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Estimating nutrient intake in comparative studies of animals: an example using dietary carotenoid content in birds

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Many different methods of reporting animal diets have been used in ecological research. These vary greatly in level of accuracy and precision and therefore complicate attempts to measure and compare diets, and quantitites of nutrients in those diets, across a wide range of taxa. For most birds, the carotenoid content of the diet has not been directly measured. Here, therefore, I use an avian example to show how different methods of measuring the quantities of various foods in the diet affect the relative rankings of higher taxa (families, subfamilies, and tribes), and species within these taxa, with regard to the carotenoid contents of their diets. This is a timely example, as much recent avian literature has focused on the way dietary carotenoids may be traded off among aspects of survival, fitness and signalling. I assessed the mean dietary carotenoid contents of representatives of thirty higher taxa of birds using four different carotenoid intake indices varying in precision, including trophic levels, a coarse-scale and a fine-scale categorical index, and quantitative estimates of dietary carotenoids. This last method was used as the benchmark. For comparisons among taxa, all but the trophic level index were significantly correlated with each other. However, for comparisons of species within taxa, the fine-scale index outperformed the coarse-scale index, which in turn outperformed the trophic level index. In addition, each method has advantages and disadvantages, as well as underlying assumptions that must be considered. Examination and comparison of several possible methods of diet assessment appears to highlight these so that the best possible index is used given available data, and it is recommended that such a step be taken prior to the inclusion of estimated nutrient intake in any statistical analysis. Although applied to avian carotenoids here, this method could readily be applied to other taxa and types of nutrients.

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Biologists have been recording information about what animals eat for hundreds of years, and descriptions of animal diets range from very basic, such as simple lists of diet items placed into broad categories (e.g. vertebrates, fruit), through to minutely detailed studies including measurements of the relative quantities of different species or food types consumed. These descriptions are useful in comparative studies where there is a hypothesised link between diet and some other aspect of ecology, life history, or behaviour. However, because the recorded diets of different species may be based on highly variable methodology, level of detail, and quantity of data, comparative analyses including dietary information are often fraught with methodological difficulties. In addition, to go out and measure diets in a consistent fashion across all of the taxa one wishes to compare can amount to several lifetimes' work in itself, and so a method allowing some standardisation of existing information is preferable.

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Here, I use an avian example to show how different methodologies rank a series of higher taxa, and species within those taxa, with regard to a single dietary nutrient, in this case carotenoids. I used this example because birds employ carotenoid pigments for a number of purposes, including the pigmentation of plumage and soft tissues (Latscha 1990, Stradi 1998), and recent literature has highlighted potential links between diet and carotenoid pigmentation, mostly in the form of tradeoffs between signalling and support for the immune and reproductive systems (Lozano 1994, 2001, Olson and Owens 1998, Hill 1999). Examples of species for which such diet-pigmentation interactions have been studied or inferred include house finches (*Carpodacus mexicanus* Müller) (Brush and Power 1976, Hill 1992, 2002), northern cardinals (*Cardinalis cardinalis* Linn.) (Linville and Breitwisch 1997, McGraw et al. 2001), American goldfinches (*Carduelis tristis* Linn.) (McGraw et al. 2001), greenfinches (*Carduelis chloris* Linn.) (Lindstrom and Lundstrom 2000), and a range of popular caged birds (Stradi 1998).

Despite the level of interest in avian carotenoid physiology and signalling, very few studies have directly measured the quantity of carotenoids in the diet of a given species (but see Partali et al. 1987, McGraw et al. 2001). Furthermore, this species-specific approach is time-consuming, so it is unlikely that researchers will be able to repeat such studies on large numbers of species in a short period of time, making this line of inquiry not conducive to broadly based comparative research. I assessed the carotenoid content of avian diets using indices that take advantage of existing avian diet descriptions and literature on the carotenoid contents of a variety of animal and plant taxa. I created four potentially useful indices varying in precision and applied these to a broad range of avian taxa. This study had several objectives. I wanted to determine whether all four indices of dietary carotenoid content resulted in the same relative ranking of higher avian taxa, and species within these taxa. I also endeavoured to make recommendations regarding the best indices for use in comparative research at either level – I considered this to be one combining adequate precision with the potential for statistically robust sample sizes.

Methods

To assess dietary carotenoid content, I first collected literature data on the carotenoid contents of various avian foods. I divided these foods into a set of categories that were defined either by taxonomic groupings or by food type (Table 1). I then gathered information about dietary constituents for various bird species using verbal descriptions and/or quantitative data presented in monographs, encyclopaedic references, and journal articles. I used 30 higher avian taxa (families, subfamilies and tribes) in this study, and within each taxon I sampled ten species for which I could obtain quantitative dietary data. The preferred data format for diets was percent composition by mass or volume, however, to be able to obtain data for ten species, in about twenty percent of cases I had to accept either frequency (i.e. numbers of items) or stomach contents (i.e. numbers of stomachs in which an item was found) data. Each species was assigned four dietary carotenoid scores that varied in precision, the least precise of which was based on trophic level, followed by a coarse-scale then a fine-scale categorical index based on the carotenoid contents of avian foods, then finally a numerical estimate of dietary carotenoid concentration based on both quantitative composition data and actual carotenoid concentrations of avian foods.

Trophic levels were ranked according to assumed relative dietary carotenoid concentration, with higher scores indicating higher carotenoid content. This index assumes that herbivores have diets with higher

Table 1. Reported carotenoid contents and fine-scale rankings of avian foods. Carotenoid concentrations are means of all available literature values (Appendix 1).

Diet item	Carotenoid content (mg kg^{-1})	Rank
Mammals, offal	2.0	1
Wood fibre	6.5	2
Seeds/nuts	10.0	3
Zooplankton	30.0	4
Notostracans, cladocerans, ostracods, copepods, isopods	47.0	5
Freshwater fishes	48.0	6
Amphibians	56.0	7
Isopterans	60.0	8
Marine fishes	78.0	9
Bivalve molluscs	83.0	10
Cirripedes (barnacles), dipterans, ants	120.0	11
Fungi	130.0	12
Reptiles, birds	198.0	13
Cephalopods	230.0	14
Arachnids, myriapods, insects general	280.0	15
Nectar, sap, pollen	300.0	16
Coleopterans, hymenopterans (not ants)	310.0	17
Molluscs general	470.0	18
Hemipterans, lepidopterans, orthopterans	520.0	19
Gastropods, decapod crustaceans	730.0	20
Crustaceans general	890.0	21
Amphipods	1590.0	22
Echinoderms	2020.0	23
Plants in general	2500.0	24
Euphausids	3020.0	25
Anostracans	3170.0	26
Foliage/leaf buds	3300.0	27
Flowers/flower buds	4100.0	28
Poriferans	4500.0	29
Fruit/berries, marine detritus	7500.0	30
Freshwater detritus	7900.0	31
Algae and diatoms	35400.0	32

carotenoid contents than carnivores, with omnivores falling in between. It also assumes that a predominance of invertebrates in a carnivorous diet provides a richer carotenoid base than a predominance of vertebrates. Therefore, a species could be herbivorous (4.0), omnivorous with either a predominance of plant (3.5), invertebrate (3.0) or vertebrate (2.5) foods, or carnivorous with a clear predominance of invertebrates (2.0), vertebrates (1.0), or some mixture of the two (1.5). I tested the assumptions associated with this index by comparing the mean reported carotenoid contents of animal versus plant foods, and of invertebrate versus vertebrate foods, using the data from Table 1.

For the coarse-scale index, a set of seven diet constituent categories arranged in order of increasing carotenoid content, and broadly reflecting natural groupings of foods, was created from the information in Table 1 (Table 2). For each species, I scored diet by assigning a weight of zero to three for each diet category, depending on its incidence and relative importance in the diet. In Table 3, I present the decision rules used to determine weighting of each category. Using the categories and decision rules described, I was able to include all but the most vague and incomplete diet descriptions in the assessment of dietary carotenoid content. I calculated a diet score based on the summed and weighted proportional contributions of each diet category to the overall diet (see Table 4 for a hypothetical example). The final score for each species was therefore between one and seven, with one representing the lowest, and seven representing the highest possible dietary carotenoid content. This index assumes that all items considered within a category are similar in carotenoid content, and that there is not a great deal of overlap among categories. The same as for the diet category index, I tested these assumptions by comparing the means of the seven food categories used, again employing data from Table 1.

The third index used also ranked avian foods, but there were 32 food categories that were more narrowly defined than for the coarse rankings (Table 1). These categories were ranked according to increasing carotenoid content. Aside from the finer scale, the calculations proceeded as for the coarse-scale index. The result was a fine-scale carotenoid intake index for each species, with possible rankings ranging from 1 (mammalian prey and offal) to 32 (algae and diatoms). Because the rankings for this index are based on all items within a rank having very similar carotenoid contents, the assumption regarding overlap of categories is not as applicable here. However, this index assumes that the literature values gathered are representative of the groups to which they pertain. I was not able to test this assumption, as for some categories very little concentration data has been published.

Table 2. Diet categories used in the assessment of dietary carotenoid intake. Typical carotenoid content is the mean recorded from available literature.

Diet category	Items included in category	Typical carotenoid content (mg kg^{-1})
1	Seeds, nuts, wood	8.3
2	Vertebrates	76.4
3	Nectar, pollen, sap, exudates, lerps	300
4	Invertebrates	1062.9
5	Foliage, flowers, fungi	3300
6	Fruit	7500
7	Algae, diatoms	35 000

The final index required quantitative dietary descriptions for species. For each food item, the proportion of the diet made up of that item was multiplied by its mean carotenoid concentration (Table 1). The resulting values were totalled to give a dietary carotenoid concentration for each species. Unlike the previous three indices, which were normally distributed, this index was skewed to low values across taxa in its raw form and covered several orders of magnitude (Komolgorov-Smirnov goodness-of-fit test: $d = 0.36$, $n = 30$, $p < 0.01$), so it was log-transformed prior to comparison with other indices. This transformation improved the distribution and therefore made this index more readily comparable with the other three. As for the fine-scale ranked index, this index assumes that the carotenoid concentration values assigned to a particular type of food are representative of the group as a whole.

I performed two separate tests on this data, one among higher taxa, and a second among the species within each taxon. To compare the rankings of higher taxa among the four indices of dietary carotenoid content, I calculated the mean score of the ten species sampled for each taxon. The 30 mean values generated were normally distributed (Kolmogorov-Smirnov goodness-of-fit test: $d > 0.10$, $p > 0.05$ in all cases), so I then calculated correlation coefficients and their associated p-values for each pair of dietary carotenoid indices. I expected a priori that all associations among indices should be positive. Sample sizes for species within higher taxa were smaller ($n = 10$) and were often not normally distributed, so I used Spearman rank correlation to compare within-taxon rankings. Again, I expected positive associations. Because the sample sizes for the categories needed to test assumptions were often small and/or highly unbalanced, I tested all assumptions using nonparametric methods. Assumptions for the trophic level index were tested using Mann-Whitney U-tests. That for the coarse-scale index was tested using a Kruskall-Wallis test. All tests were performed at the 95% significance level.

Table 3. Decision rules governing assignment of scores to diet categories, based on a diet description for a given species.

Importance in diet	Decision rules
Predominant (3)	any of: 1) in a quantitative description, any food comprising more than 50% of the diet, either numerically or by mass/volume, or comprising a smaller percentage, provided that this percentage is larger than that for any other category 2) only food listed, as long as report is considered complete 3) preceded by modifiers "mainly", "largely", "primarily", or "predominantly" 4) in any list of food items, the most frequent category into which those foods fit 5) where two or more foods are listed, and separated by the conjunction "and", all shall be considered predominant
Secondary (2)	any of: 1) in a quantitative description, any food comprising between 10 and 49% of the diet, provided that other foods are dominant either numerically or by mass/volume 2) preceded by modifier "secondarily" 3) qualified by the terms "some" or "sometimes" 4) preceded by the modifier "also" and immediately following description of dominant foods 5) in any list of food items, a category into which a smaller number of those items fit than the predominant category
Tertiary (1)	any of: 1) in a quantitative description, any food comprising less than 10% of the diet, provided that other foods are dominant either numerically or by mass/volume 2) preceded by modifiers "occasionally" or "rarely" 3) qualified by the terms "once in a while", "odd". or "from time to time" 4) specified that the food is eaten only when other foods are lacking 5) preceded by the modifier "also" and following the description of secondary foods
Exclude (0)	not present in diet

Results

The trophic level index of carotenoid intake tended to arrange taxa into broad groups with well delineated mean diet categories (Fig. 1a). Taxa comprising mostly carnivores (e.g. Falconidae, Procellariidae) tended to group together, as did omnivores eating mainly animals (e.g. Hirundinidae, Charadriidae), omnivores eating mainly plants (e.g. Icterini, Rallidae), and taxa comprising mostly herbivores (e.g. Columbidae, Estrildini). One major assumption of this index, that animal and plant foods differ in carotenoid content, was not true (Fig. 2). While plant foods had a higher mean carotenoid content than did animal foods, they also exhibited much higher variability because some of the most carotenoid-rich (e.g. fruit, algae) and least carotenoid-rich (e.g. seeds) foods are of plant origin. This higher variability resulted in there being no significant difference between the two groups of foods. The second assumption, that invertebrates are more carotenoid-rich than vertebrates, was true given the data I collected (Fig. 2).

The coarse-scale index also resulted in groupings, but these were less well delineated than for the trophic level index (Fig. 1b). The highly granivorous estrildid finches (Estrildini) and predominantly frugivorous taxa (e.g. Paradisaeini, Ramphastidae) were fairly clearly separated from other taxa. However, vertebrate-feeding carnivores, granivore/insectivores and insectivores tended to vary continuously from lower to higher mean indices. The assumption that the diet categories used for this

Table 4. Sample calculation of a coarse-scale diet score for a hypothetical family of ten species. Diet categories (from Table 2), ranked by carotenoid content, are presented in the first row. The ten numbers represent the score assigned to each species (using the decision rules from Table 3). The grand rank sum is the value used in the statistical analysis, representing the indexed mean dietary carotenoid content of the diets of the family.

Category (C)	1	2	3	4	5	6	7
Species scores	2, 2, 1, 3, 3 3, 1, 2, 3 3, 2	0, 0, 0, 0, 0, 0, 0, 0, 0	0, 0, 0, 1, 0, 0, 1, 0, 0 0	3, 3, 3, 2, 2, 3, 3, 2, 1, 3	1, 0, 0, 0, 0, 0, 0, 0, 0 0	1, 1, 2, 1, 1, 2, 1, 1, 2 0	0, 0, 0, 0, 0, 0, 0, 0, 0 0
Total (T)	22	0	2	25	1	12	0
	Grand total (GT) = sum of all category totals = 62						
T/GT	0.355	0.000	0.032	0.403	0.016	0.194	0.000
(T/GT)*C	0.355	0.000	0.096	1.612	0.080	1.164	0.000
	Grand rank sum = sum of all values in preceding row = 3.31						

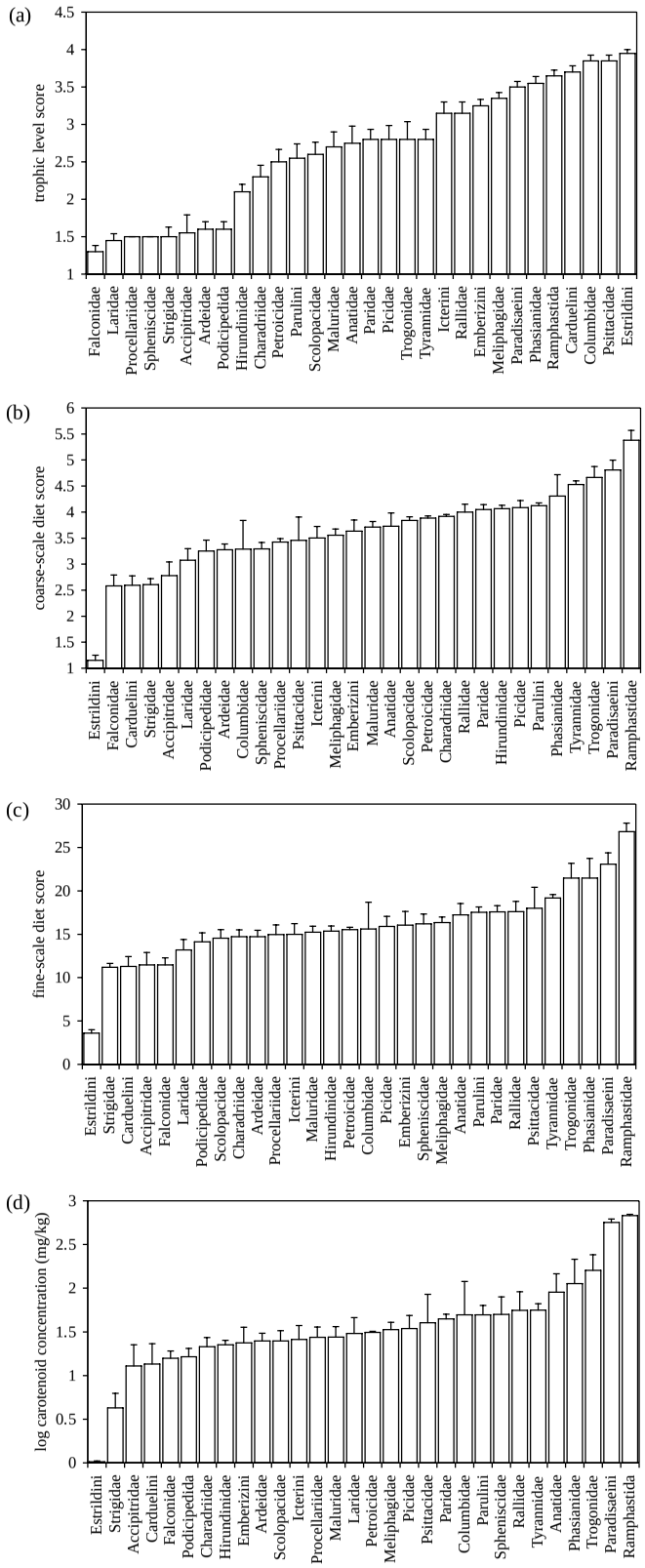

Fig. 1. Ordering of avian taxa by estimated carotenoid content of diet by four different methods: (a) trophic level categories ranked by assumed relative carotenoid content; (b) coarse (1–7) continuous scale with rankings; (c) fine (1–32) continuous scale with rankings; (d) quantitative estimate using proportional diet data and actual carotenoid concentrations. Error bars are standard errors.

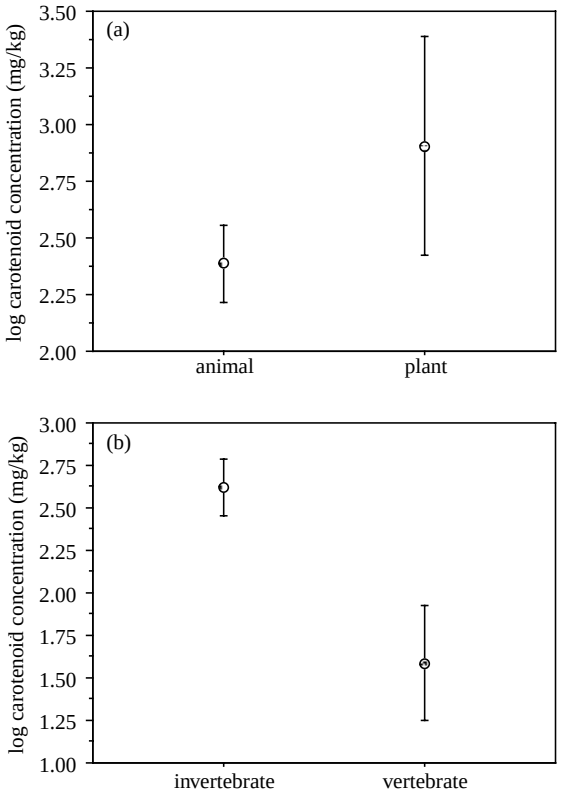

Fig. 2. Tests of the assumption that the diets of avian taxa occupying different trophic levels differ significantly in carotenoid content. Error bars are standard errors. (a) Mean carotenoid concentration of plant and animal foods did not differ significantly (Mann–Whitney U-test: $n_{animal} = 22$, $n_{plant} = 8$, $z = -1.45$, $p = 0.15$). (b) Mean carotenoid concentration of invertebrates was significantly higher than that of vertebrates (Mann–Whitney U-test: $n_{invert} = 17$, $n_{vert} = 5$, $z = -2.39$, $p = 0.02$).

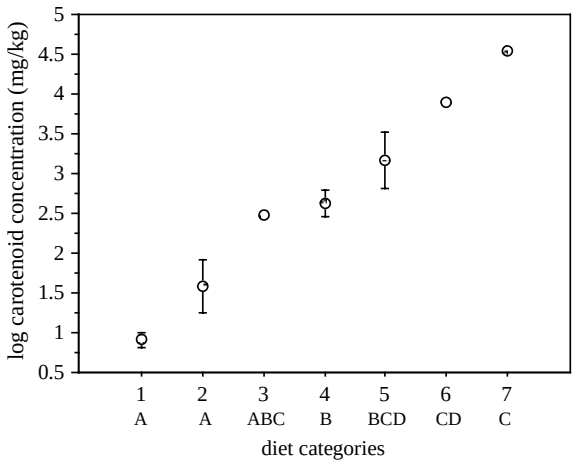

Fig. 3. Test of the assumption that coarse-scale diet categories (1–7 from Table 2) differ significantly in mean carotenoid content. Error bars are standard errors. Diet categories sharing letters (shown underneath category numbers) did not differ significantly. Means did not differ between adjacent categories, but often differed among more distant ones (Kruskall–Wallis test: $df = 6$, $H = 18.36$, $p = 0.005$).

index did not overlap significantly in carotenoid concentration was not met. Adjacent categories did not differ from one another. Categories separated by more than one rank were often, but not always, significantly different (Fig. 3). The fine-scale index exhibited more continuous variation across taxa, again with estrildids and highly frugivorous taxa standing out (Fig. 1c). Finally, the quantitative estimates based on percent diet composition and actual carotenoid concentrations showed a similar pattern, but there was much less accordance with trophic levels (Fig. 1d).

The rankings of higher taxa resulting from each dietary carotenoid index were always positively correlated, but those based on trophic levels exhibited low, non-significant, correlations with the other three indices (simple linear correlation coefficients: trophic vs coarse-scale: $r = 0.21$; trophic vs fine-scale: $r = 0.29$; trophic vs quantitative estimate: $r = 0.26$, $p > 0.10$ in all cases). The three more precise indices were highly intercorrelated (coarse-scale vs fine-scale: $r = 0.94$; coarse-scale vs quantitative index: $r = 0.88$; fine-scale vs quantitative index: $r = 0.96$, $p < 0.0001$ in all cases).

Within avian taxa, rankings of species were positively correlated across all indices of carotenoid intake in only a few cases. For most others, however, rankings were dissimilar for certain pairs of indices, particularly when comparing the trophic level index with any of the more precise indices (Table 5). For 19 of the 30 taxa used in this study, the trophic level index of dietary carotenoid intake bore little or no relationship to any of the indices in which foods were ranked by actual carotenoid content. Occasionally, rankings by trophic level were even significantly negatively correlated with at least one of the more precise indices. Among the coarse-scale, fine-scale, and quantitative indices of carotenoid intake, I observed higher levels of correlation, and no negative correlations. The coarse-scale index was at least marginally correlated with both the fine-scale and quantitative index for 20 of 30 taxa, while the fine-scale index was correlated for 29 of 30 taxa (Table 5).

Discussion

The four indices used here to assess dietary carotenoid content varied in degree of precision. The most precise index, however, required data that was often limited in availability, which meant that quantifications not based on mass or volumetric measures had to be used. Therefore, what would have been perhaps the best index of carotenoid intake may not be practical for performing wide-ranging comparative analyses where diet descriptions vary greatly in level of detail. Conversely, the tro-

Table 5. Significance of Spearman rank correlations among species within higher avian taxa for four indices of dietary carotenoid content varying in precision. T = trophic level, C = coarse-scale index, F = fine-scale index, Q = quantitative estimate. Total shown is number of significant or marginal correlations that were in the expected direction (positive).

Taxon	T vs C	T vs F	T vs Q	C vs F	C vs Q	F vs Q
Accipitridae	**	*	*	**	*	**
Anatidae	ns	ns	ns	***	*	*
Ardeidae	ns	ns	ns	**	**	*
Carduelini	ns	ns	ns	*	**	**
Charadriidae	inv m	ns	ns	ns	ns	**
Columbidae	ns	ns	ns	***	***	***
Emberizini	ns	ns	ns	*	*	***
Estrildini	inv *	inv *	inv *	***	***	***
Falconidae	m	ns	ns	ns	m	m
Hirundinidae	***	ns	ns	ns	ns	**
Icterini	inv m	inv m	inv m	*	ns	*
Laridae	*	*	*	**	**	***
Maluridae	inv ***	ns	ns	ns	ns	**
Meliphagidae	inv m	*	*	ns	ns	***
Paradisaeini	*	*	*	***	m	m
Paridae	ns	*	*	*	m	**
Parulini	**	*	ns	*	m	*
Petroicidae	inv **	ns	ns	m	m	m
Phasianidae	ns	ns	ns	**	**	**
Picidae	ns	*	*	*	**	***
Podicipedidae	**	**	*	**	*	*
Procellariidae	n/a	n/a	n/a	ns	ns	m
Psittacidae	ns	ns	m	***	***	***
Rallidae	ns	ns	ns	**	*	*
Ramphastidae	*	*	*	***	*	*
Scolopacidae	inv **	ns	ns	ns	ns	**
Spheniscidae	n/a	n/a	n/a	*	*	***
Strigidae	m	ns	ns	*	ns	ns
Trogonidae	**	*	**	**	***	***
Tyranninae	ns	ns	ns	ns	ns	*
Total	10	10	9	22	21	29

n/a = no trophic level variation, ns = not significant, inv = correlation negative (shown for marginal and significant correlations only), m = marginal ($p \leq 0.10$), * = $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.005$.

phic level index required the simplest diet descriptions, which were more easily obtainable from the literature. However, it was not correlated with the indices ranked according to carotenoid content among higher taxa, and was only occasionally correlated with these other indices among species within a taxon.

Furthermore, the carotenoid contents of plant and animal-based diets do not appear to differ in a predictable fashion, as is frequently assumed (Gray 1996, Badyaev and Hill 2000), and for a few taxa there was no variation in trophic level among the species sampled. The trophic level index was therefore considered a less reliable estimate of the true carotenoid contents of avian diets compared to the other indices. That said, where the plant-based portion of the diet within a taxon is restricted to only the low (e.g. seeds, nectar) or high (e.g. foliage, fruit) end of the carotenoid scale, the trophic level index could be considered workable. Examples of such taxa used in this study include the toucans (Ramphastinae) and trogons (Trogonidae), whose plant foods typically include only high-carotenoid items such as fruit.

Both the coarse-scale and fine-scale indices were strongly correlated with the index based on actual carotenoid concentrations and quantitative diet composition data, and so either one can be considered useful for comparative studies. However, to be able to use all of the species' diet descriptions in the fine-scale index, I had to create some rather artificial "general" categories that lumped, for example, all insects or all crustaceans, into one category that employed the mean of all of its component subcategories (Table 1). Therefore, if one were to be strict and not use general categories, some data would be lost for the fine-scale index.

Given that all but the trophic level index were strongly correlated, it must be concluded that each of the remaining three indices is suitable for comparing the dietary carotenoid contents of avian species. However, the choice of index should take into consideration the nature of the available diet data. I recommend that the coarse-scale index be employed in comparative analyses of carotenoid intake across a broad range of taxa, where the quality of diet data and/or method of data collection, are likely to be highly variable. For studies where detailed qualitative data is available for all species, a fine-scale index would likely be more appropriate. These indices could also be adapted to suit situations where quantitative data has been gathered, but the diet categories are broad. Applicable examples include a

study of several groups of Neotropical nonpasserines, where the proportions of the diet consisting of vertebrates, arthropods and fruit were recorded (Remsen et al. 1993), and also among the tanagers (Fringillidae, Thraupini), where the relative amount of fruit, seeds and insects is the most frequent form of diet record (Isler and Isler 1999). Here, the mean carotenoid contents of these food types could be multiplied by their respective proportions, rather than by an index of dietary predominance. Finally, where quantitative data regarding the proportions of various specific foods in the diet is available for all species, the quantitative index should be used, as this index is especially suitable for comparisons within a taxon where diets have been particularly well studied, for example, ducks (Anatidae) and New World warblers (Parulini).

In this paper, I have identified that reporting of animal diets and measurements of nutritional content of foods can vary considerably across studies, and that such variability needs to be assessed and, if necessary, controlled, before any form of comparative analysis can be undertaken. It must also be noted, however, that nutrients can occur in different forms depending on the dietary item in question, and so nutrient content alone is only an estimate of actual nutrient availability. For example, carotenoids can occur as free molecules and as mono-or diesters (Latscha 1990). These forms, as well as individual carotenoids (e.g. lutein, β-carotene), vary with respect to bioavailability (van het Hof et al. 1999). This study does not take into account variation in such aspects of biochemistry or digestive physiology, because at present we lack detailed information for many dietary items and most avian species. Further refinements of this technique are possible and should be attempted, but will require a deeper understanding of the ways in which birds (and other animals) process dietary carotenoids, and vary in such processing.

Using the example of carotenoids in avian diets, I have demonstrated one potential means of incorporating data varying in quality and detail into comparative analyses. Performing investigative studies such as this allows the relative advantages and disadvantages of various methods to be examined before any detailed comparative analyses are performed. I further suggest that, where possible, assumptions of relative nutrient content frequently associated with animal diets must be tested prior to analysis. Although applied to avian carotenoids in this case, this sort of method could readily be applied to other types of nutrients (e.g. proteins, vitamins, etc...) across a wide range of taxa, and could be further developed as we gain a greater understanding of foraging, nutritional biochemistry, and digestive physiology in wild populations of animals.

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References

Badyaev, A. V. and Hill, G. E. 2000. Evolution of sexual dichromatism: contribution of carotenoid- versus melanin-based coloration. – Biol. J. Linn. Soc. 69: 153–172.

Brush, A. H. and Power, D. M. 1976. House finch pigmentation: carotenoid metabolism and the effect of diet. – Auk 93: 725–739.

Gray, D. A. 1996. Carotenoids and sexual dichromatism in North American passerine birds. – Am. Nat. 148: 453–480.

Hill, G. E. 1992. Proximate basis of variation in carotenoid pigmentation in male house finches. – Auk 109: 1–12.

Hill, G. E. 1999. Is there an immunological cost to carotenoid-based ornamental coloration. – Am. Nat. 154: 589–595.

Hill, G. E. 2002. A red bird in a brown bag: the function and evolution of colorful plumage in the house finch. – Oxford Univ. Press.

Isler, M. L. and Isler, P. R. 1999. The tanagers: natural history, distribution, and identification. – Smithsonian Inst. Press.

Latscha, T. 1990. Carotenoids: their nature and significance in animal feeds. – F. Hoffmann-LaRoche, Basel.

Lindstrom, K. and Lundstrom, J. 2000. Male greenfinches (*Carduelis chloris*) with brighter ornaments have higher virus infection clearance rate. – Behav. Ecol. Sociobiol. 48: 44–51.

Linville, S. U. and Breitwisch, R. 1997. Carotenoid availability and plumage coloration in a wild population of northern cardinals. – Auk 114: 796–800.

Lozano, G. 1994. Carotenoids, parasites and sexual selection. – Oikos 70: 309–311.

Lozano, G. 2001. Carotenoids, immunity and sexual selection: comparing apples and oranges. – Am. Nat. 158: 200–203.

McGraw, K. J., Hill, G. E., Stradi, R. et al. 2001. The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American goldfinches (*Carduelis tristis*) and northern cardinals (*Cardinalis cardinalis*). – Physiol. Biochem. Zool. 74: 843–852.

Olson, V. A. and Owens, I. P. F. 1998. Costly sexual signals: are carotenoids rare, risky, or required. – Trends Ecol. Evol. 13: 510–514.

Partali, V., Liaanen-Jensen, S., Slagsvold, T. et al. 1987. Carotenoids in food chain studies II. The food chain of Parus spp. monitored by carotenoid analysis. – Comp. Biochem. Physiol. 87B: 885–888.

Remsen, J. V., Hyde, M. A. and Chapman, A. 1993. The diets of Neotropical trogons, motmots, barbets, and toucans. – Condor 95: 178–192.

Stradi, R. 1998. The colour of flight: carotenoids in bird plumage. – Solei Gruppo Editoriale Informatico, Milan.

van het Hof, K. H., Brouwer, I. A., West, C. E. et al. 1999. Bioavailability of lutein is five times higher than that of β-carotene. – Am. J. Clin. Nutr. 70: 261–268.

Appendix. List of literature sources used for carotenoid contents of avian foods.

Ambarsari, I., Brown, B. E., Barlow, R. G. et al. 1997. Fluctuations in algal chlorophyll and carotenoid pigments during solar blanching in the coral *Goniastrea aspera* at Phuket, Thailand. – Mar. Ecol. Prog. Ser. 159: 303–307.

Bernard, F., Beers, K. and Sherma, J. 1993. Thin-layer chromatographic analysis of beta-carotene and lutein in

Echmostoma trivolvis (Trematoda) rediae. – J. Parasitol. 79: 113–114.
Czeczuga, B. 1985a. Changes in the content of carotenoids in specimens *of Helix pomatia* (L.) (Mollusca: Gastropoda: Helicidae) in the period of their life activity. – Comp. Biochem. Physiol. 80B: 657–660.
Czeczuga, B. 1985b. Investigations on carotenoids in insects. VII. Contents of carotenoids in worker bees feeding on flowers of different plants. – Zool. Pol. 32: 183–190.
Czeczuga, B. and Weyda, F. 1982. Investigation on carotenoids in insects V. Archaeagnatha (Insects, Apterygota). – Zool. Pol. 29: 23–31.
Dafni, A. 1992. Pollination ecology: a practical approach. – IRL Press, Oxford.
Elmadfa, I. and Majchrzak, D. 1998. Carotenoids and vitamin A in fish. – Z. Ernahrungswiss. 37: 207–210.
Goodwin, T. W. 1980. The biochemistry of the carotenoids. Vol. I. Plants. – Chapman & Hall.
Goodwin, T. W. 1984. The biochemistry of the carotenoids. Vol. II. Animals. – Chapman & Hall.
Hertzberg, S., Bergquist, P. and Liaanen-Jensen, S. 1989. Further occurrence of sulphated carotenoids in *Ianthella* species (Demospongia). – Biochem. Syst. Ecol. 17: 57–53.
Katagiri, K., Maoka, T. and Matsuno, T. 1986. Carotenoids of shell fishes-VIII. Comparative biochemical studies of carotenoids in three species of spindleshell, *Fusinus perplexus, F.p. ferrugineus* and *F. forceps.* – Comp. Biochem. Physiol. 84B: 473–476.
Kayser, H. 1982. Carotenoids in stick insects (Phasmida). A quantitative comparison of six species at major developmental stages. – Comp. Biochem. Physiol. 72B: 427–432.
Krinsky, N. I., Mathews-Roth, M. M, and Taylor, R. F. (eds) 1989. Carotenoids: chemistry and biology. – Plenum.
Maoka, T., Yokoi, S. and Matsuno, T. 1989. Comparative biochemical studies of carotenoids in nine species of Cephalopoda. – Comp. Biochem. Physiol. 92B: 247–250.
Masson, G., Baumes, R., Puech, J.-L. et al. 1997. Demonstration of the presence of carotenoids in wood: quantitative study of cooperage oak. – J. Agric. Food Chem. 45: 1649–1652.
Matsuno, T., Maoka, T. and Toriiminami, Y. 1990. Carotenoids in the Japanese stick insect *Neophirasea japonica.* – Comp. Biochem. Physiol. 95B: 583–587.
Matsuno, T. and Tsushima, M. 1995. Comparative biochemical studies of carotenoids in sea cucumbers. – Comp. Biochem. Physiol. 11IB: 597–605.
Metusalach, J., Synowiecki, J., Brown, J. et al. 1996. Deposition and metabolism of dietary canthaxanthin in different organs of arctic charr *(Salvelinus alpinus L.).* – Aquaculture 142: 99–106.
Murugan, G., Nelis, H. J., Dumont, H. J. et al. 1995. Cis- and all-trans-canthaxanthin levels in fairy shrimps. – Comp. Biochem. Physiol. HOB: 799–803.
Noack, P. T., Laird, L. M., and Prieole, I. G. 1997. Carotenoids of sea lice *(Lepeophtheirus salmonis)* as potential indicators of host Atlantic salmon *(Salmo salar* L.) origin. – ICES J. Mar. Sci. 54: 1140–1143.
Partali, V., Liaanen-Jensen, S., Slagsvold, T. et al. 1987. Carotenoids in food chain studies II. The food chain of *Parus* spp. monitored by carotenoid analysis. – Comp. Biochem. Physiol. 87B: 885–888.
Sagi, A., Rise, M., Isam, K. et al. 1995. Carotenoids and their derivatives in organs of maturing female crayfish *Cherax quadricarinatus.* – Comp. Biochem. Physiol. 112B: 309–313.
Terra, W. R., Ferreira, C. and de Bianchi, A. G. 1980. Carotenoids from midgut and from haemolymph proteins of *Rhynchosciara* (Diptera: Sciaridae) and their metabolic implications. – Comp. Biochem. Physiol. 66B: 491–497.
Tsushima, M., Katsuyama, M. and Matsuno, T. 1997. Metabolism of carotenoids in the apple snail, *Pomacea canaliculata.* – Comp. Biochem. Physiol. 118B: 431–436.
Valadon, L. R. G. and Mummery, R. S. 1978. A comparative study of carotenoids in *Papilio* spp. – Comp. Biochem. Physiol. 61B: 371–374.
Vershinin, A. 1996. Carotenoids in mollusca: approaching the functions. – Comp. Biochem. Physiol. 113B: 63–71.
Virtue, P., Johannes, R. E., Nichols, P. D. et al. 1995. Biochemical composition of *Nyctiphanes australis* and its possible use as an aquaculture feed source: lipids, pigments and fluoride content. – Mar. Biol. 122: 121–128.
Yamashita, E. and Matsuno, T. 1990. A new apocarotenoid from the sea hare *Aplysia kurodai.* – Comp. Biochem. Physiol. 96B: 465–470.
Yamashita, E., Arai, S. and Matsuno, T. 1996. Metabolism of xanthophylls to vitamin A and new apocarotenoids in liver and skin of black bass, *Micropterus salmoides.* – Comp. Biochem. Physiol. 113B: 485–489.