Spring 2018

In Sickness and in Health: Understanding the interactive effects of immune challenge and foraging behavior on parental investment in a monogamous seabird

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Abstract

Carotenoid ornamentation is quite common in many species of birds, and often serves as an honest indicator of their fitness. The honesty of carotenoid ornamentation in the brown booby (Sula leucogaster brewsteri), a monogamous seabird, is likely driven by rarity of carotenoids in the diet but may also be regulated through additional pathways. For example, the trade-off hypothesis states that carotenoid pigments used in ornaments may also be required for the immune system as an antioxidant, thereby allowing only the healthiest birds to afford the carotenoid ornamentation. To test the trade-off hypothesis, I took advantage of a previously conducted experiment on brown boobies from the Marietas Islands, Mexico, where a subset of individuals were treated with a lipopolysaccharide to produce an immune response, while a control group was treated with a buffer solution. These seabirds were observed during their nesting period with parental behaviors and carotenoid coloration data recorded throughout the given time-frame. I studied the foraging behavior of these birds through bulk stable isotope analysis of δ^{13}C and δ^{15}N values in their breast contour feathers.

I explored foraging behavior data alongside immune responses, parental behaviors, and carotenoid coloration data in order to determine whether brown boobies foraging habits moderated the impact of immune challenge on parental effort. Results indicated that the imposed immune challenge had no effect on carotenoid ornament coloration, suggesting that the carotenoids used for ornamentation are not necessary for the bird’s immune system function, or else not limited in quantity. The results also revealed that immune-challenged birds with increased foraging ability (foraging habits associated with the preferred ornament type) provided better parental care compared to birds with decreased foraging ability. Control birds did not show this trend, and their parental care was not related to my measure of foraging behavior. My results suggest that foraging ability becomes more critical to parental effort when the parent is also producing an immune response.

Introduction

In many species, individuals choose mates based on the likelihood of receiving direct benefits: benefits directly imparting an increase in fitness to the choosier sex through shared resources such as food. While our understanding of how environmental variation impacts these benefits is often limited, we know that an animal’s ability to provide direct benefits to mates may be communicated by carotenoid ornaments (García-de Blas 2013). Carotenoid ornaments are particularly well represented among birds, occurring in various tissues such as the skin and feathers, and in varying environments including marine, freshwater, and terrestrial (Hill and Johnson 2012, Goodwin 1984). There are several examples of direct benefits in birds relating to carotenoid ornaments, particularly among monogamous species. Monogamous birds often rely on one another for direct benefits including protection, food rationing, and shared parental care (Tershy and Croll 2000). As marine systems experience high levels of environmental variability (for example, El Niño Southern Oscillation) and consequently, highly heterogeneous food sources, direct benefits are predicted to be a particularly important driver of mate selection in seabirds and other marine predators (Piacenza et al. 2015). To these species, the honesty of the sexual ornament, whether an ornament truly reflects information about an individual, may be critical to their survival and reproductive success.
The honesty of carotenoid ornamentation has been hotly debated, and multiple hypotheses attempt to explain potential costs faced by an individual when forming a high quality ornament. One such hypothesis assumes rarity of carotenoid pigments in the diet. Carotenoids cannot be synthesized by any animal and are only produced by primary producers at the base of the food web (Hill and Johnson 2012, Goodwin 1984). For higher trophic level organisms, carotenoids have to travel through multiple trophic levels and may be rare in predators’ diets, so then only the more skilled foragers will be able to obtain a sufficient amount of carotenoids. In this scenario, ornaments could reflect an individual’s foraging ability and its capacity to provide for its potential mate and chick. Expression of carotenoid pigments in many birds may also be limited by the metabolic cost of altering and depositing the consumed form of the pigments into a more derived form for the ornament, which could result in a physiological limit. The physiological limit hypothesis suggests that carotenoid ornaments are honest signals due to the physiological limits of an individual during carotenoid metabolism, preventing the production of a dishonest ornament (Inouye et.al. 2001). The last major hypothesis, the trade-off hypothesis, considers whether the carotenoids used in ornamentation are also required by the immune system as anti-oxidants, with a trade-off occurring between health and survivability of an organism, and the quality of the sexual ornament. This trade-off would suggest that only the higher quality individuals could accrue sufficient carotenoid pigment to maintain both anti-oxidant defenses and ornaments.

The rarity, physiological limit, and trade-off hypotheses have received varying degrees of attention in seabird species. For example, physiological processing limitations have not been explored in seabirds, as most carotenoids are placed directly into the integument without modification via a physiological pathway (Montoya 2015). This suggests that the maintenance of metabolic pathways does not generate the cost of ornamentation in seabirds. The traditional hypothesis of rarity has received sparse support, but recent work on the brown booby (Sula leucogaster) has suggested that rarity is the driving cost, with birds who can access more distant, offshore environments having more colorful ornaments (Michael et. al., In Preparation). The trade-off hypothesis has been explored in seabirds but has received mixed results, with unclear relationships between anti-oxidant defenses and ornament quality (García-de Blas 2013). The trade-off between depositing carotenoids in ornaments vs. using carotenoids for immune function is therefore still poorly understood in seabirds; it may be occurring but requires further investigation. It is important to note that these three hypotheses are not mutually exclusive.

The brown booby is a sexually dimorphic seabird with a carotenoid ornament on the gular skin and feet. The subspecies Sula leucogaster brewsteri can be found breeding in the Eastern Tropical Pacific, the Gulf of California, and its surrounding islands. The brown booby feeds either in coastal waters or beyond the continental shelf with a characteristic plunge-diving strategy. Their diet is highly varied, including numerous fish and squid species, but is strictly marine (Schreiber and Norton 2002). This species is reverse sexually dimorphic, with females averaging around 33% larger than males. Males also have white plumage along their head and neck that fades to dark brown, while the female’s head and neck plumage is fully brown. The white ‘hood’ found on males may be a sexual ornament, although it is not based on carotenoids (R. Torres, unpublished data). Carotenoid ornaments also differ between sexes, with males having more blue-green coloration and females having more yellow-pink colors. These traits allow for clear differentiation between the sexes (Schreiber and Norton 2002).
boobies exhibit a monogamous mating system, with mate selection partially based on carotenoid pigmentation. Stronger carotenoid pigmentation results in greener skin coloration in males, which is preferred by females over more blue coloration (Montoya and Torres, 2015). Montoya and Torres (2015) also showed that increased green coloration in males was correlated with increased feeding and chick growth, even if the chicks had been raised by non-biological fathers, indicating green coloration reflects increased male parental ability and not just superior genes. Thus, it is presumed females are selecting greener males for conferred direct benefits. As previously mentioned, the brown booby’s carotenoid ornament appears to be an honest indicator of foraging behavior and may be driven by the rarity of carotenoids in the diet. However, it is not clear if the carotenoid-based ornament of the brown booby has multiple costs. For example, it may be driven both by the rarity of carotenoids in diet and also by a trade-off between using carotenoids for antioxidant-defense vs. ornamentation (required hypothesis), in which case stressors such as disease and pollution may be impacting ornament condition and associated direct benefits.

To examine the effect of immune stress on ornamentation, Martínez-Flores and Torres (In Review) imposed an immune challenge on incubating brown boobies using a lipopolysaccharide of Escherichia coli. They found that immune activation significantly decreased parental effort. However, we do not know how or if condition or behaviors before infection, such as foraging behavior, prepared parents for this immune challenge. Using stable isotopes of carbon and nitrogen, spectrophotometer color measures and information regarding immune response and parental care, I set out to answer my main research question: How do long-term foraging patterns of brown boobies, as determined through bulk stable isotope analysis of feather, relate to the impact of an imposed immune challenge on parental investment? I hypothesized that brown boobies with more ideal foraging habitats, as associated with greener skin pigmentation, would show a smaller effect of immune challenge on parental effort. I also examined how ornament color relates to the presence/absence of an immune challenge and the long-term foraging habits of an individual.

Methodology

This research was done in collaboration with Alejandro Martínez-Flores and Dr. Roxana Torres working from the Universidad Nacional Autónoma de México, alongside Nathan Michael and Dr. Anne Wiley at the University of Akron. I analyzed a total of 104 samples, divided into 67 immune-challenged birds and 37 ‘control’ birds (birds that were observed and captured in the same way as immune-challenged birds, but injected with a buffer solution (PBS) instead of the lipopolysaccharide (LPS)). I conducted bulk carbon and nitrogen stable isotope analyses of over 300 breast contour feather samples from Martínez-Flores and Torres’ immune-challenged and control birds. This process required washing the feathers in a chloroform methanol solution (87/13 by volume), homogenizing the feather barbs, and weighing out samples from each bird to be analyzed. This analysis described the foraging patterns of the birds through the carbon and nitrogen isotope ratios (reported as $\delta^{13}$C and $\delta^{15}$N values) found in the feathers. My dependent variables consisted of the parental behavior data, specifically nest attendance, chick growth, and feeding attempts as well as spectrophotometer data on carotenoid ornamentation color. My independent variables consisted of foraging behavior, as determined through the stable isotope analysis, and the presence/absence of an immune challenge. I compared my variables using statistical analyses by JMP programs, namely bivariate analyses and one-way anovas.
Results

Figure 1: Average Green Chroma by LPS/PBS
P-Value = 0.5577
R-Squared = 0.019436
LPS Mean = 0.213473
PBS Mean = 0.212322
NO SIGNIFICANT DIFFERENCE

Figure 2: Avg. Green Chroma
Catch 1, LPS Treatment – Males Only
Mean = 0.215259
St. Dev. = 0.007461

Figure 3: Avg. Green Chroma
Catch 2, LPS Treatment – Males Only
Mean = 0.213381
St. Dev. = 0.003899

Figure 4: Avg. Green Chroma
Catch 3, LPS Treatment – Males Only
Mean = 0.211128
St. Dev. = 0.009352
Results (Continued)

**Figure 5:** Carbon isotope analysis, LPS vs PBS  
P-Value = 0.7162  
R-Squared = 0.001383  
LPS Mean = -15.222  
PBS Mean = -15.193  
NO SIGNIFICANT DIFFERENCE

**Figure 6:** Nitrogen isotope analysis, LPS vs PBS  
P-Value = 0.3544  
R-Squared = 0.008941  
LPS Mean = 16.6926  
PBS Mean = 16.5742  
NO SIGNIFICANT DIFFERENCE

**Figure 7:** Rate of chick growth compared with carbon isotope values, LPS treatment only.  
P-Value = 0.0216  
R-Squared = 0.322908  
SIGNIFICANT RELATIONSHIP

**Figure 8:** Rate of chick growth compared with carbon isotope values, PBS treatment only.  
P-Value = 0.4843  
R-Squared = 0.062987  
NO SIGNIFICANT RELATIONSHIP
Results (Continued)
Figures 1 – 8 represent the most noteworthy relationships determined from my statistical analyses, but by no means are the only ones. Parental data included comparisons of growth rate of chicks in grams per day, number of feeding attempts by parent, and minutes spent attending the nest. Comparisons among male and female birds showed several trends but none pertaining specifically to my research questions. Males tended to have greater nest attendance, while females foraged slightly farther out to sea. Coloration data was compiled from three separate catch dates and multiple areas of carotenoid ornamentation (cheeks, gular skin, and feet). The average green chroma (Figures 2-4) was calculated by averaging the values of the three body areas above for each bird for each catch.

Discussion
My initial research question asked how long-term foraging patterns of brown boobies, as determined through bulk stable isotope analysis of feather, relate to the impact of an imposed immune challenge on parental investment? I hypothesized that the brown boobies with more ideal foraging habitats (those associated with greener skin pigmentation), would show a smaller effect of immune challenge on parental effort.

I first examined the associations of carotenoid ornamentation coloration. In Figure 1, I compared the LPS treatment group’s average green chroma to the control group’s average, and found no significant difference between the two. This showed that the immune challenged birds were not facing any decrease in carotenoid ornamentation while combating their infection. In addition, Figures 2-4 compared the average green coloration of the LPS birds at three separate capture dates to determine if the immune challenge resulted in a decrease in ornamentation over time. These figures also show no significant difference between the average green coloration over time. Though they show a very slight decreasing trend over time, the control birds had an even greater decreasing trend, though still slight. These results likely indicate that the imposed immune challenge had no effect on the carotenoid ornament coloration, suggesting that the carotenoids used in ornamentation are not necessary for the bird’s immune system function, or else not limited in quantity.

Figures 5 and 6 compare the foraging data from the stable isotope analysis of $\delta^{13}$C and $\delta^{15}$N values between the experimental and control groups. Lower values of $\delta^{13}$C indicated foraging farther offshore while higher ones are associated with closer inshore foraging (Michael et. al., In Preparation, and Rubenstein and Hobson, 2004). $\delta^{15}$N values correspond to zones of upwelling surrounding the Marietas Islands, as well as trophic levels of food sources. Higher $\delta^{15}$N values indicate higher trophic levels of the birds’ prey items (Rubenstein and Hobson, 2004). However, there was no difference between the average long-term foraging behaviors of the treatment and control birds. Trends began to appear when comparing their foraging behaviors to parental behaviors. The most significant trend is displayed in Figure 7, showing that the growth rate of chicks of LPS treated parents was lowest when parents had more inshore (presumably lower quality) foraging habits. Other parental behaviors such as feeding attempts followed the trend in Figure 7, but not to a significant extent. Nest attendance on the other hand showed the opposite (non-significant) relationship, which makes sense since seabirds foraging farther away from the breeding colony (as shown by lower values of $\delta^{13}$C) will be airborne more and attending the nest less. Further research with greater sample size should clarify this.

Interestingly, the control birds did not show a relationship between chick growth rate and foraging behavior (Figure 8), or even a similar trend as the experimental birds. In all statistical
comparisons between control birds’ foraging and parental behaviors, no trends or significant relationships were found. This suggests that foraging ability becomes more critical to parental effort when the parent is also producing an immune response. In other words, nearshore (presumably lower quality) foraging habits do not appear to compromise parental ability when the parents are healthy. Based on these results, should this population of brown boobies be afflicted by disease, pollution, or some other disadvantage, we can expect selection to favor those with more offshore foraging habits.

This experiment readily displayed how foraging behaviors (measured through δ¹³C values) determined the success of immune-challenged brown boobies. However, the same cannot be said for the δ¹⁵N values. While relationships between δ¹³C and parental behavior were relatively consistent, showing increased parental efforts with more extensive offshore foraging, δ¹⁵N values showed a mix of relationships when compared to parental behaviors, none of which were significant. While not significant, the nitrogen results often conflicted with the expected relationship for foraging behaviors. In some statistical analyses they indicated that LPS-treated birds foraging from higher trophic levels actually provided better parental care. This is confusing since higher trophic levels yield less carotenoids which would not serve to indicate this increased parental effort. Needless to say, more data is required in future research to determine if the foraging behaviors reflected by δ¹⁵N values hold significance for brown booby ornaments or parental care.

In the future, I am hoping more research will be dedicated to understanding the brown booby. Should this experiment be repeated, I would significantly increase the sample size of the control group. This experiment was conducted with significantly uneven sample groups (67 experimental and 37 control), which may have hidden some differences between them.

The main conclusions I can come to from this work are that the imposed immune challenge had no effect on carotenoid ornamentation coloration, suggesting that the carotenoids used in ornaments are not necessary for the bird’s immune system function, or else not limited. The results also revealed that LPS-treated birds with increased foraging ability showed increased parental care compared to LPS-treated birds with decreased foraging ability. Control birds did not show this trend: parental care was unaffected by foraging behavior. This suggests that foraging ability becomes more critical to parental effort when the parent is also producing an immune response.
Works Cited


Martínez-Flores, Alejandro and Torres, Roxana. “Immune Activation Induces a Decline in Parental Effort and Compensation by the Mate.” Journal of Avian Biology, Currently in Review.


doi:10.2173/bna.649