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## Nitrogen Isotope Ratios Identify Deserted Seabird Colonies

Hiroshi Mizutani,<sup>1</sup> Yuko Kabaya,<sup>1</sup> P. J. Moors,<sup>2,5</sup> T. W. Speir,<sup>3,6</sup> and Graeme L. Lyon<sup>4</sup>

<sup>1</sup>Laboratory of Biogeochemistry and Sociogeochemistry, Mitsubishi Kasei Institute of

Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan;

<sup>2</sup>Department of Conservation, P.O. Box 10-420, Wellington, New Zealand;

<sup>3</sup>New Zealand Soil Bureau, Private Bag, Lower Hutt, New Zealand; and

\*Institute of Nuclear Sciences, DSIR, P.O. Box 31-312, Lower Hutt, New Zealand

The breeding population of Rockhopper Penguins (*Eudyptes chrysocome*) on subantarctic Campbell Island (52°33'S, 169°09'E), New Zealand, in the Australasian quadrant of the Southern Ocean has experienced a dramatic decline (Moors 1986). Field observations and photographs taken intermittently since 1941 document the time course of desertion of some colonies. We found this situation ideal to test the ability of the nitrogen isotope ratio ( $\delta^{15}$ N) and other chemical parameters in determining the locations of past seabird colonies.

There are two stable isotopes of nitrogen: <sup>14</sup>N and <sup>15</sup>N. In various biogeochemical reactions, they react at different rates; this results in a range of values for the ratio, <sup>15</sup>N/<sup>14</sup>N, of various nitrogen reservoirs. Among these reactions, the fractionation during the evaporation of ammonia greatly enriches the remain-

<sup>&</sup>lt;sup>5</sup> Present address: Royal Australasian Ornithologists Union, 21 Gladstone Street, Moonee Ponds, Victoria 3039, Australia.

<sup>°</sup> Present address: DSIR Land Resources, Private Bag, Lower Hutt, New Zealand.

Sample tune	\$15NI (07)	No. of	\$13C (0%)	No. of
Sample type	0-11 (700)	sampies	0 C (/00)	sampies
Guano	$7.0 \pm 0.4$	3	$-18.0\pm0.9$	3
Soil				
Occupied colonies	$23.8 \pm 3.3$	4	$-23.0 \pm 0.5$	5
Deserted colonies	$14.0 \pm 1.5$	10	$-22.2 \pm 0.6$	10
Suspected colonies	$12.4 \pm 1.3$	6	$-24.8 \pm 0.4$	6
Control sites	$-0.5\pm0.2$	2	$-27.1 \pm 0.2$	2

TABLE 1.  $\delta^{15}$ N and  $\delta^{13}$ C<sup>a</sup> for soils and guano from Campbell Island, with particular reference to occupied and deserted colonies. Values are reported ±SD.

<sup>a</sup> A portion of  $\delta^{13}$ C data is from Moors et al. (1988).

ing nitrogen in <sup>15</sup>N (Kirshenbaum et al. 1947, Kreitler 1975). Because of this effect, the  $\delta^{15}$ N for soils of seabird colonies is considerably higher than for any other type of soil (Mizutani et al. 1985a). Local heterogeneity of the elevated  $\delta^{15}$ N for soil organic nitrogen is much less than for other chemical characteristics of soil that may reflect seabird activity (Mizutani 1984, Mizutani et al. 1986, Mizutani and Wada 1988). Combined with the slow turnover of soil organic matter (Jenkinson and Rayner 1977), the unusually high  $\delta^{15}$ N and its relatively small variation within a rookery had led to the prediction that it could serve as a long-term indicator of past seabird colonies (Mizutani et al. 1986).

We collected four soil samples in January 1986 from the west coast of Campbell Island at Penguin Bay, where about 4,600 pairs of Rockhopper Penguins were breeding. We collected 4 samples at 2 sites still occupied by penguins in 1951; 2 samples from a site active in 1974, and 4 samples from a site still occupied in 1979. Each colony was probably deserted only a few years after the date of its photograph. We also collected 3 guano samples, each of which consisted of guano from 2 birds, in bags strapped to individuals.

We collected two additional soil samples from each of three sites >100 m away from, and ca. 20 m above, the closest known rockhopper colony. The sites are on steep vegetated slopes with no known records of colonization. They appeared unsuitable for Rockhopper Penguin colonies. Earlier, Moors et al. (1988), who examined soil characteristics of these soils, suspected that Sooty Shearwaters (*Puffinus griseus*), which once bred in very large numbers on Campbell Island (Bailey and Sorensen 1962) and favor steep tussocky coastal slopes for their nesting burrows, were likely to have used them in the past. We termed these sites suspected colonies.

As a control, we collected 2 soil samples in February 1987 from a flat ridge-top ca. 750 m away from the rockhopper colonies, where seabirds probably never nested. These comprised "grab" samples of peat taken at approximately 15 cm and approximately 25 cm depth. Further details of the samples and the results of relevant chemical analyses are given in Moors et al. (1988).

The preparation of N<sub>2</sub> for isotope measurements

from soil was carried out according to Mizutani et al. (1985b). The purified  $N_2$  was introduced to a Hitachi RMU-6R mass spectrometer for ratiometry. The nitrogen isotope ratio was obtained as per mil (‰) deviation from atmospheric nitrogen as defined by the following equation:

$$\delta^{15}N \ (\%) = \frac{({}^{15}N/{}^{14}N)_{sample} - ({}^{15}N/{}^{14}N)_{air}}{({}^{15}N/{}^{14}N)_{air}}$$

#### × 1000.

The standard deviation of the nitrogen isotope measurements was less than 0.2‰. The  $\delta^{13}$ C values were measured after converting organic carbon to CO<sub>2</sub> according to Mizutani and Wada (1985a) and analyzing the CO<sub>2</sub> in a manner similar to Moors et al. (1988). Analytical precision for the  $\delta^{13}$ C measurements was better than 0.1‰. Details of these experimental procedures are given in Mizutani and Wada (1988). The results of the isotope measurements are summarized (Table 1).

The  $\delta^{15}$ N for the soils changed as a function of the time elapsed after desertion (Fig. 1). Once abandoned as a colony, the  $\delta^{15}$ N decreased rapidly over 10 yr, then continued to decrease much more slowly. By 30 yr after desertion, the  $\delta^{15}$ N was still higher than at the control sites. Soil samples from the suspected colonies had significantly higher  $\delta^{15}$ N than the control sites. In fact, it was similar to the known deserted colonies.

The soils at the control sites gave the  $\delta^{15}N$  values <0%. Other than the direct inflow of organic nitrogen, common sources of nitrogen to a terrestrial ecosystem are nitrate and ammonium in precipitation and in situ N<sub>2</sub> fixation. These forms of nitrogen normally have  $\delta^{15}N$  of about 0‰ or less (Wada et al. 1975, Peters et al. 1978). The  $\delta^{15}N$  values for the control sites show that the soils probably received nitrogen from these common sources. On the contrary, the high  $\delta^{15}N$ for soils from the suspected colonies must have been caused by the degradation of large amounts of allochthonous, fresh organic matter, and subsequent ammonia volatilization. This organic matter is likely to have been deposited by seabirds as is the case in both the presently active and the deserted colonies. 962



Fig. 1. Elevated  $\delta^{15}$ N of rookery soil, and its decrease after desertion. The interval after desertion is the time between the last photograph of the occupied colony and the time of sampling. Abbreviations: S = soil samples from the suspected colonies; \* = the soils from the sites never used as a seabird colony; vertical bars show the standard deviation (SD). The SD for the control sites is much smaller than the size of the asterisk).

In seabird colonies, birds are the major source of organic nitrogen and phosphorus. Organic carbon, however, can be produced autochthonously by vegetation. Therefore, parameters that incorporate N, P, or both, would more successfully indicate past avian activity than would the organic carbon content. The soils at suspected colonies contained  $0.85 \pm 0.22\%$  P. This was less than the average P content (2.3  $\pm$  0.5%) of the known deserted colonies, but was relatively high compared with the control sites (0.06  $\pm$  0.02%). Similar differences hold for the C/N ratio. The soil from the suspected colonies had C/N ratios of 14.0  $\pm$  1.4, the C/N for the known deserted rookeries averaged 5.8  $\pm$  0.8, and that for the control sites 31.0  $\pm$  6.5. Together with these independent chemical data (Moors et al. 1988), the  $\delta^{15}N$  confirms that the suspected soil came from once active seabird colonies.

The geographical features of the suspected colonies eliminate the possibility that Rockhopper Penguins once nested there. The most likely occupants of the suspected colonies would be Sooty Shearwaters. A large number of Sooty Shearwaters once bred on Campbell Island (Bailey and Sorensen 1962). The introduction of rats in the last century eliminated the possibility of the birds successfully fledging young. Since the early 1960s, small colonies of Sooty Shearwaters remain, but few have bred successfully. Birds still breed in large numbers on nearby rat-free islands.

Among the three parameters previously identified (Moors et al. 1988) as an indicator of past colonization, the  $\delta^{13}$ C gave the lower correlation ( $r^2 = 0.38$ , n = 22)



Fig. 2. Nitrogen to carbon ratio of rookery soil, and its decrease after desertion. The interval after desertion is the same as in Figure 1; explanation of abbreviations and symbols is in Figure 1.

with  $\delta^{15}$ N and the C/P the second ( $r^2 = 0.45$ , n = 22), while the C/N ratio showed the highest correlation ( $r^2 = 0.65$ , n = 22). The correlation between  $\delta^{15}$ N and the reciprocal of the C/N ratio was still higher ( $r^2 =$ 0.71). The N/C ratio decreases with time elapsed after desertion (Fig. 2). This indicates that the N/C value would be also useful for identifying deserted nesting sites. The N/C value for the suspected colonies was close to the control value. This is probably due to the fact that the suspected sites are vegetated and the carbon content (12.6 ± 7.2%) is more than twice as high as either the presently active (5.9 ± 2.7%) or the known deserted (4.6 ± 2.4%) colonies. It appears that the N/C ratio is more susceptible than  $\delta^{15}$ N to the presence of vegetation.

The high  $\delta^{15}N$  for the rockhopper colony soils indicates that the nitrogen was of avian origin and later enriched in <sup>15</sup>N through ammonia volatilization (Table 1). On the other hand, the  $\delta^{13}$ C value for the colony soils is about midway between guano and the control, which indicates that the organic carbon in the colony soils probably originated from both higher plants and seabird colonization. As  $\delta^{13}$ C values for seabird body tissue are higher than for its excreta (Mizutani and Wada 1988, Mizutani et al. 1991a), vegetation probably accounts for more than half of the organic carbon input to the present soil. Because most of the carbon comes from higher plants and nitrogen from seabirds, the Campbell Island colonies are more like the Blacktailed Gull (Larus crassirostris) rookery in Kabushima (40°32'12"N, 141°33'41"E), Hachinohe, Japan, than the Adélie Penguin (Pygoscelis adeliae) rookery in Cape Bird (77°12'S, 166°28'E), Ross Island, Antarctica (Mizutani and Wada 1988).

A dense ornithocoprophilous plant community exists at the Kabushima rookery (Ishizuka 1966). The soil organic matter there had  $\delta^{13}$ C of -23.  $2 \pm 1.0\%$ . For gull droppings, the  $\delta^{13}$ C was  $-19.4 \pm 0.6\%$  and,

for rape (Brassica campestris), the dominant plant species in the colony, it was  $-25.4 \pm 0.6\%$ . At the Cape Bird rookery, where higher plants are absent,  $\delta^{13}$ C for soil ( $-27.9 \pm 0.6\%$ ) was much closer to the penguin guano ( $-28.1 \pm 0.2\%$ ) than to the green algae (*Prasiola cripsa*) ( $-18.7 \pm 2.3\%$ ). The N/C ratio at the occupied colony in Campbell Island is 0.26, 0.49 at Cape Bird, and 0.15 at Kabushima. If Campbell Island lacked vegetation,  $\delta^{13}$ C would be a good indicator of past colonization.

Although both  $\delta^{13}$ C and N/C are possible indicators of past colonization and work best in the absence of vegetation, they differ in other ways. For instance, the utility of  $\delta^{13}$ C is dependent on differences between terrestrial and marine organic carbon. It is useful to categorize avian food sources as marine or terrestrial (Hobson 1987, Mizutani et al. 1990, Hobson and Sealy 1991). However,  $\delta^{13}$ C may not be able to indicate sites that were used as a nest or a dwelling, if the birds depended on the land plants or if their prey used organic carbon from terrestrial primary producers. In the case of the N/C ratio, this limitation would be inapplicable so far as the ratio for bird droppings is different from that for the plants.

The  $\delta^{15}$ N level is a relatively robust indicator and, unlike the N/C ratio and  $\delta^{13}$ C, is free from the limitation due to the presence of vegetation. It is also free from differences of primary production and would not be greatly influenced by external fluctuations. For instance, the variability of chemical parameters of soil nitrogen in gull rookeries is as follows: uric acid N content > ammonium N content > Kjeldahl N content > soil organic N content > $\delta^{15}$ N for Kjeldahl N (Mizutani and Wada 1985b, Mizutani et al. 1986). A high  $\delta^{15}N$  value for colony soils results from a relatively high  $\delta^{15}$ N for incoming nitrogen due to the  $^{15}$ N enrichment along the food chain (Miyake and Wada 1967, DeNiro and Epstein 1981, Schoeninger and DeNiro 1984, Schoeninger et al. 1983), and from the enrichment that occurs with ammonium volatilization. The extent of the enrichment through the volatilization appears similar within a locality and seems to be determined basically by the latitude of the rookery (Mizutani et al. 1991b).

We believe that sites where a large input of allochthonous, fresh organic nitrogen and its subsequent degradation took place in the past can be identified by  $\delta^{15}N$ . It would be more effective for soils at high latitude. It is applicable to studies of past breeding distribution and abundance of animals. The rate of decrease in  $\delta^{15}N$  is related to the turnover rate of soil organic matter, and the elevated values appear to last >100 yr (Fig. 1).

Further investigations of the annual rate of change in  $\delta^{15}N$ , using sites for which older dates of abandonment are known, may enable the time since desertion to be estimated for the suspected colonies. Among the indicators of the past colonization,  $\delta^{15}N$  appears to have a wide applicability. For many abandoned colonies, photographic or other records are inadequate or absent. The nitrogen isotope ratio is a reliable, accurate long-term indicator of past colonization.

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# On the Validity of Bubo virginianus occidentalis Stone

ROBERT W. DICKERMAN

American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024 USA

Stone (1896) described the geographic variation in, and provided the first revision of, the Great Horned Owls (Bubo virginianus) of North America. Prior to his study, the name subarcticus had been used for the pale birds of the interior of the United States. Stone (in consultation with Ridgway) found the type of subarcticus to represent the boreal nesting population, and they considered the name to be a synonym of Strix arcticus Swainson, then in use for the pale subarctic nesting population. Stone (1896) then provided a key to the forms he recognized, and he introduced the name Bubo virginianus occidentalis for the pale birds of the Great Plains. He used for the type a pale, unsexed specimen from Mitchell County, Iowa, taken in winter of 1880 and then available in the Academy of Natural Science of Philadelphia. He did not attempt to define the range of occidentalis.

Almost immediately he apparently reached the conclusion that his type "proves to be intermediate between *B. virginianus* and *arcticus* and does not belong to the race which I had intended to rename; *the latter not extending that far east* [italics mine]" (Stone 1897). Using a specimen from the Watson Ranch, 18 miles southwest of San Antonio, Texas, he renamed the smaller and pale western birds *pallescens*.

In 1904 H. C. Oberholser published his classic revision of the species. The revision was uniformily accepted—with minor exceptions—and has been followed since. Oberholser gave *occidentalis* subspecific status, apparently ignoring Stone's recognition that the type was a migrant of the boreal population, although Oberholser (1904) cited Stone's 1897 paper as the source of *pallescens*. Oberholser assigned *occidentalis* a range from western Minnesota to southeastern Oregon, and south in the prairies to Kansas. In his introduction Oberholser wrote that "... with the exception of *occidentalis* and *wapacuthu*, all seem to be strictly nonmigratory ...," (Oberholser 1904) but he did not indicate which specimens of *occidentalis* he considered to be migrants.

With the characters of *occidentalis* being only size (the type is probably a female) and darkness relative to *pallescens*, I was confused as I attempted to identify pale birds from the east (New York, New Jersey, and Connecticut) and even specimens from South Dakota, and more recently as I attempted to resolve the status of *occidentalis* vs. *pallescens* in the prairie nesting populations of eastern New Mexico. An east-west range transecting the middle of North America and spanning the northern prairies and the Rocky Mountains from western Minnesota to northeastern California (AOU 1957) is improbable biogeographically. There is a series of good dark and ochraceous specimens typical of *virginianus* from Nebraska in the Denver