BEHAVIORAL ECOLOGY - ORIGINAL PAPER

Yolk carotenoids and stable isotopes reveal links among environment, foraging behavior and seabird breeding success

J. Mark Hipfner · James Dale · Kevin J. McGraw

Abstract Nutrients that are limited in availability, such as carotenoids, are potentially involved in trade-offs between homeostasis and reproduction. Despite their importance, factors that affect the capacity of female birds to meet their carotenoid requirements are poorly understood. We used $\delta^{15}N$ stable isotope analysis to relate foraging behavior to yolk carotenoid deposition in two seabirds, Cassin's auklet (*Ptychoramphus aleuticus*) and rhinoceros auklet (*Cerorhinca monocerata*), during each of five years. As expected from their narrower trophic range, Cassin's auklets produced yolks with fewer carotenoid types than did rhinoceros auklets (one vs. three). Cassin's auklets also fed on a lower trophic level diet richer in carotenoids, yet had lower total yolk carotenoid levels, which suggests a role for species-specific adaptations for carotenoid uptake and utilization. Within both species, lower trophic-level feeding was linked to higher yolk carotenoid levels, but through different mechanisms. In Cassin's auklets, it was due to a population-wide response to environmental variation: in warm-water years, all females fed at a low trophic level and produced carotenoid-rich yolks. In rhinoceros auklets, it was due to individual differences similarly expressed in all years: females fed across a wide trophic range, and those that fed at a low trophic level produced carotenoid-rich yolks. Rhinoceros auklets bred more successfully in years when their yolks were rich in carotenoids, probably due to a correlated response to stronger marine primary production. Our results are novel because they link avian yolk carotenoid deposition to behavioral and environmental variations.

Keywords Breeding success · Carotenoids · Macronutrients · Foraging · Trophic range

Introduction

Proximate modulators of life-history variation are of considerable interest to evolutionary ecologists (Monaghan et al. 2008; Ricklefs and Wikelski 2002; Zera et al. 2007). For example, environmentally limited nutrients are potentially involved in trade-offs between homeostasis and reproduction (Stearns 1992). Carotenoids, lipid-soluble pigments synthesized de novo only by primary producers, constitute a promising group of candidate nutrients for fitness trade-offs because consumers obtain them exclusively from dietary sources (Volker 1938), and because they play important roles as antioxidants and in immune system function, vision, and coloration (Blount et al. 2000).

Female birds deposit carotenoids into egg yolks to protect embryos against oxidative stress and boost their developing immune systems (Blount et al. 2002a; Saino et al. 2003). However, this maternal investment in offspring could carry a cost to the female's own immunological and oxidative needs if the availability of

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carotenoids is limited (Blount et al. 2002b). Indeed, both experimental studies (Biard et al. 2005; Blount et al. 2004) and multi-year observational studies (Hargitai et al. 2006) have shown that the availability of dietary carotenoids can limit the egg-laying capacity of female birds. In response, egg-laying females could forage specifically for carotenoid-rich foods (Olson 2006), although this might require them to alter their behavior (Hargitai et al. 2006). However, few studies have related foraging strategies to carotenoid acquisition (McGraw et al. 2006), despite the fact that investigations of this nature could highlight critical constraints on individual fitness and life-history evolution.

Avian tissues vary widely both within and among species in their carotenoid concentrations (Olson and Owens 2005). In contrast, the variety of carotenoid types is more consistent within species (Hargitai et al. 2006), due to general consistency in diet and physiology (McGraw et al. 2006). Much of the interspecific variation in carotenoid types and concentrations results from dietary differences, although species-specific physiological adaptations for carotenoid uptake and utilization could also be involved (Cassey et al. 2005; Tella et al. 2004). However, it is difficult to determine the relative importance of dietary versus physiological differences, because both factors tend to be similar within phylogenetic lines (Tella et al. 2004).

We examined how behavioral and environmental variation influenced the deposition of carotenoids into egg yolk in Cassin's auklets *Ptychoramphus aleuticus* and rhinoceros auklets *Cerorhinca monocerata* during each of five breeding seasons. While both species employ typical seabird life-history strategies featuring high adult survival rates, delayed recruitment, and single-egg clutches (Lack 1968), their foraging habits differ. Cassin's auklets are offshore feeders that primarily consume crustaceans (Abraham and Sydeman 2004; Hedd et al. 2002), whereas rhinoceros auklets feed in a variety of habitat types and eat a varied diet consisting mainly of small forage fish (Ito et al. 2009; Thayer et al. 2008; Thayer and Sydeman 2006). Low trophic-level prey such as crustaceans tend to be carotenoid-rich compared to high trophic-level prey such as fish (Matsuno 2001; Olson 2006).

We test a series of interspecific and intraspecific predictions. In comparing between species, we predict that: (1) the lower trophic-level Cassin's auklet will produce yolks with higher total carotenoid concentrations, whereas (2) the more trophically diverse rhinoceros auklet will produce yolks with a greater variety of carotenoid types (Matsuno 2001). In comparing among individuals within both species, we predict that: (3) individuals that feed at a low trophic level will produce yolks rich in carotenoids (Bortolotti et al. 2003); (4) yolks will contain the same types of carotenoids in all years (Hargitai et al. 2006); (5) yolks will be richer in carotenoids in years of stronger marine primary productivity; and (6) carotenoid-rich yolks will be linked to a greater probability of breeding successfully (Blount et al. 2004).

To test these predictions, we (1) measured carotenoid profiles and $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios in seabird egg yolks and a suite of prey items, and (2) monitored breeding success (i.e., whether the egg ultimately produced a fledged chick) in control pairs and those that relaid after their eggs were collected. We collected all eggs prior to the median laying date of each species, because early layers tend to be older, more experienced, and more successful birds (DeForest and Gaston 1996; Pyle et al. 2001)—those most likely to be selecting the most profitable prey (Sorenson et al. 2009). In fact, virtually all females whose eggs we collected relaid during all five years (Hipfner et al. 2008, 2010), which allowed us to relate variation in carotenoid concentration to success at both the individual (i.e., whether the specific pair fledged a chick) and the population (the proportion of all females that bred successfully in a given year) levels. Our five-year study (2002–2006) spanned a period of extreme variation in the local marine environment (Mackas et al. 2007; Wolf et al. 2009).

Materials and methods

Study area and species

We conducted field work from 2002 to 2006 on Triangle Island, British Columbia, Canada (50°52′N, 129°05′W), which supports populations of ∼500,000 Cassin's auklet pairs and ∼40,000 rhinoceros auklet pairs (Rodway et al. 1990). Both species are members of the family Alcidae. Cassin's auklets are true auklets (tribe Aethiini), and weigh ∼180 g as adults; rhinoceros auklets are actually puffins (tribe Fraterculini), and adults weigh ∼500 g. The auklet and puffin tribes are sister tribes, and our two study species are considered basal within their respective tribes (Friesen et al. 1996).

Egg and prey collections

In all five years, Cassin's auklets began laying eggs in late March (Hipfner et al. 2010), and rhinoceros auklets 4–5 weeks later (Hipfner et al. 2008). In each year, a single egg was collected from 9–12 females within two days of laying and before the median laying date of each species. Eggs were measured in the field (length and breadth to ±0.1 mm with dial calipers; mass to ±0.1 g with an electronic balance) and then placed in a freezer at −10°C

(2002–2004) or stored with shells intact in 70% ethanol (2005–2006).

We obtained fresh-frozen whole specimens of two crustacean species preyed on by Cassin's auklets during the study period (Hipfner 2009): the copepod *Neocalanus cristatus* and the euphausiid *Thysanoessa spinifera*. All specimens were collected by shipboard plankton tows along the continental shelf margin near Triangle Island (Mackas et al. 2007). We trapped rhinoceros auklets at night using long-handled nets before they could scramble into burrows to feed their chicks. In this way, we obtained a typical array of whole prey items from the birds' bills (Thayer et al. 2008): unspecified squid, Pacific saury *Cololabis saira*, Pacific herring *Clupea harengus*, young-of-year (0+) rockfish *Sebastes* sp., and adult (1+) and young-of-year (0+) Pacific sandlance *Ammodytes hexapterus*. These items were weighed and measured, and then placed in a freezer at −10°C (2002–2004) or stored whole in 70% ethanol (2005–2006).

Breeding success

We inspected 50–80 breeding burrows per species on control plots, adjacent to the plot where we collected eggs, at five-day intervals during all five years. When an egg was found, the burrow was revisited 35 days (Cassin's auklet) or 40 days (rhinoceros auklet) later to allow the birds to complete incubation undisturbed, then checked again at five-day intervals until a hatchling was found. When first found, we estimated each chick's hatching date (age 0) using a calibration of wing length against age for known-aged chicks (Hedd et al. 2002, 2006). We then measured Cassin's auklet chicks at ages of 5, 10, 25 and 35 days, and then every other day until they disappeared. Rhinoceros auklet chicks were measured at ages of 5, 10, 40 and 45 days, and then every other day until they disappeared. Chicks that survived at least 35 days (Cassin's auklet) or 45 days (rhinoceros auklet) before disappearing were considered to have fledged.

We also determined whether the pairs whose eggs we collected relaid. Briefly, we rechecked burrows at five-day intervals beginning ten days after the eggs were removed. When a second egg was found, we measured it to confirm that it had been laid by the same female whose egg we had collected previously in the same burrow, based on consistency in egg shape (Hipfner et al. 2008, 2010). We then monitored the fate of the replacement egg using the same protocol as for control pairs.

Carotenoid and isotope analyses

In the laboratory, ∼1 ml samples were extracted from the centers of all yolks, placed into Eppendorf tubes, and put into a freezer at −20°C. Yolk and prey samples were freeze-dried and powdered, and lipids were extracted by successively rinsing in a 2:1 chloroform:methanol solution and then air-drying under a fume hood. Stable isotope analysis ($\delta^{15}N$ and $\delta^{13}C$) on all prey and yolk samples collected in 2002 was performed at the University of Saskatchewan using a Robo-Prep elemental analyzer interfaced to a Europa 20:20 continuous-flow isotope ratio mass spectrometer. Stable isotope analysis on yolk samples collected in 2003–2006 was performed at the University of California Davis, using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20:20 isotope ratio mass spectrometer. All results are reported in delta notation in parts per thousand (‰) relative to air ($\delta^{15}N$) and VPDB ($\delta^{13}C$). Measurement precisions (SDs) for $\delta^{13}C$ and $\delta^{15}N$ values were estimated to be ±0.1 and ±0.3‰, respectively, for 2002 samples, and ±0.1 and ±0.2‰ for 2003–2006 samples.

Ratios of nitrogen isotopes provide a measure of the trophic level at which a consumer has fed, because ^{15}N is systematically enriched with trophic level (Peterson and Fry 1987). Ratios of carbon isotopes can be used to infer the type of marine habitat in which a consumer has fed, because ^{13}C is enriched in benthic and inshore environments relative to pelagic and offshore environments (Deniro and Epstein 1978).

We extracted carotenoids from 0.05–0.1 g yolk samples 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 Corporation, Milford, MA, USA) fitted with a Waters YMC Carotenoid 5.0 μm column (4.6 × 250 mm) and a built-in column heater set at 30°C.

We then 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 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, the mean intra-assay coefficient of variation for all carotenoids combined was 5%.

We extracted carotenoids from homogenized, whole prey items in hexane:TBME (1:1, v/v) using a mixer mill (sensu McGraw and Klasing 2006). As is typical of carotenoids from crustaceans and fish (Hudon et al. 2003), prey pigments were esterified, so we only could gauge the 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 for comparison with those detected in the egg yolks, and to 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).

Statistical analyses

For analyses of stable isotope values and carotenoid concentrations, residuals were first tested to confirm that they were normally distributed. Initially, we ran multivariate GLMs (with year, $\delta^{15}N$ and $\delta^{13}C$ as independent variables) with two-way interaction terms included, but as none were significant (all $P > 0.2$), they were dropped from the final models. Because we did not measure stable isotopes in all yolks for which we had carotenoid measurements in 2002 and 2003, sample sizes are slightly different (by 1–2 per year for both Cassin's and rhinoceros auklets) for analyses that do and do not examine relationships between carotenoid concentrations and $\delta^{15}N$ and $\delta^{13}C$. Finally, we used logistic regression to examine relaying rates and breeding

success in relation to carotenoid concentrations. Only control pairs that laid prior to the median laying date were used to analyze breeding success, because we had collected eggs from early layers.

Results

Carotenoids and isotopes in prey

Astaxanthin was present in high concentrations in the two crustaceans (N. cristatus and T. spinifera) and in squid, but only in low (Pacific saury) or very low (Pacific herring, juvenile rockfish and adult Pacific sandlance) concentrations in fish. Two other carotenoids, lutein and zeaxanthin, were detected only in juvenile Pacific sandlance. The two crustaceans were situated at a low trophic level (low $\delta^{15}N$ values), while squid and all fish were situated at a higher trophic level (Table 1). There was no consistent association between prey type (fish, squid or crustacean) and $\delta^{13}C$ (Davies et al. 2009).

Interspecific variation in yolk carotenoids and isotopes

After controlling for year effects ($F_{4,88} = 14.83$, $P < 0.001$), $\delta^{15}N$ values in Cassin's auklet yolks were lower (least square mean = 13.8 vs. 14.3‰, $F_{1,88} = 25.55$, $P < 0.0001$) and less variable (CV = 0.026 vs. 0.052) than those in rhinoceros auklet yolks. On a year-by-year basis, the mean $\delta^{15}N$ value in rhinoceros auklet yolks was 0.04–1.4‰ higher than in Cassin's auklet yolks, while the yearly

Table 1 Mean (SD) raw $\delta^{15}N$ and $\delta^{13}C$ values (‰), and their ranges, in seabird prey items and egg yolks

Item	Year (n)	$\delta^{13}C$		$\delta^{15}N$	
		Mean (SD)	Range	Mean (SD)	Range
T. spinifera	2002 (5)	−18.58 ± 2.04	−20.30 to −16.16	11.09 ± 0.84	10.18–12.01
N. cristatus	2002 (5)	−20.13 ± 1.04	−20.24 to −18.84	11.36 ± 0.72	10.21–11.93
Squid	2002 (2)	−21.95 ± 1.54	−23.04 to −20.86	13.41 ± 0.14	13.31–13.51
Rockfish	2002 (10)	−17.85 ± 0.43	−18.77 to −17.34	13.13 ± 0.89	11.84–14.47
Sandlance	2002 (10)	−17.09 ± 1.17	−19.65 to −15.56	13.65 ± 0.54	12.98–14.50
Pacific saury	2002 (10)	−20.36 ± 0.40	−21.01 to −19.78	14.31 ± 0.62	13.31–15.00
CAAU yolk	2002 (10)	−17.31 ± 0.19	−17.65 to −16.95	14.15 ± 0.33	13.82–14.68
	2003 (10)	−17.19 ± 0.30	−17.75 to −16.79	13.79 ± 0.21	13.50–14.15
	2004 (10)	−17.18 ± 0.29	−17.51 to −16.47	13.69 ± 0.32	13.17–14.20
	2005 (10)	−17.63 ± 0.55	−18.48 to −16.91	13.86 ± 0.14	13.57–14.09
	2006 (10)	−17.77 ± 0.29	−18.28 to −17.34	13.23 ± 0.20	12.76–13.54
RHAU yolk	2002 (10)	−18.94 ± 0.69	−20.03 to −18.01	14.19 ± 0.80	12.77–15.64
	2003 (9)	−17.35 ± 0.55	−18.09 to −16.49	15.20 ± 0.33	14.79–15.80
	2004 (10)	−18.00 ± 0.61	−18.92 to −17.21	14.39 ± 0.52	13.60–15.00
	2005 (9)	−19.04 ± 0.62	−19.81 to −18.15	14.23 ± 0.64	13.25–15.04
	2006 (10)	−19.07 ± 0.43	−19.81 to −18.39	13.55 ± 0.62	12.71–14.43

CAAU, Cassin's auklet; RHAU, rhinoceros auklet

range was 1.36–3.44‰ in rhinoceros auklets but only 0.52–1.03‰ in Cassin's auklets (Table 1). Similarly, δ^{13}C values in yolks were higher (least square mean = −17.41 vs. −18.49‰, $F_{1,88} = 90.72$, $P < 0.0001$) and less variable (CV = −0.022 vs. −0.046) in Cassin's auklets than in rhinoceros auklets, after controlling for year effects ($F_{4,88} = 20.83$, $P < 0.001$).

As predicted from their narrower trophic range, Cassin's auklet yolks contained fewer carotenoid types than rhinoceros auklet yolks (Fig. 1): one (astaxanthin) compared to three (astaxanthin, lutein and zeaxanthin) in all samples. This held in all five years. Cassin's auklet yolks had higher concentrations of astaxanthin than did rhinoceros auklet yolks (least square mean = 211 vs. 164 µg/g, respectively, $F_{1,88} = 8.48$, $P = 0.005$) after controlling for year effects. However, because rhinoceros auklet yolks also contained zeaxanthin and lutein, they had (despite their higher δ^{15}N values) almost three times higher total carotenoid concentrations than Cassin's auklet yolks (least square mean = 590 vs. 212 µg/g, respectively; $F_{1,88} = 73.86$, $P < 0.0001$). The pattern of interannual variation in mean total yolk carotenoid concentrations differed considerably between Cassin's and rhinoceros auklets (Fig. 1).

Intraspecific variation in yolk carotenoids and isotopes

Total yolk carotenoid concentrations varied by a factor of over four among all individuals in both Cassin's and rhinoceros auklets. Egg size varied little among years ($R^2 < 0.02$, $P > 0.9$ in both species), and explained virtually none of the variation in carotenoid levels ($R^2 < 0.001$ in both).

As predicted, concentrations of astaxanthin in Cassin's auklet yolks varied among years ($R^2 = 0.47$, $F_{4,46} = 10.10$, $P < 0.001$). Based on Tukey's post hoc tests (at $\alpha = 0.05$), concentrations were lower in 2002 than in any year from 2003 to 2006, while within the latter period only 2003 and 2006 differed significantly (Fig. 1). Likewise, concentrations of astaxanthin ($R^2 = 0.18$, $F_{4,43} = 2.54$, $P = 0.04$) and lutein ($R^2 = 0.26$, $F_{4,43} = 3.90$, $P = 0.009$) varied among years in rhinoceros auklets, but zeaxanthin did not ($R^2 = 0.09$, $F_{4,43} = 1.09$, $P = 0.37$). However, no pairwise yearly differences were significant based on Tukey's post hoc tests. Nonetheless, all three carotenoids varied congruently from year to year, being lower in 2003 and 2005 than in 2002, 2004 and 2006 (Fig. 1). Consistently positive correlations indicated that rhinoceros auklet yolks that were rich in one type of carotenoid tended to be rich in other types (Table 2).

Relationship between yolk carotenoids and isotopes

Overall, δ^{13}C was not correlated with yolk carotenoid concentrations in either study species (in full models, both $P > 0.35$), consistent with the lack of association between prey types and δ^{13}C. Therefore, we do not consider δ^{13}C values any further here.
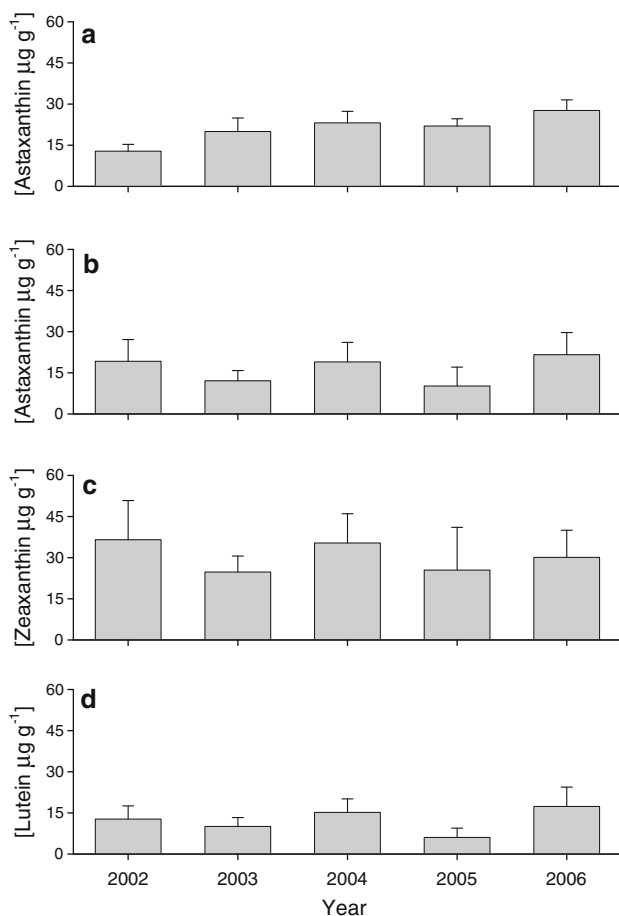

Fig. 1 Types and concentrations (µg/g) of carotenoids found in yolks of Cassin's auklets (**a**) and rhinoceros auklets (**b–d**)

Table 2 Strengths of correlations (r^2 values) relating concentrations (in µg/g) of specific carotenoid types in individual egg yolks of rhinoceros auklets within each of 5 years (all $n = 9$–12)

Year (n)	Carotenoid types		
	Astaxanthin and zeaxanthin	Astaxanthin and lutein	Lutein and zeaxanthin
2002 (12)	0.93	0.96	0.82
2003 (10)	0.66	0.81	0.29*
2004 (10)	0.89	0.93	0.74
2005 (9)	0.91	0.84	0.60
2006 (10)	0.85	0.34**	0.11*

All correlations are positive and significant at $P < 0.01$ unless indicated otherwise. * $P > 0.1$; ** $P > 0.05$ (i.e., not statistically significant)

Pooled across years, total yolk carotenoid concentrations decreased ($P < 0.01$) with $\delta^{15}N$ in both Cassin's ($r^2 = 0.20$, $\beta = -7.8 \pm 2.4$ SE) and rhinoceros auklets ($r^2 = 0.21$, $\beta = -18.6 \pm 5.2$ SE). In an ANCOVA, year and $\delta^{15}N$ explained 53% of the variation in the astaxanthin concentration in Cassin's auklet yolks ($F_{5,40} = 9.15$, $P < 0.001$, with the nonsignificant ($P > 0.2$) interaction term removed). However, while the year effect was significant ($F_4 = 7.21$, $P < 0.001$), the $\delta^{15}N$ effect was not ($P > 0.2$). Within years, there was no consistent relationship between astaxanthin and $\delta^{15}N$. In fact, only one of the correlations, which was positive rather than negative in direction, even approached statistical significance (Table 3).

Similarly, year and $\delta^{15}N$ explained 34% of the variation in total yolk carotenoid concentrations in rhinoceros auklets (ANCOVA: $F_{5,42} = 4.35$, $P = 0.003$). However, while the $\delta^{15}N$ effect was significant ($F_4 = 12.21$, $P = 0.001$), the year effect was not ($P > 0.1$). Results were similar for each of astaxanthin ($r^2 = 36\%$), lutein ($r^2 = 48\%$) and zeaxanthin ($r^2 = 23\%$) individually, although both year ($P = 0.005$) and $\delta^{15}N$ ($P < 0.001$) explained significant variation in lutein, the least concentrated carotenoid (Fig. 1). Unlike Cassin's auklets, annual relationships between total yolk carotenoids and $\delta^{15}N$ varied from zero to strongly negative in rhinoceros auklets (Table 3). Thus, year and $\delta^{15}N$ differed in their roles in explaining variation in carotenoid concentrations in the two species, with year important in Cassin's auklets (Fig. 2) but $\delta^{15}N$ important in rhinoceros auklets (Table 3).

Relationship to breeding success

In both species, 90–100% of females whose eggs we removed relaid in each of the five years. In logistic regression models, the total yolk carotenoid concentrations in the first eggs did not predict whether females in either species relaid (both Wald $\chi^2 < 1.14$, $P > 0.2$). This is not surprising given the high relaying rates. Likewise, yolk carotenoid concentrations explained little of the variation in the probability of success with the replacement egg in either species (both Wald $\chi^2 < 0.88$, $P > 0.3$), after controlling for year. Thus, yolk carotenoid concentrations could not be related to success at the level of the individual in either species.

At the population level, the annual mean carotenoid concentration in Cassin's auklet yolks had little relationship with the breeding success of either the control or the relaying females (both $r^2 = 0.01$; Fig. 3). In contrast, the mean total carotenoid concentration in rhinoceros auklet yolks had a very strong, positive relationship with the overall yearly breeding success of the relaying females ($r^2 = 0.88$, $F_{1,3} = 22.08$, $P = 0.018$), and a positive but not statistically significant relationship with that of control females ($r^2 = 0.57$, $F_{1,3} = 3.95$, $P = 0.14$; Fig. 3).

Table 3 Strengths (r^2 values) and slopes (or directions) of linear regression lines relating concentrations (in μg/g) of astaxanthin (Cassin's auklet) or total carotenoids (rhinoceros auklet) to $\delta^{15}N$ in egg yolks during each of five years

Year	Species	
	Cassin's auklet (all $n = 8$–10)	Rhinoceros auklet (all $n = 9$–10)
2002	0.01 (−)	0.33* (−26.6)
2003	0.34* (18.7)	0.02 (−)
2004	0.12 (+)	0.76** (−52.0)
2005	0.18 (−)	0.29* (−)
2006	0.02 (−)	0. 01 (−)

In Cassin's auklets, only one of the regressions even approached statistical significance, in a positive direction. In rhinoceros auklets, three of the regressions were negative in direction and either approached or achieved statistical significance, while the other two were neutral in direction
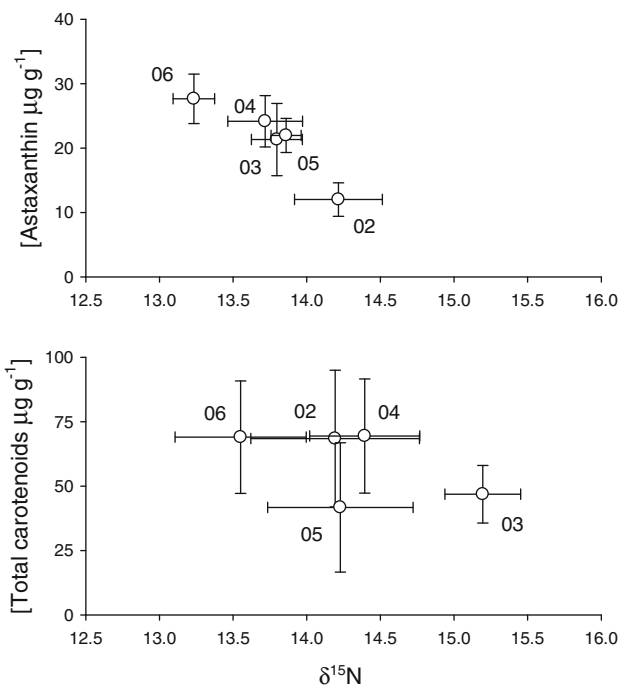
* $P < 0.1$, ** $P < 0.001$

Fig. 2 Relationships between mean $\pm$ SD $\delta^{15}N$ and yolk carotenoid concentrations (astaxanthin or total carotenoids; μg/g) measured in yolks of Cassin's auklet (*top*) and rhinoceros auklets (*bottom*) during each of the five years (2002–2006)

Discussion

Interspecific variation in yolk carotenoids

Egg-forming rhinoceros auklets fed at a higher mean trophic level, and also across a wider trophic range, compared to Cassin's auklets. As predicted from their greater trophic
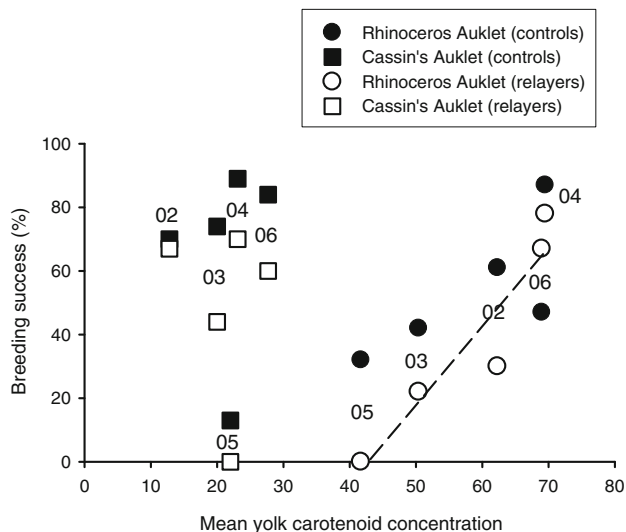
Fig. 3 Relationships between breeding success (chicks fledged per breeding pair) and annual mean carotenoid concentrations (astaxanthin or total carotenoids; μg/g) measured in yolks of Cassin's auklets and rhinoceros auklets during five years (2002–2006)

range, rhinoceros auklet yolks contained a greater variety of carotenoids. Cassin's auklets feed mainly on astaxanthin-rich crustaceans (Abraham and Sydeman 2006; Davies et al. 2009; Hedd et al. 2002), and their yolks contained only astaxanthin. Rhinoceros auklets feed on a varied diet including large amounts of fish (Davies et al. 2009; Ito et al. 2009; Thayer et al. 2008), and had astaxanthin, lutein and zeaxanthin in their yolks. We detected lutein and zeaxanthin only in juvenile (0+) Pacific sandlance, an important prey item for rhinoceros auklets at Triangle Island (Hedd et al. 2006).

The types of carotenoids present in yolks were invariant within species in each of the five years. Such consistency is normal in birds (Hargitai et al. 2006; Royle et al. 1999) and reflects consistency in diet and physiology (McGraw et al. 2006). In addition, there were positive pairwise correlations between concentrations of the three carotenoids in rhinoceros auklet yolks, as in other birds (Safran et al. 2008). Intraspecifically, it seems that accumulating more of one type means accumulating more of all others (McGraw and Gregory 2004), which also might reflect consistency in diet and/or individual variation in the physiological affinity of uptake (Blount et al. 2002a).

Despite the fact that rhinoceros auklets fed at a higher mean trophic level, however, their total yolk carotenoid concentrations averaged three times higher than those of Cassin's auklets. This suggests the existence of species-specific adaptations for the uptake and utilization of carotenoids, an antioxidant which can have complex relationships with life-history traits (Cohen et al. 2008). Interestingly, yolk androgen levels were also higher in rhinoceros auklets than in Cassin's auklets (Addison et al.

2008)—which is consistent with the hypothesis that antioxidants, including carotenoids, are allocated to offspring so as to alleviate oxidative stress caused by androgen-mediated increases in the developmental rate (Royle et al. 2001).

Intraspecific variation in yolk carotenoids

Total carotenoid concentrations varied by a factor of four or more among individuals in both species. As predicted, the interannual differences were marked in both species, as found in terrestrial birds (Hargitai et al. 2006), and feeding at a low trophic level was generally linked to production of a carotenoid-rich yolk. However, that common pattern was caused by different behavioral and environmental factors in the two species.

In Cassin's auklets, the negative relationship between carotenoids and trophic level was mainly due to a year effect. Indeed, within years, carotenoid concentrations and trophic level were unrelated across the narrow trophic range at which individuals of this species cumulatively fed. Thus, in each year, the population of egg-laying Cassin's auklets symmetrically altered its foraging behavior in response to environmental conditions. In contrast, rhinoceros auklets fed across a wide trophic range in all years, but within years showed negative relationships between total yolk carotenoid concentrations and trophic level, with little additive effect of year. This suggests that, in rhinoceros auklets, variation in yolk carotenoid concentrations was caused by among-individual variation in foraging behavior, and not by a population-wide response to environmental conditions. Nonetheless, both the interannual variation in yolk carotenoid concentrations and the positive year-to-year covariation among the three carotenoid types suggest that environment played a role in rhinoceros auklets as well.

The lack of year-to-year concordance in the two species' yolk carotenoid concentrations suggests that different environmental factors determined how available carotenoids were to them. In Cassin's auklets, two statistical periods were evident: yolk carotenoid concentrations were lowest in 2002, when the population fed at the highest trophic level, but were higher from 2003 to 2006, when the trophic level was lower. This accords well with information on the timing of the zooplankton biomass peak in surface waters off the British Columbia coast (Mackas et al. 2007). Peaks fell in early May in 2002, near the start of the auklets' hatching period, but in early-to-mid April in 2003–2006, when auklets were forming eggs and/or beginning to incubate. Thus the timing of prey blooms may have been key: yolks were carotenoid-rich in years when the birds formed eggs close to the zooplankton peak (and ate more astaxanthin-rich crustaceans as a result), but yolks

were carotenoid-poor when they formed eggs well ahead of the zooplankton biomass peak (and ate fewer crustaceans as a result).

For rhinoceros auklets, yearly variation in the concentrations of all three yolk carotenoids mirrored yearly variation in chlorophyll a concentrations in surface waters within a 100 km radius around Triangle Island, which were lower in 2003 and 2005 than in 2002 and 2004 (Wolf et al. 2009). Thus, we propose that the strength of primary production was key for rhinoceros auklets: yolks were carotenoid-rich in years in which the marine ecosystem was more productive. We emphasize that these ideas are based on a relatively short time series. Clearly, a longer time series and studies elsewhere are needed to confirm or refute them. If found to hold, then we suggest that carotenoid profiles could be a useful tool for monitoring interannual variation in marine environmental conditions and its influence on marine consumers including birds (Piatt et al. 2007).

Relationship to breeding success

In Cassin's auklets, there was no link between yolk carotenoid concentrations and breeding success at the individual or population levels. In a species that consumes large quantities of carotenoid-rich prey, and that lays a relatively small (Hipfner et al. 2004), single egg (Biard et al. 2006), females may have little difficulty in meeting their physiological requirements for carotenoids. This might be especially true of early-laying females, whose diets can be especially rich in crustaceans (Sorenson et al. 2009), and at our study site, which lies within an extremely productive marine system (Ware and Thomson 2005). Further, a threshold might exist beyond which increased levels of yolk carotenoids cease to affect embryonic survival (Tyndale et al. 2008). Nonetheless, good feeding conditions, i.e., a strong match between the timing of the spring bloom of late-stage N. cristatus copepodites and the timing of the chick-provisioning period, are key to successful breeding in Cassin's auklets at Triangle Island (Hipfner 2008). Because the phenological match between predator and prey is better for early-laying females, there is a fitness premium on early breeding onset in this population (Sorenson et al. 2009).

In rhinoceros auklets, there was no link between yolk carotenoid concentrations and an individual female's breeding successfully with the replacement egg. Again, this might indicate that females have little difficulty meeting their physiological requirements for carotenoids in any year, even though rhinoceros auklets lay larger eggs relative to body mass (Hipfner et al. 2004). In contrast to Cassin's auklets, however, we did find the predicted positive relationship between the mean total yolk carotenoid

concentration and the proportion of females in the population that raised their single offspring to fledging in a given year, especially among the relaying females themselves. Thus, in years in which yolks were rich in carotenoids, the whole population bred more successfully, and females whose eggs we removed showed a greater ability to overcome that handicap.

The positive covariation could be a reflection of the direct beneficial effects of antioxidants on offspring performance (Bize et al. 2008): the population fared better in some years *because* their yolks were richer in carotenoids. Indeed, experiments on lesser black-backed gulls *Larus fuscus* have shown that there can be strong and direct beneficial reproductive effects of yolk carotenoids (Blount et al. 2002b). Alternatively, it could be purely correlational: the birds fared better in years when their yolks were rich in carotenoids because the two are independently linked to a more productive ocean. On balance, we believe our results are more consistent with the latter explanation. First, at the level of the individual female, there was no association between carotenoid concentrations and the probability of success. Second, we included only eggs laid by early-laying females. Experiments indicate that laying early confers a direct fitness benefit at Triangle Island (Hipfner et al. 2008), and we therefore expect phenotypically better females to do so (Verhulst and Nilsson 2008). Yet even among early-laid eggs, yolks varied greatly in their carotenoid concentrations, due to trophic level. If successful breeding was causally linked to higher yolk carotenoid levels, then natural selection should favor females that feed at low trophic level while forming eggs— and we should expect to see uniformly low trophic-level feeding among early layers.

To conclude, our contrast of yolk carotenoids and isotope ratios between Cassin's auklets and rhinoceros auklets breeding over a five-year period has offered novel insights into the dynamics between carotenoids, foraging ecology and variation in environmental productivity. Nonetheless, the links between carotenoid concentrations, environmental quality and overall fitness are far from clear (Monaghan et al. 2008). We have little doubt that future work with seabirds will help to resolve them better.

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