

IDENTIFICATION OF NATAL LOCALES OF PEREGRINE FALCONS (*FALCO PEREGRINUS*) BY TRACE-ELEMENT ANALYSIS OF FEATHERS

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ABSTRACT.—Samples of secondary remiges collected from nestling Peregrine Falcons (*Falco peregrinus*) in Alaska and western Greenland were analyzed for trace-element content using instrumental neutron-activation analysis. Concentrations of 14 trace elements were subjected to a series of multivariate discriminant function analyses to ascertain whether or not these concentrations could be used to identify the geographic origins of the birds sampled. Individual falcons from the three areas studied can be placed in their proper natal locale with 100% predictability. Mercury (Hg) was the best individual discriminator for separating sample groupings. Aluminum (Al) and Vanadium (V), in conjunction with Hg, provided the most discriminant trio of elements when various groupings of element concentrations were considered as predictors. Received 2 July 1982, accepted 1 February 1983.

PRECIPITOUS declines in populations of Peregrine Falcons (*Falco peregrinus*) during the past three decades have been well documented in the eastern United States and southern Canada (Hickey and Roelle 1969) and in many parts of the North American Arctic (Cade and Fyfe 1970, Fyfe et al. 1976). Results of Arctic surveys since 1975, however, have shown upward trends in many breeding populations, dramatically so in Alaska on the Yukon River and, to a lesser extent, on the Colville River, especially in the last 3 yr (White and Fyfe in press). Nevertheless, northern breeding populations continue to be of concern, particularly with respect to their choice of wintering grounds in Latin America, where application of chlorinated pesticides is heavy and increasing in some regions.

It has been the intent of our research to develop a method of identifying the geographic origin of individual peregrines captured on migration or in wintering areas. Such identification would provide a means of assessing the status of regional populations, determining wintering areas of North American peregrines, and elucidating migration routes.

During the last decade, feather chemical analyses have developed rapidly as a means of establishing the geographic origin of birds. Pioneering studies in the early 1950's described geographic variations in feathers of upland

game birds (Campbell 1953, Campbell and McCullough 1953, Grant 1953). Much subsequent work has been accomplished with waterfowl in Canada (Hanson and Jones 1968, 1976; Kelsall 1970a, b; Kelsall and Calaprice 1972; Kelsall and Burton 1977, 1979; Ranta et al. 1978), with an emphasis on studying the geographic origin of individual birds and identifying breeding and molting grounds.

The wet chemistry techniques used in most early studies on feather minerals lacked the analytical sensitivity to measure certain elements present in only trace amounts (Kelsall 1970a). Devine and Peterle (1968) demonstrated the utility of an instrumental technique, that of neutron-activation analysis, in their investigations on populations of Canada Geese (*Branta canadensis*). Instrumental neutron-activation analysis (INAA) has proven to be a highly sensitive and versatile method for measuring trace elements in a wide variety of samples (Wainerdi and DuBeau 1963) at microgram levels (Corliss 1963). Other investigators, in studying chemical "tags" in salmon that relate directly to river of origin, employed multivariate discriminant functions for data analysis, thereby facilitating data interpretation and enhancing the prediction of natal locale (Calaprice 1970, 1971; Calaprice and Calaprice 1970; Thomson and Calaprice 1970; Calaprice et al. 1971).

In our study, we reasoned that feathers grown by nestling Peregrine Falcons should contain trace elements in an array of concentrations unique to the local geology and ecosystem. If the relative abundance of trace elements differed among breeding locales of peregrines, then the measurement of selected elements and the analysis of results by means of multivariate discriminant functions could provide an expedient method of ascertaining the population of origin of individual birds.

MATERIALS AND METHODS

Feather samples were collected in the summer of 1979 from nestling peregrines in Alaska at 7 eyries on the Yukon River ($n = 14$) and 3 eyries on the Colville River ($n = 9$) and from 3 eyries in West Greenland ($n = 6$). Two additional feather samples were obtained from immature peregrines during migration at South Padre Island, Texas in the autumn of 1979. These two birds had been banded as nestlings on Alaska's upper Yukon River that summer, but feather samples had not been obtained while the birds were still in the eyrie.

The distal 1 cm (weight 0.003–0.010 g) was collected from the fifth secondary remige. Only one sample per bird was obtained for analysis. The choice of feather and portion sampled was the result of several considerations. The choice of feather quantity was determined by the minimum amount required for analysis. Furthermore, the tip of the feather would contain trace elements incorporated into the matrix while birds were in the eyrie, thereby reflecting the chemical signature of the local area. A remex was selected for two reasons. First, in nestling peregrines, remiges emerge before other contour feathers and are often the only ones available for sampling at the time of banding in the eyrie. Second, we chose a secondary remex, because any flight impairment should be less than if a primary were sampled, and the fifth secondary is the first remex molted by immature peregrines (Mebs 1960, Stresemann and Stresemann 1966). Also, if the trace-element technique proved to be useful in diagnosing natal locale, we reasoned that we could determine wintering grounds as well, based on the known molt sequence of peregrine remiges. Differentiation of most contour feathers molted on breeding and wintering grounds would be virtually impossible.

Feather samples were collected in the field by several cooperators. Due to potential contamination from metal sampling instruments and human handling, each feather was cleaned prior to analysis. Samples were washed individually in dark bottles, agitating constantly, with a 25% solution of Radiacwash (Atomic Products Corporation), a non-ionic detergent com-

monly used in preparation of samples for neutron-activation analysis. Each sample was then rinsed five times in deionized water and dried in a dessicator under vacuum at room temperature for 2 h. Samples were placed in reactor-grade polyethylene containers and sealed for irradiation. Samples were handled with plastic forceps at all times during laboratory procedures.

Instrumental neutron-activation analysis (INAA) of feather samples was performed at Nuclear Energy Services, Inc., a private firm, located at the Department of Nuclear Engineering, North Carolina State University, Raleigh campus. Feather samples were activated in a pneumatic irradiation facility with a flux of 1.5×10^{13} neutrons \cdot cm $^{-2}$ \cdot s $^{-1}$. Radionuclides of I, Mn, Mg, Cu, V, Cl, Al, Na, Br, Se, Hg, Sc, Zn, and Co were measured following activation periods, which ranged from 20 to 2,400 min (Table 1). Induced gamma radiation from the activated feather samples was measured on a Ge(Li) gamma detector (Ortho Corporation) coupled to a computerized Nuclear Data System (ND6620). The efficiency of the detector was 24% (relative to a 7.3×7.3 cm NaI(Tl) detector measured at 1.333 MeV gamma of ^{60}Co , source to detector distance 25 cm). Quantities of trace elements were calculated from spectral data in μg element \cdot g $^{-1}$ (parts per million) of feather. Trace element values of $0.0 < X < 0.05 \mu\text{g} \cdot \text{g}^{-1}$, etc., were treated as if the values were exact. Approximate cost of multi-element scans using INAA is \$150 per sample.

Trace-element data were subjected to a series of multivariate discriminant function analyses to compare locales and to establish a priority ranking of trace elements important to this analysis. Discriminant functions (Table 2) were computed from trace-element data under Level 8 Statistical Package for the Social Sciences (Nie et al. 1975) using a Sperry UNIVAC 1100/61 computer. The level of significance was set at 0.05. Individual distances from group means (Mahalanobis's D^2 value; Rao 1952) and posterior probabilities for group membership of each sample (Table 3) were computed using concentrations of 14 trace elements as predictors. The D^2 value is a unit of statistical distance each sample deviates from the mean of each known group. Posterior probability $P(\text{group } 1/x) = \exp\{-\frac{1}{2} \cdot D_1^2(x)\} / \sum [\exp\{-\frac{1}{2} \cdot D_i^2(x)\}]$ computed from the D^2 value indicates the chance of samples belonging to each of the known groups.

RESULTS

Our initial considerations included using quantities of all 14 trace elements as predictors for group membership of the 29 feather samples collected from nestling peregrines. The result was a complete separation of individuals from all three locales. On the basis of these

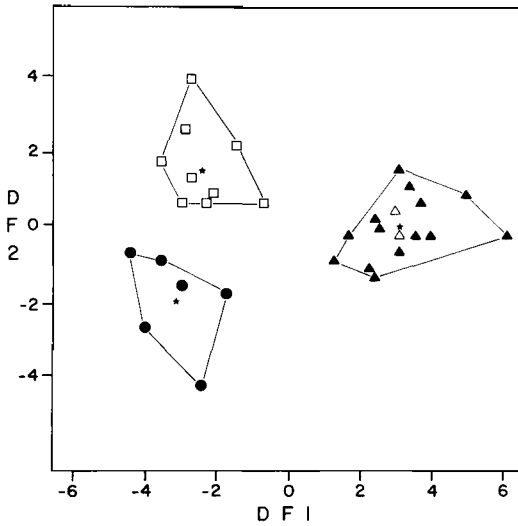


Fig. 1. Discriminant function classification of nesting peregrines from three locales and two migrant peregrines from South Padre Island, Texas. ▲ = nestlings from the Yukon River, Alaska. △ = migrant peregrines from South Padre Island, Texas. □ = nestlings from the Colville River, Alaska. ● = nestlings from western Greenland. DF 1 = discriminant function 1; DF 2 = discriminant function 2. Classification of individuals was made using 14 trace-element concentrations as predictors.

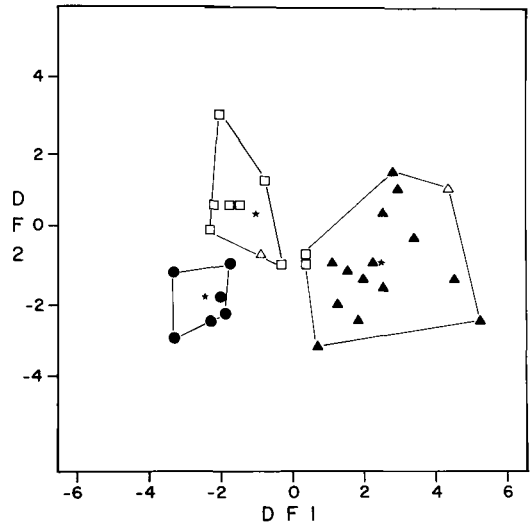


Fig. 2. Discriminant function classification of nesting peregrines from three locales and two migrant peregrines from South Padre Island, Texas. ▲ = nestlings from the Yukon River, Alaska. △ = migrant peregrines from South Padre Island, Texas. □ = nestlings from the Colville River, Alaska. ● = nestlings from western Greenland. DF 1 = discriminant function 1; DF 2 = discriminant function 2. Classification of individuals was made using concentrations of Hg, Mn, V, Na, and Br as predictors.

results, we attempted to classify the two samples collected from migrant peregrines at South Padre Island, Texas. The two samples were assigned correctly to the Yukon River group,

which we knew to be their origin (Fig. 1). Thus, our initial questions concerning the reliability of 14 trace-element concentrations as predictors for group membership had been answered.

TABLE 1. Concentrations ($\mu\text{g}\cdot\text{g}^{-1}$, mean \pm SD) of 14 trace elements measured following activation of secondary remiges of 31 Peregrine Falcons by instrumental neutron activation.

Trace element ¹	West Greenland ²	Yukon River ³	Colville River ⁴
I	0.2 \pm 0.16	2.6 \pm 5.06	0.6 \pm 0.55
Mn**	1.1 \pm 0.87	7.0 \pm 5.69	6.3 \pm 5.66
Mg	169.8 \pm 60.45	265.3 \pm 329.48	430.8 \pm 311.69
Cu	5.0 \pm 0.00	6.5 \pm 4.51	5.6 \pm 1.82
V**	0.2 \pm 0.24	0.9 \pm 0.81	0.8 \pm 0.41
Cl	714.7 \pm 234.39	631.3 \pm 183.58	526.3 \pm 147.89
Al	121.2 \pm 134.71	314.4 \pm 358.48	363.0 \pm 149.48
Na**	198.1 \pm 89.61	324.2 \pm 139.15	297.8 \pm 82.77
Se	0.6 \pm 0.26	0.9 \pm 0.61	0.9 \pm 0.36
Hg*	0.8 \pm 0.33	3.1 \pm 0.97	1.5 \pm 0.55
Br**	2.9 \pm 0.91	4.7 \pm 2.58	6.7 \pm 5.21
Sc	0.02 \pm 0.02	0.05 \pm 0.05	0.05 \pm 0.02
Zn	145.0 \pm 24.06	149.3 \pm 29.92	141.9 \pm 39.79
Co	0.05 \pm 0.00	0.07 \pm 0.09	0.05 \pm 0.01

¹ * $P < 0.001$, ** $0.05 > P < 0.10$ (one-way ANOVA).

² $n = 6$.

³ $n = 14$.

⁴ $n = 9$.

TABLE 2. Discriminant function sets defined for various groupings of trace-element quantities used as predictors for group membership of feather samples collected from 31 immature Peregrine Falcons. The most discriminant elements of each function can be identified by ignoring the sign and considering the absolute values.

Trace element	SET-1 ^a		SET-2 ^b		SET-3 ^c	
	I	II	I	II	I	II
I	-0.699	-0.254	-0.694	0.628	-0.891	0.387
Mn	-0.800	0.315			-1.088	-0.039
Mg	0.483	0.626	0.149	-0.684	1.118	0.065
Cu	-0.237	-0.311				
V	-2.894	-0.773	-2.978	0.254	-2.199	-1.326
Cl	-0.247	-0.804	-0.224	0.796	-0.289	0.806
Al	2.921	0.615	3.305	-0.913	3.433	0.147
Na	-0.241	0.681	-0.111	-0.562		
Se	-0.446	0.296	-0.382	-0.250		
Hg	-1.272	0.089	-1.335	-0.059		
Br	0.457	0.761	0.594	-0.668		
Sc	1.074	0.656				
Zn	-0.201	-0.283				
Co	-0.122	-0.562				
% of variance explained ^d	83.21*	16.79	83.54*	16.46**	68.11**	31.89***

^a Function set defined using all 14 trace elements as predictors.

^b Function set defined using nine trace elements identified in step-wise program.

^c Function set defined using six trace elements obtainable after 20-min activation time.

^d * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$ (χ^2 test).

We then decided to formulate programs that would permit the use of fewer trace elements in this type of analysis, thereby reducing time and cost.

A oneway ANOVA accomplished on the 14 trace-element concentrations resulted in significance values listed in Table 1. Mean element concentrations were tested individually for equality of group means. Mercury (Hg) was the best individual discriminator ($P < 0.001$). Four other elements, Mn, V, Na and Br, provided reasonable separation power individually ($0.05 > P < 0.10$). Considering significance values for Hg, Mn, V, Na, and Br, new discriminant functions were developed using these trace elements as predictors for group membership of the total 31 feather samples. Three of the samples, including one from a migrant peregrine, were incorrectly assigned group membership, yielding a 90.3% predictability of proper membership (Fig. 2).

In further consideration of reducing the number of variables used as predictors, a step-wise multivariate regression analysis was conducted on the 14 trace elements. The step-wise analysis identified Hg again as the best predictor, followed by Mg, Br, Cl, Na, I, Se, V, and Al in decreasing order of importance. New discriminant functions were computed using this

group of nine trace elements (Table 2). One sample from the Colville River group was incorrectly grouped, yielding a 96.7% predictability overall (Fig. 3).

Radionuclides of I, Mn, Mg, V, Cl, and Al can be obtained following a 20-min activation period of samples at the reported flux. We formulated discriminant functions to predict group membership of our feather samples using this grouping of six trace elements (Table 2). The result was 87.1% predictability of group membership (Fig. 4). When Hg concentrations were added as a predictor to this six-element grouping, the predictability was 100%. Radionuclide concentrations of Hg, however, are realized only following a 2,400-min activation period at the reported flux.

DISCUSSION

Instrumental neutron-activation analysis of feather tips provided a discriminant means of separating Peregrine Falcons from three regions into groups of similar natal origin and a potentially useful method of predicting natal origins of migrant peregrines with substantial accuracy (Fig. 1, Table 3). Our results further suggest that the array of trace elements within the feather matrix is not substantially altered

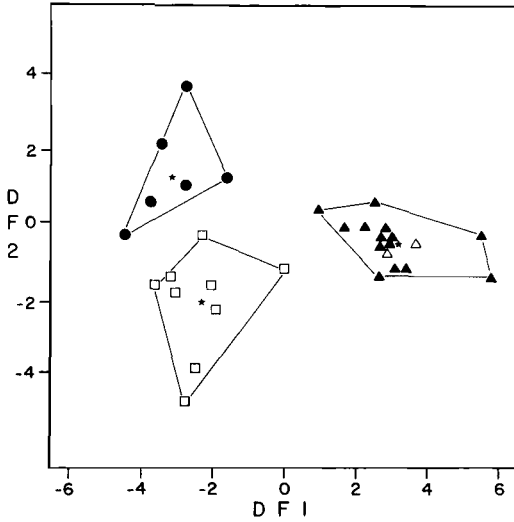


Fig. 3. Discriminant function classification of nestling peregrines from three locales and two migrant peregrines from South Padre Island, Texas. \blacktriangle = nestlings from the Yukon River, Alaska. \triangle = migrant peregrines from South Padre Island, Texas. \square = nestlings from the Colville River, Alaska. \bullet = nestlings from western Greenland. DF 1 = discriminant function 1; DF 2 = discriminant function 2. Classification of individuals was made using concentrations of Hg, Mg, Br, Cl, Na, I, Se, V, and Al as predictors.

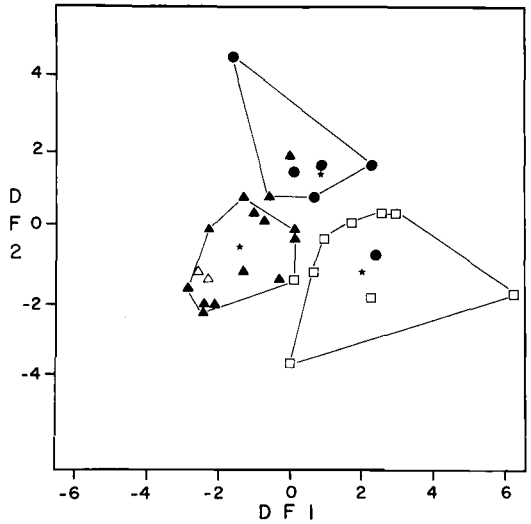


Fig. 4. Discriminant function classification of nestling peregrines from three locales and two migrant peregrines from South Padre Island, Texas. \blacktriangle = nestlings from the Yukon River, Alaska. \triangle = migrant peregrines from South Padre Island, Texas. \square = nestlings from the Colville River, Alaska. \bullet = nestlings from western Greenland. DF 1 = discriminant function 1; DF 2 = discriminant function 2. Classification of individuals was made using concentrations of I, Mn, Mg, V, Cl, and Al as predictors.

once individuals depart breeding grounds on migration. Variation in prediction capability results when groupings of particular trace-element quantities are used in formulating predictive functions (Figs. 2-4). We consider our lowest prediction capability of 87.1% using 6 trace-element quantities (Fig. 4) acceptable in some circumstances for making future predictions of a similar nature. Reducing the number of trace elements used removes the need for conducting multi-element scans of samples, thus reducing time and cost of sample irradiation.

Concentrations of Al, V, and Hg contributed the highest discriminating power to predictive functions used in separating groups of samples (Table 2). Due to the similarity in variance accounted for in function sets 1 and 2 (Table 2), we conclude that the trace elements deleted in the step-wise regression analysis would be unnecessary to use in formulating predictions for group membership. Caution must be exercised, however, in interpreting the results of a single step-wise analysis from three populations with

small sample sizes. The results of our step-wise analysis will be compared with future analyses and appropriate population statements made at a later date.

In our study, mercury content of feather samples averaged $3.1 \mu\text{g}\cdot\text{g}^{-1}$ from the Yukon River, $1.5 \mu\text{g}\cdot\text{g}^{-1}$ from the Colville River, and $0.8 \mu\text{g}\cdot\text{g}^{-1}$ from West Greenland (Table 1). We believe these concentrations are not indicative of mercury pollution, but rather reflect local background levels in the respective environments. Johnels and Westermarck (1969) measured mercury in feathers of Goshawks (*Accipiter gentilis*), Ospreys (*Pandion haliaetus*), and Great-crested Grebes (*Podiceps cristatus*) collected in Sweden between 1815 and 1966. They concluded that approximately $3\text{--}4 \mu\text{g}\cdot\text{g}^{-1}$ constituted the natural or background level in the region. Berg et al. (1966) reported that the mean level of mercury in feathers from 11 Swedish Peregrine skins collected between 1834 and 1940 was $2.6 \pm 1.1 \mu\text{g}\cdot\text{g}^{-1}$, whereas high levels of about $15\text{--}20 \mu\text{g}\cdot\text{g}^{-1}$ have been found during more recent, polluted times. In another study,

TABLE 3. Mahalanobis's distance squared (D^2) from individual group means and posterior probability (pp) for individual group membership of feather samples from 31 immature Peregrine Falcons.

Group	West Greenland		Yukon River		Colville River	
	D^2	pp^a	D^2	pp^a	D^2	pp^a
West Greenland	2.9	0.99*	38.4	0.00	12.9	0.00
West Greenland	3.7	1.00*	54.2	0.00	23.6	0.00
West Greenland	13.9	0.96*	61.3	0.00	20.4	0.03
West Greenland	14.9	1.00*	54.6	0.00	43.7	0.00
West Greenland	3.9	0.97*	43.1	0.00	10.9	0.03
West Greenland	4.7	0.99*	26.2	0.00	14.3	0.01
Yukon River	27.1	0.00	5.1	1.00*	20.9	0.00
Yukon River	50.1	0.00	19.0	1.00*	40.8	0.00
Yukon River	30.1	0.00	4.4	1.00*	31.1	0.00
Yukon River	86.2	0.00	14.8	1.00*	73.9	0.00
Yukon River	63.4	0.00	18.1	1.00*	46.2	0.00
Yukon River	63.8	0.00	20.0	1.00*	43.8	0.00
Yukon River	74.7	0.00	12.8	1.00*	57.2	0.00
Yukon River	24.3	0.00	9.0	0.99*	24.0	0.00
Yukon River	47.2	0.00	9.4	1.00*	39.1	0.00
Yukon River	36.3	0.00	11.1	1.00*	35.6	0.00
Yukon River	72.1	0.00	24.1	1.00*	62.7	0.00
Yukon River	39.7	0.00	3.5	1.00*	35.1	0.00
Yukon River	48.5	0.00	17.3	1.00*	40.4	0.00
Yukon River	55.3	0.00	7.8	1.00*	40.5	0.00
Padre Island	68.3	0.00	26.0	1.00*	60.1	0.00
Padre Island	64.1	0.00	25.3	1.00*	51.0	0.00
Colville River	44.3	0.00	54.9	0.00	13.8	1.00*
Colville River	38.5	0.00	41.5	0.00	19.7	1.00*
Colville River	17.9	0.06	44.7	0.00	12.5	0.93*
Colville River	24.3	0.04	43.3	0.00	18.1	0.95*
Colville River	25.5	0.00	53.3	0.00	11.9	0.99*
Colville River	38.3	0.00	56.3	0.00	18.7	1.00*
Colville River	18.1	0.00	38.9	0.00	6.5	0.99*
Colville River	17.1	0.01	33.1	0.00	8.2	0.99*
Colville River	17.7	0.01	20.2	0.01	9.9	0.97*

* * Denotes highest probability for group membership.

Lindberg and Mearns (1982) analyzed mercury in a total of 21 molted primary, secondary, and tail feathers from Scottish Peregrines. They concluded that the average $2.4 \pm 2.1 \mu\text{g} \cdot \text{g}^{-1}$ of mercury represented the normal background level.

The differences in mercury concentration among our three populations sampled could be due to differences in mercury deposits naturally occurring in the earth's crust, differences in the birds' diets, or a combination of both. Natural concentrations of mercury vary widely in nature. In one report, levels in soils of Sweden ranged from 0.02 to 0.92 parts per billion (ppb), with an average of 0.07 ppb; English soils had levels from 0.01 to 0.06 ppb; and ostensibly nonpolluted water samples from northeastern United States varied from 0 to 0.63 ppb mercury (Klein 1972). Appreciably higher levels, however, are usually associated with the ma-

jority of mineral deposits and, as such, do not necessarily reflect environmental contamination. Mercury's high vapor pressure allows it to diffuse constantly from mineralized zones in soil gas, even from considerable depths (Jensen and Bateman 1979). Unfortunately, no data are available on mineralized zones or natural mercury deposits in any of our three study areas.

Waterfowl, especially Northern Pintails (*Anas acuta*), Green-winged Teal (*A. crecca*) and Shovelers (*A. clypeata*), comprise about 50% by weight of the diet of Yukon River Peregrines and shorebirds about 10-12% (Cade 1960, Cade et al. 1968). Cade (1960) reported that Colville River Peregrines utilize a much smaller proportion of waterfowl (24.7%) and greater proportions of shorebirds (15.5%), with ptarmigan (*Lagopus* sp.) the preferred prey item by weight (47.9%). In West Greenland the Lapland Longspur (*Calcarius lapponicus*) is the preferred prey

item and utilization of waterfowl and shorebirds in the diet of peregrines is low (Harris and Clement 1975). Biological methylation may be significant in the mobilization and distribution of mercury into the general environment (Jensen and Jernelev 1969, Matsumura et al. 1971). Lacking baseline information on relative mercury contamination in groups of prey items, however, we can make no definitive or even presumptive statements concerning the role of dietary differences in causing different levels of mercury among the three populations of peregrines studied.

Our initial success has provided the impetus for continuing investigations. Since 1979, additional feather samples have been obtained from nestling peregrines, not only from the three study areas reported herein, but also from other northern populations. Feather samples have also been obtained from museum specimens of peregrines collected in Greenland. These additional samples will be analyzed for trace elements to enhance our data base. As studies expand in Latin America, we will attempt to develop similar "ground truth" data from feathers grown by peregrines in wintering areas. Ultimately, we hope to identify where individual peregrines originate, where they spend the winter, and what routes they use in transit.

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