online: 21 August 2013

Abstract Blood and feathers are the most targeted tissues for isotopic investigations in avian ecology, primarily because they can be easily and non-destructively sampled on live individuals. Comparing blood and feather isotopic ratios can provide valuable information on dietary shifts, trophic specialization and migration patterns, but it requires a good knowledge of the isotopic differences between the two tissues. Here, $\delta^{13}C$ and $\delta^{15}N$ values of whole blood (in blood cells of a few species) and simultaneously grown body feathers were measured in seabird chicks to quantify the tissue-related isotopic differences. Seabirds include 27 populations of 22 wild species that were sampled in 2000–2008, and a review of the literature added 8 groups (including adult birds) to the analysis. The use of a large data set that overall encompasses wide $\delta^{13}C$ and $\delta^{15}N$ ranges allowed us to depict for the first time accurate relationships between blood and feather isotopic ratios across avian taxa. Blood was impoverished in ^{13}C and generally in ^{15}N compared with feathers. Both mean $\delta^{13}C$ and $\delta^{15}N$ values of feathers and blood were highly positively and linearly related [feather $\delta^{13}C = 0.972$ (± 0.020) blood $\delta^{13}C + 0.962$ (± 0.414), and feather $\delta^{15}N = 1.014$ (± 0.056) blood $+ 0.447$ (± 0.665), respectively; both $P < 0.0001$]. The regressions should be applied to mathematically correct feather or whole blood $\delta^{13}C$ and $\delta^{15}N$ values when comparing isotopic ratios within and between ecological studies on birds.

Communicated by J. P. Grassle.

Y. Cherel (✉) · A. Maglio · A. Jaeger
Centre d'Etudes Biologiques de Chizé (CEBC),
UPR 1934 du CNRS, BP 14, 79360 Villiers-en-Bois, France
e-mail: cherel@cebc.cnrs.fr

S. Jaquemet · A. Jaeger
Laboratoire ECOMAR, Université de La Réunion,
15 Avenue René Cassin, BP 7151, 97715 Saint-Denis Cedex,
Ile de La Réunion, France

Introduction

Stable isotope analysis has been emerged as one of the primary means for examining the ecological niche of animal species (Newsome et al. 2007). Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are used routinely to study foraging ecology, feeding habits, migration patterns and resource allocation processes (Inger and Bearhop 2008; Hobson 2011). The method is especially useful because it provides time-integrated insights into trophic relationships. Comparisons of isotopic composition of tissues formed at different times can identify dietary shifts, migratory connectivity and the degree of feeding specialization at the individual and population levels (Dalerum and Angerbjörn 2005; Martinez del Rio et al. 2009; Hobson and Bond 2012). For example, blood and feathers are sampled on breeding birds to investigate their feeding strategies during the breeding and moulting periods, respectively (Cherel et al. 2008; Hedd et al. 2010), because reproduction and moult are largely separated in time in many species due to energetic constraints (Murphy and King 1991). Blood is a metabolically active tissue whose isotopic ratios reflect diet during the days/weeks before sampling, whereas feather $\delta^{13}C$ and $\delta^{15}N$ values correspond to the period of feather growth because keratin is metabolically inert after synthesis (Hobson and Clark 1992a, 1993; Bearhop et al. 2002). Blood and feathers also have the great advantage of being easily and non-destructively sampled on live individuals.

A main limitation of the use of different tissues is that a comparison of their raw isotopic data is blurred by

tissue-specific isotopic discrimination factors (Δ, differences in isotopic composition between an animal's tissues and its diet) due primarily to tissue-specific biochemical composition (Wolf et al. 2009). In birds, feathers tend to be overall enriched in ^{13}C and ^{15}N over blood (Kelly 2000; Vanderklift and Ponsard 2003; Caut et al. 2009). Indeed, when looking at the few controlled investigations involving the two tissues on the same individuals (Table 1), feathers are ^{13}C and ^{15}N enriched over blood, except in one study that reported the reverse for ^{15}N (Hobson and Clark 1992b). Thus, comparing $\delta^{13}C$ and $\delta^{15}N$ values of blood and feathers requires a good knowledge and quantification of the isotopic differences between the two tissues to calculate accurate isotopic correction factors and/or to determine mathematical relationships between tissue-specific isotopic values. This necessitates either sampling blood and feathers on captive birds raised on a constant diet, or sampling simultaneously grown feathers and blood on the wild individuals. There are three main difficulties with doing this on many species: (1) experimental investigations are time- and money-consuming; (2) ethical, conservational and practical reasons preclude raising many bird species in captivity and (3) moulting of wild species most often occurs during the inter-nesting period when adults are far from the breeding grounds and difficult to sample.

Unlike adult moult, chick moult occurs at the nest in most avian species. Hence, field sampling of blood and of simultaneously grown feathers can be easily done in many cases on the well-feathered chicks at the end of the chick-rearing period. Another positive aspect of sampling chicks is that all body feathers grow almost simultaneously, and consequently $\delta^{13}C$ and $\delta^{15}N$ values show limited inter-feather variation, which contrasts with the protracted adult moult that can cause large $\delta^{13}C$ and $\delta^{15}N$ differences among feathers from a single individual (Jaeger et al. 2010). Despite these advantages, to date, there have been relatively few studies on chicks, with only six different species of birds having been investigated (Table 2).

In the present study, we compared $\delta^{13}C$ and $\delta^{15}N$ values of feathers and blood of chicks of seabirds in the wild to quantify the isotopic differences between the two tissues. Four orders and nine families were sampled, a total of 294 individuals. We choose birds with contrasting foraging habitats and diet to encompass wide ranges in $\delta^{13}C$ and $\delta^{15}N$ values, and to help define the mathematical relationships between feather and blood isotopic ratios. Noticeably, since the diet of free-ranging birds is uncontrolled, raw $\delta^{13}C$ and

Table 1 Review of diet-tissue isotopic discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$) for feathers and whole blood (*blood cells), and of isotopic differences between feathers and blood of birds raised in captivity on a constant diet

Species	*n*	Feather $\Delta^{13}C$ (‰)	Blood $\Delta^{13}C$ (‰)	$\delta^{13}C$ differences (‰)	Feather $\Delta^{15}N$ (‰)	Blood $\Delta^{15}N$ (‰)	$\delta^{15}N$ differences (‰)	References
King penguin	10	+0.1	−0.8	+0.9	+3.5	+2.1	+1.4	Cherel et al. (2005b)
Rockhopper penguin	10	+0.1	0.0	+0.1	+4.4	+2.7	+1.7	Cherel et al. (2005b)
Spectacled eider*	11	+3.2	+2.0	+1.2	+5.6	+4.0	+1.6	Federer et al. (2010)
Mallard*	9	+0.3	−0.3	+0.7	+4.9	+3.6	+1.3	Hahn et al. (2012)
Bewick's swan*	9	+1.5	−0.7	+2.2	+4.6	+3.7	+0.9	Hahn et al. (2012)
Peregrine falcon	7	+2.1	+0.2	+1.9	+2.7	+3.3	−0.6	Hobson and Clark (1992b)
Great skua	9	+2.1 to +2.2	+1.1 to +2.3	−0.1 to +1.0	+4.6 to +5.0	+2.8 to +4.2	+0.8 to +1.8	Bearhop et al. (2002)
Ring-billed gull	14	+0.2	−0.3	+0.5	+3.0	+3.1	−0.1	Hobson and Clark (1992b)
Japanese quail	5	+1.4	+1.2	+0.2	+1.6	+2.2	−0.6	Hobson and Clark (1992b)
Yellow-rumped warbler	32	+1.9 to +4.3	−1.2 to +2.2	+2.0 to +3.1	+3.2 to +3.5	+1.7 to +2.7	+0.8 to +1.5	Pearson et al. (2003)
Garden warbler	6	+2.7	+1.7	+1.0	+4.0	+2.4	+1.6	Hobson and Bairlein (2003)
Song sparrow	28	+0.3	−1.5	+1.8	+2.9	+2.2	+0.7	Kempster et al. (2007)

Table 2 Review of simultaneously grown feathers and whole blood (*blood cells) $\delta^{13}C$ and $\delta^{15}N$ values and of isotopic differences between feathers and blood in wild birds

Species	Age	*n*	Feather $\delta^{13}C$ (‰)	Blood $\delta^{13}C$ (‰)	Paired $\delta^{13}C$ differences (‰)	Feather $\delta^{15}N$ (‰)	Blood $\delta^{15}N$ (‰)	Paired $\delta^{15}N$ differences (‰)	References
Black-browed albatross	Adults	15	−15.5 ± 0.2**	−16.8 ± 0.1**	+1.3 ± 0.1**	17.1 ± 0.4**	16.0 ± 0.4**	+1.1 ± 0.2**	Quillfeldt et al. (2008)
Spectacled petrel	Adults	21	−16.0 ± 0.6	−17.7 ± 0.7	+1.7	15.5 ± 1.0	14.0 ± 0.8	+1.5	Bugoni et al. (2008)
	Adults	21	−16.0 ± 0.1**	−17.1 ± 0.1**	+1.1 ± 0.1**	15.5 ± 0.2**	14.3 ± 0.2**	+1.2 ± 0.1**	Quillfeldt et al. (2008)
Great shearwater	Adults	15	−15.4 ± 0.1**	−16.9 ± 0.1**	+1.4 ± 0.1**	16.3 ± 0.3**	14.8 ± 0.3**	+1.5 ± 0.2**	Quillfeldt et al. (2008)
Trindade petrel	Adults	18	−15.9 ± 0.2**	−17.5 ± 0.1**	+1.5 ± 0.2**	12.1 ± 0.2**	11.3 ± 0.2**	+0.8 ± 0.1**	Quillfeldt et al. (2008)
	Chicks	15	−17.2 ± 0.1**	−18.3 ± 0.1**	+1.2 ± 0.2**	12.0 ± 0.2**	11.3 ± 0.1**	+0.7 ± 0.2**	Quillfeldt et al. (2008)
Thin-billed prion*	Chicks	17	−19.0 ± 0.2**	−19.9 ± 0.2**	+0.9 ± 0.1**	12.5 ± 0.2**	12.1 ± 0.2**	+0.3 ± 0.1**	Quillfeldt et al. (2008)
Blue-footed booby	Chicks	26	−16.1 ± 0.3	−17.2 ± 0.1	+1.1	12.8 ± 0.3	12.2 ± 0.2	+0.6	Cruz et al. (2012)
African black oystercatcher	Chicks	20	–	–	+0.9 ± 0.3	–	–	+1.2 ± 0.3	Kohler et al. (2011)
Audouin's gull	Chicks	37	–	–	+1.6	–	–	+0.9	Sanpera et al. (2007)
Little auk	Chicks	19	−21.6 ± 0.4	−22.3 ± 0.1	+0.7 ± 0.4	11.6 ± 0.3	11.9 ± 1.2	−0.3 ± 1.2	Fort et al. (2010), unpublished data

Values are mean ± SD (**SE)

$\delta^{15}N$ values were used to test linear regressions relating feather and blood data and to derive the isotopic difference between tissues, rather than the specific discrimination factor for each tissue.

Methods

Species and sampling locations are listed in Table 3. Breeding colonies were located in the Antarctic (Adélie Land), subantarctic (Marion, Crozet and Kerguelen Islands), subtropical (Amsterdam Island) and tropical (Europa and Juan de Nova) zones of the Indian Ocean and along the coast of South Africa (Ichaboe, Malgas and Bird Islands). Whole blood and feathers were collected from large well-feathered chicks in order to minimize the effect of rapid growth on blood $\delta^{15}N$ values (Sears et al. 2009). Depending on bird size, between 0.5 and 2.0 ml of blood was collected from the brachial vein using heparinized syringes. In a few cases (blue petrel, thin-billed prion and common diving petrel), whole blood was centrifuged and blood cells were subsequently used for stable isotope analysis. Previous investigation showed that blood cells have an isotopic signature almost identical to that of whole blood, because they contain much more organic matter than plasma (Cherel et al. 2005a). Blood was preserved in 70 % ethanol because the method does not alter its isotopic composition (Hobson et al. 1997). A few body feathers (hereafter feathers) were also sampled on the chicks' lower back, and they were kept dry in plastic bags. $\delta^{13}C$ and $\delta^{15}N$ values were measured on a single feather per individual.

Before isotopic analysis, blood samples were dried in an oven at +60 °C and powdered. Feathers were cleaned of surface lipids and contaminants using a chloroform/methanol solution for two min followed by two successive methanol rinses. They were then cut with scissors into small pieces. Sub-samples of homogenized feathers and blood powder were then weighed (~0.4 mg) with a microbalance and packed in tin containers. Relative abundances of C and N isotopes were determined with a continuous-flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112). Isotopic results are presented in the δ notation relative to Vienna PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicate measurement errors <0.10 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. The C:N mass ratios of samples were calculated as the ratio between the mass percentages in carbon and nitrogen. The consistently low C:N values of blood (<4.0, Post et al. 2007) verified that low lipid content did not necessitate lipid extraction (Bearhop et al. 2000; Cherel et al. 2005b).

Table 3 Statistics comparing $\delta^{13}C$ and $\delta^{15}N$ values between body feathers and whole blood (*blood cells) and isotopic differences between feathers and blood

Species	Location	Years	*n*	Pairwise t tests ($\delta^{13}C$ values)	Paired $\delta^{13}C$ differences (‰)	Pairwise t tests ($\delta^{15}N$ values)	Paired $\delta^{15}N$ differences (‰)	Pairwise t tests (C:N mass ratios)	Paired differences in C:N mass ratio
Spheniscidae									
Emperor penguin (*Aptenodytes forsteri*)	Adélie Land	2007	10	**$t = 12.54$, $P < 0.0001$**	1.05 ± 0.26	$t = 0.28$, $P = 0.787$	0.03 ± 0.38	**$t = -3.15$, $P = 0.012$**	-0.11 ± 0.11
King penguin (*Aptenodytes patagonicus*)	Crozet	2001	10	**$t = 12.30$, $P < 0.0001$**	1.27 ± 0.33	**$t = 12.94$, $P < 0.0001$**	0.99 ± 0.24	**$t = -11.09$, $P < 0.0001$**	-0.31 ± 0.09
Adélie penguin (*Pygoscelis adeliae*)	Adélie Land	2007	10	**$t = 19.79$, $P < 0.0001$**	1.49 ± 0.24	**$t = 4.49$, $P = 0.002$**	0.76 ± 0.53	**$t = -7.46$, $P < 0.0001$**	-0.27 ± 0.12
Diomedeidae									
Grey-headed albatross (*Thalassarche chrysostoma*)	Marion	2006	12	**$t = 12.74$, $P < 0.0001$**	1.95 ± 0.53	$t = -1.23$, $P = 0.245$	-0.26 ± 0.73	**$t = -9.92$, $P < 0.0001$**	-0.34 ± 0.12
Indian yellow-nosed albatross (*Thalassarche carteri*)	Amsterdam	2005	12	**$t = 11.35$, $P < 0.0001$**	1.44 ± 0.44	$t = 0.31$, $P = 0.766$	0.03 ± 0.38	**$t = -19.68$, $P < 0.0001$**	-0.36 ± 0.06
Black-browed albatross (*Thalassarche melanophrys*)	Kerguelen	2005	18	**$t = 13.66$, $P < 0.0001$**	1.99 ± 0.62	**$t = 3.98$, $P = 0.001$**	0.43 ± 0.46	**$t = -13.74$, $P < 0.0001$**	-0.53 ± 0.16
Procellariidae									
Northern giant petrel (*Macronectes halli*)	Crozet	2005	10	**$t = 16.48$, $P < 0.0001$**	1.75 ± 0.34	**$t = 15.51$, $P < 0.0001$**	0.77 ± 0.16	**$t = -9.86$, $P < 0.0001$**	-0.30 ± 0.10
Southern giant petrel (*Macronectes giganteus*)	Crozet	2005	11	**$t = 25.97$, $P < 0.0001$**	1.86 ± 0.24	**$t = 12.69$, $P < 0.0001$**	0.73 ± 0.19	**$t = -14.34$, $P < 0.0001$**	-0.33 ± 0.08
	Adélie Land	2007	12	**$t = 15.40$, $P < 0.0001$**	1.73 ± 0.39	**$t = 2.95$, $P = 0.013$**	0.44 ± 0.52	**$t = -10.39$, $P < 0.0001$**	-0.24 ± 0.08
White-chinned petrel (*Procellaria aequinoctialis*)	Kerguelen	2008	10	**$t = 12.87$, $P < 0.0001$**	1.89 ± 0.46	**$t = 4.31$, $P = 0.002$**	0.91 ± 0.67	**$t = -11.21$, $P < 0.0001$**	-0.47 ± 0.13
White-headed petrel (*Pterodroma lessoni*)	Kerguelen	2008	10	**$t = 15.86$, $P < 0.0001$**	2.29 ± 0.46	**$t = 7.91$, $P < 0.0001$**	0.97 ± 0.39	**$t = -12.74$, $P < 0.0001$**	-0.42 ± 0.10
Antarctic fulmar (*Fulmarus glacialoides*)	Adélie Land	2007	10	**$t = 18.40$, $P < 0.0001$**	2.10 ± 0.36	$t = -1.36$, $P = 0.207$	-0.37 ± 0.87	**$t = -20.54$, $P < 0.0001$**	-0.42 ± 0.07
Cape pigeon (*Daption capensis*)	Adélie Land	2007	10	**$t = 13.75$, $P < 0.0001$**	1.67 ± 0.38	**$t = -3.88$, $P = 0.004$**	-0.81 ± 0.66	**$t = -10.81$, $P < 0.0001$**	-0.35 ± 0.10
Snow petrel (*Pagodroma nivea*)	Adélie Land	2007	10	**$t = 20.43$, $P < 0.0001$**	2.60 ± 0.40	**$t = -2.44$, $P = 0.033$**	-0.45 ± 0.58	**$t = -8.51$, $P < 0.0001$**	-0.46 ± 0.17
Blue petrel* (*Halobaena caerulea*)	Kerguelen	2003	13	**$t = 12.71$, $P < 0.0001$**	1.46 ± 0.41	**$t = 7.48$, $P < 0.0001$**	0.54 ± 0.26	**$t = -3.33$, $P = 0.006$**	-0.17 ± 0.18
Thin-billed prion* (*Pachyptila belcheri*)	Kerguelen	2003	9	**$t = 11.01$, $P < 0.0001$**	1.53 ± 0.42	**$t = 5.68$, $P = 0.001$**	0.72 ± 0.38	**$t = -2.91$, $P = 0.030$**	-0.15 ± 0.15

Table 3 continued

Species	Location	Years	*n*	Pairwise t tests ($\delta^{13}C$ values)	Paired $\delta^{13}C$ differences (‰)	Pairwise t tests ($\delta^{15}N$ values)	Paired $\delta^{15}N$ differences (‰)	Pairwise t tests (C:N mass ratios)	Paired differences in C:N mass ratio
Antarctic prion (*Pachyptila desolata*)	Kerguelen	2008	10	**$t = 15.14, P < 0.0001$**	2.07 ± 0.43	**$t = 13.42, P < 0.0001$**	1.50 ± 0.35	**$t = -7.94, P < 0.0001$**	−0.54 ± 0.22
Hydrobatidae									
Wilson's storm petrel (*Oceanites oceanicus*)	Adélie Land	2007	6	**$t = 11.78, P < 0.0001$**	1.30 ± 0.27	**$t = 3.78, P = 0.013$**	0.28 ± 0.18	**$t = -10.12, P = 0.001$**	−0.31 ± 0.07
Pelecanoididae									
Common diving petrel* (*Pelecanoides urinatrix*)	Kerguelen	2003	17	**$t = 10.57, P < 0.0001$**	0.80 ± 0.31	**$t = 10.29, P < 0.0001$**	0.93 ± 0.37	**$t = 4.65, P = 0.001$**	0.07 ± 0.06
Sulidae									
Cape gannet	Ichaboe	2006	10	**$t = 36.13, P < 0.0001$**	2.03 ± 0.18	**$t = 3.60, P = 0.006$**	0.40 ± 0.35	**$t = -17.06, P < 0.0001$**	−0.26 ± 0.05
(*Morus capensis*)	Malgas	2006	10	**$t = 19.11, P < 0.0001$**	1.35 ± 0.22	**$t = 5.71, P = 0.001$**	0.37 ± 0.21	**$t = -17.85, P < 0.0001$**	−0.28 ± 0.05
	Bird Island	2006	10	**$t = 28.86, P < 0.0001$**	1.71 ± 0.19	**$t = 12.85, P < 0.0001$**	0.90 ± 0.22	**$t = -10.92, P < 0.0001$**	−0.18 ± 0.05
Phalacrocoracidae									
Kerguelen shag	Mayes	2000	10	**$t = 20.18, P < 0.0001$**	1.81 ± 0.28	**$t = 19.03, P < 0.0001$**	1.37 ± 0.23	**$t = -16.03, P < 0.0001$**	−0.25 ± 0.05
(*Phalacrocorax verrucosus*)	Pointe Suzanne	2006	10	**$t = 14.42, P < 0.0001$**	1.29 ± 0.28	**$t = 11.73, P < 0.0001$**	0.71 ± 0.19	**$t = -16.00, P < 0.0001$**	−0.18 ± 0.04
Stercorariidae									
South polar skua (*Stercorarius maccormicki*)	Adélie Land	2007	11	**$t = 14.79, P < 0.0001$**	1.21 ± 0.27	**$t = 8.06, P < 0.0001$**	0.89 ± 0.37	**$t = -12.29, P < 0.0001$**	−0.25 ± 0.07
Sternidae									
Sooty tern	Europa	2003	15	**$t = 39.83, P < 0.0001$**	2.06 ± 0.20	**$t = 14.06, P < 0.0001$**	1.04 ± 0.29	**$t = -29.90, P < 0.0001$**	−0.30 ± 0.04
(*Onychoprion fuscata*)	Juan de Nova	2003	8	**$t = 7.27, P = 0.001$**	1.28 ± 0.50	**$t = 12.19, P < 0.0001$**	1.74 ± 0.40	**$t = -21.91, P < 0.0001$**	−0.23 ± 0.03

Feathers and blood were sampled simultaneously in large chicks. Values are mean ± SD. Statistically significant results are marked in bold

Data were statistically analysed using STATISTICA 10 for WINDOWS. Values are mean ± SD.

Results

Chick isotopic ratios ranged widely, reflecting both geographical variations in baseline isotopic levels and different foraging habitats and diets of parent birds when they feed their offspring. Feather (blood) $\delta^{13}C$ values ranged from −24.7 ± 0.2 ‰ (−26.3 ± 0.3 ‰) to −10.9 ± 0.7 ‰ (−12.8 ± 0.8 ‰), corresponding to a 13.8 ‰ (13.6 ‰) difference, while feather (blood) $\delta^{15}N$ values ranged from 8.8 ± 0.7 ‰ (7.8 ± 0.3 ‰) to 15.0 ± 0.6 ‰ (14.6 ± 0.3 ‰), thus corresponding to a 6.2 ‰ (6.8 ‰) difference.

In all the 27 populations and 22 species, feathers were significantly enriched in ^{13}C compared with blood, with $\delta^{13}C$ differences (feathers minus blood) ranging from +0.8 ± 0.3 ‰ (common diving petrel) to +2.6 ± 0.4 ‰ (snow petrel) (Table 3). In the same way, C:N mass ratios were significantly lower in feathers (3.1–3.3) than in blood (3.3–3.7) for all species, except the common diving petrel. Feathers were significantly enriched in ^{15}N compared with blood in most populations and species, but feather and blood $\delta^{15}N$ values were not significantly different in four species and two of them (the cape and snow petrels) even presented the opposite pattern (Table 3; Fig. 1).

Taking into account data from both the present study and Table 2, mean feather and blood $\delta^{13}C$ values were highly positively and linearly related across populations and species [feather $\delta^{13}C = 0.972$ (±0.020) blood $\delta^{13}C + 0.962$ (±0.414), $R^2 = 0.987$, $F_{1,33} = 2{,}434$, $P < 0.0001$], as mean feather and blood $\delta^{15}N$ values were [feather $\delta^{15}N = 1.014$ (±0.056) blood $\delta^{15}N + 0.447$ (±0.665), $R^2 = 0.910$, $F_{1,33} = 334$, $P < 0.0001$] (Fig. 1). Regressions were thus used to estimate feather isotopic ratios. Most isotopic differences between calculated and measured feather values were small (<0.5 ‰; 80 and 66 % of the $\delta^{13}C$ and $\delta^{15}N$ differences, respectively); some differences were moderate (<1.0 ‰; 17 and 26 %, respectively), and two $\delta^{15}N$ differences were higher (>1.0 ‰; 3 and 8 %).

Discussion

Following Quillfeldt et al. (2008), the main interest of our approach is that it used simultaneously collected growing feathers and blood from wild birds on a natural diet in their natural habitat. Hence, it allows collecting samples on many avian species, thus contrasting with the difficulty of conducting experiments on captive birds. Overall, the study adds a substantial number of species ($n = 19$) to the previous isotopic investigations on moulting chicks ($n = 6$ species) and adults ($n = 4$) in the wild (Table 2) and on birds in controlled conditions ($n = 12$) (Table 1). Our data show that feathers are consistently ^{13}C enriched compared with blood and that they are also ^{15}N enriched in most cases. Indeed, an extensive review of the isotopic literature verified that $\Delta^{13}C$ are always higher in feathers than in blood (Table 1) and that growing feathers have higher $\delta^{13}C$ values than simultaneously sampled blood (Table 2). With a few exceptions, $\Delta^{15}N$ and $\delta^{15}N$ values were also generally higher in feathers than in blood, and we have no explanation why experimental birds from a single study consistently showed the opposite pattern (Hobson and Clark 1992b).

Differences in tissue-to-diet discrimination factors are more likely explained by tissue-specific biochemical composition, namely lipid content and amino acid composition (Wolf et al. 2009). Since lipids are depleted in ^{13}C relative to proteins and carbohydrates, some of the variation in $\delta^{13}C$ values is explained by tissues' lipid content (Post et al. 2007). Feather is a lipid-poor tissue, but blood can contain significant amounts of lipids, which affect its $\delta^{13}C$ values (Cherel et al. 2005a, c). The higher lipid content of

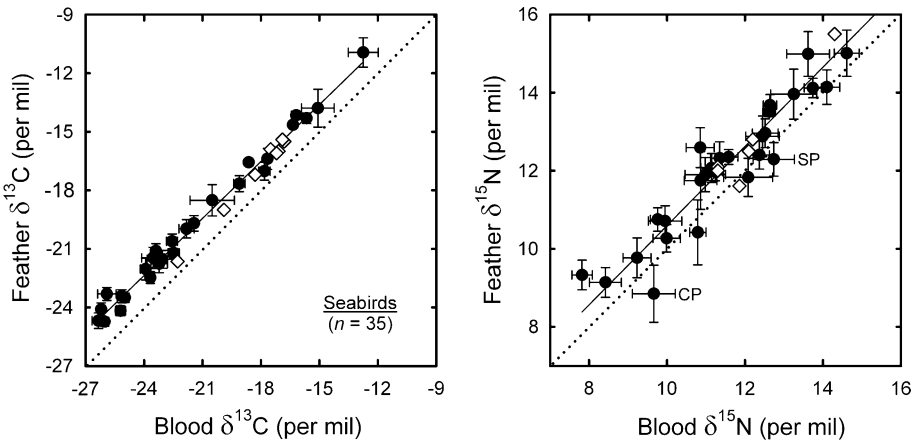

Fig. 1 Mean feather versus mean blood $\delta^{13}C$ and $\delta^{15}N$ values in seabirds, including data from present study (*filled circles*) and Table 2 (*open diamonds*). See text for regression equations and statistics. Abbreviations correspond to outliers (see "Discussion"): *CP* cape petrel, *SP* snow petrel. Values are means with and without SD for data from present study and Table 2, respectively

blood compared to feathers was here shown by its consistently higher C:N mass ratio (Table 3), a good proxy of lipid content in animal tissues (Post et al. 2007). Indeed, lipid removal decreases the C:N mass ratios and increases the $\delta^{13}C$ values of blood (Cherel et al. 2005b, c). However, the $\delta^{13}C$ difference between non-delipidated and delipidated blood is smaller ($\leq$0.5 ‰; Bearhop et al. 2000; Cherel et al. 2005b) than the isotopic difference between blood and feathers (Tables 1, 2, 3). Thus, blood lipids are not the only factor that causes $\delta^{13}C$ differences among tissues, which can also be explained by (1) the amino acid content of proteins together with (2) the isotopic composition of individual amino acids. A main biochemical characteristic of feathers is that their mass is almost exclusively keratin. Keratins are a group of structural proteins marked by a distinct amino acid composition when compared to most other body proteins. Feather keratin is notably rich in the sulphur-containing amino acid cysteine (a non-essential amino acid), and also in valine and leucine, and it is impoverished in the remaining essential amino acids (Murphy et al. 1990). Our data thus suggest that cysteine and to a lesser extent valine and leucine are ^{13}C and ^{15}N enriched. Indeed, leucine has a high $\delta^{15}N$ value, but neither $\delta^{15}N$ values for cysteine and valine nor $\delta^{13}C$ values for cysteine, valine and leucine were determined in the only study conducted on amino acids in bird tissue (Lorrain et al. 2009). Clearly, more investigations on the isotopic values of individual amino acids are needed to better understand tissue $\delta^{13}C$ and $\delta^{15}N$ differences, including that between feathers and blood.

A critical assumption of our approach is that the two chick tissues should represent similar periods of dietary intake. In birds raised on controlled diets, the isotopic half-lives of C and N in whole blood are 10–23 days and consequently C and N renewal in blood takes several weeks to a few months (Hobson and Clark 1992a; Haramis et al. 2001; Bearhop et al. 2002; Evans Ogden et al. 2004). Growth of body feathers is shorter (a few weeks) and takes place from the mid to the second half of the chick-rearing period (Bost and Jouventin 1991; Phillips and Hamer 2000). Hence, time integration of feathers is included within that of blood. Theoretically, the larger temporal window of blood could alter the isotopic comparisons between blood and feathers if significant dietary shifts occurred early during the chick-rearing period. It is thus noteworthy that the two species showing significantly higher $\delta^{15}N$ values in chick blood than in feathers were sympatric Antarctic species that were sampled at the same location in the same year, thus suggesting a dietary shift from higher to lower trophic-level prey at that time (most probably from fish to krill; Cherel 2008). Alternatively, intrinsic factors related to phylogeny could also explain the isotopic differences, because cape and snow petrels both belong to the group of fulmarine petrels, which also include the Antarctic fulmar and northern and southern giant petrels. Indeed, fulmars had non-significant higher $\delta^{15}N$ values in blood than in feathers. However, both species of giant petrels showed the usual tissue-specific $\delta^{15}N$ pattern (Table 3), suggesting that phylogeny is not a primary determinant of the isotopic signature of blood and feathers.

Linear regressions between feather and blood for both $\delta^{13}C$ and $\delta^{15}N$ values had been previously determined across individuals of two bird species, the spectacled petrel and blue-footed booby (Bugoni et al. 2008; Cruz et al. 2012). Such equations are probably the best way to arithmetically correct isotope values of different tissues for a given species, but they cannot be generalized to other avian taxa. Instead, the use of many species that overall encompass large $\delta^{13}C$ and $\delta^{15}N$ ranges allows us to depict for the first time accurate linear regressions relating feather to blood $\delta^{13}C$ and $\delta^{15}N$ values across avian taxa. As no species-specific equations are available in most cases, the among-taxa linear regressions can be applied to mathematically correct isotopic values in order to compare feather and blood $\delta^{13}C$ and $\delta^{15}N$ data of any bird. The regressions work best at the species level, but their application within species introduces additional unknown errors that could potentially be large, especially for $\delta^{15}N$. We are confident about the reliability of applying the regressions to different age classes, because isotopic values from the few investigations conducted on adult birds fit well with the chick data both between species and within species, i.e. for the only species so far investigated, namely the Trindade petrel (Quillfeldt et al. 2008). Indeed, chick $\delta^{13}C$ values are not affected by growth rate, and the growth effect on $\delta^{15}N$ is minimized on large chicks near fledging (Sears et al. 2009). Both $\delta^{13}C$ and $\delta^{15}N$ equations are highly significant, but the linear regression showed a better fit for $\delta^{13}C$ than for $\delta^{15}N$, thus inducing better corrected values for $\delta^{13}C$. Hence, despite the promising nature of our approach, more detailed studies, especially for $\delta^{15}N$, are required in order to test the validity of the equations over a larger isotopic data set, including terrestrial bird species.

Acknowledgments The authors thank the numerous fieldworkers and students for their help in the field, F. Capoulun, M. Connan and T. Cook for preparing samples, and G. Guillou, P. Richard and J. Lanham for stable isotope analysis. The present work was supported financially and logistically by the Institut Polaire Français Paul Emile Victor (Programme No. 109, H. Weimerskirch) and the Terres Australes et Antarctiques Françaises.

References

Bearhop S, Teece MA, Waldron S, Furness RW (2000) Influence of lipid and uric acid on $\delta^{13}C$ and $\delta^{15}N$ values of avian blood: implications for trophic studies. Auk 117:504–507

Bearhop S, Waldron S, Votier SC, Furness RW (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol Biochem Zool 75:451–458

Bost CA, Jouventin P (1991) The breeding biology of the gentoo penguin *Pygoscelis papua* on the Crozet Islands. Ibis 133:14–25

Bugoni L, McGill RAR, Furness RW (2008) Effects of preservation methods on stable isotope signatures in bird tissues. Rapid Commun Mass Spectrom 22:2457–2462

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46:443–453

Cherel Y (2008) Isotopic niches of emperor and Adélie penguins in Adélie Land, Antarctica. Mar Biol 154:813–821

Cherel Y, Hobson KA, Bailleul F, Groscolas R (2005a) Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. Ecology 86:2881–2888

Cherel Y, Hobson KA, Hassani S (2005b) Isotopic discrimination between food and blood and feathers of captive penguins: implications for dietary studies in the wild. Physiol Biochem Zool 78:106–115

Cherel Y, Hobson KA, Weimerskirch H (2005c) Using stable isotopes to study resource acquisition and allocation in procellariiform seabirds. Oecologia 145:533–540

Cherel Y, Le Corre M, Jaquemet S, Ménard F, Richard P, Weimerskirch H (2008) Resource partitioning within a tropical seabird community: new information from stable isotopes. Mar Ecol Prog Ser 366:281–291

Cruz LL, McGill RAR, Goodman SJ, Hamer KC (2012) Stable isotope ratios of a tropical marine predator: confounding effects of nutritional status during growth. Mar Biol 159:873–880

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658

Evans Ogden LJ, Hobson KA, Lank DB (2004) Blood isotopic (δ^{13}C and δ^{15}N) turnover and diet-tissue fractionation factors in captive dunlin (*Calidris alpina pacifica*). Auk 121:170–177

Federer RN, Hollmen TE, Esler D, Wooller MJ, Wang SW (2010) Stable carbon and nitrogen isotope discrimination factors from diet to blood plasma, cellular blood, feathers, and adipose tissue fatty acids in spectacled eiders (*Somateria fischeri*). Can J Zool 88:866–874

Fort J, Cherel Y, Harding AMA, Welcker J, Jakubas D, Steen H, Karnovsky NJ, Grémillet D (2010) Geographic and seasonal variability in the isotopic niche of little auks. Mar Ecol Prog Ser 414:293–302

Hahn S, Hoye BJ, Korthals H, Klaassen M (2012) From food to offspring down: tissue-specific discrimination and turn-over of stable isotopes in herbivorous waterbirds and other avian foraging guilds. PLoS One 7:e30242

Haramis GM, Jorde DG, Macko SA, Walker JL (2001) Stable-isotope analysis of canvasback winter diet in upper Chesapeake Bay. Auk 118:1008–1017

Hedd A, Fifield DA, Burke CM, Montevecchi WA, McFarlane Tranquilla L, Regular PM, Buren AD, Robertson GJ (2010) Seasonal shift in the foraging niche of Atlantic puffins *Fratercula arctica* revealed by stable isotope (δ^{15}N and δ^{13}C) analyses. Aquat Biol 9:13–22

Hobson KA (2011) Isotopic ornithology. J Ornithol 152(Suppl. 1):S49–S66

Hobson KA, Bairlein F (2003) Isotopic fractionation and turnover in captive garden warblers (*Sylvia borin*): implications for delineating dietary and migratory associations in wild passerines. Can J Zool 81:1630–1635

Hobson KA, Bond AL (2012) Extending an indicator: year-round information on seabird trophic ecology from multiple-tissue stable-isotope analyses. Mar Ecol Prog Ser 461:233–243

Hobson KA, Clark RG (1992a) Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor 94:181–188

Hobson KA, Clark RG (1992b) Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. Condor 94:189–197

Hobson KA, Clark RG (1993) Turnover of ^{13}C in cellular and plasma fractions of blood: implications for nondestructive sampling in avian dietary studies. Auk 110:638–641

Hobson KA, Gibbs HL, Gloutney ML (1997) Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. Can J Zool 75:1720–1723

Inger R, Bearhop S (2008) Applications of stable isotope analyses to avian ecology. Ibis 150:447–461

Jaeger A, Connan M, Richard P, Cherel Y (2010) Use of stable isotopes to quantify seasonal changes of trophic niche and levels of population and individual specialisation in seabirds. Mar Ecol Prog Ser 401:269–277

Kelly JF (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can J Zool 78:1–27

Kempster B, Zanette L, Longstaffe FJ, MacDougall-Shackleton SA, Wingfield JC, Clinchy M (2007) Do stable isotopes reflect nutritional stress? Results from a laboratory experiment on song sparrows. Oecologia 151:365–371

Kohler SA, Connan M, Hill JM, Mablouké C, Bonnevie B, Ludynia K, Kemper J, Huisamen J, Underhill LG, Cherel Y, McQuaid CD, Jaquemet S (2011) Geographic variation in the trophic ecology of an avian rocky shore predator, the African black oystercatcher, along the southern African coastline. Mar Ecol Prog Ser 435:235–249

Lorrain A, Graham B, Ménard F, Popp B, Bouillon S, van Breugel P, Cherel Y (2009) Nitrogen and carbon isotope values of individual amino acids: a tool to study foraging ecology of penguins in the Southern Ocean. Mar Ecol Prog Ser 391:293–306

Martinez del Rio C, Sabat P, Anderson-Sprecher R, Gonzalez SP (2009) Dietary and isotopic specialization: the isotopic niche of three *Cinclodes* ovenbirds. Oecologia 161:149–159

Murphy ME, King JR (1991) Nutritional aspects of avian molt. In: Beff BD, Cossee RO, Flux JEC, Heather BD, Hitchmough RA, Robertson CJR, Williams MJ (eds) Acta XX Congressus Internationalis Ornithologici. Christchurch, pp 2186–2193

Murphy ME, King JR, Taruscio TG, Geupel GR (1990) Amino acid composition of feather barbs and rachises in three species of pygoscelid penguins: nutritional implications. Condor 92:913–921

Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. Front Ecol Environ 5:429–436

Pearson SF, Levey DJ, Greenberg CH, Martinez del Rio C (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia 135:516–523

Phillips RA, Hamer KC (2000) Postnatal development of northern fulmar chicks, *Fulmarus glacialis*. Physiol Biochem Zool 73:597–604

Post DM, Layman CA, Albrey Arrington D, Takimoto G, Quattrochi J, Montana CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189

Quillfeldt P, Bugoni L, McGill RAR, Masello JF, Furness RW (2008) Differences in stable isotopes in blood and feathers of seabirds are consistent across species, age and latitude: implications for food web studies. Mar Biol 155:593–598

Sanpera C, Moreno R, Ruiz X, Jover L (2007) Audouin's gull chicks as bioindicators of mercury pollution at different breeding locations in the western Mediterranean. Mar Pollut Bull 54:691–696

Sears J, Hatch SA, O'Brien DM (2009) Disentangling effects of growth and nutritional status on seabird stable isotope ratios. Oecologia 159:41–48

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia 136: 169–182

Wolf N, Carleton SA, Martinez del Rio C (2009) Ten years of experimental animal isotopic ecology. Funct Ecol 23:17–26