Stable Isotopes Link Diet to Avian Yolk Carotenoid Allocation: A Comparative Study of Five Auk Species (Charadriiformes: Alcidae)

J. Mark Hipfner[1,*]
Keith A. Hobson[2]
James Dale[3]
Kevin J. McGraw[4]

[1]Centre for Wildlife Ecology, RR 1 5421 Robertson Road, Delta, British Columbia V4K 3N2, Canada; [2]Environment Canada, 11 Innovation Boulevard, Saskatoon, Saskatchewan S7N 3H5, Canada; [3]Department of Behavioural Ecology and Evolutionary Genetics, Max Planck Institute for Ornithology, P.O. Box 1564, D-82305 Starnberg, Germany; [4]School of Life Sciences, Arizona State University, Tempe, Arizona 85287-4501

ABSTRACT

The allocation of important but environmentally limited nutrients, such as carotenoids, often represents a trade-off between homeostasis and reproduction. However, key questions remain about how diet and species traits influence carotenoid allocation. We studied yolk carotenoid profiles and yolk color in relation to trophic level (based on $\delta^{15}N$ values) in five species of seabirds belonging to the family Alcidae: common murre (*Uria aalge*), pigeon guillemot (*Cepphus columba*), Cassin's auklet (*Ptychoramphus aleuticus*), rhinoceros auklet (*Cerorhinca monocerata*), and tufted puffin (*Fratercula cirrhata*). In three species, which ranged from low (tufted puffin) to high (pigeon guillemot) trophic level, yolks had pink to red hues and contained exclusively astaxanthin, while yolks of species from a high trophic level (common murre) and from a generalist forager (rhinoceros auklet) had yellow to orange hues and contained astaxanthin, lutein, and zeaxanthin. The absence of a systematic relationship between trophic level and carotenoid types suggests that these species possess adaptations for the uptake and use of specific carotenoids. In contrast, total yolk carotenoid concentrations were best explained by the combination of species and trophic level: lower-trophic-level feeding was linked to production of carotenoid-rich yolks, both across species and within the one generalist species. We conclude that both behavioral and physiological traits can play strong roles

* Corresponding author; e-mail: mark.hipfner@ec.gc.ca.

in the acquisition and allocation of critical nutrients from mothers to their offspring.

Introduction

Key nutrients that are limited in availability often trade-off between homeostasis and reproduction (Stearns 1992; Ricklefs and Wikelski 2002; Monaghan et al. 2008). Carotenoids are promising candidate nutrients for investigating such fitness trade-offs because (1) they are potentially limited to consumers since they are synthesized only by primary producers; yet (2) they play important roles in immune system function and as antioxidants in adults and offspring of many species (Olson and Owens 1998). For example, in many oviparous vertebrates, females deposit carotenoids into egg yolk to protect their offspring against oxidative stress and to boost their developing immune systems (Blount et al. 2000; Tyndale et al. 2008). However, this maternal investment in offspring may carry a cost to the mother's own physiological requirements for carotenoids (Blount et al. 2004; Biard et al. 2005; Bertrand et al. 2006; McGraw 2006).

Carotenoid ecology and physiology has been studied in considerable detail in birds in comparison to other free-ranging vertebrates (Surai et al. 2001*b*, 2003; Blount 2004; Olson and Owens 2005). Indeed, a number of studies have shown that the availability of dietary carotenoids can limit avian egg-laying capacity (Blount et al. 2004; Biard et al. 2005; Hargitai et al. 2006). However, it is still poorly understood exactly how breeding females differentially obtain and allocate these potentially limiting nutrients (Bortolotti et al. 2003; McGraw 2005; Isaksson et al. 2008).

The kinds and concentrations of carotenoids found in avian tissues vary widely among species. This is due to differences in diet (Olson 2006) and to species-specific ecological, behavioral, and physiological adaptations for carotenoid uptake and utilization (Surai et al. 2001*b*; Blount et al. 2002; Tella et al. 2004; Cassey et al. 2005). It can be difficult to quantify how important diet and species traits (such as ornamental carotenoid-based pigmentation) are to carotenoid allocation, however, because these characteristics are often similar within phylogenetic lineages (Cassey et al. 2005). This can confound attempts to partition the variance because of each causal factor in isolation. One approach to resolving this issue is to study carotenoid allocation in closely related species selected specifically because they differ markedly in diet (Mahler et al. 2003). Yet there have been few such studies to date (McGraw 2005), despite their

potential to highlight critical constraints on fitness and the evolution of life histories.

In this study, we investigated the relative importance of diet and (unidentified) species traits on avian yolk carotenoid allocation in five coexisting species of auks (family Alcidae). This small family of 23 exclusively marine bird species exhibits marked interspecific variation in diet (Gaston and Jones 1998). Trophic level and carotenoid concentrations tend to be negatively related in avian foods (Olson 2006), including marine-derived foods (Matsuno 2001). Therefore, we compared trophic level (based on yolk $\delta^{15}N$ values) and the variety of types and concentrations of carotenoids found in bird prey items, including both low-trophic-level crustaceans and higher-trophic-level fish, to those correspondingly found in bird egg yolks. Further, carotenoids are important factors in the pigmentation of egg yolk (Karunajeewa et al. 1984), and we have noticed that egg yolk color in auks varies dramatically from pale to bright and from yellow to orange to red (J. M. Hipfner, personal observation). We therefore also investigated relationships between egg yolk color and carotenoid profiles.

We tested a series of specific predictions: (1) that within and between species, there would be marked variation in yolk carotenoid types and amounts and that this variation would correspond to variation in yolk coloration; (2) that species feeding across a broader trophic range would produce yolks with a broader suite of carotenoid types; and (3) that species feeding on carotenoid-rich, lower-trophic-level prey (Olson 2006) would produce yolks with higher total carotenoid concentrations.

Material and Methods

Study Site and Species

Our study took place in 2002 on Triangle Island, British Columbia, Canada (50°52′N, 129°05′W), which lies within an extremely productive but annually and seasonally variable marine ecosystem (Ware and Thomson 2005). In 2002, local primary production was close to the long-term average (Mackas et al. 2007). Our study involved common murre (*Uria aalge*), pigeon guillemot (*Cepphus columba*), Cassin's auklet (*Ptychoramphus aleuticus*), rhinoceros auklet (*Cerorhinca monocerata*), and tufted puffin (*Fratercula cirrhata*). These five species include at least one member of four of the six "distinct lineages" of the Alcidae (Friesen et al. 1996).

Egg Collections

The timing of egg laying at Triangle Island varies markedly among our five study species (Davies et al. 2009). We collected single, fresh eggs laid early in each species' laying period from 8 Cassin's auklets (March 28–April 1), 10 rhinoceros auklets (April 22–May 3), 6 tufted puffins (May 9–14), 10 common murres (June 4–9), and 3 pigeon guillemots (June 8–12). The eggs were measured (length and maximum breadth to ±0.1 mm with dial calipers; mass to ±0.1 g with an electronic balance), then placed in a freezer at −10°C in the field. The frozen

eggs were later cut in half with a miter saw, and 1-mL samples were removed from the center of the yolk for laboratory analyses.

Prey Collections

We obtained whole prey items typically consumed by seabirds at Triangle Island (Hedd et al. 2006; Hipfner 2009) and spanning a range of trophic levels. These included fresh-frozen, whole specimens of two crustaceans, the copepod *Neocalanus cristatus* and the euphausiid *Thysanoessa spinifera*, which were collected on shipboard plankton tows along the continental shelf margin near the island (Mackas et al. 2007). They also included whole specimens of unspecified squid, Pacific saury (*Cololabis saira*), juvenile (young-of-year) rockfish (*Sebastes* sp.), and adult and juvenile Pacific sandlance (*Ammodytes hexapterus*), which we collected from provisioning rhinoceros auklets. These items were placed in a freezer at −10°C in the field.

Laboratory Analyses

Stable-nitrogen isotope samples were prepared at the Environment Canada lab of K. A. Hobson and isotopic measurements made on 1-mg subsamples at the Department of Soil Science stable isotope facility of the University of Saskatchewan. The yolk samples were freeze-dried and powdered. Lipids were extracted by successively rinsing in a 2 : 1 chloroform : methanol solution and then air-drying under a fume hood. Samples were loaded into tin cups and combusted in a Robo-Prep elemental analyzer at 1,200°C. The resultant N_2 gas was separated and analyzed using an interfaced Europa 20 : 20 continuous-flow isotope ratio mass spectrometer, with every fifth sample separated by two (albumin) laboratory standards. Results were reported in parts per thousand (‰) relative to air. On the basis of replicate measurements of albumin standards, measurement precision for $\delta^{15}N$ values was estimated to be ±0.3‰.

Yolk carotenoids were extracted from ca. 0.05–0.1 g thawed yolk using 500 μL ethanol and 500 μL *tert*-butyl methyl ether (TBME), vortexing the solution for 20 s after the addition of each solvent. The tube was centrifuged for 15 s at 10,000 rpm and the supernatant removed and evaporated to dryness. We resuspended the residue in 200 μL mobile phase (methanol : acetonitrile : dichloromethane, 42 : 42 : 16, v/v/v) and injected 50 μL into a Waters Alliance 2695 HPLC system (Waters, Milford, MA) fitted with a Waters YMC Carotenoid 5.0-μm column (4.6 mm × 250 mm) and a built-in column heater set at 30°C. We used a three-step gradient solvent system to analyze both xanthophylls and carotenes in a single run, at a constant flow rate of 1.2 mL/min: first, isocratic elution with 42 : 42 : 16 (v/v/v) methanol : acetonitrile : dichloromethane for 11 min, followed by a linear gradient up to 42 : 23 : 35 (v/v/v) methanol : acetonitrile : dichloromethane through 21 min, held isocratically at this condition until 25 min, and finishing with a return to the initial isocratic condition from 25 to 40 min. Data were collected from 250 to 600 nm using a Waters 2996 photodiode array detector. We identified pigments by

Table 1: Mean (SE) raw δ^{15}N values and carotenoid types and relative concentrations in seabird prey items

Species	Tissue Type	n	δ^{15}N (‰)	Carotenoid Type (Relative Concentration)
Euphausiid (*Thysanoessa spinifera*)	Whole	5	11.09 (.33)	Astaxanthin (high)
Copepod (*Neocalanus cristatus*)	Whole	5	11.36 (.33)	Astaxanthin (high)
Squid	Muscle	2	13.41 (.53)	Astaxanthin (high)
Juvenile rockfish	Muscle	10	13.13 (.23)	Astaxanthin (very low)
Pacific saury	Muscle	10	14.31 (.23)	Astaxanthin (low)
Pacific sandlance	Muscle	10	13.65 (.23)	Astaxanthin (very low); lutein, zeaxanthin (low)

comparing their respective retention times and absorbance maxima (λ_{max}) to those of reference carotenoids run as external standards. For a subsample of 12 yolks run twice in a pilot test, mean intra-assay coefficient of variation for all carotenoids combined was 5%.

Carotenoids were extracted from homogenized, whole-prey items in hexane : TBME (1 : 1, v/v) using a mixer mill (McGraw and Klasing 2006). As is the case with carotenoids from many crustaceans and fish (Hudon et al. 2003), prey pigments were esterified, so we only could gauge general elution of pigment types and did not quantify amounts of these poorly resolved peaks. Instead, we approximated relative concentrations by visually inspecting the solution's color. Our intent was simply to determine what types of carotenoids were available in the different prey types so that we could compare them with those detected in the egg yolks and assess their concentrations in a general way. More detailed quantitative assessments of carotenoid profiles in marine prey types are available elsewhere (Matsuno 2001; Olson 2006).

Yolk Coloration

We measured yolk coloration using digital image analysis. Briefly, yolk samples were photographed in their transparent eppendorf tubes against a gray card background under standardized conditions using flash photography. Digital images of the yolks were then scored for "hue" (on the hue, saturation, brightness color axis) with Adobe Photoshop's eyedropper function. Hue is traditionally measured on a 360° color wheel where pure red is defined as a hue of 0 and pure yellow as a hue of 60. Because all our hue values fell between 0 and 60, we treated hue as a linear variable, with lower values corresponding to more red, less yellow coloration.

Statistical Analysis

To analyze yolk color in relation to carotenoid profiles (types and concentrations), we used general linear models. For species that produced eggs with more than one type of carotenoid, we correlated concentrations of each type against all other types within individual eggs. In evaluating how diet and species traits shape yolk carotenoid allocation, we considered that they could potentially have independent, additive, interactive, or no effects. Therefore, we built a set of five candidate models to predict total yolk carotenoid concentrations in the five study species: (1) null or equal means model; (2) species (as a categorical variable); (3) δ^{15}N (as a continuous variable); (4) species + δ^{15}N; and (5) species + δ^{15}N + (species × δ^{15}N). These five candidate models were ranked using Akaike's Information Criterion (AIC) corrected for small samples size, based on the difference between each model's AIC_c and the lowest AIC_c from among the candidate set. In addition to selecting a single most

Table 2: Mean (SE) raw δ^{15}N values (‰) and the types and the mean (SE) concentrations of carotenoids in seabird yolks

Species	n	δ^{15}N	Carotenoid Type	Concentration (μg g^{-1})
Common murre	10	15.82 (.05)	Astaxanthin	2.3 (.3)
			Lutein	3.1 (.4)
			Zeaxanthin	10.0 (1.1)
Pigeon guillemot	3	15.83 (.12)	Astaxanthin	68.0 (9.8)
Cassin's auklet	8	14.15 (.12)	Astaxanthin	12.8 (.9)
Rhinoceros auklet	10	14.19 (.26)	Astaxanthin	17.9 (3.4)
			Lutein	11.9 (2.1)
			Zeaxanthin	34.4 (6.1)
Tufted puffin	6	12.62 (.22)	Astaxanthin	133.6 (7.7)

parsimonious model ($\Delta AIC_c = 0.0$), any models with ΔAIC_c scores ≤ 2 can be considered to have received strong support (Burnham and Anderson 2002).

A previous comparative study on passerine birds from New Zealand found that, within species, yolk carotenoid concentrations were higher in eggs laid earlier in the laying sequence and in eggs of lower fresh mass (Cassey et al. 2005). However, all but one of our study species lays a single-egg clutch (clutch size is two in the pigeon guillemot, but we collected only first-laid eggs), and there was no correlation ($r^2 < 0.01$) between total yolk carotenoid concentrations and fresh egg mass in larger samples of yolks of Cassin's and rhinoceros auklets (Hipfner et al., forthcoming). Consequently, we did not consider either factor in this study.

Results

Description of Carotenoids and Isotope Signatures in Seabird Prey

Our qualitative analysis of prey types was generally supportive of a negative correlation between trophic level and carotenoid concentrations (Table 1). Among prey species, the two crustaceans (*Neocalanus cristatus* and *Thysanoessa spinifera*) were situated at low trophic level and contained exclusively astaxanthin in high concentrations. In contrast, squid and all fish were situated at higher trophic level. Squid, like crustaceans, were rich in astaxanthin only. Astaxanthin was also detected in low concentrations in Pacific saury and in very low concentrations in juvenile rockfish and Pacific sandlance. Lutein and zeaxanthin were detected only in low concentrations in Pacific sandlance (Table 1).

Description of and Correlations among Carotenoid Types in Alcid Yolks

Within the five auk species we studied, there were two different kinds of yolks with respect to carotenoid composition, which was invariant among individuals within all five species. All Cassin's auklet, pigeon guillemot, and tufted puffin yolks contained exclusively astaxanthin, while all common murre and rhinoceros auklet yolks contained a cocktail of astaxanthin, lutein, and zeaxanthin (Table 2). In both species with multiple carotenoid types, there were very strong positive pairwise correlations (all $r^2 \geq 0.82$, all $P < 0.01$) between concentrations of the three carotenoid types in individual yolks (Fig. 1).

Yolk Coloration and Hue Scores

The color of egg yolks varied dramatically within and between species (Fig. 2a), and yolk hue corresponded strongly to both the kinds and amounts of carotenoids present (Fig. 2b). The general linear model with two independent variables, yolk carotenoid type (astaxanthin only or cocktail) and total carotenoid concentration, predicted 93% of variance in egg yolk hue (total model: $F_{2,43} = 289$, $R^2 = 0.927$, $P < 0.0001$; carotenoid type: $\beta = -7.91$ [SE $= 0.60$], $t = -13.27$, $P < 0.0001$; total con-
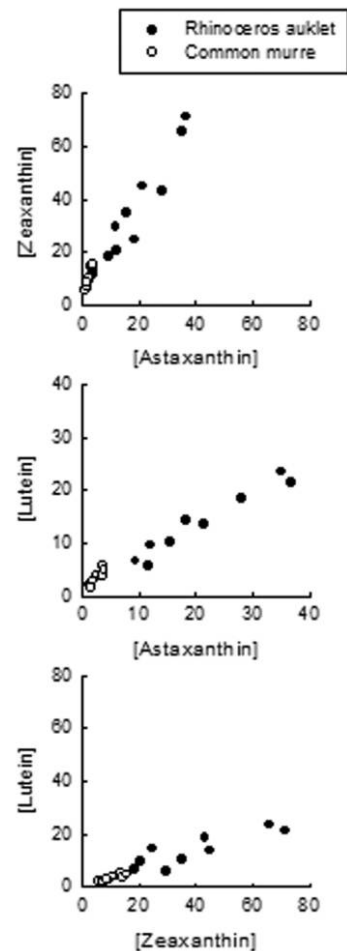

Figure 1. Pairwise correlations between concentrations ($\mu g\ g^{-1}$) of the three carotenoid types in yolks of the eggs laid by rhinoceros auklets and common murres. Note that all data are plotted with x- and y-axes scaled at 1 : 1.

centration: $\beta = -0.16$ [SE $= 0.01$], $t = -14.29$, $P < 0.0001$). Species with all three carotenoid types tended to have yellow- to orange-colored yolks, with yolks being more orange colored (i.e., lower hue values) when total concentrations were higher. In contrast, species with only astaxanthin in their yolks tended to have pink- to reddish-colored yolks, with yolks becoming more red at higher concentrations (Fig. 2a). This trend culminated in the extremely astaxanthin-rich, bright red yolks of tufted puffins.

Variation in Yolk Carotenoids versus Trophic Level

On the basis of $\delta^{15}N$ measurements, the three species with only astaxanthin in their yolks each fed within a relatively narrow trophic range, but in aggregate, they spanned the full trophic range used by all five species involved in the study (Table 2; Fig. 3). In the two species with a mix of carotenoids in their yolks, common murres fed at restricted and high trophic level, while the among-individual variation in trophic level of rhi-
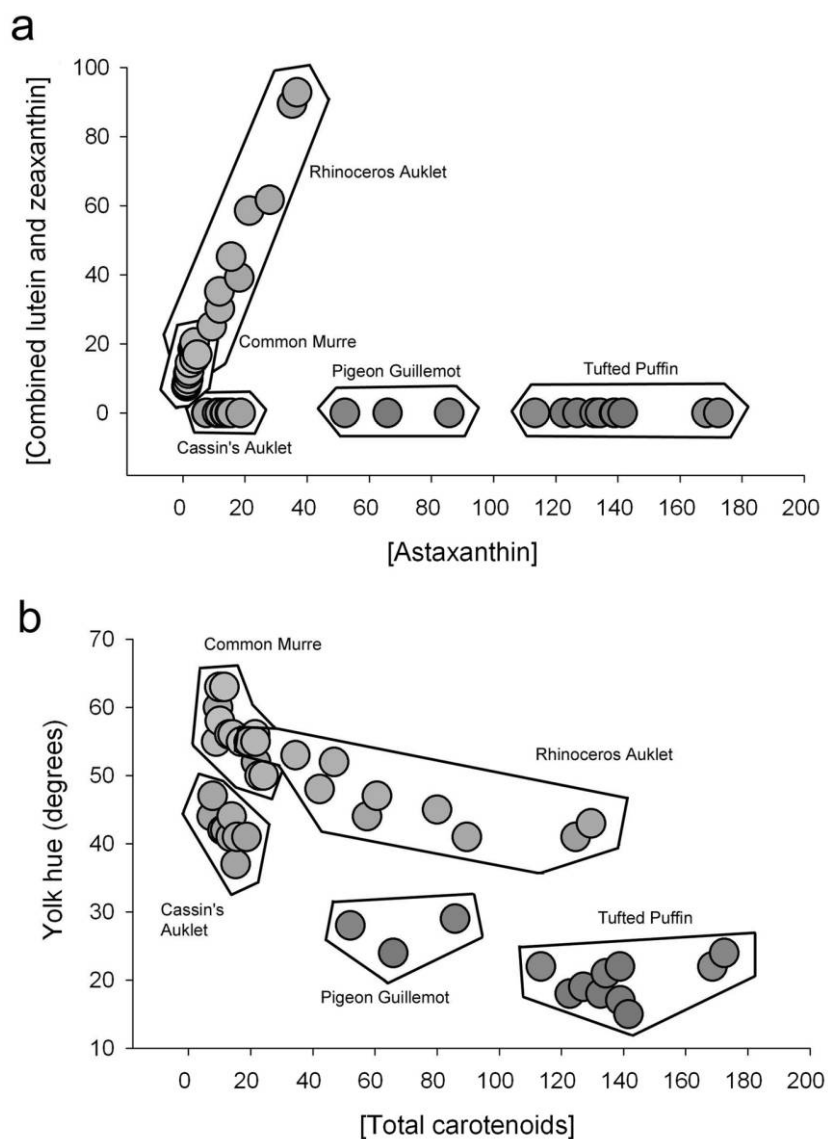
Figure 2. Carotenoid concentrations and yolk coloration in five species of auks at Triangle Island. Darker data points correspond to yolks with redder coloration. Note that the range of carotenoid concentrations vary by over an order of magnitude. *a*, Concentrations (μg g^{-1}) of lutein and zeaxanthin combined versus astaxanthin. Lutein and zeaxanthin were combined for this figure because these two carotenoids had highly correlated concentrations across all species ($r^2 = 0.94$), and they always occurred together when present. *b*, Yolk hue versus total carotenoid concentrations. Note that lower hue values correspond to more red, less yellow coloration.

noceros auklets virtually spanned that of the other four species combined.

Total yolk carotenoid concentrations varied dramatically, by a factor of 23, among the five auk species. Of the five candidate models to explain this variation, only one received strong support (ΔAIC$_c \leq 2.0$). This model included species + δ^{15}N and explained 86% of the variation in total carotenoid concentrations (Table 3). It also received five times the weight of the next best model (species alone), despite that its explanatory power was only slightly better. Further, examination of parameter estimates and their standard errors indicated strong support for both independent variables (Table 4). Thus, total yolk carotenoid concentrations were highly species specific, even

after controlling for trophic level. They were particularly high in tufted puffin and pigeon guillemot and particularly low in Cassin's auklet. But there was, in addition, an important additive effect of trophic level. As predicted, feeding at lower trophic level was associated with production of yolks richer in total carotenoids (Fig. 3). The negative relationship between carotenoid concentrations and trophic level is obvious interspecifically but also intraspecifically in the one species that spanned a wide trophic range, the rhinoceros auklet (Fig. 3).

Discussion

Yolk color varied markedly among the five auk species included in our study because of the interspecific variation in the types
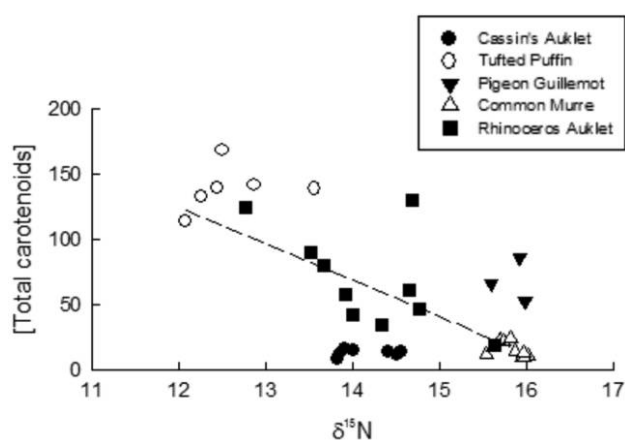
Figure 3. Total carotenoid concentrations (μg g^{-1}) in relation to trophic level (δ^{15}N) in the yolks of eggs laid by five species of auks at Triangle Island.

and amounts of carotenoids the yolks contained. In contrast, the types of carotenoids found were invariant within all species. In three auks that had pink- to red-colored yolks—pigeon guillemot, Cassin's auklet, and tufted puffin—astaxanthin was the only carotenoid detected. In the other two species that had pale yellow to orange yolks—common murre and rhinoceros auklet—astaxanthin was found in combination with lutein and zeaxanthin. These results are as expected, because it is well known in birds that lutein and zeaxanthin tend to produce yellow orange yolks, while astaxanthin tends to produce red yolks. Moreover, the high intraspecific consistency in carotenoid types is also expected in avian species (Royle et al. 2003; Hargitai et al. 2006; Isaksson et al. 2008) and likely reflects general consistency in diet and physiology (McGraw 2005). The same factors (Blount et al. 2002) likely generated the strong and positive intraspecific pairwise correlations between concentrations of the three carotenoid types in yolks (Safran et al. 2008) in the two species that had multiple carotenoid types.

In general, carotenoid profiles in the prey types we examined varied in the manner expected of marine organisms at different trophic levels (Matsuno 2001). Astaxanthin was highly concentrated in low-trophic-level crustacea but was detected in lower concentrations in high-trophic-level fish, while lutein and zeaxanthin were detected in only one fish, Pacific sandlance. The only prey type that deviated from the pattern was squid, which was situated at high trophic level yet was also rich in astaxanthin. Despite these general trends, and contrary to prediction (Olson 2006), there was no indication of any systematic interspecific relationship between consumer trophic level and yolk carotenoid types. Astaxanthin was the only carotenoid found in three species that varied in trophic level from low (tufted puffin) to intermediate (Cassin's auklet) to high (pigeon guillemot). Further, the same three carotenoid types (astaxanthin, lutein, and zeaxanthin) were found in all yolks of both a consistently high-trophic-level feeder (common murre) and

a generalist feeder (rhinoceros auklet). Finally, there was no intraspecific variation in the yolk carotenoid types of rhinoceros auklets, even though individuals varied dramatically in trophic level.

On the basis of these observations, we conclude that there must be important species-specific physiological traits, such as strong affinities for specific carotenoid type(s) (Hill 2006), by which all individual females allocate the same specific carotenoid type(s) to yolk. Further, that two species that belong to the same tribe (the rhinoceros auklet and tufted puffin, members of the Fraterculini) each had one of the two kinds of yolks (astaxanthin and the cocktail) is consistent with previous studies that indicate that yolk carotenoid composition can be evolutionarily labile (Cassey et al. 2005; Biard et al. 2009).

Stable-nitrogen isotope values approximate nutrient sources available to females during egg formation (Hobson 1995). They provided us with a useful indicator of diet in our study species (Davies et al. 2009), demonstrating again the potential utility of stable isotope analysis in comparative ecophysiological research (Sabat et al. 2006). However, there are mechanisms that could potentially decouple the isotope data from the yolk carotenoid data. For example, assuming little endogenous routing, lipid-free yolk δ^{15}N really represents the dietary protein (i.e., N) source for yolk formation. Because carotenoids are lipid soluble, carotenoids present in eggs may have been deposited from dietary lipids, such as VLDL (Bortolotti et al. 2003) that could have differed in dietary source from the origin of proteins. Such differential metabolic routing of macronutrients to eggs needs to be considered in all interpretations of egg isotope data (Hobson 1995). For species with a narrow diet, this would not be an issue, as protein and lipid sources would be identical. However, for species with broad diets, such as rhinoceros auklet, it is possible that lipid sources to eggs were not identical (in proportion) to protein sources. That mechanism may account for additional noise in the regression between carotenoids and egg yolk δ^{15}N for this and other species.

As with yolk carotenoid types, yolk carotenoid concentra-

Table 3: Results of Akaike's Information Criterion (AIC) model comparisons to explain interspecific variation in total yolk carotenoid concentrations in eggs laid by five species of auks

Model	R^2	ΔAIC$_c$	AIC$_w$	k
Species + δ^{15}N	.86	.00	.83	7
Species	.82	3.20	.17	6
δ^{15}N	.49	17.14	.00	8
Species + δ^{15}N + species × δ^{15}N	.86	24.15	.00	12

Note. The only model that received strong support (ΔAIC$_c \leq$ 2) included both species and δ^{15}N, and this model received almost five times the support of the second-ranked model. No other model, including the null model, had AIC$_w > 0.00$ (AIC$_w$ measures the weight of evidence in favor of a particular model given the data and candidate model set).

Table 4: Parameter estimates and standard errors (SE) for variables in the top-ranked AIC model (species + δ^{15}N) explaining variation in total yolk carotenoid concentrations in five auk species

Parameter	Category	Estimate (SE)
Species	Common murre	32.1 (15.5)
Species	Pigeon guillemot	84.9 (18.4)
Species	Rhinoceros auklet	56.6 (10.0)
Species	Tufted puffin	99.3 (15.7)
δ^{15}N	…	17.6 (7.1)

Note. The reference category for species is Cassin's auklet.

tions varied among species. However, unlike carotenoid types, the carotenoid concentrations in yolks varied in the manner predicted from trophic level (Olson 2006). In general, most species fed within a very restricted trophic range, and those that fed at a low trophic level produced carotenoid-rich yolks. The one exception was rhinoceros auklet, in which individuals spanned virtually the full trophic range of the other four species combined (crustaceans to fish). In that species, there was a negative relationship between total yolk carotenoids and trophic level, and it closely paralleled the negative relationship for all species. This suggests that there is a functional association between the two factors. Within this one small avian family then, as found in two more broadly based comparative avian studies (Tella et al. 2004; Cassey et al. 2005), species-specific physiological and behavioral traits, as well as an individual's choice of diet, are important factors that underlie carotenoid accumulation.

The dramatic variation in yolk concentration among the different species raises important questions about the physiological functions of carotenoids and about how limited carotenoids actually are to female auks. For example, it is noteworthy that, among species with only astaxanthin in their yolks, this carotenoid was richly concentrated in both a low-trophic-level feeder (tufted puffin) and a high-trophic-level feeder (pigeon guillemot) but not in an intermediate-trophic-level feeder (Cassin's auklet). Further, total yolk carotenoid concentrations in the auks were comparable to those in yolks laid by marine and freshwater piscivorous birds elsewhere (Surai et al. 2001a).

The question remains about why the interspecific variation in total yolk carotenoid concentrations should be so marked. We speculate that diets and species traits associated with carotenoid allocation in the auks reflect physiological needs related to expression of secondary sexual characters (McGraw 2005). Previously, male carotenoid color signals were found to be more prevalent in species with high yolk carotenoid levels in a broad-based comparative study of birds (Biard et al. 2009). The tufted puffin is probably the most ornate auk, displaying bright red eye rings and rosettes and orange legs and bills (Gaston and Jones 1998), and produced the most carotenoid-rich yolks for trophic level in our study. This species exhibits a dramatic trophic shift from the prebreeding period, when it takes predominantly astaxanthin-rich crustacea while developing breeding ornaments (and eventually producing eggs), to its chick-provisioning period, when it takes predominantly fish (Williams et al. 2008; Davies et al. 2009). In contrast, Cassin's auklet is the least ornate of the auks, is basal within a tribe (Aethiini) that otherwise includes only ornate species (Friesen et al. 1996), and produced the least carotenoid-rich yolks for trophic level in our study. Clearly, further studies on the mechanisms of color expression in the auks, and its relationship to diet, are needed. Moreover, why embryos destined to become highly ornate adults might benefit through developing on a carotenoid-rich yolk is a mystery.

Acknowledgments

Literature Cited

Bertrand S., C. Alonso-Alvarez, G. Devevey, B. Faivre, J. Prost, and G. Sorci. 2006. Carotenoids modulate the trade-off between egg production and resistance to oxidative stress in zebra finches. Oecologia 147:576–584.

Biard C., D. Gil, F. Karadaş, N. Saino, C.N. Spottiswoode, P.F. Surai, and A.P. Møller. 2009. Maternal effects mediated by antioxidants and the evolution of carotenoid-based signals in birds. Am Nat 174:696–708.

Biard C., P.F. Surai, and A.P. Møller. 2005. Effects of carotenoid availability during laying on reproduction in the blue tit. Oecologia 144:32–44.

Blount J.D. 2004. Carotenoids and life-history evolution in animals. Arch Biochem Biophys 430:10–15.

Blount J.D., D.C. Houston, and A.P. Møller. 2000. Why egg yolk is yellow. Trends Ecol Evol 15:47–49.

Blount J.D., D.C. Houston, P.F. Surai, and A.P. Møller. 2004. Egg-laying capacity is limited by carotenoid pigment availability in wild gulls *Larus fuscus*. Proc R Soc Lond B 271: S79–S81.

Blount J.D., P.F. Surai, D.C. Houston, and A.P. Møller. 2002. Patterns of yolk enrichment with dietary carotenoids in gulls:

the roles of pigment acquisition and utilization. Funct Ecol 16:445–453.

Bortolotti G.R., J.J. Negro, P.F. Surai, and P. Prieto. 2003. Carotenoids in eggs and plasma of red-legged partridges: effects of diet and reproductive output. Physiol Biochem Zool 76: 367–374.

Burnham K.P. and D.R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer, New York.

Cassey P., J.G. Ewen, R.L. Boulton, T.M. Blackburn, A.P. Møller, C. Biard, V. Olson, and F. Karadaş. 2005. Egg carotenoids in passerine birds introduced to New Zealand: relations to ecological factors, integument coloration, and phylogeny. Funct Ecol 19:719–726.

Davies W.E., J.M. Hipfner, K.A. Hobson, and R.C. Ydenberg. 2009. Seabird seasonal trophodynamics: isotopic patterns in a community of Pacific alcids. Mar Ecol Prog Ser 382:211–219.

Friesen V.L., A.J. Baker, and J.F. Piatt. 1996. Phylogenetic relationships within the Alcidae (Charadriiformes: Aves) inferred from total molecular evidence. Mol Biol Evol 13:359–367.

Gaston A.J. and I.L. Jones. 1998. The Auks. Oxford University Press, Oxford.

Hargitai R., Z. Matus, G. Hegyi, G. Michl, G. Tóth, and J. Török. 2006. Amtioxidants in the egg yolk of a wild passerine: differences between breeding seasons. Comp Biochem Physiol B 143:145–152.

Hedd A., D.F. Bertram, J.L. Ryder, and I.L. Jones. 2006. Effects of interdecadal climate variability on marine trophic interactions: rhinoceros auklets and their fish prey. Mar Ecol Prog Ser 309:263–278.

Hill G.E. 2006. Environmental regulation of ornamental coloration. Pp. 507–560 in G.E. Hill and K.J. McGraw, eds. Bird Coloration: Mechanisms and Measurements. Harvard University Press, Cambridge, MA.

Hipfner J.M. 2009. Euphausiids in the diet of a North Pacific seabird: annual and seasonal variation and the role of ocean climate. Mar Ecol Prog Ser 390:277–289.

Hipfner J.M., J. Dale, and K.J. McGraw. Forthcoming. Yolk carotenoids and stable isotopes reveal links among foraging behavior, environment, and breeding success. Oecologia.

Hobson K.A. 1995. Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components—patterns of isotopic fractionation and turnover. Condor 97: 752–762.

Hudon J., G.F. Grether, and D.F. Millie. 2003. Marginal differentiation between the sexual and general carotenoid pigmentation of guppies (Poecilia reticulata) and a possible visual explanation. Physiol Biochem Zool 76:776–790.

Isaksson C., A. Johansson, and S. Andersson. 2008. Egg yolk carotenoids in relation to habitat and reproductive investment in the great tit Parus major. Physiol Biochem Zool 81: 112–118.

Karunajeewa H., R.J. Hughes, M.W. McDonald, and F.S. Shen-stone. 1984. A review of factors influencing pigmentation of egg-yolks. World Poult Sci J 40:52–65.

Mackas D.L., S. Batten, and M. Trudel. 2007. Effects on zooplankton of a warmer ocean: recent evidence from the Northeast Pacific. Prog Oceanogr 75:223–252.

Mahler B., L.S. Araujo, and P.L. Tubaro. 2003. Dietary and sexual correlates of carotenoid pigment expression in dove plumage. Condor 105:258–267.

Matsuno T. 2001. Aquatic animal carotenoids. Fish Sci 67:771–783.

McGraw K.J. 2005. Interspecific variation in dietary carotenoid assimilation in birds: links to phylogeny and color ornamentation. Comp Biochem Physiol B 142:245–250.

———. 2006. Dietary carotenoids mediate a trade-off between egg quantity and quality in Japanese quail. Ethol Ecol Evol 18:247–256.

McGraw K.J. and K.C. Klasing. 2006. Carotenoids, immunity, and integumentary coloration in red junglefowl (Gallus gallus). Auk 123:1161–1171.

Monaghan P., N.B. Metcalfe, and R. Torres. 2008. Oxidative stress as a mediator of life-history trade-offs: mechanisms, measurements, and interpretations Ecol Lett 11:1238–1251.

Olson V.A. 2006. Estimating nutrient intake in comparative studies of animals: an example using dietary carotenoid content in birds. Oikos 112:620–628.

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

———. 2005. Interspecific variation in the use of carotenoid-based coloration in birds: diet, life history, and phylogeny. J Evol Biol 18:1534–1546.

Ricklefs R.E. and M. Wikelski. 2002. The physiology/life-history nexus. Trends Ecol Evol 17:462–468.

Royle N.J., P.F. Surai, and I.R. Hartley. 2003. The effect of variation in dietary intake on maternal deposition of antioxidants in zebra finch eggs. Funct Ecol 17:472–481.

Sabat P., K. Maldonado, M. Canals, and C.M.D. Rio. 2006. Osmoregulation and adaptive radiation in the ovenbird genus Cinclodes (Passeriformes: Furnariidae). Funct Ecol 20: 799–805.

Safran R.J., K.M. Pilz, K.J. McGraw, S.M. Correa, and H. Schwabl. 2008. Are yolk androgens and carotenoids in barn swallow eggs related to parental quality? Behav Ecol Sociobiol 62:427–438.

Stearns S.C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford.

Surai A.P., P.F. Surai, W. Steinberg, W.G. Wakeman, B.K. Speake, and N.H.C. Sparks. 2003. Effect of canthaxanthin content of the maternal diet on the antioxidant system of the developing chick. Br Poult Sci 44:612–619.

Surai P.F., G.R. Bortolotti, A.L. Fidgett, J.D. Blount, and B.K. Speake. 2001a. Effects of piscivory on the fatty acid profiles and antioxidants of avian yolk: studies on eggs of the gannet, skua, pelican and cormorant. J Zool (Lond) 255:305–312.

Surai P.F., B.K. Speake, N.A.R. Wood, J.D. Blount, G.R. Bortolotti, and N.H.C. Sparks. 2001b. Carotenoid discrimination

by the avian embryo: a lesson from wild birds. Comp Biochem Physiol B 128:743–750.

Tella J.L., J. Figuerola, J.J. Negro, G. Blanco, R. Rodríguez-Estrella, M.G. Forero, M.C. Blázquez, A.J. Green, and F. Hiraldo. 2004. Ecological, morphological, and phylogenetic correlates of interspecific variation in plasma carotenoid concentration in birds. J Evol Biol 17:156–164.

Tyndale S.T., R.L. Letcher, J.W. Heath, and D.W. Heath. 2008. Why are salmon eggs red? egg carotenoids and early life survival of chinook salmon (*Onchorhynchus tshawtscha*). Evol Ecol Res 10:1187–1199.

Ware D.M. and R.E. Thomson. 2005. Bottom-up ecosystem trophic dynamics determine fish production in the Northeast Pacific. Science 308:1280–1284.

Williams C.T., S.J. Iverson, and C.L. Buck. 2008. Stable isotopes and fatty acid signatures reveal age- and stage-dependent foraging niches in tufted puffins. Mar Ecol Prog Ser 363: 287–298.