Animal Behaviour 108 (2015) 9–16

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

# Regulation of breeding expenditure in the blue-footed booby, *Sula nebouxii*: an experimental approach

Erick González-Medina [a, b, 1], José Alfredo Castillo-Guerrero [c, 2], Francisco Santiago-Quesada [d, 3], Auxiliadora Villegas [d, 4], José A. Masero [d, 5], Juan M. Sánchez-Guzmán [d, 6], Guillermo Fernández [a, *]

[a] *Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Sinaloa, Mexico*
[b] *Posgrado de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mexico*
[c] *Centro de Investigación en Alimentación y Desarrollo A.C., Unidad Mazatlán en Acuicultura y Manejo Ambiental, Sinaloa, Mexico*
[d] *Conservation Biology Research Group, Área de Zoología, Universidad de Extremadura, Badajoz, Spain*

## ARTICLE INFO

*Article history:*
Received 18 February 2015
Initial acceptance 7 May 2015
Final acceptance 15 June 2015
Published online
MS. number: A15-00132

*Keywords:*
creatine kinase
foraging effort
leucocytes
parental care
reproductive effort

In the short-term, reproductive expenditure has repercussions for survival and future reproductive output. In long-lived seabirds, parents are expected to adjust their reproductive investment based on their physiological and immune status and then respond to the needs of their offspring without incurring additional costs. However, the impacts of parental expenditures on physiological and immune status have not been well explored. We compared the foraging effort (number and duration of foraging trips), time at nest and physiological status (plasma metabolites, heterophil/lymphocyte (H/L) ratio, creatine kinase (CK) activity) and body condition index (BCI) of blue-footed boobies subjected to experimentally increased or decreased breeding demands. When parental reproductive demands were increased, adults made more foraging trips and decreased their time at the nest. The increases in adult physical activity were reflected in higher levels of creatine kinase/total protein (CK/TP, U/g) and higher H/L ratios, indicating that adjustments in foraging effort were reflected in their physiological status. When parental reproductive demands were reduced, parents adjusted their level of effort according to the lower needs of their offspring; as a result, their overall physiological status was greater than that of the controls, showing lower CK/TP levels and H/L ratios. Our findings indicate that parents modified their foraging effort in response to variation in the food demands of the brood to maintain reproductive value, but this adjustment had consequences for physiological status in both costs and benefits. Blue-footed boobies inhabit upwelling systems where they experience high environmental variability throughout their life span. Thus, the ability to adjust breeding effort may buffer breeding success under different scenarios, with short-term physiological expenditures possibly related to long-term survival.

In long-lived species, current reproductive investment is likely to be regulated by the impact of reproductive effort on parental survival (Linden & Møller, 1989). The breeding effort threshold in a particular animal species may vary depending on environmental conditions and the physiological state of each individual (Velando & Alonso-Alvarez, 2003). Therefore, during reproduction, parents must decide how much parental care to provide to their offspring (Varpe, Tveraa, & Folstad, 2004). To maximize their return on a reproductive event, parents should adjust their reproductive decisions according to their physiological and immune status to meet the needs of their offspring while taking into account their own long-term breeding success (Clutton-Brock, 1991; Velando & Alonso-Alvarez, 2003). In long-lived seabirds, knowledge of individuals' physiological and immune statuses is critical for understanding individuals' strategies for reproduction, foraging and survival (Navarro, González-Solís, Viscor, & Chastel, 2008). However, these characteristics and their effects on parental investment

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[*] Correspondence: G. Fernández, Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Apartado Postal 811, Mazatlán, Sinaloa, C.P. 82040, Mexico.
*E-mail address*: gfernandez@ola.icmyl.unam.mx (G. Fernández).
[1] *E-mail address*: gmedinaerick@gmail.com (E. González-Medina).
[2] *E-mail address*: alfredocas@gmail.com (J. A. Castillo-Guerrero).
[3] *E-mail address*: fquesada@unex.es (F. Santiago-Quesada).
[4] *E-mail address*: villegas@unex.es (A. Villegas).
[5] *E-mail address*: jamasero@unex.es (J. A. Masero).
[6] *E-mail address*: jsanchez@unex.es (J. M. Sánchez-Guzmán).

http://dx.doi.org/10.1016/j.anbehav.2015.06.025

have received little attention (Dehnhard et al., 2011; Navarro & González-Solís, 2007).

Seabirds have different investment strategies depending on the reproductive capacity of the species. For most bird species, the chick-rearing period is assumed to be a critical stage for decisions that influence the balance of parental effort (e.g. Drent & Daan, 1980; Riechert, Chastel, & Becker, 2014; Weathers & Sullivan, 1993; but see Gruebler & Naef-Daenzer, 2010; Tarwater & Brawn, 2010). Experimental studies of seabirds in which the demands of the offspring are manipulated show one of three basic patterns: (1) parents can adjust their effort in response to the changing demands of the offspring (Granadeiro, Bolton, Silva, Nunes, & Furness, 2000; Phillips & Croxall, 2003); (2) individuals exhibit a partial adjustment, such that the demands of the offspring are not completely met (González-Medina, Castillo-Guerrero, & Mellink, 2010; Velando, 2002); or (3) parents do not adjust their parental care efforts and are inflexible to the demands of their offspring (Hamer, Lynnes, & Hill, 1998; Weimerskirch, Chastel, & Ackermann, 1995). Therefore, it is of interest to investigate how seabirds adjust their parental effort and foraging behaviour and the subsequent effects of these adjustments on their physiological and immune status.

Here, using a cross-fostering experiment, we tested how male and female blue-footed booby parents adjust their parental effort and foraging behaviour in response to changes in the reproductive expenditure of attending to younger chicks (i.e. lower demand for food) or older chicks (i.e. higher demand for food) and investigated the resulting effects on their physiological and immune status. The blue-footed booby is a long-lived seabird with a long period of biparental care (up to 6 months, Nelson, 2005). Both parents feed their nestlings (Nelson, 2005), and parental feeding continues after chicks have completed plumage development (86–90 days; Drummond, Osorno, Torres, García-Chavelas, & Merchant Larios, 1991). Parents with older chicks (>10 days old) provide up to twice as much food as do those with younger chicks (5 days old; Guerra & Drummond, 1995). A single measurement of plasma metabolite levels can be used as a reliable indicator of body condition (Guglielmo, Cerasale, & Eldermire, 2005; Jenni-Eiermann & Jenni, 1998). Specifically, triglycerides and β–OH–butyrate (BUTY) levels are good indicators of increases or decreases in body reserves, respectively (Quillfeldt, Masello, & Möstl, 2004), whereas creatine kinase (CK) is an indicator of the physical exertion associated with muscle activity and foraging (Ramírez et al., 2010). The heterophil/lymphocyte (H/L) ratio reflects stress in response to various stressors: inflammatory processes or infectious diseases, parasite infestation, food or water deprivation, or temperature extremes (e.g. Davis, Maney, & Maerz, 2008; Lobato, Moreno, Merino, Sanz, & Arriero, 2005; Vleck, Vertalino, Vleck, & Bucher, 2000). In birds, the H/L ratio may correlate with the production of antibodies against novel antigens (Krams et al., 2013).

We predicted that if adults adjust their behaviour (i.e. investment in parental care) based on needs of their offspring, this would affect their own physiological state. Thus, when brood demand increases, parents are expected to increase the number and/or duration of foraging trips (foraging effort) to obtain more food, thus reducing their time spent in the nest. Furthermore, because of an increase in physical activity, CK and BUTY concentrations and the H/L ratio (physiological and immune statuses) should increase, and triglyceride levels and body condition (BCI) should decrease. In contrast, when brood demand decreases, parents are expected to spend less time out of the nest and to have improved body condition and be less stressed. In the blue-footed booby, previous studies have shown that when the reproductive effort of adults is increased by manipulating flight cost and brood size, males maintain their body mass at the expense of their offspring's growth, whereas females increase their investment in offspring and lose body mass (Velando & Alonso-Alvarez, 2003). It appears that the sexes have different trade-offs between investment in current reproduction and body maintenance, with males apparently working at some physiological maximum or being unable to pay increased demands and with females having a flexible maternal strategy (Velando & Alonso-Alvarez, 2003). Thus, females might have a buffer of nutritional reserves that they use based on the needs of their offspring. The cross-fostering experiment of the present study allowed us to test for sex-specific differences in parental ability to adjust behaviour and in the physiological flexibility of parents in terms of plasma metabolites, immune status (H/L ratio), BCI, foraging behaviour and time at nest. Therefore, taking into account sex-related differences in investment in current reproduction and body maintenance in the blue-footed booby, we expected females to show greater modifications to their foraging behaviour and time at nest compared with males, especially when brood demand increases, but without impairing their physiological status to a greater extent than that observed in males.

## METHODS

### Study Area

Fieldwork was conducted on the Isla El Rancho (25°10′N, 108°23′W; 327 ha) in the northern mouth of Bahía Santa María-La Reforma, which is the largest coastal wetland of Sinaloa, Mexico (Engilis, Oring, Carrera, Nelson, & Martinez-Lopez, 1998). The blue-footed booby colony is on the northwestern part of the island, among 4 m high sand dunes. Approximately 3000 pairs of blue-footed booby nest in a 1.7 ha area, with an average nest density of 0.26 ± 0.12 nests/m$^2$ (González-Medina, n.d.).

### Experimental Design: Manipulation of Brood Demands

Before conducting the cross-fostering experiment, we selected a homogeneous group of early layers (median laying date: 24 days after the first egg laid, 3 December; only two nests contained eggs that were laid 5 days after the median date) to undergo the experimental manipulations; we assumed that the quality of blue-footed booby parents with similar laying dates would be similar. During the breeding season, the median laying date was 5 December (26 days after the first egg laid, $N = 154$; range 10 November–14 February). Moreover, we did not detect a significant effect of laying date ($P > 0.05$) in our analyses. We conducted a cross-fostering experiment using broods with two chicks: 19 nests with 1-week-old chicks (mean age of the two chicks: 5.7 ± 0.4 days) and 20 nests with 2-week-old chicks (mean age of the two chicks: 13.0 ± 0.6 days). For the experimental treatments, we exchanged the two-chick broods between pairs of nests consisting of one nest of each type, placing 2-week-old chicks into the former nests of 1-week-old broods and vice versa. The ages of the chicks were estimated through comparison of chick plumage and external body measurements with those of chicks of known age (the difference in age between offspring of known age and the age estimated by external measures was 0.25 ± 0.1 days).

The brood exchanges produced four groups: (1) 1-week-old broods replaced by 2-week-old broods (Increased Demands treatment, $N = 13$); (2) 2-week-old broods replaced by 1-week-old broods (Decreased Demands treatment, $N = 13$); (3) a control group for the Increased Demands treatment (1-week-old chicks exchanged with chicks from another nest of the same age; Increased Control treatment, $N = 6$); and (4) a control group for the Decreased Demands treatment (2-week-old chicks exchanged with chicks from another nest of the same age; Decreased Control treatment, $N = 7$). The adoptive parents did not reject the chicks