Notornis, 2021, Vol. 68: 65-71
0029-4470

SHORT NOTE

Individual long-distance migrant *Chrysococcyx* cuckoos repeat carbon and nitrogen stable isotope ratios after moulting in non-breeding range on successive migrations

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The New Zealand shining cuckoo (*Chrysococcyx lucidus lucidu*s Gmelin, 1788) (Fig. 1) breeds only in the New Zealand archipelago (other subspecies breed in Australia and New Caledonia; Higgins 1999). Post-breeding, New Zealand cuckoos migrate 5,000 km, across the Tasman and Coral Seas, to the Bismarck Archipelago (New Britain, New Ireland) and the Solomon Islands, near the Equator (Fig. 1) (Higgins 1999). At least some New Zealand birds migrate via south-eastern Queensland, Australia (Gill 1983b). Mayr (1932) described the 10,000+ km trans-oceanic migration by this small (<30 g) bird as "amazing", a sentiment echoed, in essence, by banding pioneer A. Landsborough Thomson, who described the migration as "perhaps the most remarkable trans-oceanic migration by a land bird" (Thomson 1964).

Received 21 January 2020; accepted 30 September 2020
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In their breeding range, shining cuckoos inhabit a wide range of habitats, including temperate rain forest, forest remnants, riparian woodlands, exotic plantations, and farmland with trees, as long as there are populations of its host species, the grey warbler (*Gerygone igata* Quoy & Gaimard, 1830) (Acanthizidae) (Higgins 1999). Shining cuckoos still breed in most areas of New Zealand (Robertson *et al.* 2007; Robertson *et al.* 2017) despite dramatic losses of natural vegetation since human settlement began (McWethy *et al.* 2010, 2014) and the species is currently not considered at risk (Robertson *et al.* 2007). However, their habitats are threatened by deforestation in their non-breeding range (Bayliss-Smith *et al.* 2003; Buchanan *et al.* 2008) potentially threatening their survival. The vegetation of the stopover area in southern Queensland is also now heavily modified, with 'brigalow' woodland especially fragmented (Dwyer *et al.* 2009).

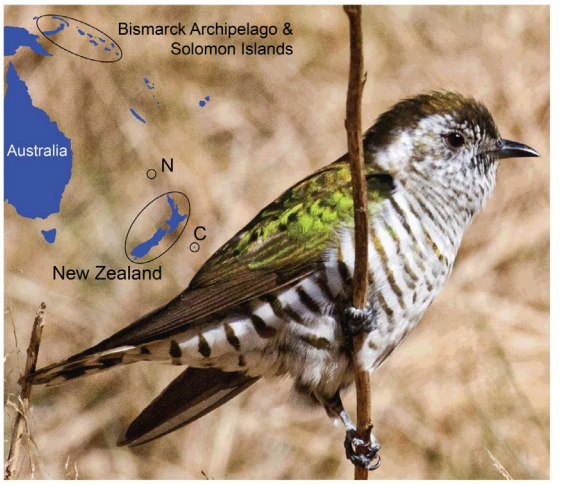

Figure 1. Shining cuckoos (*Chrysococcyx lucidus lucidus*), weighing <30 grams, breed in New Zealand, Chatham Island (C), and Norfolk Island (N) and migrate to the Bismarck Archipelago and Solomon Islands, where they moult. At least some birds migrate via eastern Australia, in a migration involving four ocean crossings totalling *c.* 10,000 km. Image: courtesy of Nathan Hill. © Nathan Hill; accessed via New Zealand Birds Online.

Increasing environmental change raises questions about the future conservation of the shining cuckoo, as it does for most migratory species. However, the Australasian *Chrysococcyx* cuckoos are notoriously cryptic when not calling, which they do only during the breeding season, and their distributions, ecology, and habitat requirements are poorly known away from their breeding areas (Higgins 1999; Noske 2019). Information on the habitat requirements of the species outside the breeding range is essential to understanding its threat status and for any management (Bowen *et al.* 2009).

Determining the diet and habitat of a species when it is not directly observable (Higgins 1999) can be achieved indirectly by measuring its isotopic niche (Bearhop *et al.* 2004; Newsome *et al.* 2007). For migratory species that moult in the non-breeding range, isotopic values can be measured in a relatively non-invasive fashion by sampling feathers. Stable isotopic measurements of the feathers of migratory birds have been used to determine the broad location of non-breeding areas for species inhabiting difficult and remote environments (Hobson *et al.* 2010). Where the bird's location has been established by other means, measurements of carbon and nitrogen stable isotopic ratios ($\delta^{13}C$, $\delta^{15}N$, respectively) can provide information on habitat use and diet and hence provide a remotely-sensed window into a bird's biology in the area where the feathers are moulted and replaced.

New Zealand shining cuckoos moult in their non-breeding ranges in the Bismarck Archipelago, Bougainville, and the Solomon Islands in the tropical southwest Pacific, but their juvenile plumage is based on the diet provided by the host species in the breeding range in New Zealand (Gill 1983a, 1998). The carbon and nitrogen stable isotopic ratios of feathers of adults caught in New Zealand should reflect the diet and isotopic niche occupied by the adults in their non-breeding range, while isotopic ratios of feathers of juveniles caught before their first migration should reflect the diet and local habitat of the host species.

Animals have $\delta^{15}N$ values higher than their food, the difference between consumer and prey being typically *c.* 3–4‰, where the prey are not water-stressed (Ambrose & DeNiro 1986): differences in $\delta^{13}C$ values are about one-third of those of $\delta^{15}N$ (Ambrose & DeNiro 1986). Baseline values for both ratios depend on the structure of the environment and of the food web (Ambrose & DeNiro 1986; Cerling *et al.* 2004; Hawke & Holdaway 2005; Hawke & Holdaway 2009; Holdaway *et al.* 2013; Holdaway & Rowe 2020), with each species' values reflecting its isotopic niche. Taxa with very constrained isotopic niches are likely to be vulnerable to environmental change, including availability of preferred foods and habitat structure; those with wider isotopic niches should be more resilient against change (Holdaway *et al.* 2013). Thus, an examination of the isotopic values of shining cuckoos should reveal both their isotopic niche on the wintering grounds and its variability.

Twenty-two adults and three juveniles were individually metal-banded and their feathers sampled at two sites in New Zealand between December 2014 and mid-November 2017. Birds were captured by mist-netting individuals attracted to song playback. Nets were erected with their top panels as close as possible to the canopy top, as birds rarely came lower in response to playback. Nineteen adults (9 Dec 2014 [2], 9 Nov 2016 [2], 1 Dec 2016 [1], 2 Dec 2016 [1], 3 Dec 2016 [1], 7 Dec 2016 [1], 12 Oct 2017 [3], 13 Oct 2017 [1], 24 Oct 2017 [1], 29 Oct 2017 [1], 31 Oct 2017 [1], 2 Nov 2017 [1], 5 Nov 2017 [1], 7 Nov [2]) were sampled at Kowhai Bush, Kaikoura, north-eastern South Island (42°22'37'S, 173°36'58'E), and five (16 Dec 2016 [1], 17 Dec 2016 [1], 14 Dec 2017 [1], 17 Dec 2017 [2]) at Milnthorpe Park Scenic Reserve, in the far northern South Island (40°42'47'S, 172°40'55'E) (Fig. 2).

Two of the adults at Kowhai Bush were sampled twice, in successive years (Band B122248, 7 December 2016, 5 November 2017; Band BP11568, 14 December 2016, 31 October 2, 1017). All three juveniles were sampled at Kowhai Bush on 30

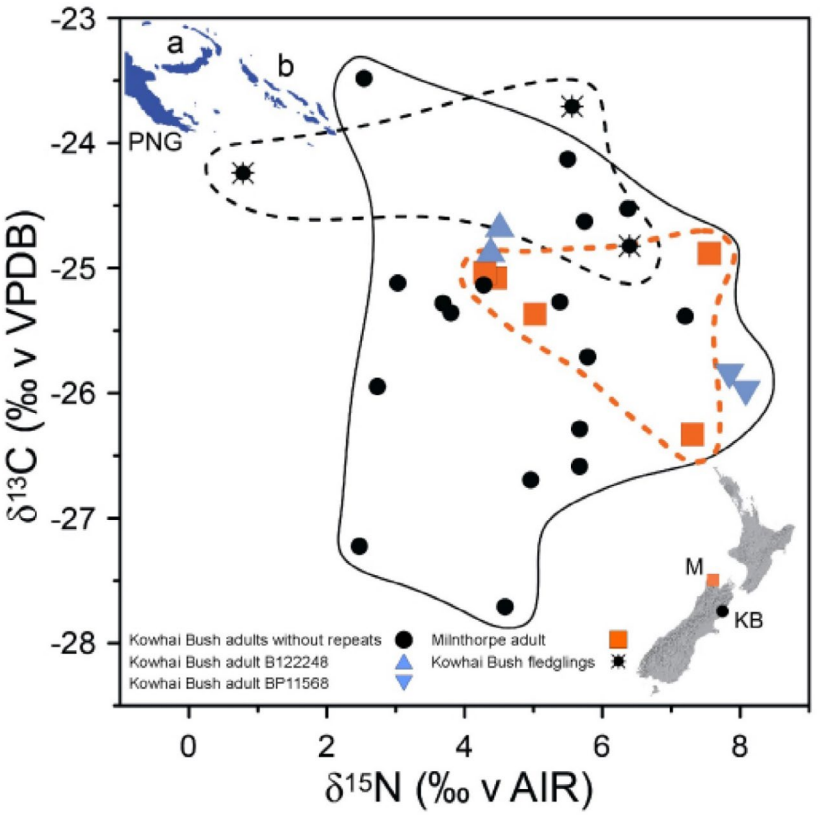

Figure 2. New Zealand shining cuckoos (*Chrysococcyx lucidus lucidus*) return to particular moulting sites within a much broader range of habitats occupied by the species during the non-breeding season. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopic ratios for feathers replaced during moult in the non-breeding range in the Southwest Pacific by 21 adult shining cuckoos sampled once, two sampled twice (in consecutive years and listed by band number in figure), and of three juveniles raised by grey warbler (*Gerygone igata*) foster parents in New Zealand. Solid circles, solid line: adult cuckoos sampled once at Kowhai Bush. Black stars, black broken line: juvenile cuckoos sampled in breeding area at Kowhai Bush. Orange squares, broken orange line: birds sampled once at Milnthorpe Reserve. Erect and inverted triangles, bi-isotopic values for two adults sampled in successive years at Kowhai Bush. Top left: Bismarck Archipelago (a) and Solomon Islands (b); Papua New Guinea (PNG). Bottom right: New Zealand; M, Milnthorpe Reserve; KB, Kowhai Bush (Kaikoura). Outline maps not to common scale.

January 2018. Two or three breast feathers were removed from each bird under University of Canterbury Animal Ethics permit (2014/26R) and New Zealand Department of Conservation permit (39907-FAU), and stored in separate labelled glass vials until analysed.

Carbon and nitrogen stable isotopic ratios were measured at the National Institute of Water & Atmospheric Research Ltd laboratory, Greta Point, Wellington, New Zealand. Each feather was washed in 70% ethanol, and rinsed 3x in ultra-pure water, before oven drying at 60°C. Material from one side of the shaft was cut off using sterile scissors, cut further into small pieces, and mixed, before subsampling for stable isotopic analysis. Isotopic ratios were measured in a Delta Plus automated continuous-flow isotope ratio mass spectrometer, linked to a NA1500 elemental analyser (both Thermo-Fisher Scientific, Bremen, Germany), with 2-point normalisation, using an AS 200 autosampler, against international standards. Reference materials (National Institute of Standards and Technology, NIST; United States Geological Survey, USGS) were used to determine isotopic values, following Paul *et al.* (2007).

Sample $\delta^{15}N$ values were 2-point normalised using isotopic data from the daily analysis of NIST 8573 USGS40 L-glutamic acid and NIST 8548

Table 1. Carbon and nitrogen stable isotopic ratios of feathers of adult and juvenile shining cuckoos (*Chrysococcyx lucidus lucidus*) sampled at Kaikoura and in Golden Bay. $\delta^{15}N$ reported relative to air; $\delta^{13}C$ reported relative to the Vienna PeeDee Belemnite. Mass, feather mass analysed; -a, -b, repeat recoveries; rpt, repeated measurement (B122255 repeat measurement of same sample).

Band no.	Mass (mg)	%N	$\delta^{15}N$ (‰)	%C	$\delta^{13}C$ (‰)	C:N
B104572	1.122	12.61	2.47	47.34	-27.22	3.8
B104575	0.711	13.35	4.45	47.26	-25.08	3.5
B104576	0.872	13.47	5.67	46.74	-26.29	3.5
B104577	0.673	13.26	4.96	46.12	-26.69	3.5
B104578	0.834	13.97	7.20	46.16	-25.39	3.3
B104579	1.017	13.65	5.38	46.71	-25.27	3.4
B104581	0.774	14.00	4.29	45.71	-25.04	3.3
B109463	0.753	13.59	3.03	46.87	-25.12	3.4
B109464	0.854	14.16	5.74	46.09	-24.63	3.3
B120851	0.702	13.74	5.50	46.20	-24.13	3.4
B120853	0.754	14.07	3.80	45.84	-25.36	3.3
B122245	0.683	13.79	5.79	45.87	-25.71	3.3
B122247	0.938	12.35	4.59	48.23	-27.71	3.9
B122248-a	0.675	14.14	4.51	46.55	-24.65	3.3
B122248-b_rpt	0.69	13.47	4.38	45.67	-24.85	3.4
B122249	0.726	14.55	2.54	46.00	-23.48	3.2
B122250	0.718	13.80	4.28	45.99	-25.14	3.3
B122251	0.739	13.18	5.67	45.81	-26.59	3.5
B122252	0.709	14.18	2.73	47.14	-25.95	3.3
B122253	0.887	13.76	3.68	45.83	-25.28	3.3
B122254	0.788	13.41	6.37	45.96	-24.53	3.4
B122255_rpt	0.743	13.18	5.02	45.89	-25.37	3.5
B122256	0.914	13.69	7.31	46.71	-26.33	3.4
B122257	0.789	14.26	7.55	46.59	-24.88	3.3
B122258	0.764	14.15	0.78	45.46	-24.24	3.2
B122259	0.731	13.80	6.39	45.37	-24.82	3.3
B122260	0.707	14.36	5.56	45.31	-23.71	3.2
BP11568-a	1.082	13.67	8.08	47.33	-26.00	3.5
BP11568-b	0.721	13.89	7.84	43.97	-25.87	3.2

Table 2. Measurements of nitrogen isotope ratio $\delta^{15}N$ standards against reported values. NIST, (US) National Institute of Standards and Technology; USGS, US Geological Survey; NC, not certified; ND, not determined. *, material used for data normalisation.

	Reported		Normalised measured		IRMS	
Standard	$\delta^{15}N \pm SD$ (‰ v Air)	%N	$\delta^{15}N$ Mean ± SD	*n*	%N Mean ± SD	*n*
NIST RM8573 USGS40 L-glutamic acid *	-4.52 ± 0.12	9.52	-4.52 ± 0.03	5	9.44 ± 0.09	5
NIST RM8548 IAEA-N-2 Ammonium sulphate*	20.40 ± 0.2	21.2	20.41 ± 0.11	6	20.97 ± 0.41	6
USGS65 Glycine	20.68 ±0.06	18.67	20.85 ± 0.18	5	18.46 ± 0.30	5
DL Leucine	NC	10.57	13.62 ± 0.24	12	10.64 ± 0.09	11
Squid laboratory std	NC	ND	13.31 ± 0.20	6	12.53 ± 0.29	6

Table 3. Measurements of carbon isotope ratio $\delta^{13}C$ standards against reported values. NIST, (US) National Institute of Standards and Technology; USGS, US Geological Survey; IAEA, International Atomic Energy Agency; NC, not certified; ND, not determined. *, material used for data normalisation.

	Reported		Normalised measured		IRMS	
Standard	**$\delta^{13}C$ ± SD (‰ v Air)**	**%C**	**$\delta^{13}C$ Mean ± SD**	***n***	**%C Mean ± SD**	***n***
NIST RM8573 USGS40 L-glutamic acid *	-26.39 ± 0.09	40.82	-26.37 ± 0.14	5	40.27 ± 0.34	5
NIST RM8542 IAEA-CH-6 Sucrose*	-10.45 ± 0.07	42.11	-10.45 ± 0.12	6	41.68 ± 0.30	6
USGS65 Glycine	-20.29 ± 0.04	32	-20.20 ± 0.14	5	31.46 ± 0.17	5
DL Leucine	NC	54.38	-28.53 ± 0.14	12	54.61 ± 0.48	11
Squid laboratory std	NC	ND	-18.22 ± 0.10	6	42.23 ± 0.42	6

IAEA-N2 ammonium sulphate. Sample $\delta^{13}C$ values were 2-point normalised using isotopic data from the daily analysis of NIST 8573 USGS40 L-glutamic acid and NIST 8542 IAEA-CH-6 Sucrose. Precision was determined by the repeat analysis of the working laboratory standard DL-Leucine (DL-2-Amino-4-methylpentanoic acid, $C_6H_{13}NO_2$, Lot 127H1084, Sigma, Australia). Data from the daily analysis of USGS65 Glycine was used to check accuracy and precision. Repeat analysis of Squid lab standard provided a further check on precision.

Carbon and nitrogen stable isotopic ratios of the feather samples are shown in Table 1, and the results for the nitrogen and carbon standards in Table 2 and Table 3, respectively.

Our results display two contrasting features of cuckoo isotopic niche use (Fig. 2). Firstly, the species as a whole has a broad isotopic niche. Single factor ANOVA showed no differences between the isotopic values for any combination of site and age. The $\delta^{15}N$ values were consistent with the birds' consuming the same diet, primarily herbivorous caterpillars, recorded in New Zealand (Higgins 1999), and in the subcanopy or canopy of a tropical rain forest (Cerling *et al.* 2004). The $\delta^{13}C$ values suggested a range of habitats, from the undergrowth of closed forest (*c.* -27.8‰) to open forest and shrubland (*c.* -23.5‰ to -24‰) (Ambrose & DeNiro 1986; Cerling *et al.* 2004; Hawke & Holdaway 2009; Holdaway *et al.* 2013; Johnston 2014; Hawke *et al.* 2017; Holdaway & Rowe 2020). The $\delta^{13}C$ value for the three juveniles sampled at Kowhai Bush reflected the known natal habitat of low-canopied kanuka (*Kunzea robusta* de Lange et Toelken, 2014) open woodland. The isotopic spectrum of our samples, from two populations, suggests that the species as a whole has the capacity to cope with significant environmental change, so long as sufficient woody vegetation remains to support its preferred foods.

Despite the broad range of habitats occupied by the species in general, individuals appear to occupy only specific and narrow isotopic habitats. Both adults sampled at Kowhai Bush in successive years moulted in the same isotopic space they had occupied the previous year, but at remarkably different positions in the species' isotopic range (Fig. 2). This implies that the individuals either returned to exactly the same geographic (and habitat) location, or to different locations with the same isotopic values. Either way, both birds reached, and stayed in, the same isotopic niche for the duration of their moult period. These results imply that, while the species may be resilient, local populations in the non-breeding areas are vulnerable to environmental change. Both birds were from the same breeding area which suggests that the local population can be subject to losses occurring in different areas in the non-breeding areas in Australasia and the South-west Pacific. The similarity between the isotopic values of three cuckoos sampled at Milnthorpe to that of a repeat-sampled individual from Kowhai Bush, suggests that populations at both sites in New Zealand could be adversely affected by events elsewhere. A similar reduction in the breadth of its isotopic niche was associated with the near-extinction of the New Zealand brown teal (*Anas chlorotis* G.R. Gray, 1845) (Holdaway *et al.* 2013).

Our results show that small migrant birds may have broad habitat requirements as a species but that individuals may be tied to particular habitats, and possibly locations, at each end of their migration path, and at any stopover point. Other taxa may occupy non-breeding habitats structurally similar to their breeding habitats but whose isotopic values are different as a result of different climate (e.g. cloudiness [Helama *et al.* 2018]) or vegetation (e.g. C3 versus C4). Hence, they may shift their

isotopic niche (Hahn *et al.* 2013) without changing their physical niche. The relationship between isotopic and conventional niche is still unclear (Flaherty & Ben-David 2010).

Removal of particular favoured habitats, such as that represented by the group of individuals occupying the moulting habitat represented by the isotopic space near $\delta^{13}C$ ~ -25‰ and $\delta^{15}N$ ~ +4‰ (Fig. 2), would jeopardize the survival of birds breeding in two different areas in New Zealand. Site specificity away from the breeding grounds is difficult to detect. However, it may be general, showing that more research is needed into what may be a significant factor in the declines of small terrestrial migrant birds.

ACKNOWLEDGEMENTS

For their help in catching cuckoos, we thank Lorna Deppe, Mailee Stanbury, Gabriela Schalemberger, Robyn White, and David Lloyd-Jones. Access to Milnthorpe Park was approved by the Milnthorpe Park Society (Chairperson Mik Symmons) and field assistance in Golden Bay was provided by Ken George (Takaka) and Tim Eckert, and Jane Greatrex (Milnthorpe). Josette Delgado and Julie Brown carried out the stable isotope analyses overseen by Sarah Bury, at the NIWA Environmental Stable Isotope Laboratory, New Zealand. We thank David Hawke for discussion of stable isotopic data and helpful comments on the draft manuscript. Funding was provided by a grant from the Brian Mason Scientific and Technical Trust and by the University of Canterbury.

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Keywords: isotopic site fidelity, migration, shining cuckoo, *Chrysococcyx lucidus lucidus*, New Zealand, Bismarck Archipelago, Solomon Islands, habitat loss, moult, stable isotope analysis, isotopic niche