# SHORT COMMUNICATIONS

## Carbon Isotope Ratio of Feathers Reveals Feeding Behavior of Cormorants

HIROSHI MIZUTANI,[1] MICHIO FUKUDA,[2] YUKO KABAYA,[1] AND EITARO WADA[1]
[1]*Laboratory of Biogeochemistry and Sociogeochemistry, Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan, and*
[2]*Ueno Zoological Gardens, Ueno, Daito-Ku, Tokyo 110, Japan*

A colony (ca. 2,000 birds) of the Great Cormorant (*Phalacrocorax carbo*) is present in Shinobazunoike Pond, Tokyo, Japan. The cormorants feed in rivers and in Tokyo Bay. The major direction of their daily flight in winter is to the rivers, and in summer to the Bay (Fukuda 1985). The relative importance of the two feeding sites in terms of dietary contribution is unknown. Dietary studies conventionally depend on stomach contents, cast pellets, and field observations. Although useful to reveal birds' choice of prey species, such details cannot determine the relative dependence of cormorants on the two feeding areas. Stable carbon isotope analysis of bird tissues is a convenient way to measure such dependence, as it tells if the organic carbon in the tissues is terrestrial or marine in origin. Compared with conventional dietary studies, isotope analysis is less susceptible to day-by-day variation of prey species and is in principle free of numerical bias for easily identifiable foods.

The two stable isotopes of carbon are $^{12}C$ and $^{13}C$. In various biogeochemical reactions, they react at different rates, and the ratio $^{13}C/^{12}C$ of various carbon reservoirs becomes different (Mizutani and Wada 1985a). For instance, organic carbon produced by $C_3$ plants that constitute the major terrestrial producer differs by ca. 20‰ in this ratio from atmospheric $CO_2$. In marine ecosystems, primary carbon fractionation occurs during planktonic photosynthesis and the resultant organic carbon is ca. 7‰ higher than the terrestrial organic carbon. This difference is passed through the food chain, and tissues of consumers of both terrestrial and marine organic carbons will have a carbon isotope ratio intermediate between the two sources. Thus, the measurement of the ratio for tissues may elucidate questions of the origin of carbon compounds in nature.

There have been a number of successful applications of isotope analysis to determine relative proportion of terrestrial and marine organic matters in various samples (e.g. Sackett 1964, Tauber 1981, Wada et al. 1987). Hobson (1987), the first to apply isotope analysis to avian dietary studies, measured the carbon isotope ratio of bone collagen of gulls (*Larus* spp.) to estimate the relative contributions of marine and terrestrial protein in their diets. Moors et al. (1988) used the carbon isotope ratio to identify the deserted colonies of the Rockhopper Penguin (*Eudyptes chrysocome*).

We chose feathers as samples to estimate the relative dietary carbon inputs to the birds. Feathers are readily available in the field without sacrifice of birds. The isotope values of feathers represent the diet during their growth period, which enables the recognition of seasonal changes. Each feather reflects the diet of an individual bird. Finally, the breeding season of the cormorants at Shinobazunoike Pond begins in September and ends in the following June (Fukuda 1980), and the shed feathers are available all year.

To relate the isotope ratios of feathers to diet, isotope fractionation during the assimilation of organic carbon into the body tissue must be considered (DeNiro and Epstein 1978, Minagawa and Wada 1985). We studied the fractionation of carbon isotopes into feathers of captive cormorants on a constant diet of mackerel (*Pneumatophorus japonicus*). The number of captive cormorants varied, but the average was ca. 30. In 1975 and 1976, we captured birds that were less than one-month-old. Since then, they have eaten a constant diet of mackerel.

Mackerels and newly shed primary feathers of the captive birds were collected periodically from May 1984 to June 1989. The samples were treated (Mizutani and Wada 1988) and combusted to form $CO_2$ (Mizutani and Wada 1985b). The $CO_2$ gas was introduced for ratiometry to either a Hitachi RMU-6R mass spectrometer with dual inlet and double collector systems or a Finnigan MAT-251 mass spectrometer with dual inlet and triple collector systems. The carbon isotope ratio was expressed in ‰ deviation ($\delta^{13}C$) from PDB carbonate standard, which is a Cretaceous belemnite (*Belemnitella americana*) from the Peedee Formation of South Carolina, where

$$\delta^{13}C\ (‰) = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{PDB}}{(^{13}C/^{12}C)_{PDB}} \times 1{,}000.$$

The carbon isotope data from the Hitachi RMU-6R were corrected for $^{17}O$. Results from the triple-collecting Finnigan MAT-251 were compared with those from the Hitachi RMU-6R to modify the correction equation of Craig (1957). Working standards of carbon were calibrated against U.S. National Bureau of Standards isotope reference material No. 20 and No. 21. The $\delta^{13}C$ values for the two working standards were −19.4‰ and −12.0‰. Standard deviations of the carbon isotope measurements were less than 0.1‰.

Keratin, the major component of avian feathers, has a high sulfur content. Because the high sulfur content may interfere with complete combustion of feather

TABLE 1. The $\delta^{13}C$ values of food items regurgitated by Great Cormorants.

| Food items | No. of measurements[a] | $\delta^{13}C$ of organic carbon (‰) | |
|---|---|---|---|
| | | $\bar{x} \pm SD$ | Range |
| Roach | 9 | $-23.5 \pm 1.4$ | $-26.6$ to $-21.6$ |
| Immature gray mullet | 4 | $-24.0 \pm 2.0$ | $-27.4$ to $-22.7$ |
| Dragonet | 3 | $-17.3 \pm 0.4$ | $-17.8$ to $-16.9$ |
| Flathead | 5 | $-18.6 \pm 0.7$ | $-19.3$ to $-17.3$ |
| Goby | 7 | $-17.6 \pm 1.6$ | $-20.2$ to $-15.7$ |
| Gizzard shad | 1 | $-15.7$ | — |
| Flatfish | 6 | $-18.3 \pm 0.5$ | $-19.0$ to $-17.6$ |

[a] Each fish was analyzed only once.

samples and produce incorrect $\delta^{13}C$ values, we performed an incomplete combustion of L-cystine (lot #274, Amino Acid Kit, Kyowa Hakko Kogyo Co. Ltd., Tokyo) and feathers of the Nankeen Night Heron (*Nycticorax caledonicus*). For both samples, measurements were within 0.1‰ of complete combustion values. The high sulfur content in feathers did not seem to interfere with an accurate measurement of the isotope ratios.

The $\delta^{13}C$ for the whole bodies of the mackerel was $-20.1 \pm 1.2$‰ (19 samples), and for the feathers was $-16.1 \pm 0.6$‰ (11 samples). Therefore, the apparent enrichment of $^{13}C$ relative to diet in cormorant feathers was 4.0‰. The enrichment factor is only apparent because the food item (i.e. mackerel) is composed of various organic compounds (including lipids) whose individual enrichment factors may be different.

When wild cormorants returned to Shinobazunoike Pond after feeding, they sometimes regurgitated their stomach contents. Regurgitated items included a roach (*Carassius auratus*), an immature gray mullet (*Mugil cephalus*), a dragonet (*Repomucenus richardsonii*), a flathead (*Suggrundus meerdervoorti*), a goby (*Acanthogobius flavimanus*), a gizzard shad (*Konosirus punctatus*), and a flatfish (*Limanda yokohamae*) (Fukuda 1985). As mentioned above, regurgitations only partly reflect the actual diet. They indicate the utilization of both marine and riverine resources by cormorants. Therefore, we collected regurgitated material during May 1984 and April 1989, froze them immediately, and treated them in the same manner as the mackerels (Table 1).

The $\delta^{13}C$ values for the dragonet, flathead, goby, gizzard shad, and flatfish indicate their marine origin. The roach and the immature gray mullet are from freshwater (Schoeninger and DeNiro 1984). The average $\delta^{13}C$ for oceanic fish ($-23.8$‰) was 6.3‰ lower than for the riverine fishes ($-17.5$‰). The difference is similar to the difference between primary producers of the marine and terrestrial organic carbons, and the two averages can be assumed to represent the ratio for the two food sources.

When cormorants feed on these fish, carbon in their feathers should be enriched in the heavy isotope by ca. 4.0‰ relative to the fish. For instance, if the gray mullet (the lowest in $^{13}C$ content) was the only food for a cormorant, $\delta^{13}C$ of its feather should on average be $-20.0$‰. Similarly, the $\delta^{13}C$ of its feather would be $-11.7$‰ if a cormorant depends solely on the gizzard shad, which has the highest $\delta^{13}C$ values. The actual $\delta^{13}C$ values for cormorant feathers should be between these two extremes, and the values depend on the combination of these food items.

We collected naturally shed primary feathers of adult cormorants in the pond from May 1984 to May 1989. Feathers were treated in the same manner as the feathers of cormorants in captivity. These feathers represent a maximum of 46 individuals out of ca. 2,000 cormorants in the pond. Their average $\delta^{13}C$ was $-16.0$‰, and it indicated an uneven utilization of terrestrial and oceanic resources by the cormorants (Fig. 1). If the roach and the immature gray mullet represent the terrestrial organic carbon source and the other fish are oceanic, the cormorants in the pond get on average ca. six tenths of their organic carbon from Tokyo Bay and the rest from rivers.

The distribution of $\delta^{13}C$ in the feathers of individual birds was broad (Fig. 1). The range for feathers (9.1‰) was wider than that for foods (8.3‰). Because carbon in feathers is likely to represent the diet of individuals only during feather formation, the distribution suggests that seasonal or individual variation in diet is maximal.

The broad distribution of $\delta^{13}C$ does not appear to result from seasonal differences of the flight direction. Of the 46 feathers (Fig. 1), 18 were collected when the cormorants were flying mostly to the Bay (from 15 June to 15 October), and 14 were collected when they were feeding in the rivers (from 15 December to 15 April). If adult cormorants normally shed their feathers approximately one year after formation, feathers collected in summer are likely to reflect summer diet, and those collected in winter reflect winter diet. The mean $\delta^{13}C$ value for the feathers was $-16.3 \pm 2.6$‰ in summer, and $-17.1 \pm 2.6$‰ in winter. The average for summer was higher than that for winter (as expected from the seasonal flight direction); however, the difference of 0.8‰ is small and statistically insignificant. Although the life span of feathers may vary considerably, the broad distribu-

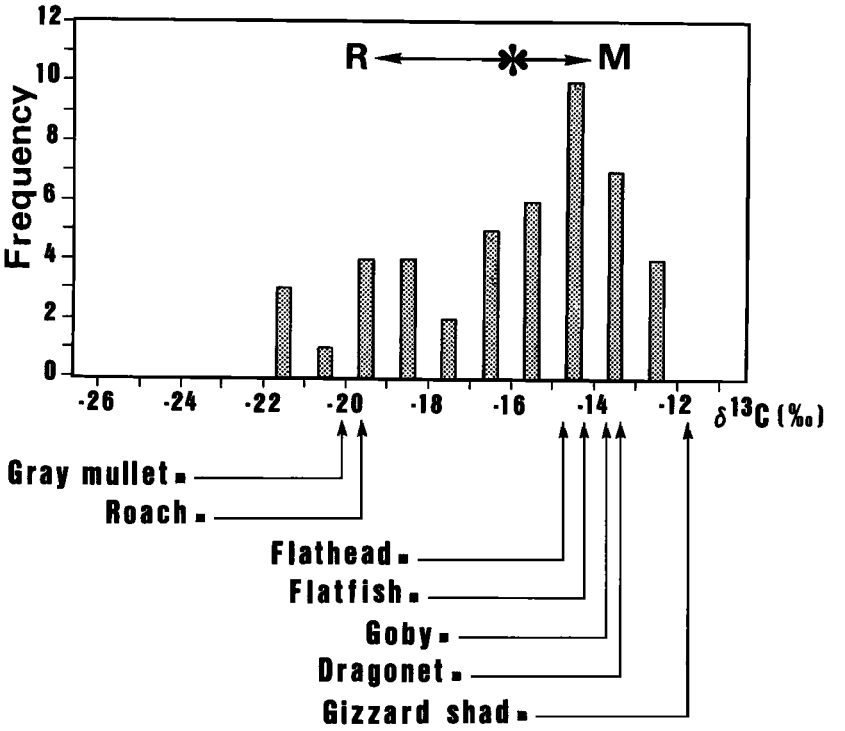


Fig. 1. Frequency distribution of $\delta^{13}C$ in cormorant feathers ($n$ = 46). The $\delta^{13}C$ values for the foods are indicated on the horizontal axis. The length of horizontal portions of the arrows in the lower half represents the enrichment of 4.0‰ obtained from feathers of cormorants on a constant diet. The positions of R and M are at expected $\delta^{13}C$ values of feathers of cormorants that feed only on the riverine (R) or the marine (M) fish. The asterisk indicates the average $\delta^{13}C$ for the 46 cormorant feathers.

tion was not a composite of two seasonally different distributions.

I suggest the difference might result from the particular feeding behavior of the cormorants. There were four feathers with $\delta^{13}C$ below the average of the two riverine fishes, and eight with $\delta^{13}C$ greater than that of the five oceanic fishes. If we assume these feathers are from cormorants that preyed at the time of feather formation upon riverine fish and marine fish, respectively, a few hundred cormorants out of the total of 2,000 would have depended mostly on riverine resources, whereas approximately one fifth of the total depended on marine resources. The rest, approximately three quarters of the total, depended on both in various proportions. Many cormorants were observed to move daily between rivers and Tokyo Bay in their search for prey. I believe that the majority of the cormorants must alternate their daily feeding sites.

Black-tailed Gulls (*Larus crassirostris*) at Kabushima, Aomori Prefecture, Japan, present a different situation (Mizutani and Wada 1988). The average $\delta^{13}C$ of available foods there ranged from −22.8‰ (sardine) to −17.8‰ (larvae of Japan soldier fly) (range: 5.0‰). The range of $\delta^{13}C$ values for seven gull feathers collected in 1983 was 0.7‰. The narrow distribution in gull feathers suggests a narrow variation in their diet. An additional 17 gull feathers collected in 1988 and 1989 increased the $\delta^{13}C$ range of the 24 feathers to 1.5‰ with little change in either the average or the standard deviation (Fig. 2).

When Fig. 2 is compared with Fig. 1, the narrow distribution is apparent. Presumably the gulls feed heavily on a single foodstuff or each individual selects its food resources in a similar way. An alternative explanation is that gulls molt more synchronously than the cormorants. Further study is necessary to understand the cause of the narrow distribution, but the diet of the cormorants appears far more variable than that of the gulls. The cormorants are apparently more individualistic in feeding behavior than the gulls.

The application of carbon stable isotopes to ornithology and ecology is not without some complications (Fry 1988). With caution, however, the technique could be of value for ornithology. My results yield valid information concerning both diet content and feeding behavior. Combination with other elements such as nitrogen would further enhance the value of the stable isotope technique.

The Ueno Zoological Gardens kindly allowed us to

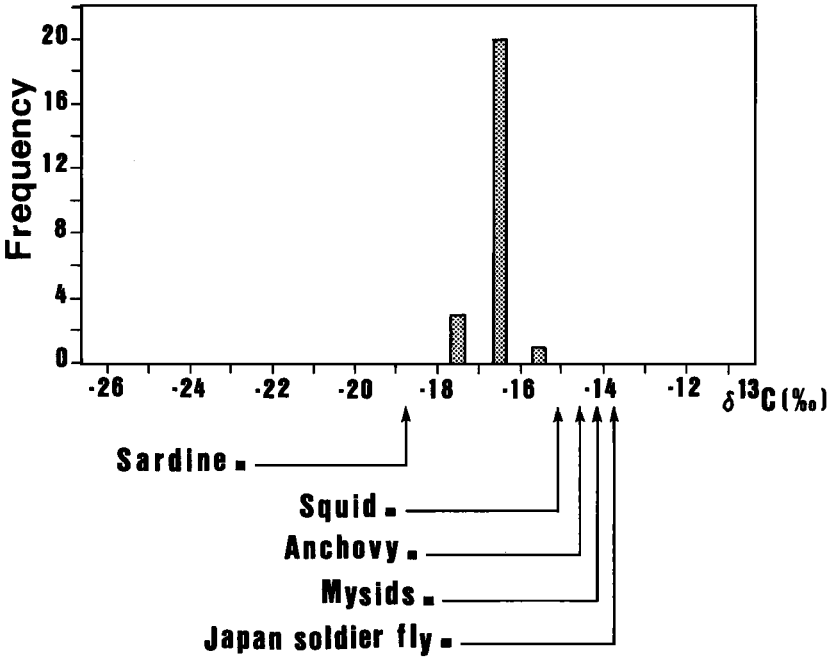


Fig. 2. Frequency distribution of $\delta^{13}C$ in gull feathers ($n$ = 24). The $\delta^{13}C$ of various foods and the extent of the $^{13}C$ enrichment is indicated on the horizontal axis. The length of horizontal portions of the arrows represents the enrichment of 4.0‰ obtained from gulls on a constant diet (Mizutani et al. unpubl. data).

collect samples. Kiichi Narita of the Hachinohe Science Museum for Children collected and donated some gull feathers. We thank Kyoko Karasawa for assistance with some of the measurements.

## Literature Cited

Craig, H. 1957. Isotopic standards for carbon and oxygen isotopes. Geochim. Cosmochim. Acta 3: 53-92.

DeNiro, M. J., & S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42: 495-506.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnol. Oceanogr. 33: 1182-1190.

Fukuda, M. 1980. Life of the Common Cormorants. Wild Birds 45: 679-684.

———. 1985. Notes on the Common Cormorants in Tokyo. Animals and Nature 15: 11-15.

Hobson, K. A. 1987. Use of stable-carbon isotope analysis to estimate marine and terrestrial protein content in gull diets. Can. J. Zool. 65: 1210-1213.

Minagawa, M., & E. Wada. 1985. Biogeochemical implication of H, C, N, O stable isotope compositions in plant and animal. Chikyukagaku 19: 39-52.

Mizutani, H., & E. Wada. 1985a. Carbon dioxide and the biosphere. Viva Origino 13: 23-49.

———, & ———. 1985b. Combustion of organic samples by infrared furnace for carbon isotope analysis. Anal. Biochem. 146: 90-95.

———, & ———. 1988. Nitrogen and carbon isotope ratios in seabird rookeries and their ecological implications. Ecology 69: 340-349.

Moors, P. J., T. W. Speir, & G. L. Lyon. 1988. Soil analyses and $^{13}C/^{12}C$ ratios identify sites of deserted Rockhopper Penguin colonies. Auk 105: 796-799.

Sackett, W. M. 1964. The depositional history and isotopic organic carbon compositions of marine sediments. Marine Geology 2: 173-185.

Schoeninger, M. J., & M. J. DeNiro. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim. Cosmochim. Acta 48: 625-639.

Tauber, H. 1981. $^{13}C$ evidence for dietary habits of prehistoric man in Denmark. Nature 292: 332-333.

Wada, E., M. Minagawa, H. Mizutani, T. Tsuji, R. Imaizumi, & K. Karasawa. 1987. Biogeochemical studies on the transport of organic matter along the Otsuchi River Watershed, Japan. Estuarine Coastal Shelf Sci. 25: 321-336.

*Received 31 May 1989, accepted 20 September 1989.*