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FEATHER IDENTIFICATION BY MEANS OF KERATIN PROTEIN ELECTROPHORESIS

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SUMMARY

Identification of feathers by visual means leaves a percentage of unidentifiable samples, particularly at the lower taxonomic levels. Optical or scanning electron microcopy can improve results but about 25% of the samples cannot be identified below the family level. Electrophoresis of proteins extracted from feather keratin, used previously in taxonomic research, can provide reliability and repeatability in identifying feather remnants from any source. Protein extraction has been refined and standardized, as well as the methodology for electrophorizing feather protein concentrates. Current results indicate that identifications to the species level provided the sample is at least 10 mg can be obtained in most cases. There is little individual variation and differences between species are significant and can often be assessed visually. When gels are scanned with a laser densitometer, the differences between each keratin profile are more obvious and can be measured. The values of the curve can be used for separating closely related species. Our results indicate a high success rate and precision in identifications exceeding the results obtained by other means for the samples that cannot be identified visually.

1. INTRODUCTION.

The identification of bird remains resulting from collisions with aircraft can be a difficult task particularly when remnants are very small or have been seriously fragmented on impact. When the samples are sufficiently large, direct comparison with specimens in research and reference collections coupled with the use of macroscopic visual clues is the usual procedure to insure identifications. In this manner no less than 50% of the samples can be identified by an experienced ornithologist. It is necessary to employ different techniques to identify the remaining 50% of the samples with various levels of success. Although a number of feather identification techniques have been described elsewhere (Robertson et al. 1986), the procedure in use at the Canadian Museum of Nature for bird strike remains identification is outlined briefly before detailing the methodology and procedure for feather identification using protein electrophoresis of keratin.

2. IDENTIFICATION BY VISUAL MEANS.

2.1 Basic Visual Means.

A project was initiated to improve upon the identification performance of undetermined samples by straight visual comparisons or with the use of low optical magnification and to obtain identifications at the Species level in higher proportions. One of the first objectives was to devise identification keys for two families of North American birds based on the extensive visual analyses of certain feathers selected on body parts. These feathers were selected because they display homogeneity in form, colour, or coloration pattern. Only thoroughly cleaned feathers, free of any dirt, oil or other deposits were used for comparison purposes with reference specimens in the research collection. Two Families, the Anatidae (ducks, geese, and swans) and the Charadriidae (waders and plovers), were selected because of their regular occurrence in bird strikes in Canada. Feathers were obtained from the upper and lower parts of the body [crown, neck, upper and lower dorsum, throat, breast, flanks, and abdomen] for all the Canadian species of Anatidae (12 feathers) and Charadriidae (11 feathers). Each species is represented by adult

males and females, and immatures of both sexes when sexual dimorphism occurs. Each feather was meticulously examined and described using various criteria such as colour, coloration patterns, the presence of characteristic markings, or other distinctive features. Comparisons of these characters were then made for all the feathers from each area and for each species. This long and tedious process was followed for each feather represented in the sample.

The results permitted to isolate individual feathers or groups of feathers with common characteristics, thus allowing for the identification of a single species or a group of species sharing similar characters. Identification keys were then constructed manually based on a dichotomous choice of characters. The keys were later verified on a computer programme. It was then estimated that approximately 70% of the unidentified samples taken in these two families could be identified to the species level. The remaining 30% which comprises feathers without characteristic markings such as colour, bars, stripes, etc., could not be identified to the Species or even the Genus level. Feathers with a uniform coloration such as sandy beige, drab, buff, or white, form the bulk of this category. For example, nearly all the species of plovers have white feathers on the under parts. Similarly nearly all waterfowl species have white in their plumage. Having been unable to isolate specific differences in the structure of those feathers we found it impossible to provide identification beyond the Family level. The problem appears to be similar for other families of birds found in Canada although it has not been studied extensively. For such groups as the Laridae (gulls) and many species of songbirds in which several species share large numbers of common characters in a very large number of feathers, the specific determinations are usually impossible to make when using only visual clues.

2.2 Optical Microscopy.

The microscopic structure of feathers (Chandler, 1916; Sick, 1937; Voitkevich, 1966) provides as well important clues and is very useful in the identification of a significant number of samples that cannot be identified readily upon basic visual examinations but seldom provides the essential clues for identifications to the Species level. Using the structure of downy barbules, Brom (1986) stated that

97% of the feather samples can be identified at the Order or Family levels. Our results concur but our success rate for any identification below the Family level (Genus and Species) using this technique is very low and does not improve significantly the overall identification performance.

2.3 Scanning Electron Microscopy.

Another phase of the study consisted in trying to detect differences and identify at a more precise taxonomic level the samples of the Anatidae and Charadriidae which could not be identified by other means. Feathers of all the species studied earlier by other techniques were examined by means of a scanning electron microscope. With this powerful tool it was possible to study accurately (Davies 1970) the microscopic structure of feathers. Magnification was either at 500 or 1000 times, which permits a detailed examination of the barbs, barbules, nodes, and internodes. Precise measurements of the various components of the feathers were recorded for comparative purposes and analysis. Within each family, only insignificant differences were found between the various species but these differences are not sufficient to allow identification, even at the Genus level.

The distance between the various components of the feather structure (nodes, internodes, etc.) or the shape of these elements (nodes, barbs, hooks, etc.) are so overlapping in size or form, even on the same feather that it is impossible to obtain an identification on that basis. However, the differences recorded in the structure of the feathers in the Laridae and Charadriidae can be used to identify feathers at the Family level.

These results are in agreement with Brom (1987) and Chandler (1916) who used standard microscopic techniques in their studies. It can be concluded that satisfactory results can be obtained using Brom's (1986) technique for identification at the Order and Family levels and that the utilisation of the scanning electron microscope is an expensive and labour intensive technique which, although it reveals more information than standard light microscopy in the study of feather structures, is not satisfactory for the identification of feathers.

2.4 Summary.

A critical evaluation of these results, including series of tests to verify the accuracy of identification keys, techniques of macroscopic visual examination, conventional dissection microscopy, and light transmission microscopy, indicates that the detailed and precise identifications of nondescript feathers, which constitute approximately 25% of the samples submitted for determination, can only be achieved through other techniques.

3. ELECTROPHORESIS OF FEATHER KERATIN.

Electrophoresis of proteins obtained from the keratin of feathers has been used in a limited way in taxonomic work and indicate that the proteins of keratin have similarities among themselves, have small molecular weights, and vary from species to species (Brush 1976; Brush and Witt 1983; Busch and Brush 1979; Knox 1980a, 1980b). These results suggested that the technique could have a useful potential in the identification of feather samples that could not be identified by any of the other means as outlined earlier.

3.1 Protein Extraction.

Whole feathers are cleaned in batches by washing in hot detergent and rinsing in hot tap water. When dry, they are rinsed in two changes of naphtha (hexanes, Fisher), once in distilled water, and in two changes of denatured alcohol. After final drying they are packaged and stored for future use (Knox 1980a).

Keratin is extracted from 10 mg of finely cut feather samples. To each of these samples 1.0 ml of extracting solution consisting of 0.05M THAM (Fisher T-370), 8M urea (Fisher 4204-1), and, at the last minute, 0.05M dithiothreitol [DTT] (Pharmacia) is added (Marshall et al. 1986). The samples are stirred overnight under an atmosphere of nitrogen at room temperature. Each reaction mixture is centrifuged [12,000 rpm, 10 min; Eppendorf 5415 Microfuge] to sediment the residual feather fragments. The supernatant is removed and stored at -20° C.

To 25μL of each extracted sample 5μL of 0.1M DTT is added at least 10 min before typing (Carracedo et al. 1986). The samples are added to Pharmacia polyacrylamide Phastgel IEF 3-9 presoaked for 15 min in 1.0μl Pharmacia Pharmalyte 3-10, 250μl 20% NP-40, 12% sucrose, and 5.0ml distilled H_2O.

3.2 Protein Electrophoresis.

Isoelectric focusing proceeds at 2000V, 25mA, 4W, 20° C for 400Vh with a prefocusing phase at 2000V, 25mA, 2W, 20° C for 50Vh using the Pharmacia Phast System Separation and Control Unit. The gels are stained in the Pharmacia Phast System Development Unit using the protocol for Phast Gel IEF silver staining techniques (Phast System Owner's Manual) except that an extra step using 0.0125% DTT (Pharmacia) for 10 min at 40° C is added between steps Nos. 8 and 9 of the protocol.

4. GEL ANALYSIS.

After drying the gels are examined visually and differences between the bands of the tracks are compared. Then each track (8 on each gel) is processed on a laser densitometer (LKB Ultro Scan XL) in order to obtain quantitative values for each of the bands shown on the tracks as well as a curve of these values. Some of the bands may be undetected visually but can be separated by the beam of the densitometer. In this manner, it is possible to obtain distribution curves of the values recorded for each sample and compare these with curves of unknown samples. We designate these curves as "KERATIN PROFILES". The comparison of unknown keratin profiles against profiles obtained from known samples permits the identification of unknown feathers. An exact match can be obtained in most cases although a slight variation has been observed and is interpreted as individual variation.

5. RESULTS.

5.1 Protein Variation in Body Regions.

It was originally suspected that some variation could occur in

keratin profiles corresponding to the nature of the feathers and their region of origin on the body. This possibility was tested twice. Feathers from different parts of the body of a single bird were selected and subjected to the entire electrophoresis process and the results indicate that there is no significant variation in the protein bands of the gels nor in the keratin profiles of the feathers obtained from the same individual, regardless of the parts of its body. However, when dealing with feathers longer than 15 to 20 mm or with a thick shaft, only the vanes are used.

5.2 Intersexual Variation.

Similarly, no intersexual differences in keratin profiles were noted between individuals of the same species upon extensive comparisons of keratin profiles of over 300 species.

5.3 Individual Variation.

Our results indicate that there is little individual variation in a given species. As an example, eight different individuals of the Ring-billed Gulls (Larus delawarensis) representing different sexes and ages are compared to each other and later verified by comparing large samples of keratin profiles from several species. The tracks of the gels show a great uniformity in the location of the various bands and the keratin profiles are very similar.

5.4 Interspecific Differences.

Interspecific differences between closely related congeners can be important in some cases and can often be estimated visually as indicated in Figure 1. In this case the gel shows the protein electrophoretic patterns of seven species of gulls (Larus delawarensis, L. argentatus, L. glaucoides [kumlieni], L. hyperboreus, L. marinus, L. heermanni, L. californicus, L. glaucescens). It can be evaluated visually, without the aid of any equipment, that a number of bands on each track have a common position and that the others are situated in a different place on the track. Bands that occupy a similar position in a series of tracks can be interpreted as characteristics common to species in a same taxonomic category such as the Genus, Family, or

even the Order. The other bands, located in different places on the track, are considered to be Species characteristics. These differences are more obvious on the keratin profiles obtained through densitometric scanning than from the examination of the gels as shown for the eight species of gulls of Figures 2 to 9. In addition, the differences between each curve can be measured as well as any point on the curve. The values obtained can then be used for separating closely related Species or Species that have small differences such as is the case with the eight Species of gulls. Certain differences between species are small but are sufficient to distinguish between species, particularly when the values are computed and averaged for the peaks or highest values of the curves.

6. CONCLUSIONS.

The methodology described above provides a technique that allows the accurate identification of feather samples even of small size. The amount of feathers available for analysis should be in excess of 10 mg and the feathers should not have been altered by excessive heat or degenerated by chemical products. For any feather meeting these basic requirements and unsuitable for identification by visual methods, our results indicate that a high success rate, combined with a high degree of precision, exceeding by far the results secured by any other identification techniques can be attained through keratin electrophoresis and subsequent analysis of the gel patterns with a scanning densitometer. It is hoped that protein profiles for the bird species known to occur in Canada will be available during the next few months and that the results can be computerized to eventually generate rapid and accurate comparisons of unidentified samples against the known protein profiles of a data bank.

7. ACKNOWLEDGMENTS.

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microscopy

8. REFE

Brom, T. G
fragm
181-2

Brush, A.
taxon

Brush, A. H
Pelica
keratin

Busch, N. E
aspec
Zoolog

Carracedo,
Isoele
Journa

Chandler, A
referer
Publica

Davies, A.
electro
165-17

Knox, A. G.
informa

Knox, A. G
relation

available the results of his work on identification using optical microscopy and scanning electron microscopy.

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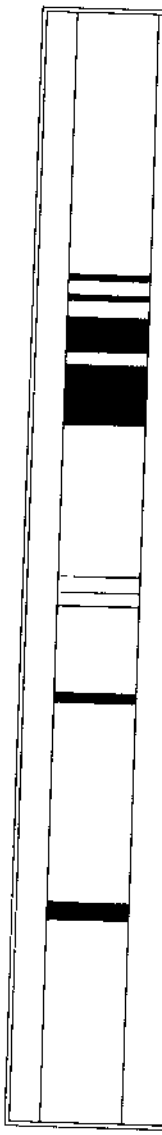
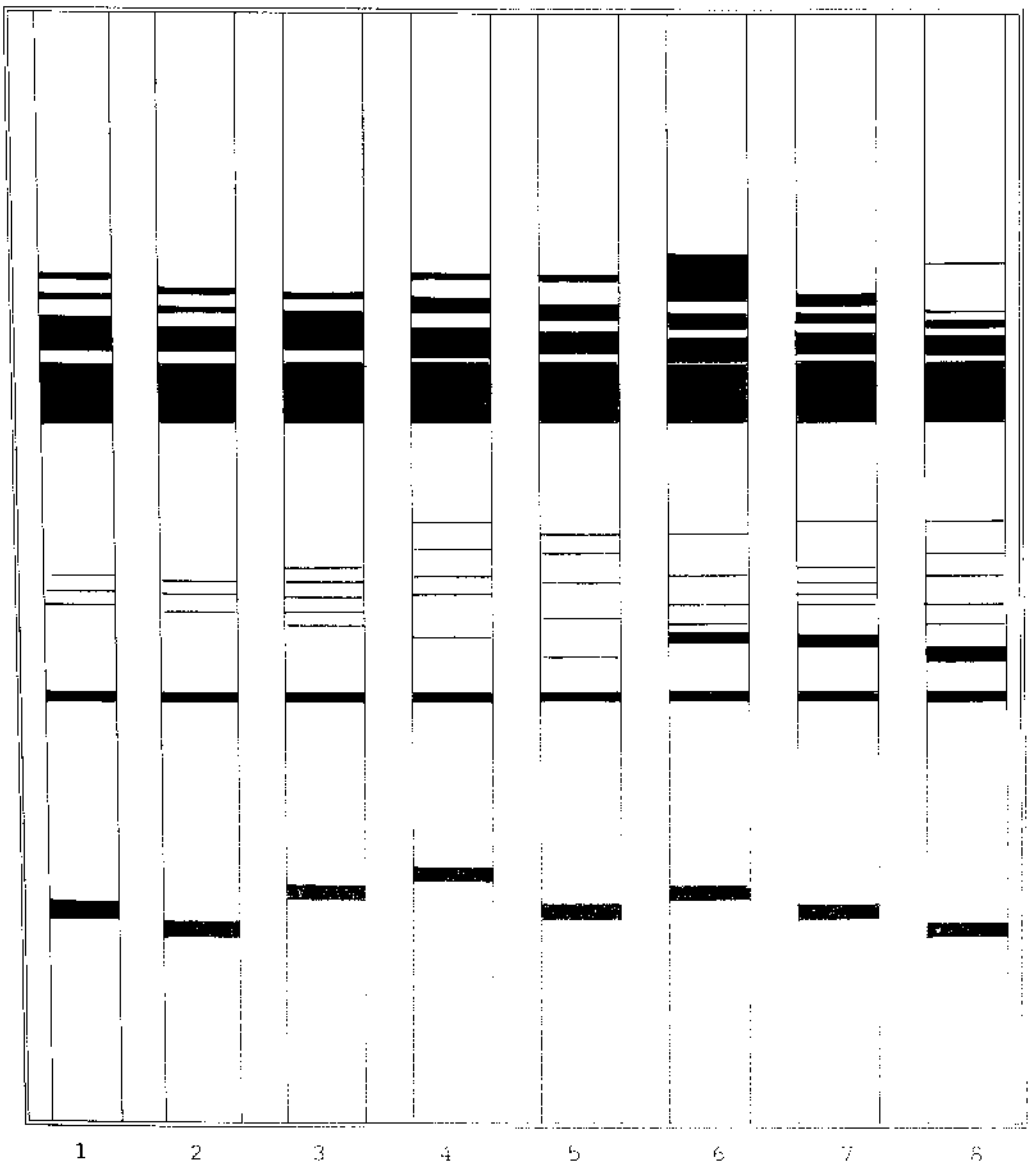
Figure 1.



1. Larus
2. Larus
3. Larus
4. Larus

Figure 1. Schematized gel of eight species of gulls (Laridae) showing the position of the bands on the tracks in relation to each other.



1. Larus delawarensis
2. Larus argentatus
3. Larus glaucoides [kumlieni]
4. Larus hyperboreus
5. Larus marinus
6. Larus heermanni
7. Larus californicus
8. Larus glaucescens

Figure 2. Keratin profile of the Ring-billed Gull (*Larus delawarensis*).

Figure 3

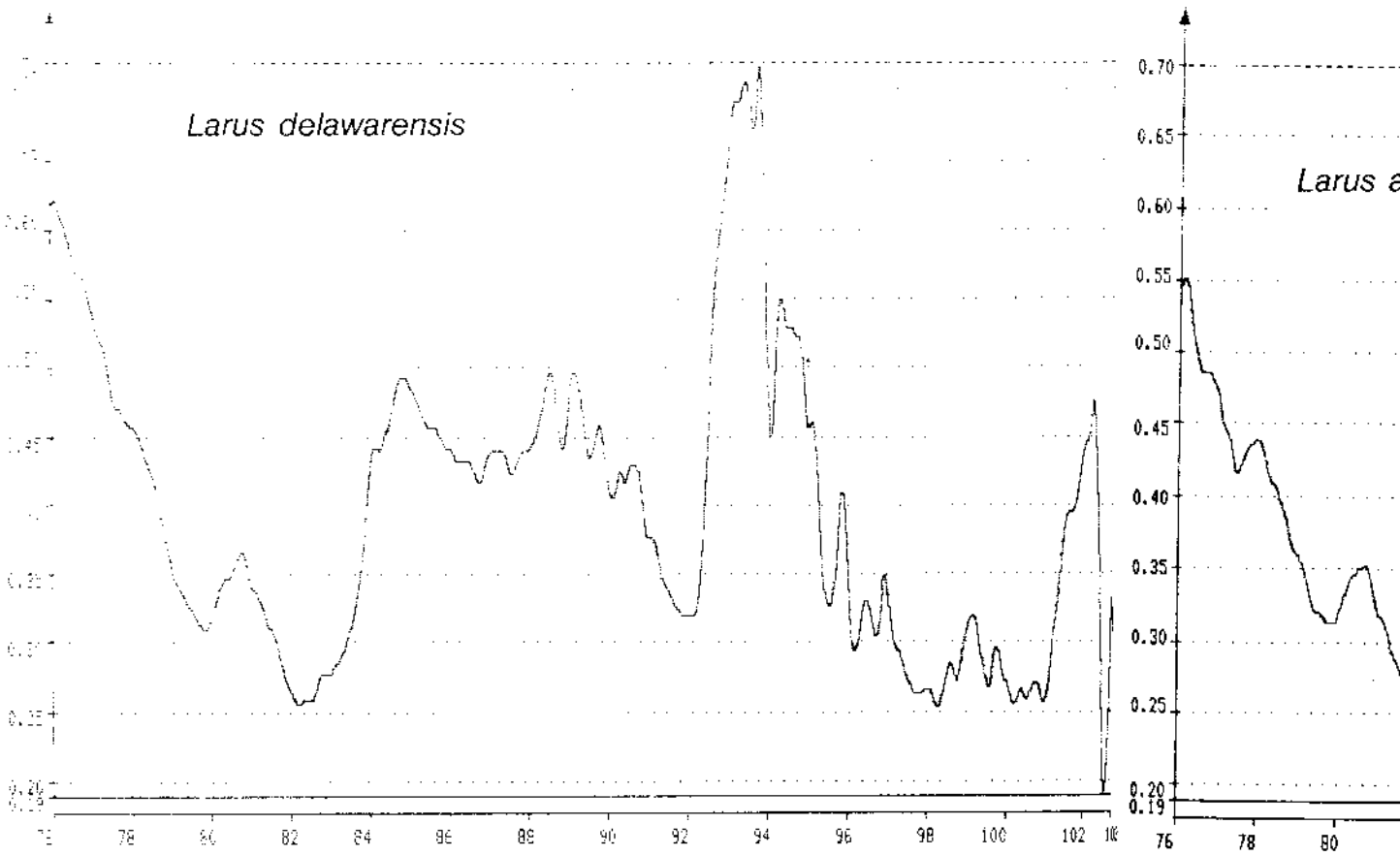


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Figure 3. Keratin profile of the Herring Gull (*Larus argentatus*).

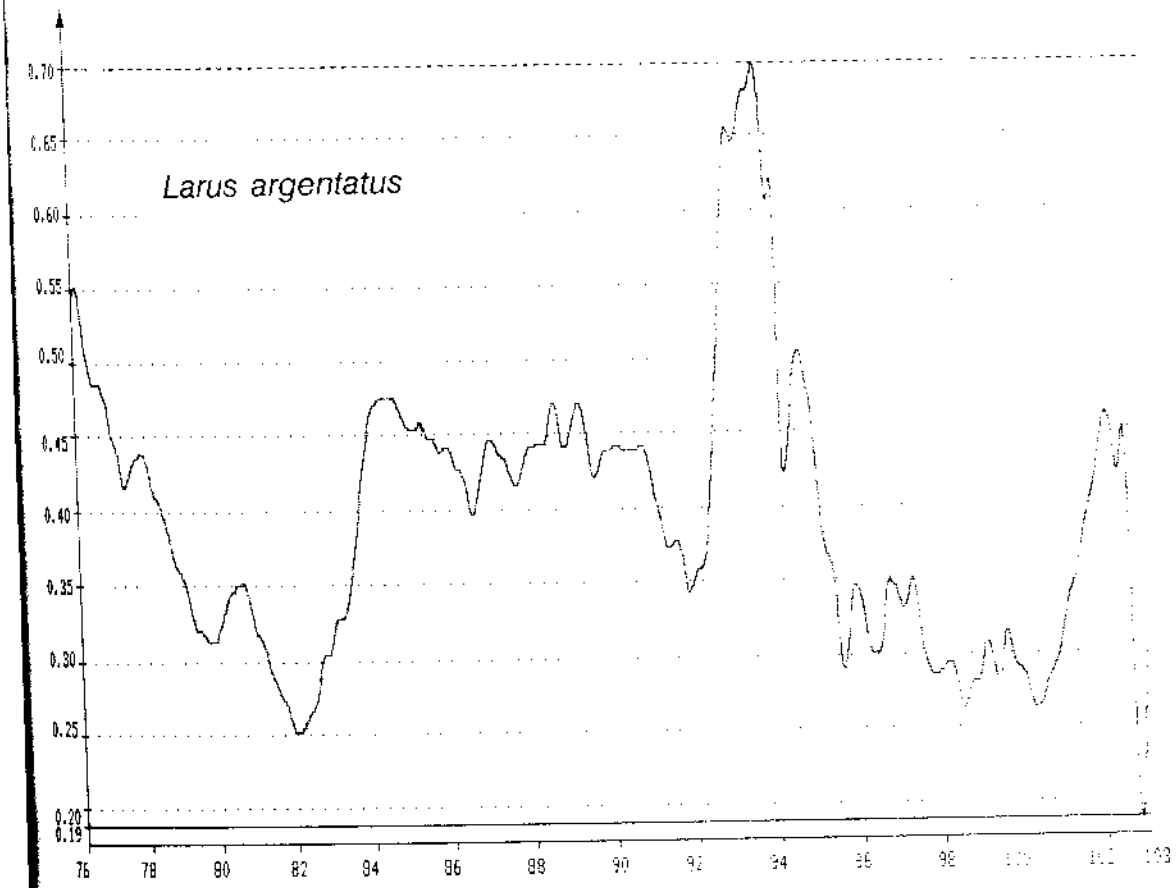
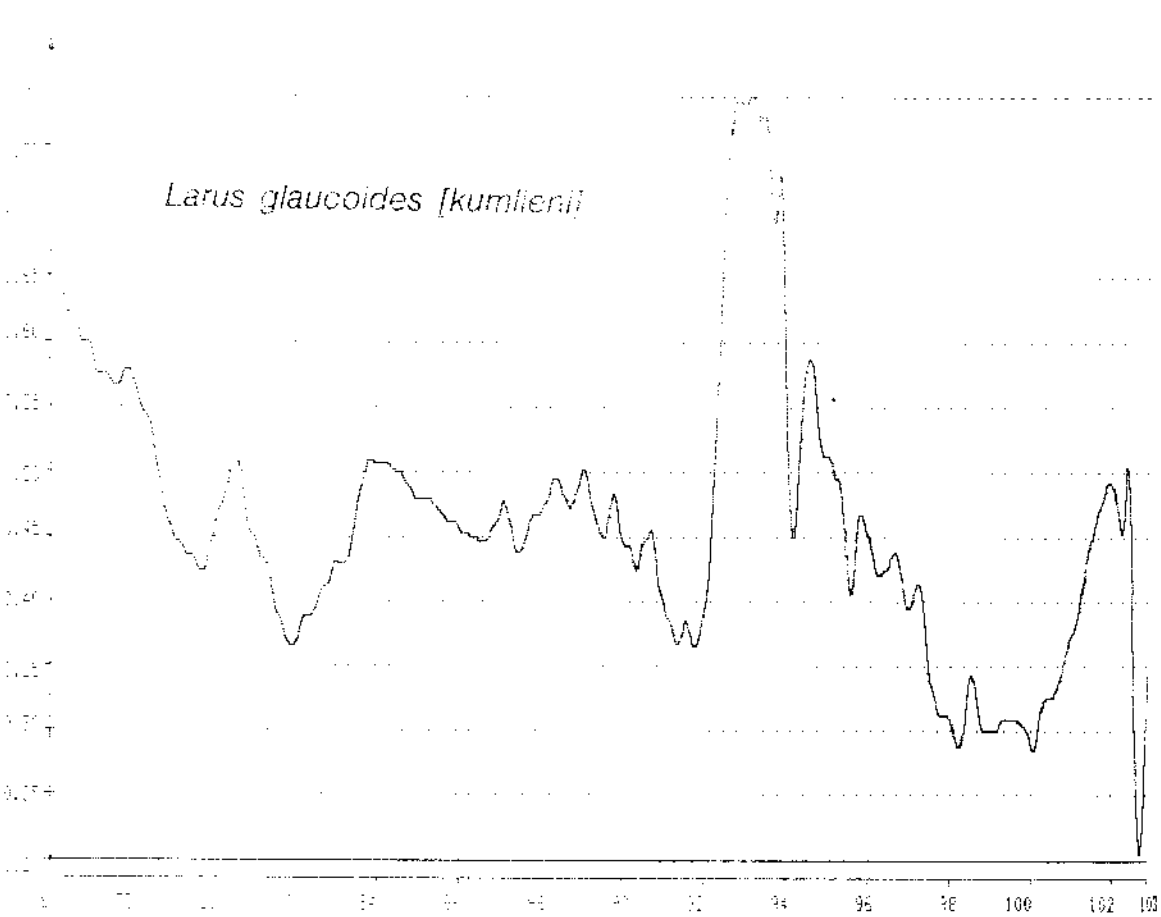


Figure 5. Ke


Larus glaucoides [kumlieni]

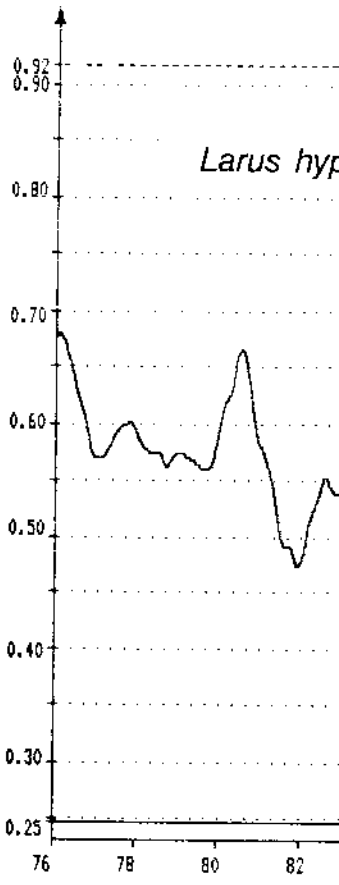
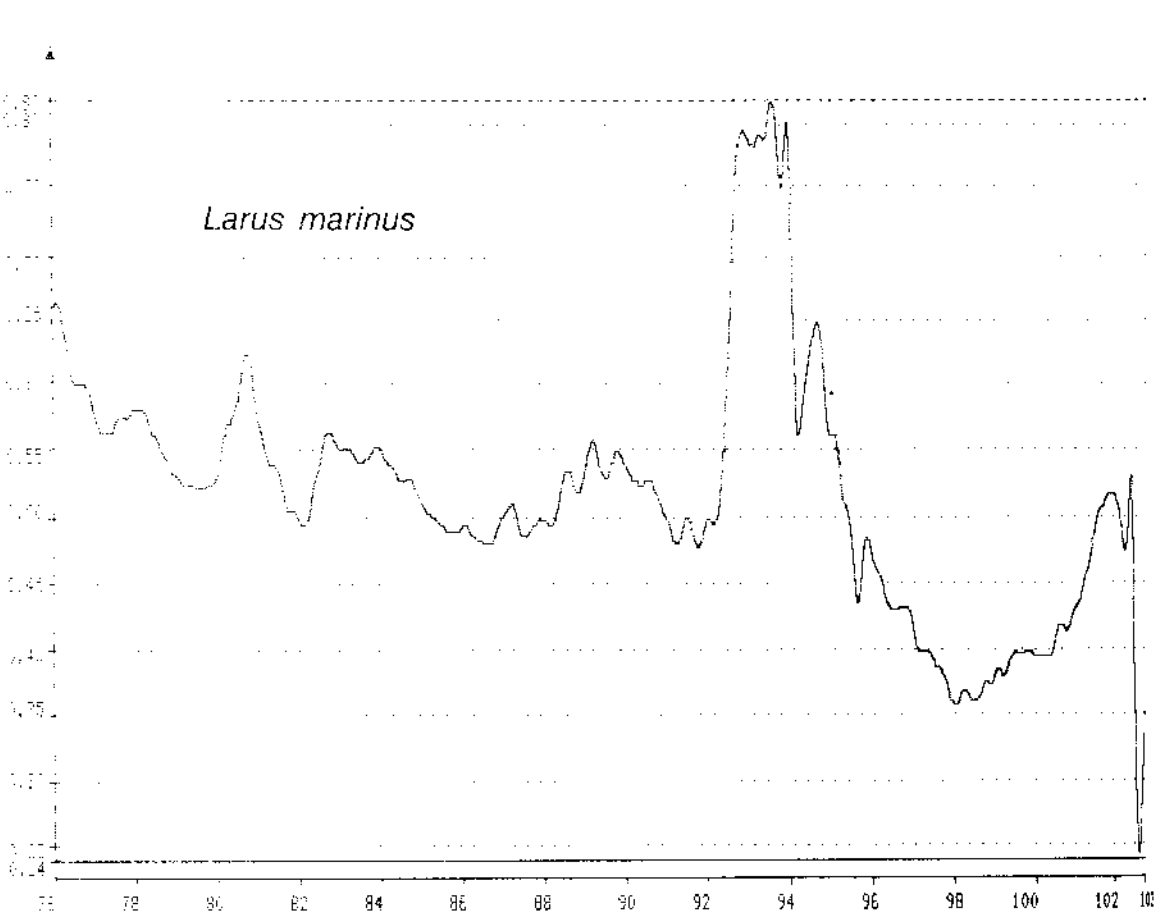

Larus hyp
0.92
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.25
76
78
80
82

Figure 6. Keratin profile of the Great Black-backed Gull (*Larus marinus*).



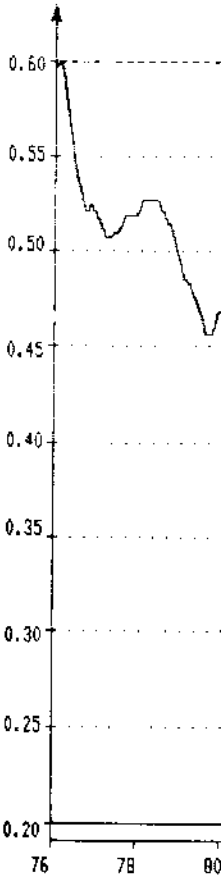


Figure 7. Keratin profile of Heermann's Gull (*Larus heermanni*).

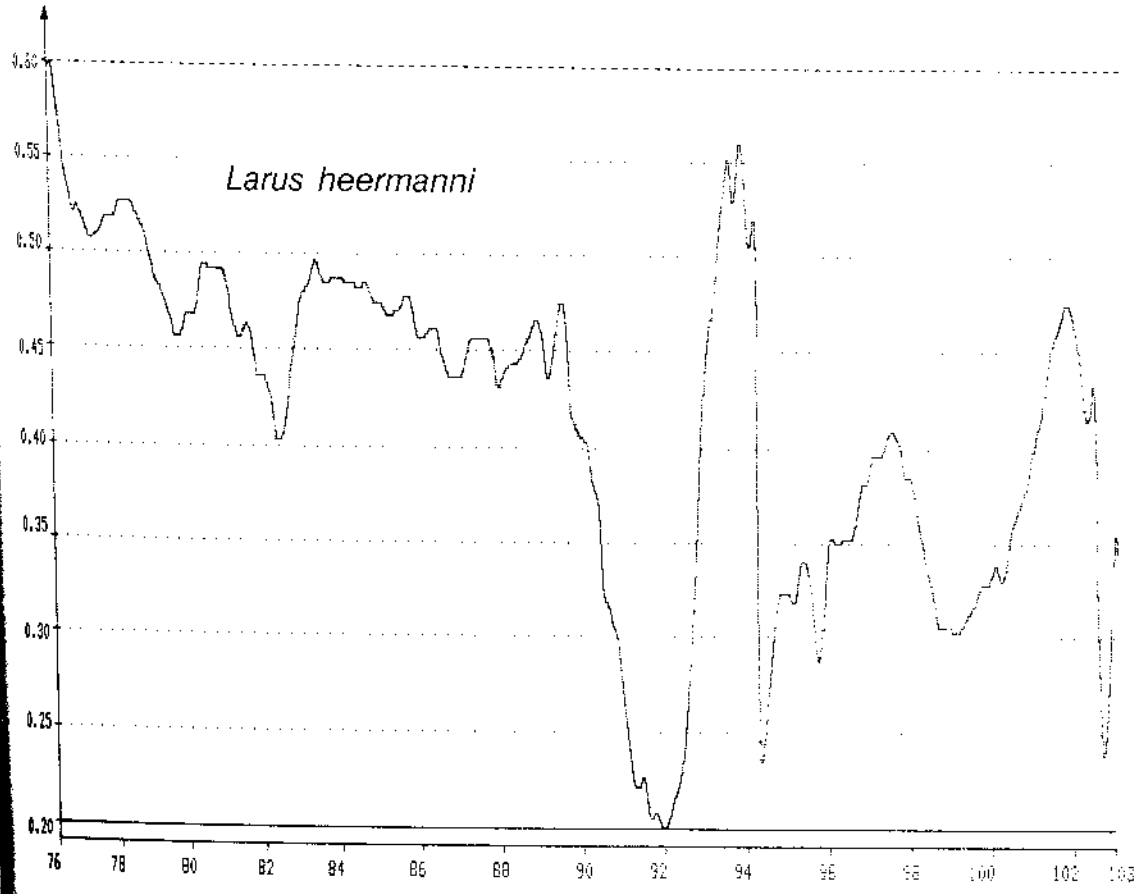
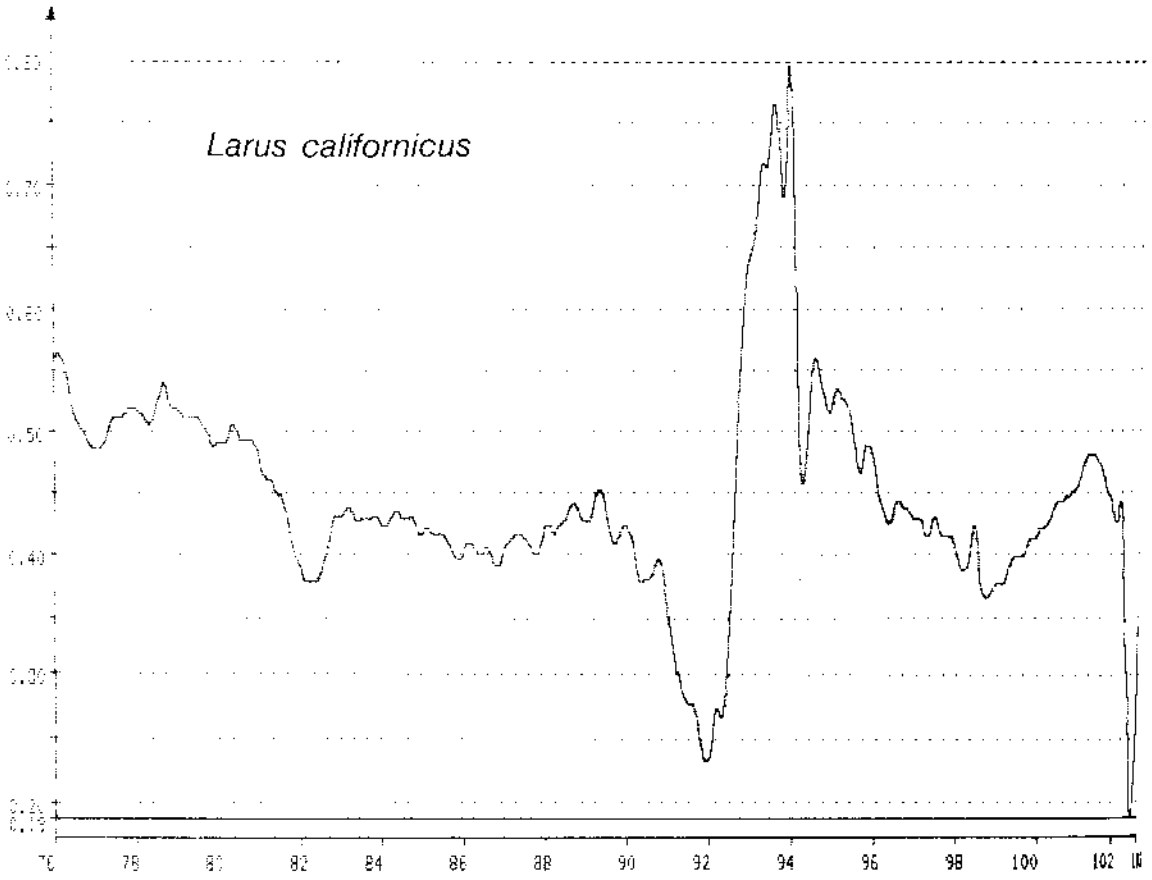


Figure 8. Keratin profile of the California Gull (*Larus californicus*).



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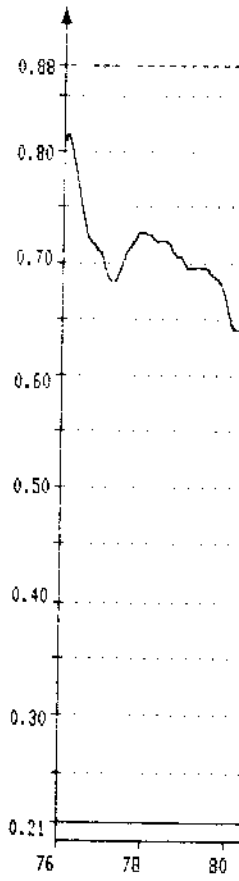

Figure 9. Keratin profile of the Glaucous-winged Gull (*Larus glaucescens*).