Carotenoid-based skin ornaments reflect foraging propensity in a seabird, *Sula leucogaster*

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Abstract

Carotenoid-based ornaments are common signaling features in animals. It has long been proposed that such ornaments communicate information about foraging abilities to potential mates. However, evidence linking foraging with ornamentation is largely missing from unmanipulated, free-ranging populations. To investigate this relationship, we studied a coastal population of brown booby (*Sula leucogaster brewsteri*), a seabird with a carotenoid-based gular skin ornament. $\delta^{13}C$ values from both feathers and blood plasma were negatively correlated with male gular color, indicating birds that consumed more pelagic prey in offshore locations had more ornamented skin than those that fed on nearshore, benthic prey. This relationship was supported by our GPS tracking results, which revealed longer, more offshore foraging trips among highly-ornamented males. Our data show that brown booby ornaments are honest indicators of foraging propensity; a link consistent with the rarity hypothesis and potentially driven by the concentration of carotenoids found in phytoplankton versus benthic algae. Carotenoid-based ornaments may reflect foraging tendencies in animals such as coastal predators that utilize food webs with distinct carotenoid profiles.

Keywords: Seabirds, Stable Isotopes, Carotenoid Ornamentation, Rarity Hypothesis, GPS Tracking, Foraging

1. Introduction

Carotenoid-based sexual ornaments are common signaling features in animals and are proposed to be honest indicators of individual condition and ultimately, reproductive value [1–3]. While the mechanistic link between individual condition and production of carotenoid-based ornaments remains uncertain, hypotheses variously implicate diet, vitamin A production and redox state, and efficiency of cellular respiration [2–4]. Critically, carotenoid pigments cannot be produced *de novo* by animals and therefore, it has long been suggested that ornament formation is linked to foraging choices and ability [1]. For example, the rarity hypothesis states that carotenoids may be difficult to obtain from the diet, and indeed, carotenoid-depleted or supplemented diets can alter ornament condition [2,5]. However, the link between foraging and carotenoid-based ornaments has seldom been elucidated in free-ranging animals without manipulation. Therefore, it is important to understand how the natural range of foraging choices and ability may constrain ornament condition in wild populations.

If carotenoid ornaments are communicating information about foraging abilities of potential mates, foraging behavior may provide a mechanistic link between ornamentation and direct benefits gained by mates, such as increased offspring provisioning. In other words, high quality individuals with optimized foraging behavior might obtain more carotenoids in their diets, display more intense carotenoid-based ornaments, and feed offspring more often, potentially with higher-quality foods (e.g. more carotenoid and antioxidant-rich) [6,7]. To investigate the potential link between foraging behavior and ornament quality, we examined a coastal population of brown boobies (*Sula leucogaster brewsteri*), a seabird with a carotenoid-based gular skin ornament advertised during courtship [8]. A previous study showed that females gain direct benefits such as increased chick growth by mating with males that have greener, more carotenoid-rich-skin, rather than with presumably-lower quality blue-skinned males [6]. Notably, the major carotenoids in male brown booby gular skin (lutein, zeaxanthin, and 13-cis beta-carotene) [8] are derived directly from diet, with little metabolic transformation [3].

Our study takes advantage of two minimally invasive techniques, GPS tracking and stable isotope analysis, to investigate whether ornament quality is related to dietary tendencies and at-sea behaviors, which we term foraging propensity. Stable isotope ratios of animal tissues reflect assimilated diet and can differentiate between individuals using distinct ecosystems or nutrient sources. For example, in coastal environments, stable carbon isotope values ($\delta^{13}C$) are predictably lower in pelagic and offshore versus benthic and near-shore food webs, all of which are utilized by brown boobies [9]. By employing carbon isotopes and GPS tracking, techniques previously unused in carotenoid studies, we aim to shed light on the signal content of carotenoid-based ornaments in wild populations.

2. Material and Methods

We studied courting male brown boobies from Isla Larga, Parque Nacional Islas Marietas, Mexico (20.69°N, 105.58°W). We collected white breast feathers from 13 birds in July 2012 and blood plasma from 12 different individuals in July 2016 (supplementary materials). We transferred blood samples to heparin-coated vials, centrifuged to separate red blood cells, and preserved plasma with 70% ethanol. Whereas isotope data from blood plasma reflect the previous ca. 10 days [10], isotope data from breast feathers represent the period of feather growth, likely concentrated during the non-breeding season, based on examination of brown booby specimens at the National Museum of Natural History (supplementary materials). Color measurements of the gular skin were taken from all birds during capture, using a handheld spectrophotometer (MINOLTA CM 2600d, Osaka, Japan) that measured reflectance in 10 nm intervals from 360 to 740 nm. We used reflectance data to calculate green chroma: the sum of values within the green spectrum (520–560 nm) divided by total reflectance [6]. In 2016, we obtained two color measures, once upon initial capture and again upon GPS retrieval. As expected [8], these two measures were highly correlated (R^2=0.77, p=0.0017, df=8) and were therefore averaged for each individual.

Using modified i-gotU GT-120 GPS loggers (Mobile Action Technologies, New Taipei City, Taiwan), we tracked nine male birds during 2016 that were also sampled for isotope analysis. We sealed tags in waterproof heat-shrink tubing and attached with waterproof tape (Tesa® 4651, Norderstedt, Germany) to the underside of three to four central rectrices. We removed tags 7–10 days after deployment. We developed an inshore versus offshore foraging index by combining three tracking parameters into one principal component (PC1): average azimuth of foraging trip centroids, maximum ocean depth averaged among all trips, and maximum range (figure 1a–c; supplementary materials).

Before isotope analysis, we washed feather barbs in solvent (87:13 chloroform:methanol by volume), dried under vacuum, and homogenized. We dried blood plasma under vacuum to remove ethanol and then lyophilized and powdered samples (supplementary materials). We analyzed our isotope samples using a Vario PYRO Cube elemental analyzer (Elementar Americas) interfaced to an Isoprime 100 stable isotope ratio mass spectrometer (Isoprime). We report stable isotope values in per mil (‰) according to delta notation: $\delta^{13}C=([R_{sample}/R_{standard}]-1)x1000$, where R denotes the ratio of $^{13}C/^{12}C$ and the standard is Vienna Pee-dee belemnite. Precision for $\delta^{13}C$ was ≤ 0.1‰.

Principal components and linear regression analyses were completed using JMP Pro 13.

3. Results

During courtship, maximum range among male brown boobies varied from 168.8 to 315.3 km (mean ± SD = 261.1 ± 39.8). Azimuth ranged from 0.9° to -71.3° (-33.1° ± 21.8), and maximum ocean depth ranged from 535 m to 2868 m (1719 ± 638) (figure 1d: kernel density plot representing all tracked individuals). PC1 (hereafter, "coastal foraging index") accounted for the majority (82.8%) of observed variation in our focal tracking parameters. Gular green chroma was negatively correlated with δ^{13}C values of blood plasma (2016) and feather (2012) (2012, R^2=0.58, p=0.004; 2016, R^2=0.38, p=0.03;figure 2a&b). The coastal foraging index increased significantly with blood plasma δ^{13}C (linear regression, R^2=0.48, p=0.03) (figure 2c), and was negatively correlated with green chroma (R^2=0.46, p=0.04) (figure 2c&d).

4. Discussion

Sexual ornament coloration varied with foraging propensity among male brown boobies, as indicated by both stable isotope results and our coastal foraging index, derived from GPS tracking data. As expected, brown boobies with lower δ^{13}C values had more offshore foraging habits (as indicated by lower coastal foraging index values; figure 2c) and were therefore more likely to depend on pelagic prey. Because males with greener ornaments had both lower δ^{13}C and coastal foraging index values (figure 2b&d), our data indicate that ornament quality increases with the degree of offshore and pelagic-based foraging. This relationship was robust, as it was visible in isotope data from two tissues reflecting different time scales: in blood plasma representing a ca.10-day period in the courtship phase, and in feathers, likely representative of the non-breeding season. Furthermore, the relationship between $\delta^{13}C$ and ornament color persisted across distinct El Niño Southern Oscillation phases, including a neutral phase (2012) and an El Niño event (2016).

The importance of offshore and pelagic foraging in the development of high-quality ornaments may lie in the carotenoid pigments found in the brown booby gular ornament. Carotenoids are generally produced at higher concentrations in phytoplankton, relative to the concentrations observed in benthic algae [11]. For example, marine phytoplankton such as *Dunaliella*, a widespread and well-studied genus, contain high concentrations of the pigments found in brown booby ornamentation, including 13-cis beta carotene, lutein, and gamma carotene [8,12]. Therefore, while brown boobies are known to feed on both pelagic and benthic fish and squid, pelagic prey such as Pacific sardine and flying fish may yield increased dietary carotenoids [13].

Notably, our coastal foraging index incorporated the maximum range of a bird, meaning that individuals with greener skin who foraged more offshore also traveled greater distances. As typical sulids, brown boobies have greater wing-loading than many other seabirds and a relatively high cost of long-distance flight [14,15]. Hence, our results suggest that individuals in poor condition may be constrained to low-cost, short-distance foraging trips and therefore, unable to obtain the pelagic diet necessary for production of carotenoid-rich ornaments.

Gular ornament coloration in the brown booby appears to be an honest indicator of foraging propensity—a link potentially driven by the rarity of dietary carotenoids and the cost to participating in more distant, carotenoid-rich pelagic food webs. The relationship between diet and ornamentation deserves further study, particularly among coastal predators (e.g. fish, seabirds), whose conspecifics vary in their utilization of benthic versus pelagic food webs [16] and where pelagic-based foraging may be widely important for carotenoid consumption. Many organisms forage within multiple ecosystems and exhibit forging dichotomies that may present trade-offs between carotenoid access and the imposed costs of acquiring them. We suggest that the potentially widespread link between foraging and ornaments be revisited, especially in the context of environmental variance.

Ethics. We obtained appropriate IACUC approval and field permits (University of Akron Institutional Animal Care and Use Committee: Protocol#: 16-06-13-WBC; SEMARNAT Dirección General de Vida Silvestre SGPA/DGVS/04708/16). The use of trade, product or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Data Accessibility. Michael N, Torres R, Welch A, Adams J, Bonillas-Monge ME, Felis J, Lopez-Marquez L, Martínez-Flores A, Wiley A. Carotenoid-based skin ornaments reflect foraging propensity in a seabird, *Sula leucogaster*. Dryad Digital Repository. https://doi.org/10.5061/dryad.8v9d969[17]

Author Contributions. NPM, RT, AJW, and AEW conceived the study. NPM, RT, AJW, MEBM, LL-M, AM-F, and AEW conducted field work. NPM and AEW wrote manuscript, with edits and final approval from all authors. NPM and AEW conducted stable isotope analyses. JA and JF developed GPS tracking methodology, data processing, and mapping. NPM conducted all statistical analyses. All authors agree to be held accountable for the content therein and approve the final version of the manuscript.

Competing Interests. We have no competing interests.

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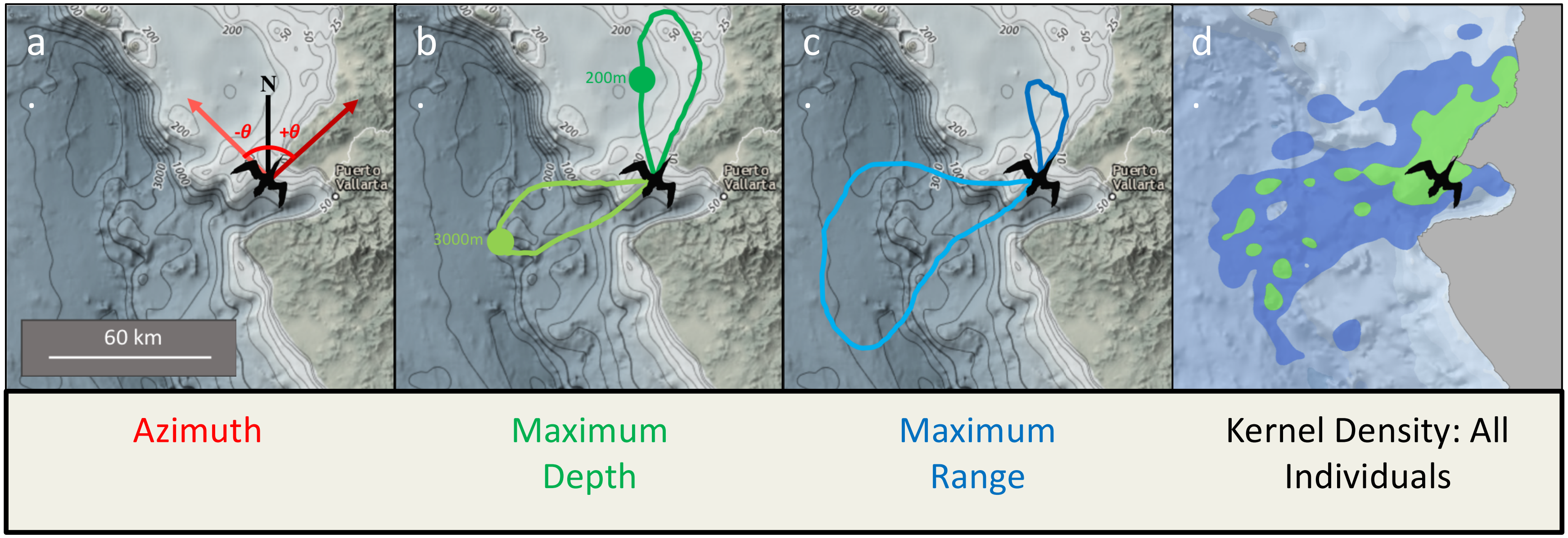
figure 1. Depictions of the three variables used to create an coastal foraging index (A–C; light-shaded lines represent more negative offshore values and dark-shaded lines, positive inshore values), and (D) the foraging distribution of male brown boobies from the Marietas Islands, Mexico, during courtship (kernel density plot of foraging trips, 50% contour shown in green, 95% contour in blue). In all panels, the bird silhouette marks the location of the Marietas Islands breeding colony.



Azimuth

Maximum Depth

Maximum Range

Kernel Density: All Individuals

figure 2. Male brown booby, showing the blue to green-colored skin ornamentation (A), and the relationship between stable carbon isotope values, coastal foraging index values (decreasing index values correspond with more offshore foraging), and gular skin green chroma (B–D). Open circles denote blood plasma and closed circles, body contour feathers. B. plasma p-value=0.03, feather p=0.004, C. p=0.03, D. p=0.04.