# Reproduction and Immune Homeostasis in a Long-lived Seabird, the Nazca Booby (*Sula granti*)

BY

VICTOR APANIUS, MARK A. WESTBROCK, AND DAVID J. ANDERSON

*Department of Biology, Wake Forest University, P.O. Box 7325, Winston Salem, North Carolina 27109, USA*

ORNITHOLOGICAL MONOGRAPHS NO. 65

PUBLISHED BY
THE AMERICAN ORNITHOLOGISTS' UNION
WASHINGTON, D.C.
2008

TABLE OF CONTENTS

## LIST OF TABLES



APPENDICES



## LIST OF FIGURES

# *From the Editor*

This monograph deals with reproduction in the Nazca Booby (*Sula granti*), using a population of breeding birds on the Galápagos Islands. Thanks to *National Geographic* and the BBC in the past, and numerous cable channels more recently, everyone knows about the Galápagos and its tortoises, finches, and marine iguanas. The islands' fame started with Charles Darwin's stay there, of great importance to the development of his theory of evolution by natural selection. In particular, Darwin learned much from inter-island variation in tortoises and mockingbirds; the fairly complex groups of finches found on each island were not particularly helpful to his theories, even though they soon were known as "Darwin's finches."

Numerous studies have been done on Galápagos birds in the modern era. The eminent British ecologist David Lack started this off with his detailed look at the ecology and evolution of Darwin's finches about 60 years ago. Peter and Rosemary Grant and a group of exceptional graduate students have provided wonderful long-term studies of finch populations on Isla Daphne for more than 30 years. How many researchers have popular books written about their work, as Jonathan Weiner's *The Beak of the Finch* covered that of the Grants and their students? Of course, some of those students studied other things, including mockingbirds and other finch populations. Tjitte de Vries initiated studies on the endemic Galápagos Hawk (*Buteo galapagoensis*), a bird with an unusual cooperatively polyandrous mating system. I was fortunate enough to continue these studies, which are currently being handled by Patricia Parker and James Bednarz. Expanding from her work on hawks, Patty also studies a variety of avian diseases on the islands, a subject that is very interesting evolutionarily and of great potential conservation importance. Other groups, including the authors of this monograph, have started long-term studies on the ecology and behavior of some of the breeding seabirds.

Darwin, the Grants, de Vries, and most of the other ornithologists who have focused on Galápagos landbirds were attracted to these islands because their extreme isolation made them unusual natural experiments on the adaptation and radiation of species. Birds colonized infrequently enough that most of the species on the Galápagos originated within that island system. Even among such groups as the mockingbirds and hawks, where only one species can exist on each island, one can see how different forms have evolved from an original colonist in these unusual circumstances.

Of course, for seabirds, the Galápagos Islands are not a particularly unusual breeding site, given that seabirds typically find remote oceanic islands for nesting. Why are seabird studies easier to do on the Galápagos Islands than elsewhere? Check out Figure 1 for the answer. For some reason, animals on the Galápagos are incredibly tame. This might be expected for the animals on land that have not coexisted with humans until quite recently, but it is also true of the many seabird species that nest on these islands. One can easily walk up to a nesting seabird, capture it (sometimes by hand, or perhaps with a stick and a noose or something simple like that), gather whatever samples are needed, then let it go. It will not be pleased by the circumstances (though you will note in Figure 1 how little the booby seems to resist being measured), but it will not leave the nest or respond as many other normal birds do to such disturbance. Thus, one can take samples from the same bird over and over again without having negative effects on the process of reproduction.

Even the endemic hawks are tame. With the proper incentive (such as a dead goat), we could attract numerous Galápagos Hawks to a site for banding. Some we would catch with a noose on a broomstick; others we could sometimes simply grab by hand! We discovered that our plastic hawk bands did not last long in the Galápagos environment, but it was generally easy to walk up to a bird and read the number on a metal band with binoculars. This tameness certainly made our research much easier in what was an otherwise difficult work environment, though one still had to be careful around an active nest, because the hawks were as aggressive as any raptor in that situation. As you read about the various adaptations used by Nazca Boobies to produce high-quality young, keep in mind how the tameness of these birds aids in the measurements needed for this work. Don't we wish that every bird species was so easy to study.

*John Faaborg*

*Ornithological Monographs*
Volume (2008), No. 65, 1–46

Printed in USA.

# REPRODUCTION AND IMMUNE HOMEOSTASIS IN A LONG-LIVED SEABIRD, THE NAZCA BOOBY (*Sula granti*)

VICTOR APANIUS, MARK A. WESTBROCK, AND DAVID J. ANDERSON

*Department of Biology, Wake Forest University, P.O. Box 7325, Winston Salem, North Carolina 27109, USA*

ABSTRACT.—The evolution of longevity requires that the marginal investment in self-maintenance at the expense of reproductive effort is favored by realizing a longer reproductive lifespan. This can occur when extrinsic mortality factors (weather, predators, etc.) are less important than intrinsic mortality factors, such as the physiological cost of reproduction. Long-lived pelagic seabirds have low annual reproductive output and prolonged offspring growth periods that are thought to have evolved to accommodate marine resource variability. The life-history theory of senescence predicts that these same taxa should minimize *per diem* reproductive costs and shift effects of resource variability to the offspring. To address this prediction, we measured parental effort, offspring growth, and one aspect of self-maintenance (serum immunoglobulin G concentration [IgG]) in a long-lived pelagic seabird, the Nazca Booby (*Sula granti*). We collected data on 38 families in the 2002–2003 breeding season on Isla Española, Galápagos Islands, Ecuador. Offspring body-mass growth showed variable trajectories, but a variable nestling period allowed similar (sex-specific) fledging mass to be attained. Growth of two structural traits was most variable when the traits were growing most rapidly, but again attained sex-specific targets at fledging. Offspring [IgG] showed marked inter-individual variation, but the ontogeny of [IgG] was unrelated to morphological growth. Mothers spent more time at sea than fathers, and both parents spent more time at sea for offspring of the larger (female) sex at the time of peak body mass. Foraging effort did not show consistent inter-individual variation but was correlated between pair members. Sex-specific body mass of the parents showed consistent inter-individual variation as it declined across the nestling period, with a greater decline in parents raising daughters. In parents, [IgG] was stable across the nestling period and was correlated among family members. The plasticity of offspring growth and the consistency of self-maintenance of the parents accord with the predictions of the life-history theory of senescence. To our knowledge, this is the first study to use a longitudinal analysis to assess intra- and inter-individual variation in parental effort, offspring growth, and a measure of immune-mediated self-maintenance in a wild vertebrate population. *Received 7 December 2006, accepted 6 July 2007.*

RESUMEN.—La evolución de la longevidad requiere que la inversión marginal en auto mantenimiento vs. el esfuerzo reproductivo, sea favorecida por tener una larga vida reproductiva. Esto puede ocurrir cuando los factores extrínsecos de mortalidad (clima, depredadores, etc.) son menos importantes que los factores intrínsecos de mortalidad, como los costos fisiológicos de la reproducción. Las aves marinas longevas tienen una baja producción reproductiva anual y un prologando periodo de crecimiento de la descendencia, se cree que esto ha evolucionado así para acomodarse a la variabilidad en los recursos marinos. La teoría de la historia de vida del envejecimiento predice que los mismos taxa deberían minimizar per diem los costos reproductivos y cambiar los efectos de la variabilidad de recursos a la descendencia. Para determinar esta predicción medimos el esfuerzo parental, el crecimiento de la progenie y un aspecto de auto mantenimiento (concentración de inmunoglobulina G en suero [IgG] en un ave marina longeva, *Sula granti*. Colectamos datos de 38 familias en la temporada de reproducción 2002–2003 en la Isla Española, Islas Galápagos, Ecuador. El aumento de la masa corporal de la progenie mostró

[1]E-mail: apaniuv@wfu.edu

*Ornithological Monographs*, Number 65, pages 1–46. ISBN: 978-0-943610-80-1.  DOI:10.1525/om.2008.65.1.1.

trayectorias variables, pero un periodo de anidación variable permitió alcanzar una masa corporal similar (específica al sexo) en los volantones. El crecimiento de dos rasgos estructurales fue mucho más variable cuando estos crecieron más rápidamente, pero también lograron obtener el tamaño específico de cada sexo como volantones. La progenie mostró una marcada variación entre individuos de la [IgG], pero la ontogenia de [IgG] no se relacionó con el crecimiento morfológico. Las madres gastaron más tiempo en el mar que los padres, y ambos padres gastaron más tiempo en el mar en el momento del pico de la masa corporal, si su progenie era del sexo más grande (hijas). El esfuerzo de forrajeo no mostró una variación entre individuos consistente pero estaba correlacionado entre los miembros de la pareja. La masa corporal específica de cada sexo de la pareja mostró una variación consistente que disminuyó durante la alimentación de los polluelos, con un mayor declive en el caso de padres criando una hija. En los padres, la [IgG] fue estable durante el periodo de polluelos y estaba correlacionada entre los miembros de la familia. La plasticidad del crecimiento de la progenie y la consistencia del auto mantenimiento de los padres está de acuerdo con las predicciones de la teoría de historia de vida del envejecimiento. Hasta donde tenemos conocimiento, este es el primer estudio que emplea un análisis longitudinal para evaluar la variación intra y entre individuos en el esfuerzo parental, el crecimiento de la progenie y una medida del auto mantenimiento inmunológico en una población silvestre de vertebrados.

## Introduction

The life-history theory of senescence provides an evolutionary explanation for the extensive variation in lifespan among organisms, invoking the balance between extrinsic and intrinsic sources of mortality to determine the allocation of limited somatic resources between reproductive output and parental self-maintenance (Medawar 1952, Goodman 1974). A central premise of the theory is that a reduction in extrinsic mortality factors (e.g., predation, unfavorable weather) favors increased physiological investment in self-maintenance, delaying mortality due to somatic deterioration and prolonging reproductive lifespan. An individual's allocation of resources toward tissue renewal and repair, as opposed to parental care and offspring production, will be profitable only in relatively benign environments, where the fitness benefits of investment in self-maintenance will accrue via an extended reproductive lifespan.

Pelagic seabirds that breed on remote oceanic islands experience little predation, and their demographic syndrome provides indirect evidence of the predicted shift toward enhanced self-maintenance (long pre-reproductive period, willingness to abandon eggs or broods, exceptionally high annual survival) and reduced short-term reproductive effort (small clutch sizes, prolonged reproductive cycles; Ricklefs 1984, Weimerskirch 2002). Thus, these long-lived seabirds—especially those that raise one offspring per annual or biennial breeding attempt—may occupy one end of the self-maintenance–reproductive-effort tradeoff axis that is central to life-history theory and evolutionary analysis of senescence (Goodman 1974, Sæther et al. 1993, Charlesworth 1994, Moreno 2003). In these long-lived avian taxa, the physiological evidence that their demographic syndrome is entrained by enhanced self-maintenance is only beginning to be assembled (Esparza et al. 2004, Apanius and Nisbet 2006).

Abundant demographic data indicate the extent to which seabirds incur costs of reproduction (Reid 1987; Jacobsen et al. 1995; Pyle et al. 1997; Golet et al. 1998, 2004; Kalmbach et al. 2004). A common approach is to augment or reduce the number of young per brood and document survival and breeding of marked adults in the future. In Golet et al.'s (2004) study of Black-legged Kittiwakes (*Rissa tridactyla*), for example, adults whose eggs were removed did not breed and were more likely to survive to the next year than unmanipulated controls. Behavioral studies document the manner in which seabirds respond to naturally or artificially increased reproductive effort (Sæther et al. 1993, Weimerskirch et al. 1995, Lorentsen 1996, Erikstad et al. 1998), for example by adjusting the mix of trips that increase parental condition at the expense of offspring condition (Weimerskirch et al. 1995). These studies enrich an earlier paradigm based on environmentally constrained and stochastic food availability (Ashmole 1963, 1971; Nelson 1978), challenging the assumption that a fixed schedule of reproductive investment can avoid significant costs of reproduction (Ricklefs 1987, 1992). Within this revised paradigm that incorporates behavioral flexibility in a stochastic environment (Erikstad et al. 1998), allocation between reproductive effort and self-maintenance in pelagic seabirds will differ from that in short-lived birds in the manner in which reproductive

costs are borne by the parents versus the offspring.

In general, long-lived, but not short-lived, birds are predicted to consistently allocate nutritional resources to sustain self-maintenance processes, such as immune function and antioxidant protection, and to adjust reproductive effort accordingly (Moreno 2003, Apanius and Nisbet 2006). Because of high rates of mortality from extrinsic factors, short-lived taxa cannot effectively convert current self-maintenance into future reproduction, and they should instead allocate resources to high annual fecundity and rapid offspring growth. In these taxa, resource limitation during reproduction causes both parents and their offspring to down-regulate self-maintenance, as measured by immune function (Deerenberg et al. 1997, Nordling et al. 1998) or antioxidant levels (Wiersma et al. 2004). The negative relationship between reproductive effort and immune function has generated a great deal of discussion in the literature (Sheldon and Verhulst 1996, Owens and Wilson 1999, Norris and Evans 2000, Zuk and Stoehr 2002), but the discussion has focused mainly on short-lived passerines, with the assumption that all birds face tradeoffs between parental effort and immune function. For pelagic seabirds, the small brood size and slow offspring growth may allow parents to reduce (or may impose a safeguard against increasing) the daily physiological exertion associated with reproduction (Daan et al. 1996) and spare them the need to redirect a significant portion of nutrients from self-maintenance to sustain a high level of reproductive effort.

Visser's (2002) review of available data on parental daily energy expenditure (DEE, adjusted for body mass) provides the beginning of a comparative test of this idea. Average DEE of long-lived seabird species ($n = 10$ species) raising single-offspring broods was 54% higher than that of short-lived species ($n = 8$) raising three to five offspring per brood. This increased energy expenditure is most likely related to the cost of foraging in the pelagic environment (Ellis and Gabrielsen 2002) and not to the energy demands of the offspring: peak offspring energy demand was only 31% of the seabird parent's energy budget, whereas it was 140% of the parent's energy budget in short-lived species (Visser 2002). It appears that short-lived species must subsidize their peak rate of parental energy expenditure by reallocating nutrients from self-maintenance to reproductive effort. Pelagic seabirds may forgo the need for substantial reallocation by reducing *per diem* reproductive costs through single-egg clutches and a prolonged nestling period. This leads to the prediction that pelagic seabirds will not show the dramatic down-regulation of self-maintenance, *vis-à-vis* immune function, during reproductive challenges that is observed in birds with shorter lifespans.

The life-history theory of senescence also predicts that long-lived parents will shift the effects of resource limitation to the offspring, resulting in offspring whose quality varies substantially with resource availability (Sæther et al. 1993, Mauck and Grubb 1995) or by sex, as in the case of dimorphism in size and food requirements of offspring (Anderson et al. 1993, Townsend et al. 2007). Accordingly, the variance in offspring body condition is expected to exceed that of the parents. Because somatic growth and immune function compete for nutrients in nestlings, resource limitation exposes this tradeoff in short-lived birds (Christe et al. 1998). It is an open question whether the same constraint operates in the offspring of pelagic seabirds, with preliminary evidence from a coastal seabird suggesting that the tradeoff between growth rate and immune function is not inevitable (Apanius and Nisbet 2006).

In pelagic seabirds, parents should exhibit low intra-individual variance in reproductive effort and self-maintenance while, in complementary fashion, the offspring should show greater phenotypic variance, greater compensatory growth plasticity, or both. Previous tests of these predictions have typically focused on the means, but not the variances, of these traits, and have used body mass (or size-adjusted mass) as a measure of self-maintenance. In the present study, we focused on within-individual variance in body mass and used an immunological trait as a measure of self-maintenance. We employed a powerful repeated-measures design that allowed us to partition the trait variance into within- and between-individual components, recognizing that individuals in long-lived species may show consistent trajectories across time (Cam et al. 2002) in relation to a latent variable called "individual quality" (Wendeln and Becker 1999, Lewis et al. 2006).

## Assessing Self-Maintenance in Field Studies

The cost of reproduction is typically measured with demographic parameters, such as annual

survival and future reproductive success, because they are fundamentally related to lifetime reproductive success. The connection between these demographic parameters and the underlying physiological processes that are subsumed in the term "self-maintenance" are receiving increasing scrutiny (Ricklefs and Wikelski 2002). Body mass, body condition index (body mass adjusted for body size), and fat scores have been used as proxies for self-maintenance status, but whether the loss of body mass represents a physiological cost or an aerodynamic adjustment is debatable (Jones 1994, Rands et al. 2006). Hematological and immunological parameters have come into focus in the past decade, with many studies linking parental immune function with reproductive effort, success, and annual survival in a bidirectional manner (Table 1). In short-lived birds, experimentally increased reproductive effort is usually associated with decreased immune function. Conversely, immunologically challenged birds often, but not always, have reduced reproductive effort, success, and survival. It is thought that the physiological costs of immune function are sufficiently large that reallocation of nutrients toward or away from the immune system can have significant effects on reproduction and survival (Sheldon and Verhulst 1996; but see Råberg et al. 1998). The costs and benefits of down-regulating immunity depend on the specific mechanism that is being modulated (Demas 2004, Klasing 2004), and compensatory effects by different components of the immune system cannot be discounted (Apanius 1998b).

Assays of immune function that challenge the animal by injecting foreign material, such as antigens (e.g., veterinary vaccines), mitogens (e.g., phytohemagglutinin or PHA), or pyrogens (e.g., lipopolysaccharide or LPS) have been preferred over observational approaches, because the injected compounds represent an experimental treatment, thereby allowing stronger inference of cause and effect (Sheldon and Verhulst 1996, Norris and Evans 2000). However, interpreting the outcome of these invasive treatments is not always straightforward. The choice of antigen and its dose will determine the magnitude of antibody responses (Staszewski and Boulinier 2004). Despite the widespread use of PHA-induced skin swelling, interpretation of swelling size is not straightforward, because of the complexity of the cascade of inflammatory processes underlying the morphological response (Martin et al. 2006). Interpretation of repeated PHA treatments is also problematic (Kennedy and Nager 2006). Because the dose–response relationships of invasive treatments are seldom documented, it is difficult to establish that the administered agent is inducing a physiologically realistic response, especially for LPS (Viney et al. 2005). With these caveats, it seems safe to generalize that increased parental effort is associated with decreased immune function in short-lived passerine birds. The relationship in long-lived birds remains an open question, which motivated the present study.

We assessed self-maintenance with an immunological trait that is well characterized, is amenable to repeated measurements, and shows parallel responses with more invasive assays (Table 1). Immunoglobulin G (IgG = IgY) is the most abundant isotype of antibody in circulation and is synthesized by bursally derived (B–) lymphocytes (Warr et al. 1995). The concentration of IgG in serum ([IgG]) reflects the systemic production of natural (nonspecific) and antigen-specific antibodies directed against viral, microbial, fungal, and parasite antigens that breach body surface barriers (Hanson 1979, Lemke et al. 2004). In birds and mammals, [IgG] reflects the persistent antigenic pressure from the diet and the external environment (Lemke et al. 2004), and levels are notably increased in human populations living in unhygienic conditions (McFarlane 1973). At the same time, [IgG] can be reduced by stress-induced increases in corticosterone in mammals (Barnard et al. 1994, de Vries et al. 1997). Extensive metabolic studies in laboratory animals and humans show that [IgG] is maintained around a homeostatic set-point by independent control of synthesis and degradation rates (Waldmann et al. 1970). The homeostatic levels in domestic chicken (*Gallus gallus domesticus*) strains show heritable variation (Rees and Nordskog 1981) and respond to artificial selection (Sarker et al. 1999). Selection for an elevated [IgG] set-point was correlated with increased specific antibody responses (Sarker et al. 2000). Because the population of B-cells that synthesize IgG undergoes affinity maturation over the course of natural antigenic stimulation, the protective ability of the IgG pool improves with an individual's age (Lemke et al. 2004). The B-cells that produce IgG are capable of retaining long-term immunological memory (Hanson 1979), a property that has been shown, in theoretical models, to be relevant to the evolution of longevity (Boots and Bowers 2004).

TABLE 1. Relationship between reproductive effort and self-maintenance as measured by immune function in birds. Average life expectancy ($=0.5+1/[1-s]$, where $s$ is the maximum adult survival rate; Gaillard et al. 1989) is shown for illustrative purposes and may not pertain to the referenced study. AB response = antibody titer or index; AB responders = percentage of birds showing detectable antibody responses; $\gamma$-globulin = percentage of $\gamma$-globulins in protein electrophoresis; H:L = heterophil to lymphocyte ratio, [IgG] = immunoglobin G (= Y) concentration; PHA response = phytohemagglutinin-induced skin-swelling. "Females" and "males" refer to sex of the parents.

| Species (Average life expectancy years) | Predictor/**Treatment** | Response | References |
|---|---|---|---|
| Common Eider | Across incubation | ↑H:L (females) | Hollmén et al. 2001 |
| *Somateria mollissima* (10.5)[a] | period | ↓$\gamma$-globulin (females) | |
| | | ↓[IgG] (females) | Bourgeon et al. 2006 |
| | | ↓PHA response (females) | |
| | ↑Body mass loss | ↑H:L (females) | Hanssen et al. 2003 |
| | ↑**Clutch size** | —H:L (females) | Hanssen et al. 2005 |
| | | ↓AB responders (females) | |
| | **AB response** (females) | ↓Annual survival | Hanssen et al. 2004 |
| Common Tern | Across nestling period | ↓/—[IgG] (both) | Apanius and Nisbet 2006 |
| *Sterna hirundo* (11.6)[b] | ↓Fledging rate | ↓[IgG] (both) | |
| Great Tit | Across reproductive | ↑H:L (females) | Hõrak et al. 1998 |
| *Parus major* (2.4)[b] | period | ↓$\gamma$-globulin (both) | |
| | ↓**Brood size** | ↓H:L (both) | Ots and Hõrak 1996 |
| | **Male removal** | —AB response (females) | Snoeijs et al. 2005 |
| Blue Tit | **AB response** (females) | — Fledging rate | Råberg et al. 2000 |
| *Cyanistes caeruleus* (2.0)[b] | ↑**Brood size** | ↓[IgG] (females) | Merino et al. 2006 |
| Barn Swallow | Across reproductive | ↓$\gamma$-globulin (females) | Saino et al. 2001 |
| *Hirundo rustica* (2.8)[b] | period | —$\gamma$-globulin (males) | Saino et al. 1997a |
| | ↓**Brood size** | ↑PHA response (males) | Saino et al. 2002 |
| | ↑**Brood size** | ↓PHA response (females) | Saino et al. 1997b, Pap and Markus 2003 |
| Tree Swallow | ↑**Brood size** | ↑H:L (both) | Shutler et al. 2004 |
| *Tachycineta bicolor* (2.7)[c] | ↑**Brood size** | ↓2° AB response (females) | Ardia et al. 2003 |
| | ↑**Workload** (females) | ↓AB response (females) | Hasselquist et al. 2001 |
| | ↓Fledging rate | ↑PHA response (both) | Lifjeld et al. 2002 |
| Collared Flycatcher | ↑**Brood size** | ↓AB response (females) | Nordling et al. 1998, Cichón et al. 2001 |
| *Ficedula albicollis* (1.9)[d] | | | |
| | ↓**Clutch size** | ↑AB response (females) | Cichón 2000 |
| Pied Flycatcher | **AB response** (females) | ↓Fledging rate | Ilmonen et al. 2000 |
| *F. hypoleuca* (2.0)[e] | ↑**Brood size** | —PHA response (females) | Ilmonen et al. 2002 |
| | ↑**Brood size** | ↑PHA response (females) | Moreno et al. 2001 |
| European Starling | **AB response** (females) | —Fledging rate | Williams et al. 1999 |
| *Sturnus vulgaris* (3.0)[b] | | ↓2nd clutch size | |
| Dark-eyed Junco | Across reproductive | ↓[IgG] (both) | Greives et al. 2006 |
| *Junco hyemalis* (2.0)[f] | period | | |

Sources of life-expectancy data:
[a] Yoccoz et al. 2002.
[b] Gaillard et al. 1989.
[c] Robertson et al. 1992.
[d] Merilä and Hemborg 2000.
[e] Sanz 2001.
[f] Nolan et al. 2002.

## Study System

Our empirical model, the Nazca Booby (*Sula granti*), is a tropical seabird with a low annual reproductive rate (Humphries et al. 2006), a monogamous mating system (Maness and Anderson 2007), and biparental care (Anderson and Ricklefs 1992) that breeds on remote islands (Anderson 1993) like other pelagic seabirds, such as albatrosses (Diomedeidae) and penguins (Spheniscidae; Weimerskirch 2002). With high annual survival and slow actuarial senescence, the Nazca Booby provides a suitable model to investigate the reproductive–self-maintenance tradeoff in a long-lived species (Anderson and Apanius 2003). Demographic, physiological, and behavioral studies have been conducted on a large population on Isla Española, Galápagos Archipelago, Ecuador, since 1984 (Fig. 1).

No more than one chick is raised per year, though two-hatchling broods are frequently produced and rapidly reduced to one by obligate siblicide (Anderson 1989a, Humphries et al. 2006). Both males and females exhibit survival costs of reproduction after raising their single-chick broods (Townsend and Anderson 2007a). This taxon was formerly considered a subspecies of the Masked Booby (*S. dactylatra*) and was recently elevated to species status (Pitman and Jehl 1998, American Ornithologists' Union 2000, Friesen et al. 2002).

Females are larger than males as adults (Nelson 1978, Townsend and Anderson 2007b), and females make longer foraging trips during brooding and deliver larger loads of food and larger prey items to the nest (Anderson 1989b, Anderson and Ricklefs 1992). Females are also underrepresented in the adult population (Townsend and Anderson 2007a), though hatching and fledging sex ratios are unbiased (Maness et al. 2007), which suggests excess mortality of females at some point after fledging.

This information motivated investigation of sex-specific patterns of reproductive effort, body condition, and self-maintenance of the parents as well as offspring growth rate and self-maintenance. Reproductive effort was inferred from foraging time budgets derived from hourly observations of parental attendance that were made every day of the 120-day nestling period. Parental body condition was inferred from the dynamics of body mass measured at 20-day intervals across the nestling period. Parental self-maintenance was inferred from changes in [IgG] that were measured in blood samples taken at the same 20-day intervals. Blood samples to determine [IgG] were taken from each offspring on the same schedule as for its parents. Offspring body mass, culmen, and tarsus were measured at 10-day intervals. We used these data to test the hypothesis that reproductive costs will be expressed not as reduced self-maintenance of the parents but as variation in offspring condition, which presumably varies with resource availability and possibly between smaller sons and larger daughters (Townsend and Anderson 2007b) in this sexually dimorphic species.

## Assumptions and Predictions

### Parents will Regulate Foraging Effort at Consistent Levels

We assumed that parental foraging effort will be reflected by time budgets. Given that increasing foraging effort tracks increasing offspring food requirements across the nestling period, we predicted that parents would maintain consistent, individual-specific foraging effort and body condition, especially when challenged by maximal offspring food-demand late in the nestling period (Anderson 1990). Whether parental foraging effort matches the differential requirements of dimorphic sons and daughters is an open question.

### Offspring Growth will Buffer Stochastic Variation in Food Provisioning

We assumed that offspring growth would be highly variable between nestlings because of variation among parents in their foraging proficiency, with inefficient foragers limiting their physical exertion to protect their health. Parents should maintain their body reserves, as reflected by body mass, and should not subsidize the growth rate of their offspring. On the basis of these expectations, we predicted that morphological traits would not be correlated between parents and offspring at the end of the growth period and that offspring would show greater trait variances. However, the capacity for compensatory growth, either by accelerated rates or prolongation of the nestling period, is an alternative buffering mechanism that minimizes reproductive costs of the parents.

FIG. 1. At our study site, D.J.A. measures the culmen of an adult Nazca Booby. Some birds show temporary marks applied during an annual census. (Photograph by Sebastian Cruz.)

### Parents and Offspring will Maintain [IgG] Homeostasis

By shunting costs of reproduction that exceed a threshold to the offspring, parents should be able to maintain consistent self-maintenance during the reproductive cycle. Therefore, we expected to find stable [IgG] across the nestling period, especially during the peak of offspring demand and, hence, parental effort. In addition, we assumed that immune function represented a critical developmental investment for long-lived species and considered whether morphological growth would be uncoupled from the ontogeny of [IgG].

## MATERIALS AND METHODS

### Study Site and Population

We studied the Nazca Booby population at Punta Cevallos, Isla Española, Galápagos Islands (1°23′S, 89°37′W), Ecuador, during the breeding season of 2002–2003, in conjunction with other long-term research on this species. Approximately 3,500 Nazca Booby pairs breed at Punta Cevallos, most eggs are laid from October to February, and fledging occurs from March until June (Anderson 1993). For the present study, we monitored all nests intensively between 25 September 2002 and 28 May 2003 in a subsection of the main study colony (Fig. 2). Of 65 clutches in this focal subsection, 47 produced at least one chick. Four of these died before an initial blood sample at age 10 days, and two died later. This yielded a fledging success in families that produced a hatchling of 41 of 47 (mean = 0.872; 95% confidence interval [CI]: 0.748–0.939), which was not different from that of the rest of the study colony (807 of 946; mean = 0.853; 95% CI: 0.829–0.874). We restricted our analyses to 38 families that successfully fledged their offspring, omitting 3 of the 41 families because our data on the parents were incomplete. Clutches contained either one or two eggs, but offspring data represent the single chick that was reared, either a single-egg clutch or the siblicidal victor. Our offspring data set included an exceptional case in which brood reduction was delayed until the younger chick's day 51, but this family was not an outlier for the parameters we measured.

In the focal subsection of the colony, eggs hatched between 8 November 2002 and 11 February 2003, with one exception on 9 March 2003. The focal families hatched eggs between 8 November 2002 and 23 January 2003, thus spanning a representative range of hatching dates. In the study colony, laying dates—and, consequently, hatching dates—were trimodally distributed, and they were not related to offspring

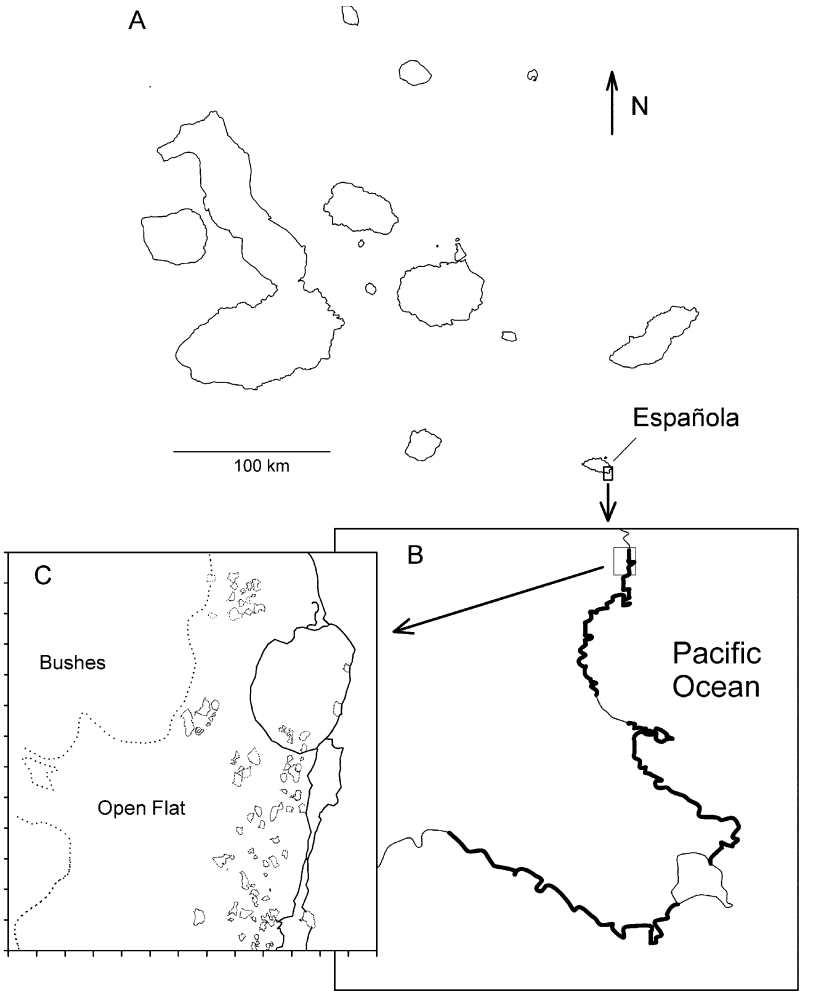


FIG. 2. (A) The Galápagos Islands. (B) Enlargement of the Punta Cevallos area, with heavy lines showing the three subcolonies of Nazca Boobies monitored in our long-term work. (C) Section (referred to as the "mini-area" in other publications) of Subcolony 1 (Huyvaert and Anderson 2004) used for the present study, with the bush line marked with a dotted line, elevational topographical changes with heavy solid lines (including a roughly circular elevated rock pile), and smaller rocks with lighter lines. Axes are scaled in 5-m increments. Most Nazca Booby nest sites are among the rocks.

sex (Westbrock 2005). In the focal subsection, hatching dates were uniformly distributed and the variance of hatching dates was greater for sons than for daughters (Levene's $F = 5.51$, $df = 1$ and 36, $P = 0.025$). Furthermore, 5 of 18 sons hatched before the first of 20 daughters, so offspring sex was related to hatching date (Fisher's exact $P = 0.017$). Acknowledging this interaction, we analyzed hatching date only in cases where it might have confounded analyses of sex-specific traits (see below).

### Offspring Growth Rate

Preambulatory nestlings were identified by their nest location and later by a uniquely numbered steel leg band. Nestlings were measured every 10 days from day 0 (hatch day) until day 120: "age-class" refers to these 10-day time-points. We measured body mass with a Pesola spring balance (days 0–30: ±1 g; days >30: ±20 g), culmen length with vernier calipers (±0.1 mm), and flattened wing chord with a wing rule (±1.0 mm).

Offspring body mass was log-transformed to stabilize the variance in statistical analyses. Fledging date was assigned by daily visual inspection to determine whether guano had been cleaned from the feet by immersion at sea. After their first flight around day 110, all offspring remained based in the colony until at least day 120, at which time 95% (19 of 20) of the daughters and 89% (16 of 18) of the sons had fledged. In previous studies, we have used 99% completion of pennaceous plumage as an end-stage for measuring developmental rate (Clifford and Anderson 2001, Anderson and Apanius 2003); this variable was correlated with fledging age ($r_s = 0.771$, $P < 0.0001$, $n = 38$). Fledging age was not related to hatching date ($r_s = 0.183$, $P = 0.27$, $n = 38$). Because of right-skewness of the fledging-date distribution, we classified families with a binary variable ("nestling period": short–long) based on whether they fledged before, on, or after the median fledging age (day 107 for this sample). Offspring sex and nestling-period group samples were reasonably balanced, and the two factors were not confounded (daughters–short: $n = 10$; sons–short: $n = 10$; daughters–long: $n = 12$; and sons–long: $n = 6$; $\chi^2 = 1.08$, df = 1, $P = 0.30$).

### Molecular Sex-Determination

Adults were sexed during prior encounters when their sex-specific vocalizations were recorded. Nestlings were sexed in the laboratory from a blood sample by amplification of an intron region of the CHD gene (Fridolfsson and Ellegren 1999). DNA was extracted from the samples with a proteinase K digestion followed by phenol-chloroform extraction according to standard protocols (Sambrook et al. 1989). Polymerase chain reactions (PCR; modified from Fridolfsson and Ellegren 1999) using an Amplitron II (Barnstead-Thermolyne, Dubuque, Iowa) thermal cycler were performed in 15-$\mu$L volumes containing 0.15 U RedTaq Genomic DNA Polymerase (Sigma-Aldrich, St. Louis, Missouri), 10 mM dNTPs (Promega, Madison, Wisconsin), 1.0X PCR Buffer (Sigma), 2.5 mM $MgCl_2$, 3.3% DMSO, 0.05–1.0 $\mu$g template DNA, and 0.15 $\mu$g each of primers 2550F and 2718R (Fridolfsson and Ellegren 1999). After an initial denaturing step at 94°C for 2 min, we ran 40 cycles of denaturation at 94°C for 45 s, annealing at 46.5°C for 1 min, and extension at 72°C for 1.5 min, followed by a final extension at 72°C for 5 min. The PCR products were separated on 2% agarose gels (BMA SeaKem LE, Lonza, Rockland, Maine, and Synergel Agarose Clarifier, Diversified Biotech, Boston, Massachusetts), run in 1X TBE buffer at 180V for 100–110 min. The PCR products were stained with ethidium bromide and visualized under ultraviolet light using GENESNAP (Hitachi, Alameda, California). Maness et al. (2007) verified the accuracy of the procedure by blind sex-determination of 100 known-sex adults, all of which were correctly classified. Because extrapair fertilizations have not been detected (Anderson and Boag 2006, D. Anderson et al. unpubl. data) and are estimated to be extremely rare if they occur at all (<0.14%; Anderson and Boag 2006), we refer to social parents as "mothers" and "fathers," and to their offspring as "daughters" and "sons," with a high degree of confidence.

### Offspring Mortality Rates

Daily nest checks in the study colony were conducted in the 1992–1993 to 2004–2005 breeding seasons. Nestling mortality rates were calculated for 10-day intervals after hatching based on the age of the nestling when it died. We used these data to determine the temporal pattern of nestling mortality across a span of years that showed highly variable breeding success.

### Parental Foraging Effort

Each adult in the focal subsection had a uniquely numbered steel leg band and a field-readable plastic leg band (Pro Touch Engraving, Saskatoon, Saskatchewan). Parental foraging effort was estimated by monitoring the presence or absence of the parents in the study colony—given that they typically attend their nest when in the colony—between hatching and fledging. On each day of the breeding season, each nest was visited hourly between 0600 and 1800 hours, and additionally at 0530 hours (sunrise), 1830 hours (sunset), and 2000 hours to record the presence of each parent (this species is indifferent to the close presence of humans; Fig. 1). Foraging effort was scored as the number of daylight hours spent at sea across a 20-day interval that was centered on blood sampling time-points (see below). Time spent away from the nest was used as a measure of parental foraging effort because (1) parents were not observed elsewhere in the colony (D. Anderson et al. unpubl. data), (2) preliminary data from dive monitors indicated minimal rest periods on the

sea surface during daylight (D. Anderson et al. unpubl. data), and (3) absence from the nest site was positively correlated with foraging effort on the basis of radiotracking (Anderson and Ricklefs 1987).

### Parents' Morphological Measurements

Body mass, culmen length, and wing length were measured in the same manner as for offspring. Parents were weighed every 20 days (±2 days) from nestling days 10 to 110 at the circadian phase (0200–0530 hours) when mass of ingested food was lowest. On one occasion, culmen and wing length were also measured. The sex of parents was determined by their vocalizations (Nelson 1978, Anderson 1993). For mothers and fathers, body mass was not correlated with culmen or wing length (all $P > 0.19$) and, therefore, size-adjusted body mass was not used as a measure of body condition (Green 2001).

### Blood Sampling

Blood was collected by brachial venipuncture (100–300 $\mu$L) from nestlings and parents every 20 days between nestling days 10 and 110. Blood sampling was standardized to the same circadian phase (0200–0530 hours). Blood was placed in 1.5-mL polypropylene microcentrifuge tubes and allowed to clot at ambient temperature for 2–4 h, then centrifuged at 10,000 rpm for 10 min. Ten microliters of serum were quantitatively transferred into 100 $\mu$L of sodium-dodecyl-sulphate (SDS) buffer typically used in polyacrylamide gel electrophoresis of proteins (see below). Serum proteins were denatured by immersion in a 100°C water bath for 4 min within 6 h of collection. Preserved samples were stored at ambient temperature in the field for a maximum of eight months, then stored at –20°C until laboratory analysis. An additional blood sample (50 $\mu$L) was collected from each nestling on its hatch day and stored in lysis buffer (Longmire et al. 1992) for molecular sex-determination.

### Serum IgG Assay

Serum IgG of Nazca Boobies was identified from the molecular weight of the native protein and of the subunits after reductive dissociation in two-dimensional electrophoresis following Apanius et al. (1983). Serum [IgG] was measured by electrophoretic separation from other serum proteins in 7.5% polyacrylamide gels followed by quantitative staining and densitometry (Apanius and Nisbet 2003; Fig. 3). Purified chicken IgG (I4881; Sigma) was used to construct a standard curve (2, 4, 6, 8, 10 mg $mL^{-1}$) in each gel. This concentration range produced a linear standard curve with $r^2 > 0.95$ for each gel. The repeatability (intraclass correlation coefficient) of [IgG] measurements from 11 randomly chosen serum samples analyzed in duplicate, but in different gels, was 0.907 ($F = 20.47$, df = 10 and 11, $P < 0.0001$).

To characterize the dynamics of [IgG] in newly hatched chicks, [IgG] was measured at 0, 5, 10, and 15 days of age in the first-hatched offspring of 18 nests that were not part of the main study.

### Statistical Analyses

Exploratory data analysis used individual profile plots and plots of the means, variances, coefficients of variation (CV), and correlations as a function of time between measurements to guide model selection (Verbeke and Molenberghs 2000). For the correlation functions, observations were centered by subtracting the mean and standardized by dividing by the standard deviation, and then the Pearson correlation coefficient was calculated for all pairwise combinations as a function of time between measurements (i.e., "lag length"; Verbeke and Molenberghs 2000). Correlations plotted as a function of lag length show the autocorrelation structure of the repeated measurements. Because sample sizes decreased with lag length, we displayed correlation functions with symbol sizes that were proportional to sample sizes (Verbeke and Molenberghs 2000).

Repeated-measures analyses were performed using linear mixed models (Littell et al. 1996; PROC MIXED in SAS, version 9.1.3, SAS Institute, Cary, North Carolina) following the formalism presented in Appendix 1. Linear mixed models allow inference about the mean structure of fixed effects and consider the within- and between-individual covariance parameters to be nuisance terms and treat them as random effects (Littell et al. 2000). Here, we focus on these covariance matrices to estimate the within- and between-individual sources of variation (Fitzmaurice et al. 2004), which allows us to test predictions regarding how tightly self-maintenance is regulated in parents and offspring. We used the structure of the covariance matrix to infer the

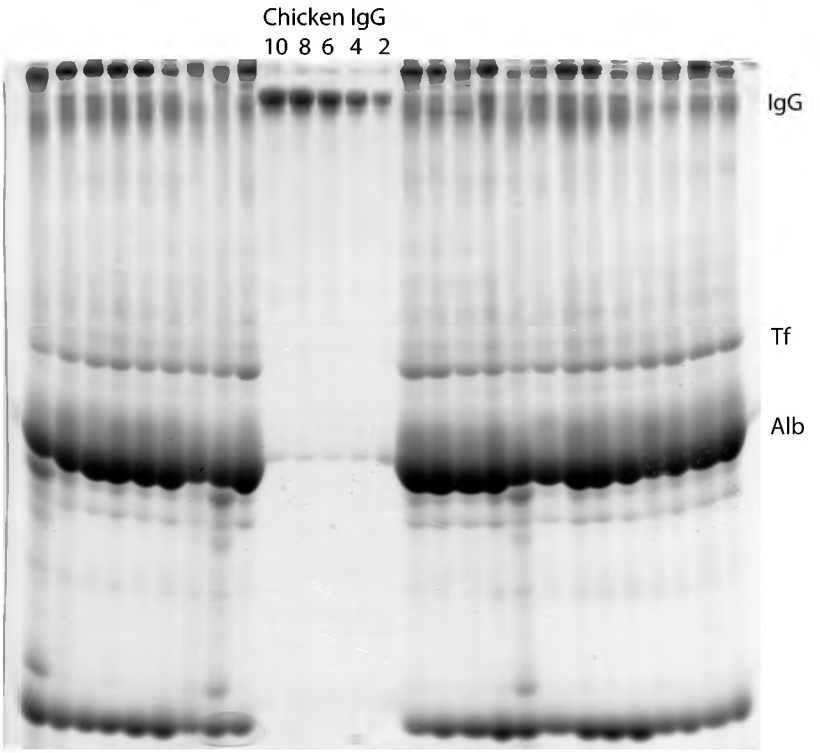


FIG. 3. Electropherogram of serum proteins of adult Nazca Boobies (see text for details). Chicken IgG standards are shown at 10, 8, 6, 4, and 2 g $L^{-1}$. IgG = immunoglobulin G, Tf = transferrin, and Alb = albumin.

mode of regulation of each trait, following the precedent in human clinical chemistry (Harris et al. 1980, Queraltó 2004). Appendix 1 provides a description of model notation, covariance structures, and their interpretation.

We first present results for the mean trends based on the fixed-effects analysis, using the best-fit covariance structure. The significance tests for *post-hoc* comparisons of means at differing time-points used simulation-adjusted critical values (Westfall et al. 1999). Then, we examine the estimated covariance parameters to see how the residual (nonfixed effects) variation is partitioned into between- ($var_b$) and within-individuals ($var_w$) variance components and, in some cases, an additional between-families ($var_f$) component (see below). We then consider the magnitude of the estimated autocorrelation coefficient ($\rho$) to infer the degree of homeostatic regulation, with a higher autocorrelation implying tighter regulation (Harris et al. 1980, Queraltó 2004).

Finally, we examine the correlations of traits between family members in each age-class. In cases where correlations between family members are evident, we use an additional random effect, indexed to family, to account for this covariation, and we refer to this covariance parameter as $var_f$. In those cases, the random effect representing between-individual variation ($var_b$), indexed to band number, is nested within the family effect.

Nestling mortality rates for 10-day intervals were calculated with PROC LIFETEST in SAS, and differences in survival curves were tested using the Kaplan-Meier estimator and the log-rank test (Allison 1995).

## RESULTS

### OFFSPRING GROWTH

#### Body Mass

Mean (log-transformed) body mass increased asymptotically from days 0 to 50 and peaked at day 70 (Fig. 4A), when daughters were 81.8 g (4.4%) heavier than sons, but this difference was

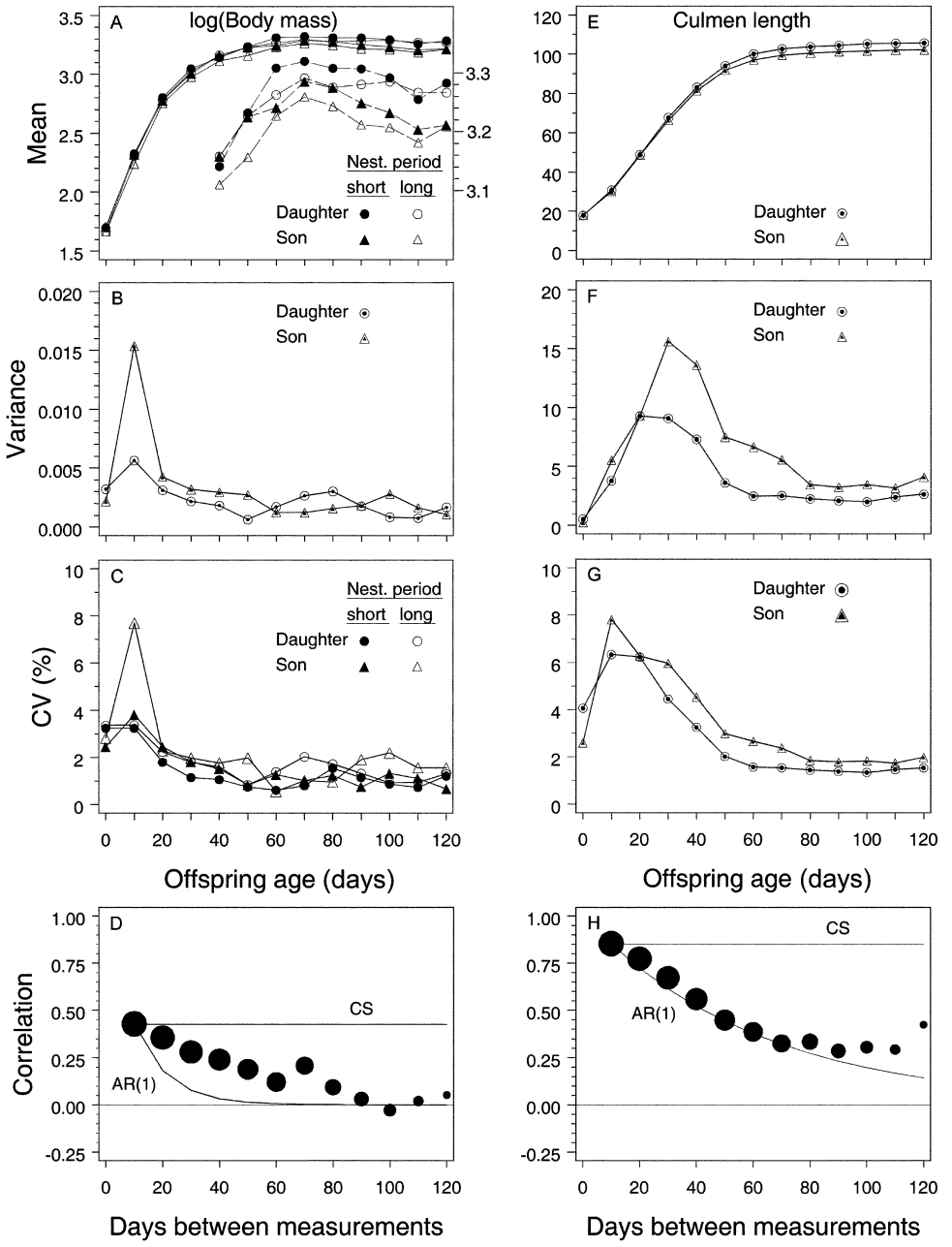


FIG. 4. Offspring traits of Nazca Boobies as a function of offspring age-class. $Log_{10}$-transformed body-mass (A) mean, (B) variance, and (C) coefficient of variation (CV). Culmen-length (E) mean, (F) variance, and (G) coefficient of variation. For (A) mean body mass, dashed lines correspond to the expanded scale (right side). Separate lines are shown for groups that differ significantly in means or variances according to linear-mixed-model analyses (Appendix 2, Tables 1–4).Also shown is the exploratory analysis of correlation structure based on the correlation between measurements from the same individuals as a function of interval between measurements for $log_{10}$-transformed (D) body mass and (H) culmen length. For the analysis of correlation structure, symbol size is proportional to the sample size. Units: body mass (g), culmen length (mm). (*Continued on the next page.*)

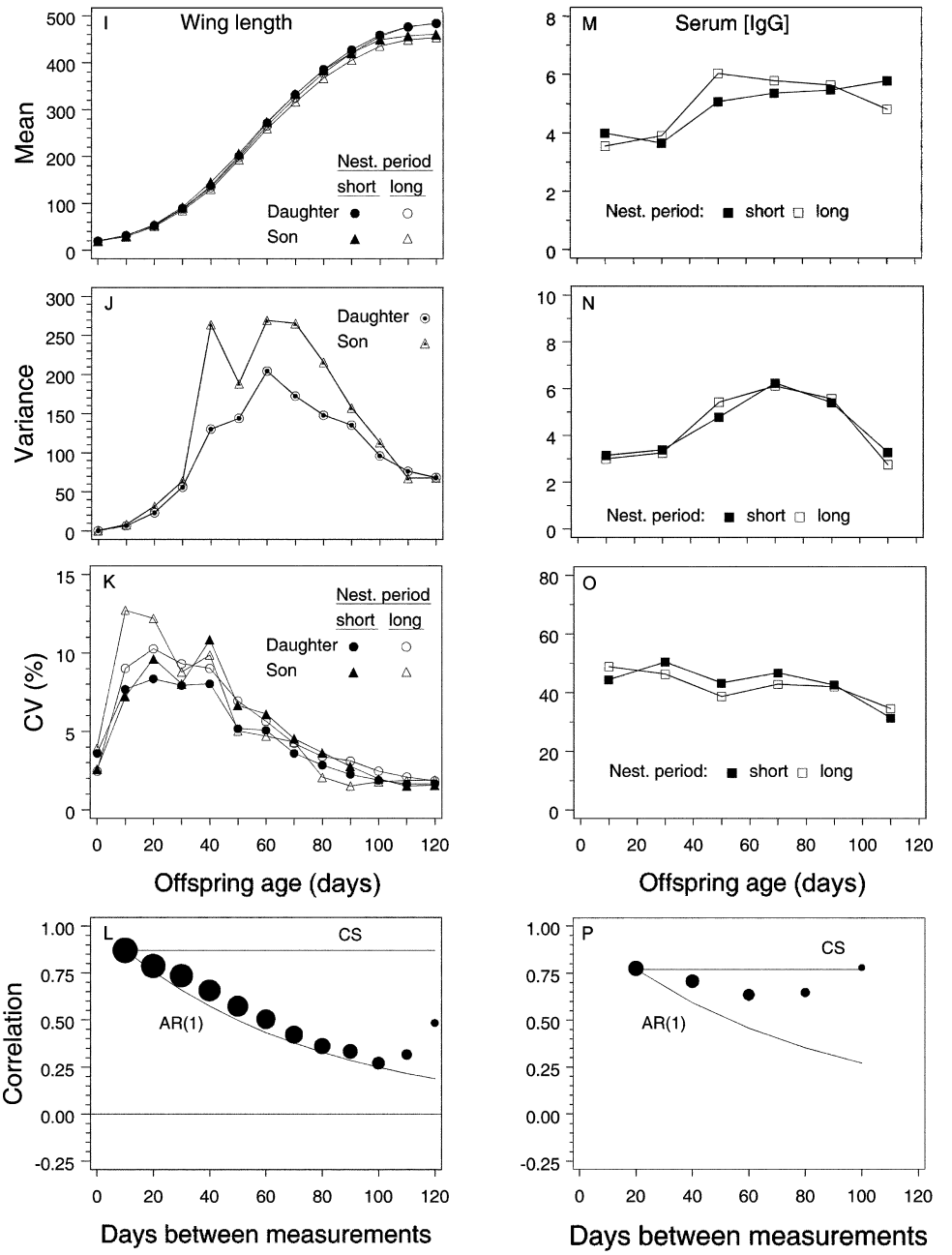


FIG. 4. (*Continued.*) Offspring traits of Nazca Boobies as a function of offspring age-class. Wing-length (I) mean, (J) variance, and (K) coefficient of variation. Serum [IgG] (M) mean, (N) variance, and (P) coefficient of variation. Exploratory analysis of correlation structure for (L) wing length and (P) [IgG]. Units: wing length (mm), [IgG] (g $L^{-1}$).

not significant (adj. $P = 0.29$). From the peak, body masses decreased by 8.0% for daughters and 12.4% for sons at fledging. Although the age at fledging was similar for daughters (mean = 109 days, 95% CI: 100–124) and sons (mean = 109, 95% CI: 95–149; $F = 0.01$, df = 1 and 36, $P = 0.92$), the mean fledging body mass of daughters was 209 g (11.1%) greater than that of sons ($F = 16.85$, df = 1 and 36, $P = 0.0002$). Offspring that fledged at a younger age had significantly greater body mass across all age-classes ($F = 29.25$, df = 1 and 35, $P < 0.0001$); nonsignificant two-way and three-way interactions of sex with nestling period (Appendix 2: Table 1) indicated that this relationship did not vary by sex. Overall, body mass across the growth period was 5.9% greater for offspring with shorter nestling periods (least-squares mean = 1,023 g, 95% CI: 997–1,049) than for those with longer ones (least-squares mean = 963 g, 95% CI: 935–994). Near the end of growth, around the time of fledging (days 90–120), this effect of nestling-period disappeared for both sexes (all $P > 0.17$). Consequently, we conclude that the length of the nestling period was shorter for offspring that were heavier throughout the growth period and that all offspring converged on sex-specific fledging body-mass targets, with sons showing a greater mass recession than daughters.

In exploratory data analysis, the variance of (log-transformed) body mass spiked on day 10 because of three sons that had depressed body mass (Fig. 4B); despite this transient increase, covariance parameters did not differ significantly as a function of offspring sex or nestling period (Appendix 2: Table 1). The CV of offspring body mass showed a corresponding spike at age 10 days but was below 2% for most of the nestling period (Fig. 4C). Thus, variation in mass was nearly constant when logarithmically scaled to body size across the growth period.

Correlation of consecutive (lag = 10 days) body-mass measurements was $r = 0.430$ (Fig. 4D) and decreased with increasing lag length to effectively zero for lags $\geq$50 days (Fig. 4D). Visual inspection of the correlation matrices revealed considerable heterogeneity in the correlation between consecutive measurements, ranging from −0.272 to 0.768 for daughters and −0.421 to 0.762 for sons. For both sexes, strong positive correlations were observed in early age-classes and weak negative or nonsignificant correlations in older age-classes. This heterogeneous correlation structure was most consistent with the VC covariance structure (see Appendix 1 for details of model notation), with negligible between-individual variance and autocorrelation. It was clearly the best-fit model for body-mass growth for both sons and daughters across all age-classes (Appendix 2: Table 1). This implied that body mass was not regulated around individual-specific set-points. Instead, we observed a nestling period of variable length that allowed offspring to reach their sex-specific growth target.

### Culmen Length

Growth of the culmen was initially rapid and became sexually size-dimorphic near the growth asymptote (Fig. 4E), as indicated by a significant age-class * offspring-sex interaction ($F = 9.46$, df = 12 and 404, $P < 0.0001$). Culmen length of daughters first exceeded that of sons at day 50 (adj. $P = 0.023$) and remained longer thereafter (all $P < 0.0002$). Mean culmen length of daughters was 3.6 mm (3.4%) longer than that of sons at fledging. Nestling period was not related to mean culmen length (Appendix 2: Table 2).

In exploratory data analysis, variance in culmen length was low on day 0, increased rapidly by day 30, and then decreased to stable values upon reaching asymptotic size (Fig. 4F). The CV for culmen length peaked between 5% and 9% on days 10–30, then declined to 3–4% when asymptotic culmen length was attained (Fig. 4G). The variance and CV appeared to be greater for sons than for daughters between days 30 to 80. In summary, variation in culmen length was greatest early in the growth period and before the emergence of sexual size-dimorphism (SSD).

Correlation of consecutive (lag = 10 days) culmen measurements was higher ($r = 0.854$) than for mass (Fig. 4H). Inspection of the correlation matrices showed that, except for the day-0 to day-10 correlation (daughters: $r = 0.549$; sons: $r = -0.059$), the correlation of consecutive measurements ranged from 0.833 to 0.974 across age-classes. The correlation between culmen measurements at hatching and fledging (lag = 120 days) was significant for daughters ($r = 0.455$, $P = 0.044$, $n = 20$) but not for sons ($r = 0.393$, $P = 0.11$, $n = 18$). For lags >10 days, the correlations between culmen measurements followed a first-order autoregressive decline (Fig. 4H).

Accordingly, the ARH1 + RE covariance structure, which accounted for the high autocorrelation

as well as age-dependent heterogeneity, unambiguously provided the best-fit covariance structure for culmen growth (Appendix 2: Table 2). Using this model, the estimated autocorrelation coefficient across age-classes was relatively high and did not differ between daughters ($\rho = 0.902$, 95% CI: 0.853–0.951) and sons ($\rho = 0.925$, 95% CI: 0.883–0.971). From the exploratory analysis, the variance in culmen length was greatest on days 20 and 30 for daughters, and the peak at day 30 for sons was higher than that of daughters (Fig. 4F). This is reflected in the best-fit covariance structure (ARH1 + RE), which estimated separate covariance parameters for each sex and age-class (Appendix 2: Table 2). The sex- and age-class-specific covariance parameters reflected the age-dependent pattern of variance shown in Fig. 4F, with sons having higher covariances than daughters (Appendix 2: Table 2). In summary, the high autocorrelation coefficients implied tight regulation of culmen growth. The pattern of age-dependent variances (and CVs) indicated that variation increased during rapid growth and subsequently decreased as all offspring reached sex-specific asymptotic size.

### Wing Length

Wing growth also showed sexual dimorphism (Fig. 4I), based on a significant age-class * offspring-sex interaction ($F = 14.04$, df = 12 and 404, $P < 0.0001$). Daughters developed significantly longer wing length than that of sons by day 90 (adj. $P = 0.0024$) and remained longer afterward (all $P < 0.0001$). Mean wing length of daughters was 21.0 mm (4.4%) longer than that of sons at fledging. Nestling period was related to wing length as a main effect ($F = 8.42$, df = 1 and 35, $P = 0.0064$) and as an interaction with age-class ($F = 1.96$, df = 12 and 404, $P = 0.026$). On average, wing length was 7.1 mm (2.7%) longer for offspring in the shorter-nestling-period group (least-squares mean = 258 mm, 95% CI: 254–261) than for those in the longer-nestling-period group (least-squares mean = 251, 95% CI: 247–255). Between days 60 and 100, wing length was ~10 mm longer in offspring with shorter nestling periods than in those with longer ones. At fledging age, wing length was 11.6 mm (2.4%) shorter in daughters with a shorter nestling period ($F = 6.01$, df = 1 and 18, $P = 0.025$), with no difference in sons ($F = 0.03$, df = 1 and 16, $P = 0.87$). In conclusion, offspring with faster growth in wing length after day 60 fledged at an earlier age but with slightly shorter wing length, at least in daughters.

In exploratory data analysis, variance in wing length was low at hatching, peaked around day 60, and then decreased at fledging (Fig. 4J). The CV for wing length peaked at 8% and 13% between days 10 and 40 and gradually declined to 2% at fledging (Fig. 4K). As with culmen growth, variation in wing growth was greatest when growth was most rapid and decreased with the emergence of sexual dimorphism.

Correlation between consecutive (lag = 10 days) wing measurements was high ($r = 0.870$; Fig. 4L). Inspection of correlation matrices showed that, except for days 0–10 (daughters $r = 0.409$; sons $r = 0.321$), the correlation of consecutive measurements ranged from 0.833 to 0.987 across age-classes. The correlation between wing measurements at hatching and fledging (lag = 120 days) was significant for daughters ($r = 0.664$, $P = 0.0014$, $n = 20$) but not for sons ($r = 0.305$, $P = 0.21$, $n = 18$). For lags >10 days, the correlations of wing measurements decreased as a first-order autoregressive process for both sexes (Fig. 4L).

As with culmen growth, the ARH1 + RE covariance structure unambiguously provided the best-fit model for wing growth. The autocorrelation for wing growth across age-classes was relatively high ($\rho = 0.910$, 95% CI: 0.886–0.944) and did not differ between sexes (Appendix 2: Table 3). The peak variance around day 60 observed in the exploratory analysis (Fig. 4J) was reflected in the estimated age-specific covariance parameters, which did not differ by sex or nestling-period groups (Appendix 2: Table 3). In summary, the high autocorrelation coefficients implied tight regulation of wing growth. The pattern of age-dependent variances (and CVs) indicated that variation increased during rapid growth and subsequently decreased as offspring reached sex-specific asymptotic sizes.

### Serum [IgG] of Offspring

Serum [IgG] was measured in a group of offspring that were not part of the main study on days 0, 5, 10, and 15. In this group, [IgG] (mean ± SE) monotonically declined from days 0 (5.62 ± 0.85) to 10 (3.06 ± 0.44; $F = 21.63$, df = 1 and 31, $P < 0.0001$) and was not significantly different between days 10 and 15 (2.85 ± 0.29; $F = 2.26$,

df = 1 and 15, $P = 0.15$). In the main study, offspring [IgG] was lowest at days 10 and 30, then increased significantly between days 30 and 50 ($F = 91.57$, df = 5 and 142, $P < 0.0001$), and remained relatively constant thereafter (Fig. 4M). [IgG] was not related to offspring sex directly or as an interaction with age-class (Appendix 2: Table 4). [IgG] was not related to nestling period as a main effect but was related as a significant interaction with offspring age-class ($F = 3.12$, df = 5 and 142, $P = 0.011$). However, significant differences could not be identified at any particular offspring age-class by nestling-period combination (all $P > 0.10$), which indicates that the interaction was attributable to minor differences across multiple age-classes. [IgG] was not related to the growth of morphological traits during the early phase when [IgG] was stable (days 10 and 30), during the phase of increasing [IgG] (days 30 and 50), or during the final phase, when [IgG] was stable again (days 50–110; Appendix 2: Table 5).

In exploratory data analysis, variance in offspring [IgG] showed a gradual increase and then decrease across the growth period (Fig. 4N). The CV for [IgG] was the highest for the offspring traits studied and gradually decreased from 50% to 30% (Fig. 4O).

The correlation between consecutive (lag = 20 days) measurements was $r = 0.775$ (Fig. 4P). For lags >20 days, the correlation function appeared to be intermediate between CS and AR1 + RE (Fig. 4P). The AR1 + RE covariance structure was the most parsimonious covariance structure for offspring [IgG], with an estimated autocorrelation coefficient ($\rho = 0.461$, 95% CI: 0.153–0.768) that was less than that observed for culmen and wing length. The age-dependent variances observed in the exploratory analyses were not deemed significant because the ARH1 + RE and CSH structures, with additional age-dependent covariance parameters, were not significant improvements over the AR1 + RE covariance structure (Appendix 2: Table 4). Furthermore, the estimated covariance parameters did not differ between offspring sexes or nestling-period groups (Appendix 2: Table 4). In summary, 62% of the variance in [IgG] was attributable to differences between individuals, and the modest autocorrelation implied relatively weak regulation around individual set-points for this self-maintenance trait.

### Parental Foraging Effort

Foraging effort of mothers and fathers was negatively correlated at days 10 ($r = -0.523$) and 30 ($r = -0.549$) and was positively correlated at day 50 and afterwards ($r = 0.355$ to 0.652, all $n = 38$). This correlation pattern was similar for parents of daughters (days 10 and 30: $r = -0.684$ to $-0.736$; days 50–110: $r = 0.424$ to 0.623; all $n = 20$) and sons (day 10 and 30: $r = -0.287$ to $-0.461$; days 50–110: $r = 0.176$ to 0.680; all $n = 18$). Although the significance tests of these correlations are unreliable because of pseudoreplication, the correlations suggested a need to model this source of covariance. Further analyses were conducted separately in age-class groups (days 10–30 or 50–110) and with an additional random effect indexed to nest identification number to account for the correlation within pairs.

Considering days 10 and 30, fathers rearing sons spent more time at sea on day 10 than fathers rearing daughters and compared to mothers (Fig. 5A, E), as indicated by a significant interaction of age-class * parent-sex * offspring-sex ($F = 4.88$, df = 1 and 71, $P = 0.030$). The between-individual variance (99.45, 95% CI: 58.56–205.05) was less than the within-individual variance (144.50, 95% CI: 106.87–206.32). Thus, 41% of the variance in foraging time in early age-classes was attributable to between-individual differences.

Considering days 50–110, mothers spent an average of 22.8 ± 3.3 more hours at sea (per 20-day interval) than fathers ($F = 56.00$, df = 1 and 68, $P < 0.0001$) and consistently spent more time at sea than fathers across all offspring age-classes (nonsignificant parent-sex * age-class interaction; Appendix 2: Table 6). Parents spent 8.4% and 5.5% more time at sea for daughters than for sons at days 70 and 90, respectively (age-class * offspring-sex interaction, $F = 3.03$, df = 3 and 204, $P = 0.031$). Parental foraging effort was not related to nestling period as a main effect or as an interaction with age, parent sex, or offspring sex (Appendix 2: Table 6).

In exploratory data analysis, the variance in foraging effort appeared to increase with the mean across offspring age-classes (Fig. 5A, B, E, F). Log-transformation did not homogenize the variances or improve the model fit based on the analysis of residuals. The CV for foraging effort varied between 5% and 20%, with no clear pattern

for parents of sons versus parents of daughters (Fig. 5C, G).

For days 50–110, the correlation of foraging effort between consecutive (lag = 20 days) measurements was $\rho = 0.392$ (Fig. 5D, H). For lags >20 days, the correlations decreased, which is consistent with an autoregressive process. Accordingly, the AR1 + RE covariance structure was the best-fit model; the time-dependent heterogeneity incorporated in the ARH1 + RE covariance structure was not justified (Appendix 2: Table 6). Thus, the increased variance around day 70 observed in the exploratory analysis was not reflected in the estimated covariance parameters (Appendix 2: Table 6), which also did not differ by parent or offspring sex or nestling-period (Appendix 2: Table 6). Combined and separate analyses of mothers and fathers were unable to estimate positive covariance parameters for individual random intercepts, which indicates that the between-individual covariance was zero (i.e., no consistent differences between individuals). The weak autocorrelation between consecutive measurements implied a modest level of homeostatic regulation within individuals. Overall, 17.5% of the variance was attributable to differences between pairs and not to individual differences (Appendix 2: Table 6).

### Parental Body Mass

Body mass of mothers was 256 ± 20 g (13%) greater than that of fathers ($F = 165.12$, df = 1 and 68, $P < 0.0001$; Fig. 5I, M). Body masses of mothers and fathers were not correlated at any age-class (all $P > 0.12$), so parental measurements were treated as statistically independent. Body masses of mothers and fathers decreased monotonically with increasing offspring age ($F = 29.43$, df = 5 and 259, $P < 0.0001$; Fig. 5I, M). Body mass of parents rearing daughters decreased at a greater rate than that of those rearing sons (age-class * offspring-sex interaction, $F = 2.98$, df = 5 and 259, $P = 0.012$), and the decrease was similar for mothers and fathers (nonsignificant parent-sex * offspring-sex * age-class interaction; Appendix 2: Table 7). Parents of daughters lost 189 ± 20 g between days 10 and 90, compared with 130 ± 32 g for parents raising sons. This corresponds to 9.8% and 6.7% of body mass for mothers and 11.3% and 7.8% for fathers, respectively. The parents of offspring that fledged in the shorter nestling period at an earlier age were, on average, 49.3 g (2.7%) heavier than parents with longer nestling periods ($F = 8.33$, df = 1 and 68, $P = 0.0052$). The significant age-class * offspring-sex * nestling-period interaction indicated that nestling period also influenced the relationship of parental mass loss as a function of age-class and offspring sex ($F = 3.00$, df = 4 and 259, $P = 0.019$), though a clear pattern was difficult to discern.

Across offspring age-classes, variance in parental body mass appeared to decrease in mothers and to be greater in fathers raising sons (Fig. 5J, N). The CV for parental body mass varied between 3% and 9% and reflected the pattern of variances (Fig. 5K, O).

The correlation between consecutive measurements (lag = 20 days) appeared to be higher for mothers ($\rho = 0.587$) than for fathers ($\rho = 0.327$; Fig. 5L, P) and remained at that value or declined slightly for lags >20 days. Accordingly, the CS covariance structure was the most parsimonious model. Although ARH1 + RE and AR1 + RE were also plausible choices (Appendix 2: Table 7), the estimated autocorrelation coefficient included zero ($\rho = 0.139$, 95% CI: −0.024 to 0.302). Regardless of the structure, covariance parameter estimates did not differ as a function of parent or offspring sex or nestling period (Appendix 2: Table 7). Across age-classes, 42.6% of the residual variance in body mass could be apportioned to inter-individual differences, and repeated measurements had negligible autocorrelation (Appendix 2: Table 7). Analyses of balanced subsets of the data were consistent with the complete data set, indicating that the imbalance created by partial data sets from late-breeding parents did not influence the estimation of covariance matrices or the mean trends (not shown).

In summary, body mass declined across the offspring age-classes in both parents, with a greater decrease in parents raising daughters. Parents of offspring with shorter nestling periods were heavier than parents with longer ones. Although inter-individual variation was consistent, body-mass measurements had low autocorrelation.

### Parental [IgG]

Parental [IgG] averaged 7.77 ± 0.16 g $L^{-1}$ and was significantly greater than offspring

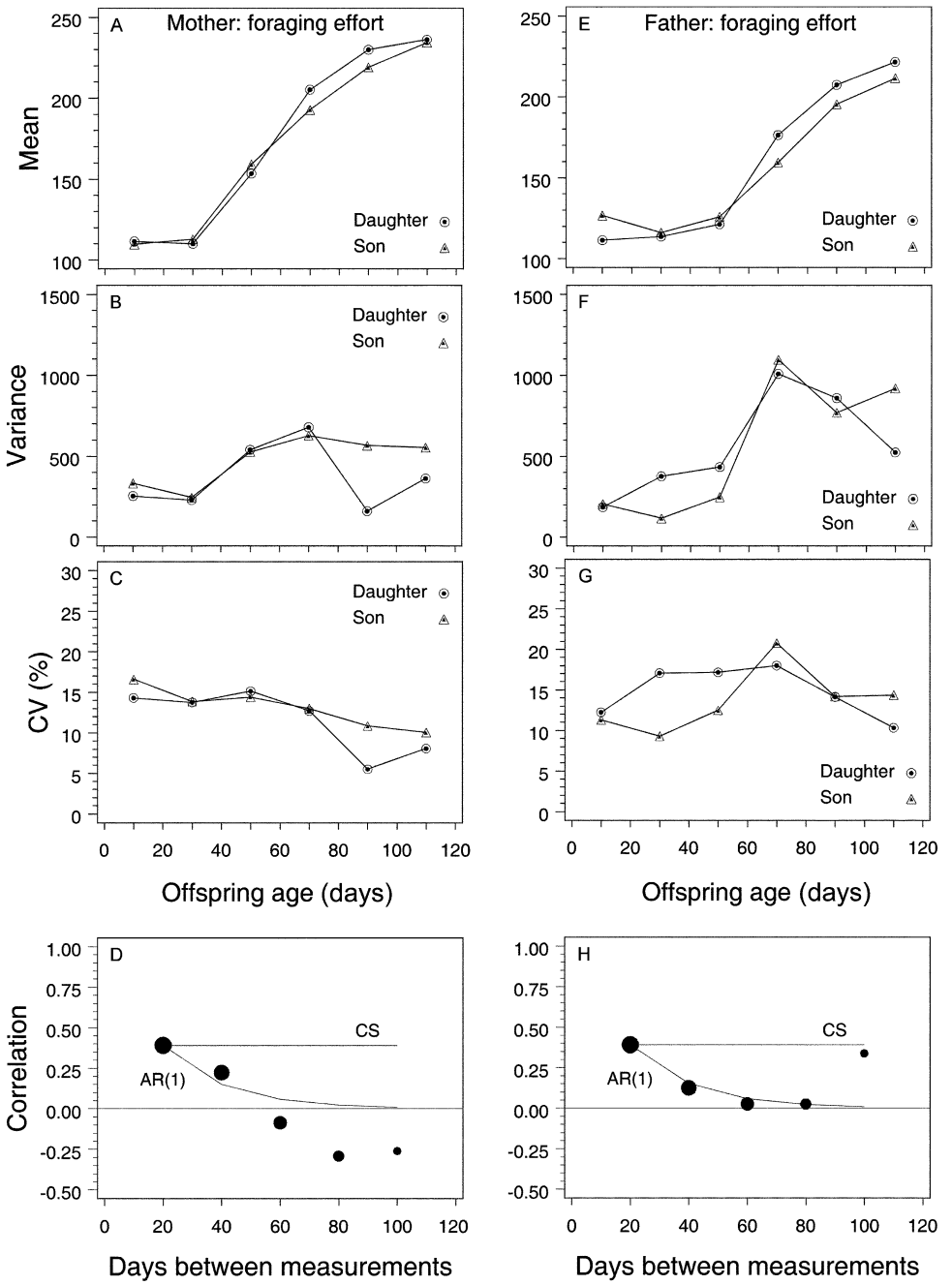


FIG. 5. Parent traits of Nazca Boobies as a function of offspring age-class. Mother's foraging effort: (A) mean, (B) variance, and (C) coefficient of variation (CV). Father's foraging effort: (E) mean, (F) variance, and (G) coefficient of variation. Separate lines are shown for groups that differ significantly in means or variances according to linear-mixed-model analyses (Appendix 2, Tables 6 and 7). Also shown is the exploratory analysis of correlation structure based on the correlation between measurements from the same individuals as a function of interval between measurements for foraging effort of (D) mothers and (H) fathers. For the analysis of correlation structure, symbol size is proportional to sample size. Units: foraging effort (number of daylight hours absent per 20-day interval). (*Continued on the next page.*)

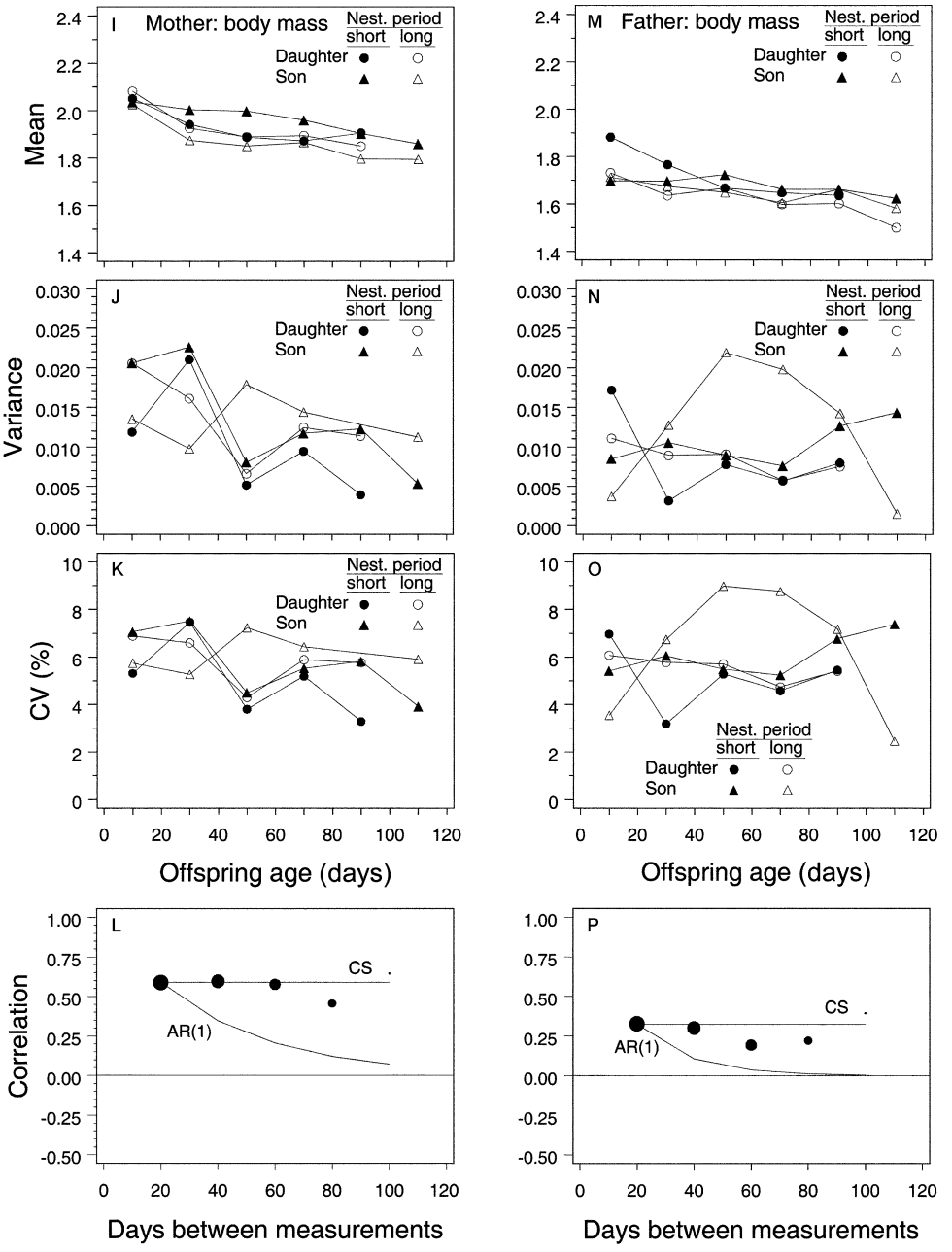


FIG. 5. (*Continued.*) Parent traits of Nazca Boobies as a function of offspring age-class. Mother's body mass: (I) mean, (J) variance, and (K) coefficient of variation. Father's body mass: (M) mean, (N) variance, and (O) coefficient of variation. Exploratory analysis of correlation structure for body mass of (L) mothers and (P) fathers. Units: body mass (kg).

[IgG] at all age-classes ($F = 8.39$, df = 2 and 119, $P = 0.0004$). Across all age-classes, [IgG] was positively correlated between mothers and fathers ($r = 0.550$–$0.360$), mothers and offspring ($r = 0.601$–$0.403$), and fathers and offspring ($r = 0.591$–$0.525$), except at day 110, when only the father–offspring correlation was significant ($r = 0.492$). This correlation motivated an additional random effect indexed to nest to account for the correlation among family members. As with foraging effort, we partitioned the residual variance into between-family, between-individual, and within-individual components (Fig. 6).

Parental [IgG] was not significantly related to offspring age-class, sex of the parent or offspring, nestling period, or their interactions (Fig. 6A, E; Appendix 2: Table 8). The parent-sex * offspring-sex interaction approached significance ($F = 3.07$, df = 1 and 34, $P = 0.089$) and indicated lower [IgG] in mothers of sons than in mothers of daughters ($F = 4.54$, df = 1 and 34, $P = 0.040$), with no corresponding difference in fathers ($F = 0.10$, df = 1 and 34, $P = 0.75$). Separate analyses also indicated that [IgG] declined with age-class in mothers ($F = 2.19$, df = 5 and 128, $P = 0.059$) but not fathers ($F = 0.62$, df = 5 and 133, $P = 0.69$), though with marginal significance and without support in the combined analysis (nonsignificant offspring-age-class * parent-sex interaction, Appendix 2: Table 8). The offspring-age-class * offspring-sex * nestling-period interaction was marginally significant ($F = 2.16$, df = 5 and 141, $P = 0.062$) and hinted at higher [IgG] in parents of older daughters with longer nestling periods.

In exploratory analysis, the variance in parental [IgG] showed no systematic pattern across age-classes (Fig. 6B, F) and the CV was consistently between 30% and 40% (Fig. 6C, G).

For consecutive measurements (lag = 20 days) of [IgG], the correlation was high ($r = 0.842$) and declined slightly for longer lags (Fig. 6D, H). The AR1 + RE covariance structure provided the best-fit against competing CS and ARH1 + RE models; all models had a random effect for nest, and the random effect representing individuals was nested within this factor (Appendix 2: Table 8). The covariance parameters differed as a function of parent sex ($G^2 = 7.6$, df = 2, $P = 0.022$), but not of offspring sex or nestling period (Appendix 2: Table 8). The autocorrelation of [IgG] was negligible for mothers ($\rho = 0.105$, 95% CI: −0.142 to 0.351) and intermediate for fathers ($\rho = 0.552$, 95% CI: 0.268 to 0.837). Separate analyses by parent sex showed that the CS and AR1 + RE structures fit equally well for mothers ($G^2 = 0.8$, df = 1, $P = 0.37$), but AR1 + RE fit significantly better than CS for fathers ($G^2 = 16.2$, df = 1, $P < 0.0001$). The analyses of the mean trends presented above employed an AR1 + RE covariance matrix with separate parameters for mothers and fathers. For parental [IgG], 48% of the variance was attributed to differences between pairs, and 37% of the variance was apportioned to differences between individuals within pairs. Thus, the relatively high CV observed for [IgG] reflected the variation between, and not within, individual parents.

### Parent–Offspring Comparison

At fledging, mean body mass of daughters was 103 g (5.8%) greater than that of their mothers, the variances were comparable, and body masses were uncorrelated (Table 2). Body masses of sons and fathers at fledging did not differ in mean or variance and they were also uncorrelated. Thus, daughters were slightly heavier than their parents, and offspring did not show greater variation or parent–offspring correlations in body mass.

Mean culmen length at fledging was 1.7 mm (1.6%) shorter in daughters than in mothers and 1.7 (1.6 %) shorter in sons than in fathers (Table 2), possibly indicating that culmen growth was incomplete. Variance in culmen length did not differ between daughters and mothers, but was marginally greater in sons than in fathers (Table 2). Culmen length was uncorrelated between daughters and mothers and between sons and fathers (Table 2).

Mean wing length at fledging was similar for daughters and mothers and for sons and fathers (Table 2). Variance in wing length was marginally greater in daughters than in mothers, but was not greater in sons than in fathers (Table 2). Wing length was not correlated between daughters and mothers, but was positively correlated between sons and fathers (Table 2).

As shown earlier, [IgG] was significantly lower in offspring than in the parents and [IgG] was positively correlated between offspring and parental midpoint (Table 2). The variances of [IgG] were similar for offspring and parents (Table 2).

In summary, it appears that the morphological traits of offspring at fledging were similar to—and, generally, were not more variable than—those of their parents. By contrast, [IgG] was

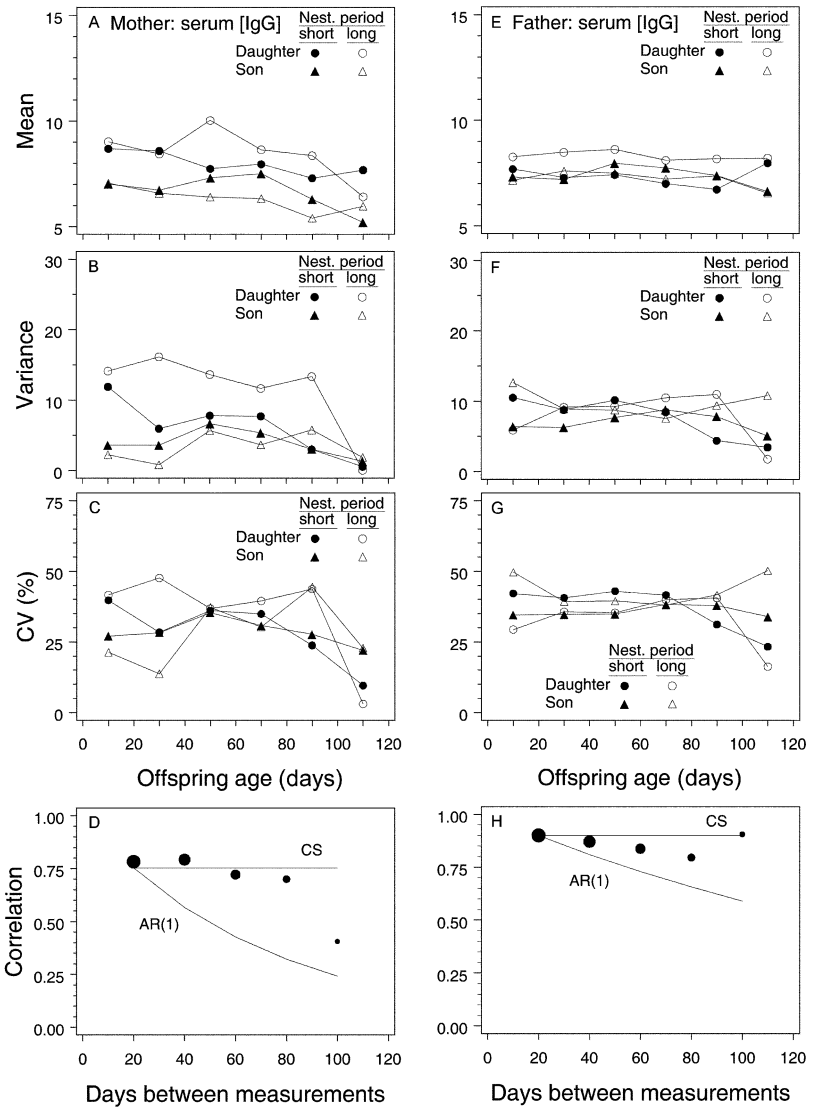


FIG. 6. Parent Nazca Booby [IgG] as function of offspring age-class. For mothers: (A) mean, (B) variance, and (C) coefficient of variation (CV). For fathers: (E) mean, (F) variance, and (G) coefficient of variation. Separate lines are shown for groups that differ in means or variances according to linear-mixed-model analyses (Appendix 2, Table 8). Also shown is the exploratory analysis of correlation structure based on the correlation between measurements from the same individuals as a function of interval between measurements of [IgG] for (E) mothers and (H) fathers. For the analysis of correlation structure, symbol size is proportional to sample size. Units: [IgG] (g $L^{-1}$).

significantly lower in the fledglings and was correlated with levels in the parents.

### OFFSPRING MORTALITY RATE

Nestling mortality rate in the study area (including the focal subsection) typically fell over the course of the nestling period, except during the unusual conditions of the El Niño–Southern Oscillation Event (ENSO) of 1997–1998 (Fig. 7). Age-specific mortality rate during this study was low compared with other non-ENSO years (log rank $\chi^2 = 25.9$, df = 1, $P < 0.0001$; Fig. 7).

TABLE 2. Comparison of offspring and parent traits of Nazca Boobies: body mass, culmen length, wing length, and IgG. Homogeneity of means tested with one-way analysis of variance (ANOVA) and variances with Levene's test (df = 1 and $n - 2$). Correlation tested with Pearson correlation coefficient.

| Trait | Offspring Mean ± SD | Parent Mean ± SD | Means | | Variances | | Correlation | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $F$ | $P$ | $F$ | $P$ | $n$ | $r$ | $P$ |
| Body mass (day 110; g) | | | | | | | | | |
| Daughter–Mother | 1878 ± 150.3 | 1775 ± 103.9 | 6.28 | 0.017 | 2.32 | 0.14 | 20 | 0.052 | 0.83 |
| Son–Father | 1660 ± 177.0 | 1580 ± 137.0 | 2.28 | 0.14 | 2.18 | 0.15 | 18 | 0.210 | 0.40 |
| Culmen length (day 120, mm) | | | | | | | | | |
| Daughter–Mother | 105.7 ± 1.62 | 107.4 ± 2.23 | 7.67 | 0.0087 | 3.07 | 0.088 | 19 | 0.025 | 0.92 |
| Son–Father | 102.2 ± 2.02 | 103.9 ± 2.67 | 5.03 | 0.032 | 2.58 | 0.12 | 18 | 0.274 | 0.27 |
| Wing length (day 120, mm) | | | | | | | | | |
| Daughter–Mother | 483.5 ± 8.27 | 479.2 ± 8.66 | 2.43 | 0.13 | 0.3 | 0.87 | 18 | 0.303 | 0.22 |
| Son–Father | 458.4 ± 8.23 | 459.8 ± 10.32 | 0.20 | 0.66 | 0.84 | 0.37 | 18 | 0.503 | 0.033 |
| Serum IgG (mean of days 70 to 110, midpoint value for parents, g $L^{-1}$) | | | | | | | | | |
| | 5.77 ± 2.17 | 7.65 ± 2.29 | 13.54 | 0.0004 | 0.14 | 0.71 | 38 | 0.757 | <0.0001 |

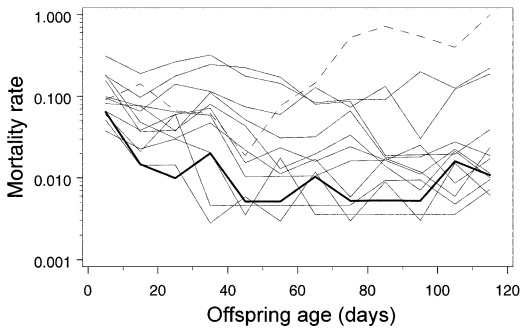


FIG. 7. Interval mortality rate of offspring as a function of age-class for the study year 2002–2003 (thick solid line), El Niño year 1997–1998 (dashed thin line), and all other years between the 1992–1993 and 2004–2005 breeding seasons (thin solid lines).

## DISCUSSION

Costs of reproduction are clearly demonstrated in short-lived bird species with high annual reproductive output, where the parents appear to reallocate nutrients from self-maintenance to fuel the physical activity needed to sustain a high level of parental effort (Daan et al. 1996). These studies suggest that the parent's consistent provisioning of offspring promotes uncompromised offspring growth, but the latter appears to be subsidized by the reduction in the parent's self-maintenance activity, as assessed by immune function (Sheldon and Verhulst 1996). This life-history strategy is observed in environments in which high extrinsic mortality (e.g., predation pressure) discounts investment in self-maintenance by the parents. In environments with low extrinsic mortality, the life-history theory of senescence predicts that sustained investment in self-maintenance would benefit individuals by extending their reproductive lifespan (Goodman 1974). As a corollary, parents in long-lived taxa, such as our study organism, should regulate reproductive effort at a level compatible with sustained self-maintenance, and not subsidize their offspring's fitness with their own personal health and survival (Moreno 2003). Offspring growth and body condition are expected to absorb stochastic resource fluctuations as a result, because the parent's self-maintenance has primacy for limited nutritional resources (Sæther et al. 1993, Mauck and Grubb 1995, Navarro and González-Solís 2007).

We tested these predictions in a long-lived seabird, using a longitudinal sampling design and linear mixed models to estimate the within- and between-individual variance components of parent and offspring traits. This approach allowed us to test the hypothesis that individual parents maintained stable levels of self-maintenance ([IgG]) and body condition (parent body mass) as foraging effort (time spent at sea) and offspring food demand (offspring body mass) increased dramatically across the nestling period. We also examined patterns of variation in offspring growth to infer whether parents relayed resource variability to the offspring. The multiple repeated measurements (6–13 per individual) allowed testing the fit of alternative covariance

structures that model the trajectories of these traits over time and from which we can infer the mode of regulation. Next, we summarize the salient results, considering each in more detail in the following sections.

In individual Nazca Booby parents, [IgG] was stable across the nestling period, after accounting for considerable inter-individual variation. Autocorrelation was significant for fathers, but not for mothers, which implies a certain degree of homeostatic regulation in the former. By contrast, parental body mass showed a modest (7–11%), but consistent, decline across the nestling period, also after accounting for considerable inter-individual variation. Overall, mean body mass was lower in parents that were rearing offspring that had longer nestling periods, and mass loss was greater in parents rearing the larger-sized offspring (daughters). Repeated measurements of body mass of individuals were uncorrelated, which implies either weak regulation or considerable measurement error of body mass. As parental foraging effort doubled across the nestling period, consistent differences between individuals did not emerge, after controlling the sex of the parent and their offspring. However, the modest level of autocorrelation of foraging effort within individuals seemed to indicate that parents were showing a limited degree of consistency despite the lack of significant differences in foraging effort between individuals.

In summary, our measure of immune function ([IgG]) supported the expectation of sustained self-maintenance as demands on the parents increased in this long-lived pelagic seabird. Our proxy for body condition (body mass) provided equivocal support for sustained self-maintenance, in that body mass decreased slightly but with a consistent trajectory within individuals. Our measure of reproductive effort (time spent at sea) provided weak evidence for a fixed individual-specific schedule of foraging effort: parents spent more time foraging for offspring of the larger sex (female) but appeared to do so with a modest degree of consistency.

Parents in short-lived species increase their workload to compensate for mismatched offspring demand and food availability. In contrast, parents in long-lived species should not increase parental effort in response to resource shortfalls but, instead, should produce offspring whose quality reflects current resource availability. In these species, the body condition of offspring, and not that of the parents, should reflect the deficit between food demand and delivery (Sæther et al. 1993, Mauck and Grubb 1995). As in other long-lived pelagic seabirds, offspring body-mass growth in Nazca Boobies lacked autocorrelation except over short measurement intervals, and it appeared that offspring buffered resource variability with plasticity in body-mass growth to a greater degree than in the growth of skeletal traits. A biologically significant component of buffer capacity turned out to be the length of the nestling period. Chronically lightweight offspring had longer nestling periods but still attained an apparent sex-specific body-mass target at fledging. We were surprised to find that the variances of offspring traits did not exceed those of the parents. Nonetheless, the high intra-individual variance in body-mass growth and a variable development period support the view that parents sustained their own self-maintenance by relaying resource variability to the offspring.

Further evidence for variable offspring growth was observed in the striking peak of variance in two structural traits at the time of peak growth rate of each trait and not during the time of peak food demand. Although offspring showed significant structural heterogeneity (CV = 5–13%) during the rapid growth phase, the variable length of the nestling period allowed all fledglings to reach their sex-specific sizes with less structural variation (CV < 3%) at fledging. This plasticity of structural growth provides additional evidence that offspring buffered parental reproductive costs.

These patterns of variation in morphological growth were not coupled to the ontogeny of [IgG] in the offspring. This suggests that whatever food limitation the parents may have imposed on the morphological growth of the offspring, it did not influence the ontogeny of this immunological trait in the offspring, in contrast to the tradeoff between growth and immune function observed in short-lived species (Table 3). This component of immune function may be critical for survival in the dense, fecally contaminated breeding colony (Fig. 8), especially with offspring sustaining frequent injurious attacks by adults (Anderson et al. 2004).

### Regulation of Self-maintenance in Long-lived Species

In Nazca Boobies, our measure of parental self-maintenance ([IgG]) did not decrease with

TABLE 3. Relationship between offspring growth conditions and immune function. $\gamma$-globulin = percentage of $\gamma$-globulins in protein electrophoresis; H:L = heterophil to lymphocyte ratio, [IgG] = immunoglobin G (= Y) concentration; PHA response = phytohemagglutinin-induced skin-swelling.

| Species | Predictor/**Treatment** | Response | References |
|---|---|---|---|
| Black-legged Kittiwake | ↑**Food available to** | —[IgG] | Gasparini et al. 2006 |
| *Rissa tridactyla* | **parents** | —PHA response | |
| Common Tern | ↑Brood size | ↑[IgG] | Apanius and Nisbet 2006 |
| *Sterna hirundo* | | | |
| Eurasian Kestrel | ↑**Brood size** | —PHA response | Fargallo et al. 2002 |
| *Falco tinnunculus* | | | |
| American Kestrel | ↑Body condition | ↑PHA response | Tella et al. 2000 |
| *F. sparverius* | ↑First-year survival | | |
| Great Tit | ↓**Brood size** | —H:L | Ots and Hõrak 1996 |
| *Parus major* | ↓**Brood size** | ↑PHA response | Hõrak et al. 1999 |
| | **Male removal** | ↓PHA response | Snoeijs et al. 2005 |
| Blue Tit | ↑**Brood size** | —[IgG] | Merino et al. 2006 |
| *Cyanistes caeruleus* | | | |
| Barn Swallow | ↑**Brood size** | ↓PHA response | Saino et al. 1997b, Pap and Markus 2003 |
| *Hirundo rustica* | ↑Growth rate | ↓PHA response | Pap and Markus 2003, Saino et al. 2001 |
| Tree Swallow | ↑**Brood size** | —H:L | Shutler et al. 2004 |
| *Tachycineta bicolor* | | | |
| House Martin | ↑Body condition | ↑PHA response | Christe et al.1998, 2001 |
| *Delichon urbica* | ↑First -year survival | —PHA response | Christe et al. 2001 |
| | | ↑$\gamma$-globulin | |
| | ↑Body mass | ↑PHA response | Merino et al. 1999 |
| Bank Swallow | ↑**Brood size** | ↓$\gamma$-globulin | Szép and Møller 1999 |
| *Riparia riparia* | | | |
| European Magpie | ↑Methionine in diet | ↑PHA response | Soler et al. 2003 |
| *Pica pica* | | ↓Growth rate | |
| House Sparrow | ↑Body mass | ↑PHA response | Westneat et al. 2004 |
| *Passer domesticus* | | | |

increasing parental foraging effort and decreasing body mass across the nestling period. It did not vary significantly with parent sex, offspring age-class or sex, the duration of the nestling period, or their interactions. We found weak evidence (marginal statistical significance) for a decline in [IgG] across the reproductive cycle in mothers and for lower mean [IgG] in mothers raising sons. However, 85% of the residual variance that was not explained by the fixed effects was attributable to differences between pairs (48%) and to differences between individuals within pairs (37%). Of the remaining variance, autocorrelation of [IgG] was significant for fathers but not mothers, which implies more stringent regulation in the former, given that measurement error should be the same for both sexes. Although the stability of [IgG] in parents across the nestling period supports the life-history theory of senescence, data from mothers provide a hint that their [IgG] was less stable.

The stability of [IgG] that we observed in Nazca Boobies contrasts with results from other avian taxa (Table 1). Significant declines in [IgG] and $\gamma$-globulins (a less precise measure of [IgG]) across various phases of the reproductive cycle have been observed in female Common Eiders (*Somateria mollissima*), female Barn Swallows (*Hirundo rustica*), female Blue Tits (*Cyanistes caeruleus*), and both sexes of Great Tits (*Parus major*) and Dark-eyed Juncos (*Junco hyemalis*). Changes in body mass and plasma metabolite levels in Great Tits and Common Eiders supported the interpretation that decreased $\gamma$-globulins accompanied declining physiological condition (Hõrak et al. 1998, Hollmén et al. 2001). The observed decline in [IgG] across the reproductive cycle is consistent with experimentally increased parental effort inducing decreased immune function in the parents, as evidenced by increased heterophil:lymphocyte ratios, decreased antibody responses, and decreased PHA responses (Table 1). In short-lived passerine birds, the tradeoff between reproductive effort and self-maintenance, as measured by immune function, is amply demonstrated.

FIG. 8. Representative section of the study site. The native substrate is black or dark gray; all white surface is an overlay of guano.

In contrast, this potential tradeoff is poorly resolved in long-lived species. Long-lived Common Eiders showed an unequivocal decline in [IgG] and other measures of immune function (Table 1) during incubation that was associated with fasting and depletion of body reserves (Hanssen et al. 2005). This reproductive tactic appears to be a response to intense predation pressure on this ground-nesting species (Yoccoz et al. 2002). Long-lived Common Terns (*Sterna hirundo*) showed an equivocal ($P = 0.05$, $n = 13$) decline in [IgG] during nestling growth (Apanius and Nisbet 2006). Common Terns do not fit the pelagic-seabird model, because they experience both extended sibling competition and nest predation and, as a result, show relatively rapid growth for a seabird (Nisbet 2002). Nazca Boobies experience minimal predation on nestlings (Anderson 1993), and their brood size is effectively a single chick because of obligate siblicide (Humphries et al. 2006). The breeding-related decline of [IgG] in some long-lived seabirds (Common Eider and Common Tern) contrasts with the stability of [IgG] in a long-lived pelagic seabird (Nazca Booby) and supports, for the first time, the hypothesis that sustained self-maintenance during reproduction depends on the magnitude of extrinsic mortality, namely nest predation. In this view, the absence of nest predation (Lack 1968) and the lack of extended sibling competition (Werschkul and Jackson 1979, Ricklefs 1982) provide a selective regime whereby an extended and flexible offspring-development period allows long-lived parents to sustain their own self-maintenance during reproduction (see also Martin et al. 2001).

### PARENTAL FORAGING EFFORT

In long-lived species, foraging effort of parents is expected to track increasing offspring demand, but without entailing reallocation of resources from self-maintenance. If individuals differ in foraging proficiency, one would expect to observe individual-specific foraging effort. Several studies

provide examples of individual-specific foraging schedules based on the amount of time spent at sea (Hamer et al. 2001, Gray et al. 2005, Cook et al. 2006; but see Burger and Piatt 1990). We use a similar metric and extend its analysis by examining the covariance structure to address whether this behavior appears to be regulated in an individual-specific manner.

In our study, Nazca Boobies did not show significant inter-individual variation in foraging effort, as measured by the amount of time spent at sea. This may refute the idea that an individual's foraging time is commensurate with its proficiency. One might expect that efficient foragers are able to spend less time at sea and have offspring with average or above-average growth rates. We did not find a significant relationship between foraging time and offspring mass or size at any particular age-class or as an age-class (i.e., growth rate) interaction. Rather, it appeared that parental foraging effort depended on the sex of the parent (mothers spent more time at sea than fathers) and the offspring (more time at sea for daughters) and the foraging time of each bird's partner (see below). Within these categories, parents foraged with similar effort, though the modest autocorrelation and autoregressive covariance structure of individual foraging effort is compatible with the expectation that individuals have fixed foraging schedules, albeit not markedly different from other individuals. It is conceivable that differences in individual quality, which may be manifest in foraging efficiency, are not perceivable from foraging-time-budget data alone in this species.

Individual-specific foraging schedules may not have been detected in this study because the spatiotemporal heterogeneity of marine food resources obscured variation in foraging proficiency of individuals. Additionally, the amount of time spent at sea may not accurately measure parental workload, because the principal foraging tactic of this species (plunge diving) requires repeated and energetically costly lift-offs from the water, which were not measured in this study. Finally, it is possible that foraging efficiency (food acquired per unit effort) is the critical parameter that shows individual-specific consistency, but we did not measure the amount of food delivered in this study.

Although individual-specific foraging schedules were not apparent, we observed modest autocorrelation ($\rho = 0.37$) of foraging effort. Within individuals, time spent at sea was correlated across 20-day intervals, but the autoregressive (exponential decay) covariance structure indicated that the correlation between measurements over a 60-day interval was nil ($r = 0.05$; Fig. 5D, H). This implied a modest degree of regulation of foraging effort within individuals across the short spans of the nestling period.

As foraging effort increased for both parents in the middle of the nestling period, it was positively correlated between members of a pair. The positive correlation indicated that parents did not compensate for any reduced effort by their mate, which is contrary to the prediction of most current provisioning models (Houston and Davies 1985, McNamara et al. 1999). A parsimonious explanation for the correlation notes that each pair of parents shares a common food demand from the offspring (such as parents of daughters requiring more food than parents of sons) and faces the same spatial distribution of food resources. Therefore, they spend corresponding, sex-specific amounts of time at sea. Offspring SSD may contribute to, but cannot account for all of, the correlation, because time spent at sea was correlated within pairs raising daughters and within pairs raising sons. This correlation could also stem from assortative mating based on foraging ability, with more capable birds requiring less foraging time to satisfy a given level of offspring food demand. However, foraging time did not show consistent differences between individuals and so was not a repeatable trait, as required if assortative mating drove the correlation.

Parental foraging effort was not related to the length of the nestling period. Regardless of their sex, offspring that were heavier across age-classes had a shorter nestling period than lighter offspring. Thus, it appeared that some pairs were able to deliver more food for the same level of foraging effort (or with greater regularity) and that their offspring had heavier body masses, developed relatively rapidly, and fledged at an earlier age as a consequence. This suggests that length of the nestling period may be a suitable proxy for (joint) parental quality in this species, which is ultimately based on foraging efficiency.

### Parental Body Mass

Seabird parents commonly lose body mass during breeding, and experimentally increased parental effort induced a greater decrease in body mass in some pelagic seabird species (Jacobson

et al. 1995, Golet et al. 1998, Weimerskirch et al. 2000), but not in others (Sæther et al. 1993, Mauck and Grubb 1995, Weimerskirch et al. 1999, Duriez et al. 2000). In Nazca Boobies, fathers and mothers both lost 7–11% of their body mass across the nestling period, with inter-individual differences in sex-specific body mass accounting for ~43% of the residual (nonfixed effect) variance, with minimal autocorrelation. Although the covariance structure for parental body mass was problematic to identify, the best-fit models were consistent in showing significant long-term differences between individuals, with no autocorrelation within individuals or correlation between pair members. The lack of autocorrelation might be expected for a species that consumes few large prey items unpredictably through the daily cycle. Our indicator of body mass included gut contents, which were variable in mass and potentially large, which would lead to large errors in body-mass measurements. We measured body mass just before dawn, which minimized but probably did not eliminate measurement error attributable to gut contents. This error may also account for the lack of correlation between (sex-specific) body mass and (sex-specific) structural size. Although body mass varied in the manner of a random walk within individuals, the consistent differences between individuals suggested that parents were regulating body mass within individual-specific envelopes. Whether the 7–11% decrease in body mass over the 100-day nestling period represented a decrease in body reserves (i.e., a physiological cost of reproduction) or is linked to increased flight time and wing-loading considerations (Jones 1994, Ritz 2007; see below) is not known. Both ideas predict a correlation between degree of mass loss and time spent aloft at sea, thus making body-mass dynamics difficult to interpret in cost-of-reproduction studies of seabirds.

The length of the nestling period was negatively related to the sex-specific (least squares mean) body mass of the parents, but not to the sex-specific (least squares mean) foraging time. This suggested that parental body mass was linked to provisioning ability, with lightweight parents providing less food for their offspring with a subsequent extension of the nestling period. Given that length of the nestling period was not related to foraging effort, this suggested that parents were not expending more foraging effort for offspring with a long nestling period, further reinforcing the connection between foraging ability (rather than effort), parental body mass, and length of the nestling period.

Sex-specific body masses were uncorrelated between parents and offspring, and a correlation is expected if lightweight parents produced lightweight offspring. Instead, the variable nestling period may have allowed lightweight parents to eventually produce offspring with a body mass comparable to that of offspring of heavier parents.

### Seabird Growth Plasticity

The growth of offspring included marked peaks in variability in size of structural traits, but not in mass (ignoring the variance induced on day 10 by three outlier males; see above), early in growth (Fig. 3). The CV of mass declined somewhat during growth, indicating a canalization of the growth process within sex as growth proceeded. Body-mass measurements were moderately correlated ($\rho = 0.430$) when measured 10 days apart but were uncorrelated after 50- to 60-day intervals. Except over short intervals, offspring body mass was not a repeatable trait, given that the between-individual variance was negligible compared to the within-individual variance.

The lack of consistent inter-individual differences in mass between offspring could be attributable to erratic delivery of large meals, because engorgement and assimilation of large food boluses could mask individual differences in body mass. Previous studies have used daily weighing and three- or five-day moving averages to smooth body-mass fluctuations resulting from large meal sizes in seabirds. Leach's Storm-Petrels (*Oceanodroma leucorhoa*) have relatively large meal sizes with unpredictable food-delivery schedules and $\rho \approx 0.5$ for (5-day moving average) body mass at 10-day intervals; experimental evidence indicated that parents, not offspring, control provisioning rates (Ricklefs 1992). Manx Shearwaters (*Puffinus puffinus*) have consistent meal sizes and delivery rates and $\rho \approx 0.6$ for (3-day moving average) body mass at 10-day intervals; experimental evidence suggested that both parents and offspring influence provisioning rate (Hamer et al. 1999). For both of these species with single-offspring broods, autocorrelations became nonsignificant at measurement intervals of 15–25 days (60-day parental care period) and 12–15 days (70-day parental-care period) for Leach's

Storm-Petrels and Manx Shearwaters, respectively (Ricklefs 1992, Hamer et al. 1999). In general, offspring body masses are autocorrelated for only short intervals of the nestling period, regardless of provisioning mode or frequency of measurements. These patterns of autocorrelation conform to the view that offspring body-mass growth has a stochastic element that presumably reflects variability of marine resources, and it also implies that parents of some species do not adjust their foraging effort to maintain consistent offspring growth.

A significant outcome of the present study was the observation that the variances in offspring culmen and wing lengths were greatest in the younger (days 20–50) and middle (days 40–80) age-classes, respectively. The variance peaked before sexual dimorphism was evident in these traits (culmen: day 50; wing: 90). Furthermore, variation as a function of mean trait size (CV) peaked at even younger age-classes. Peak structural variation occurred when body mass was growing rapidly, but before the expected peak in food demand by the offspring (late in the nestling period; Anderson 1990), and at the time-point where mothers, but not fathers, started to increase their foraging effort. This suggests that the regularity of food delivery was responsible for this variation. We found a notable sex difference in morphological variability also: culmen and wing lengths were more variable in sons than in daughters. The greater variation in growth of sons may be attributable to an association of the probability of producing a son and parental quality, with sons being more likely to be raised by parents with poor provisioning ability (i.e., lower quality; Kalmbach et al. 2004). This preliminary conclusion deserves further attention in the future.

Did parental time budgets and the highly variable offspring growth covary? The twofold increase in foraging effort across the nestling period was associated with a nearly constant CV of ~15%. Inspection of Figures 1 and 2 indicated that the variance in foraging effort of parents was unrelated to the age-dependent heterogeneity observed in culmen and wing growth of the offspring. Thus, the pattern of morphological variance in the offspring did not appear to be related to the mean or variance of parental foraging effort. This points to the possibility that regularity of food delivery, which could not be resolved with our time-budget data, may be a causal link between parental foraging effort and the pattern of morphological variation of the offspring. This reinforces our interpretation that offspring growth, and not parental effort, reflects short-term resource variation in the marine environment.

Following the period of peak variance, both structural traits converged to CVs of ~2% at fledging. Given that no offspring died in our focal group, the variable nestling period may have diminished structural variation by the time of fledging. For the offspring at fledging, the variances of wing and culmen length were not greater than the variances of these traits for the parents. Thus, structural traits exhibited growth plasticity that afforded the opportunity for offspring to buffer unpredictable food delivery but without apparent consequences for their fledging morphology.

Does the peak variance in the offspring's structural size portend increased mortality of offspring with stunted growth? Patterns of age-specific mortality rates across a span of 12 years did not correspond with those of variance in offspring structural size (Figs. 3F, G, J, K, and 7). Regardless of overall level of offspring mortality in different years, the highest mortality rates for the survivor of siblicide occurred between days 0 and 10 and decreased thereafter. Therefore, it is difficult to argue that the variance in structural size was temporally associated with increased risk of mortality in the year of our study. It seems more likely that the pattern of variance reveals the plasticity of offspring growth and does not herald offspring mortality, at least in years with high fledging success such as the year of our study.

The absence of both nest predation and extended sibling competition in Nazca Boobies may be key factors that permit a prolonged and elastic developmental period of the offspring to reduce reproductive costs to the parents (Fig. 9). In Common Terns and Arctic Terns (*S. paradisaea*) with sibling competition and relatively short nestling periods, inclement weather caused body-mass growth, but not structural growth, to be severely retarded, and normal fledging body mass was achieved by extending the nestling period (Robinson et al. 2002). These two species showed no evidence of compensatory acceleration of body-mass growth as might be expected if parents increased food delivery. Instead, the period of parental care was prolonged (Robinson et al. 2002). In Horned Puffins (*Fratercula corniculata*) and Tufted Puffins (*Lunda cirrhata*) with no sibling competition and somewhat longer nestling

FIG. 9. Nazca Booby parents and their one-chick brood. The exceptionally slow growth of pelagic seabird chicks is reflected in the relative body sizes of the family members. This well-fed chick is ~45 days old, yet has reached only two-thirds the mass of the parents. Its primary feathers have barely begun growth. Parental care is one-third complete at this stage. (Photograph by Tui de Roy.)

periods, food restriction retarded growth of body mass and tarsus, but not that of culmen or feathers (Kitaysky 1999). In Fork-tailed Storm-Petrels (*O. furcata*) that have no sibling competition and a prolonged nestling period, the growth rates of mass and wing length were negatively related to the duration of the nestling period (Boersma and Parrish 1998). Thus, flexibility of growth rate in seabirds may depend on the particular trait and the length of the nestling period, which in turn may be driven by predation pressure and sibling competition. The lack of extended sibling competition and predators in our study species appeared to favor a prolonged nestling period.

Although a prolonged developmental period in seabirds is typically believed to buffer resource variability, a variable nestling period can also permit the parents to pass the costs associated with resource variability to the offspring and thereby spare the parents additional foraging effort when resources are scarce. We recognize the diversity of seabird foraging tactics and how it relates to offspring growth dynamics (Visser 2002). In this context, we stress that our predictions and results are based on the pelagic seabird model, emphasizing (1) minimal predation, (2) unpredictable prey-resource levels, (3) lack of extended sibling competition, and (4) slow offspring growth. We suspect that the theoretical prediction of sustained self-maintenance during reproduction, coupled with plastic offspring growth, will be most prominent in this type of long-lived bird, and we await results from comparable studies of other long-lived birds to test the generality of our conclusions.

### SEXUAL SIZE-DIMORPHISM

Fledgling Nazca Boobies showed the female-larger SSD of adults, with the magnitude of the sex difference similar in the two groups for mass (13% and 12%, respectively), culmen length (3.4% and 3.4%), and wing length (5.5% and 4.2%). The cube root of body mass and square root of length measurements are also used to assess dimorphism, and these parameters showed similar trends for mass (offspring: 4.2%, parent: 4.0%), culmen length (offspring: 1.7%, parent: 1.7%), and wing length (offspring: 2.7%, parent: 2.1%; Table 2). Other members of the Sulidae also show relatively different body masses but similar structural sizes (Lewis et al. 2005, Weimerskirch et al. 2006). Wing-loading of females thus exceeds that of males in several booby species (Townsend et al. 2002, Weimerskirch et al. 2006) and may be related to sex-specific foraging and provisioning behaviors (Guerra and Drummond 1995, Tershy and Croll 2000, Lewis et al. 2005, Lormee et al. 2005, Weimerskirch et al. 2006). Our time-budget data showed that Nazca Booby mothers spent more time at sea than fathers during much of their chicks' growth, which is consistent with earlier results indicating that mothers have a greater provisioning role than fathers: mothers make longer foraging trips during chick rearing and return with larger loads (Anderson and Ricklefs 1992). By contrast, fathers spend more time at the nest attending chicks, thereby protecting them from intrusive visits by nonbreeding adults, which can cause injuries and death to nestlings (Curry and Anderson 1987, Anderson et al. 2004). This trade-off confronted by parents, between food delivery (requiring absence at sea) and protection (requiring presence at the nest), puts a premium on foraging efficiency. Although the higher wing-loading of mothers imposes costs during take-off (Townsend et al. 2002) and probably during flight, it is associated with a higher efficiency (mass of food per time at sea) of food delivery to the nest (Anderson and Ricklefs 1992). The larger average prey size of mothers (Anderson 1989a) probably contributes to higher efficiency, and access to larger prey may be a consequence of larger body size if larger size permits deeper plunge-dives (Lewis et al. 2005). Under this reasoning for

the evolution of SSD in Nazca Boobies, one sex has a large mass to increase delivery efficiency while the other has a low-requirement, smaller mass that facilitates nest attendance.

Evidence for this idea comes from the more highly dimorphic Blue-footed Booby (*S. nebouxii*), which experiences nestling predation by hawks in the Galápagos Islands (Anderson and Hodum 1993) and in which fathers are present at the nest more than mothers (Anderson and Ricklefs 1992, Guerra and Drummond 1995), but support is not universal. Red-footed Boobies (*S. sula*) have a similar degree of SSD to Nazca Boobies, yet have low predation at the nest (Nelson 1978, Anderson 1991) and little division of labor (Lewis et al. 2005, Lormee et al. 2005). Brown Boobies (*S. leucogaster*) have greater SSD than Nazca Boobies, yet have few nest predators in much of their range (Nelson 1978). The ultimate causation of SSD in boobies is, thus, unclear, but accumulating information continues to link it to the foraging biology of adults. We found it curious that wing length was correlated for fathers and sons but not for mothers and daughters. Our data offer no new insight into why the benefits of larger mass for one sex are not accompanied by larger structural size, which should increase flight efficiency by decreasing wing-loading (Pennycuick 1989). The question of which sex should be the larger is also left unanswered.

Sexual size-dimorphism in nestlings leads to the expectation of higher parental effort required for daughters than for sons, because offspring of the larger sex typically require more food to reach their target body size than members of the smaller sex (reviewed by Anderson et al. 1993). Data from a different breeding season (2000–2001) for our study population showed that the SSD was established during the period of peak food intake in that year (Townsend et al. 2007). By contrast, our analysis indicated that sons and daughters followed similar growth trajectories until reaching peak body mass in 2002–2003 (Fig. 4A) but that body-mass recession was greater in sons than in daughters. Breeding conditions were apparently relatively favorable in 2002–2003, judging from the 11-year comparison of offspring mortality rates (Fig. 7). The probability that a hatchling survived to fledging was greater in 2002–2003 (0.853, 95% CI: 0.830–0.900) than in 2000–2001 (0.555, 95% CI: 0.484–0.626); perhaps limited resource availability constrained offspring growth in 2000–2001. These two data sets show that SSD is established during the period of parental care and that both differential growth and differential mass loss during parental care can contribute to its ontogeny. They also show that the plasticity of offspring growth is expressed in response to presumed variation in food-resource levels between years.

Parents spent more time at sea as chicks grew, and more for daughters than for sons. Given the value of presence at the nest, to guard against intrusions by nonbreeders (Anderson et al. 2004), parents probably minimize absence; under this reasoning, the difference in absence by offspring sex reflects the excess foraging effort of producing daughters. Parents of daughters also lost more mass during chick rearing, which could reflect an excess physiological cost to parents of daughters. However, this difference may also reflect a beneficial, progressive reduction in wing-loading that occurred more rapidly when caring for a daughter because parents of daughters spend more time at sea (and, presumably, more time flying). Any higher effort induced by daughters did not impinge on our measure of parental self-maintenance [IgG]. In fact, suggestive evidence of impaired immune self-maintenance in mothers was associated with sons, not daughters.

### Ontogeny of Immunity

Offspring [IgG] decreased after hatching as maternally derived IgG was used and degraded before the onset of significant endogenous synthesis (Apanius 1998a). Surprisingly, offspring [IgG] remained at its lowest levels at days 10 and 30. The observation that offspring [IgG] did not attain adult values by the end of the long nestling period is also noteworthy. Generally, [IgG] reaches adult levels in birds when the offspring reach adult body mass or size (reviewed in Apanius 1998a), as was observed in offspring of long-lived Common Terns (Apanius and Nisbet 2006).

Offspring [IgG] increased during the phase of rapid morphological growth between days 30 and 50, when body mass, culmen, and wing were growing at their most rapid rate and structural traits showed the greatest variability. During this period, [IgG] was not related to morphological growth, providing another notable contrast with results from short-lived species that have rapidly growing offspring (Table 3). Levels of $\gamma$-globulin in passerine nestlings are negatively related to morphological growth. In Barn Swallows, first-hatched chicks grew faster than later chicks but

had lower $\gamma$-globulin levels at 12 days of age (Saino et al. 2001). In Bank Swallows (*Riparia riparia*), $\gamma$-globulin levels in chicks were negatively related to the number of chicks in the nest (Szép and Møller 1999). These studies suggest that morphological growth and the development of [IgG] compete for limited nutrients. Results based on H:L ratios, and on antibody and PHA responses, indicate generality in the existence of this tradeoff between somatic growth and the ontogeny of immune function in short-lived birds (Table 3). On the other hand, the development of [IgG] in nestlings of long-lived Common Terns was relatively rapid but was not negatively related to morphological growth (Apanius and Nisbet 2006). Supplementary feeding of long-lived Blacklegged Kittiwake parents did not increase [IgG] in their 10-day-old offspring, which suggests that food limitation did not modulate growth of this trait (Gasparini et al. 2006). Therefore, the ontogeny of [IgG] does not invariably show a tradeoff with morphological growth but is more likely linked to life-history strategy. Unlike morphological traits, which appeared to buffer variable food-provisioning by the parent, the development of this self-maintenance trait appears to be decoupled from the plasticity of offspring growth. These results support the idea that insulating the development of complex traits, such as nervous and immune systems, from environmental perturbations may be central to the evolution of longevity (Ricklefs and Wikelski 2002).

The correlation of [IgG] within families was an unprecedented outcome of the present study, because it has not been previously observed (Apanius and Nisbet 2006). Parents showed positively correlated [IgG], not only with each other, but also with their offspring. The shared environment of the nest site provides a possible explanation of this family-specific [IgG]. This would be curious, because the sharing of environment decreased considerably across the nestling period, with parents increasingly absent (Fig. 5A, E; Anderson et al. 2004) and fecal contamination of the nest site by family members and neighbors increasing considerably across the nestling period (Fig. 8). Parents should diverge from each other—and, especially, both should diverge from the nest-bound chick—at later chick ages. Alternatively, the within-family correlation could be produced by assortative mating based on [IgG] (or a correlated trait) and by high heritability of [IgG]. Our observation of a high autocorrelation for parental [IgG] and its repeatability in individual adults across years (V. Apanius et al. pers. obs.) suggest that intrinsic factors govern the homeostatic set-point for [IgG]. Further investigation is needed to determine whether the similarity in [IgG] within families is a prominent feature of long-lived colonial seabirds and how this similarity arises.

## ACKNOWLEDGMENTS

We thank the Galápagos National Park Service for permission to work in the park, the Charles Darwin Research Station, and TAME Airline for logistical support; K. Fischer, S. Freeman, C. Zambrano, and E. Porter for field assistance; A. D'Epagnier for laboratory assistance; T. Maness for laboratory mentoring; F. Estela, S. Cruz, and C. Proaño for abstract translation; and the members of the Anderson lab group and anonymous reviewers for comments on an earlier draft. This material is based on work supported by the National Science Foundation under grants DEB 93045679, DEB 9629539, DEB 98 06606, and DEB 0235818 to D.J.A and by a Sigma Xi grant-in-aid of research to M.A.W. and Wake Forest University. This work was approved by the Institutional Use and Care of Animals Committee and conducted with permits from the Galápagos National Park Service, and specimens were transported into the United States with permits from U.S. Department of Agriculture-Animal and Plant Health Inspection Service.

## LITERATURE CITED

ALLISON, P. 1995. Survival Analysis Using the SAS System: A Practical Guide. SAS Institute, Cary, North Carolina.

AMERICAN ORNITHOLOGISTS' UNION. 2000. Forty-second supplement to the American Ornithologists' Union *Check-list of North American Birds*. Auk 117:847–858.

ANDERSON, D. J. 1989a. Differential responses of boobies and other seabirds in the Galápagos to the 1987 El Niño–Southern Oscillation event. Marine Ecology Progress Series 52:209–216.

ANDERSON, D. J. 1989b. The role of hatching asynchrony in siblicidal brood reduction of two booby species. Behavioral Ecology and Sociobiology 25:363–368.

ANDERSON, D. J. 1990. Evolution of obligate siblicide in boobies. 2: Food limitation and parent–offspring conflict. Evolution 44:2069–2082.

ANDERSON, D. J. 1991. Apparent predator-limited distribution of Galápagos Red-footed Boobies. Ibis 33:26–29.

ANDERSON, D. J. 1993. Masked Booby (*Sula dactylatra*). *In* The Birds of North America, no. 73 (A. Poole and F. Gill, Eds.). Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, D.C.

ANDERSON, D. J., AND V. APANIUS. 2003. Actuarial and reproductive senescence in a long-lived seabird: Preliminary evidence. Experimental Gerontology 38:757–760

ANDERSON, D. J., AND P. T. BOAG. 2006. No extra-pair fertilization observed in Nazca Booby (*Sula granti*) broods. Wilson Journal of Ornithology 118:244–247.

ANDERSON, D. J., AND P. J. HODUM. 1993. Predator behavior favors clumped nesting in an oceanic seabird. Ecology 74:2462–2464.

ANDERSON, D. J., E. T. PORTER, AND E. D. FERREE. 2004. Non-breeding Nazca Boobies (*Sula granti*) show social and sexual interest in chicks: Behavioural and ecological aspects. Behaviour 141:959–978.

ANDERSON, D. J., J. REEVE, J. E. MARTINEZ GOMEZ, W. W. WEATHERS, S. HUTSON, H. V. CUNNINGHAM, AND D. M. BIRD. 1993. Sexual size dimorphism and food requirements of nestling birds. Canadian Journal of Zoology 71:2541–2545.

ANDERSON, D. J., AND R. E. RICKLEFS. 1987. Radio-tracking Masked and Blue-footed boobies in the Galápagos Islands. National Geographic Research 3:152–163.

ANDERSON, D. J., AND R. E. RICKLEFS. 1992. Brood size and provisioning in Masked and Blue-footed boobies (*Sula* spp.). Ecology 73:1363–1374.

APANIUS, V. 1998a. Ontogeny of immune function. Pages 203–222 *in* Avian Growth and Development: Evolution within the Altricial–Precocial Spectrum (J. M. Starck and R. E. Ricklefs, Eds.). Oxford University Press, Oxford, United Kingdom.

APANIUS, V. 1998b. Stress and immune response. Pages 133–154 *in* Stress and Behavior (P. J. B. Slater, J. S. Rosenblatt, C. T. Snowden, and M. Milinski, Eds.). Academic Press, San Diego, California.

APANIUS, V., AND I. C. T. NISBET. 2003. Serum immunoglobulin G levels in very old Common Terns *Sterna hirundo*. Experimental Gerontolology 38:761–764.

APANIUS, V., AND I. C. T. NISBET. 2006. Serum immunoglobulin G levels are positively related to reproductive performance in a long-lived seabird, the Common Tern (*Sterna hirundo*). Oecologia 147:12–23.

APANIUS, V., S. A. TEMPLE, AND M. BALE. 1983. Serum proteins of wild Turkey Vultures (*Cathartes aura*). Comparative Biochemistry and Physiology B 76:907–913.

ARDIA, D. R., K. A. SCHAT, AND D. W. WINKLER. 2003. Reproductive effort reduces long-term immune function in breeding Tree Swallows (*Tachycineta bicolor*). Proceedings of the Royal Society of London, Series B 270:1679–1683.

ASHMOLE, N. P. 1963. The regulation of numbers of tropical oceanic birds. Ibis 103:458–473.

ASHMOLE, N. P. 1971. Seabird ecology and the marine environment. Pages 223–286 *in* Avian Biology, vol. 1 (D. S. Farner and J. R. King, Eds.). Academic Press, New York.

BARNARD, C. J., J. M. BEHNKE, AND J. SEWELL. 1994. Social behaviour and susceptibility to infection in House Mice (*Mus musculus*): Effects of group size, aggressive behaviour and status-related hormonal responses prior to infection on resistance to *Babesia microti*. Parasitology 108:487–496.

BOERSMA, P. D., AND J. K. PARRISH. 1998. Flexible growth rates in Fork-tailed Storm-Petrels: A response to environmental variability. Auk 115:67–75.

BOOTS, M., AND R. G. BOWERS. 2004. The evolution of resistance through costly acquired immunity. Proceedings of the Royal Society of London, Series B 271:715–723.

BOURGEON, S., F. CRISCUOLO, Y. LE MAHO, AND T. RACLOT. 2006. Phytohemagglutinin response and immunoglobulin index decrease during incubation fasting in female Common Eiders. Physiological and Biochemical Zoology 79:793–800.

BURGER, A. E., AND J. F. PIATT. 1990. Flexible time budgets in breeding Common Murres: Buffers against variable prey abundance. Pages 71–83 *in* Auks at Sea (S. G. Sealy, Ed.). Studies in Avian Biology, no. 14.

CAM, E., W. A. LINK, E. G. COOCH, J.-Y. MONNAT, AND E. DANCHIN. 2002. Individual covariation in life-history traits: Seeing the trees despite the forest. American Naturalist 159:96–105.

CHARLESWORTH, B. 1994. Evolution in Age-structured Populations. Cambridge University Press, Cambridge, United Kingdom.

CHRISTE, P., F. DE LOPE, G. GONZÁLEZ, N. SAINO, AND A. P. MØLLER. 2001. The influence of environmental conditions on immune responses, morphology and recapture probability of nestling House Martins (*Delichon urbica*). Oecologia 126:333–338.

CHRISTE, P., A. P. MØLLER, AND F. DE LOPE. 1998. Immunocompetence and nestling survival in the House Martin: The tasty chick hypothesis. Oikos 83:175–179.

CICHÓN, M. 2000. Costs of incubation and immunocompetence in the Collared Flycatcher. Oecologia 125:453–457.

CICHÓN, M., A. DUBIEC, AND M. CHADZI~SKA. 2001. The effect of elevated reproductive effort on humoral immune function in Collared Flycatcher females. Acta Oecologica 22:71–76.

CLIFFORD, L. D., AND D. J. ANDERSON. 2001. Experimental demonstration of the insurance value of extra eggs in an obligately siblicidal seabird. Behavioral Ecology 12:340–347.

COOK, T. R., Y. CHEREL, AND Y. TREMBLAY. 2006. Foraging tactics of chick-rearing Crozet Shags: Individuals display repetitive activity and diving patterns over time. Polar Biology 29:562–569.

CURRY, R. L., AND D. J. ANDERSON. 1987. Interisland variation in blood drinking by Galápagos Mockingbirds. Auk 104:517–521.

DAAN, S., C. DEERENBERG, AND C. DIJKSTRA. 1996. Increased daily work precipitates natural death in the kestrel. Journal of Animal Ecology 65:539–544.

DEERENBERG, C., V. APANIUS, S. DAAN, AND N. BOS. 1997. Reproductive effort decreases antibody responsiveness. Proceedings of the Royal Society of London, Series B 264:1021–1029.

DEMAS, G. E. 2004. The energetics of immunity: A neuroendocrine link between energy balance and immune function. Hormones and Behavior 45:173–180.

DEVRIES, A. C., J. M. GERBER, H. N. RICHARDSON, C. A. MOFFATT, G. E. DEMAS, S. E. TAYMANS, AND R. J. NELSON. 1997. Stress affects corticosteroid and immunoglobulin concentrations in male House Mice (*Mus musculus*) and Prairie Voles (*Microtus ochrogaster*). Comparative Biochemical Physiology A 118:655–663.

DURIEZ, O., H. WEIMERSKIRCH, AND H. FRITZ. 2000. Regulation of chick provisioning in the Thin-billed Prion: An interannual comparison and manipulation of parents. Canadian Journal of Zoology 78:1275–1283.

ELLIS, H. I., AND G. GABRIELSEN. 2002. Energetics of free-ranging seabirds. Pages 359–408 *in* Biology of Marine Birds (E. A. Schreiber and J. Burger, Eds.). CRC Press, Boca Raton, Florida.

ERIKSTAD, K. E., P. FAUCHALD, T. TVERAA, AND H. STEEN. 1998. On the cost of reproduction in long-lived birds: The influence of environmental variability. Ecology 79:1781–1788.

ESPARZA, B., A. MARTÍNEZ-ABRAÍN, S. MERINO, AND D. ORO. 2004. Immunocompetence and the prevalence of haematozoan parasites in two long-lived seabirds. Ornis Fennica 81:40–46.

FARGALLO, J. A., T. LAAKSONEN, V. POYRI, AND E. KORPIMÄKI. 2002. Inter-sexual differences in the immune response of Eurasian Kestrel nestlings under food shortage. Ecology Letters 5:95–101.

FITZMAURICE, G. M., N. M. LAIRD, AND J. H. WARE. 2004. Applied Longitudinal Analysis. Wiley, Hoboken, New Jersey.

FRIDOLFSSON, A. K., AND H. ELLEGREN. 1999. A simple and universal method for molecular sexing of non-ratite birds. Journal of Avian Biology 30:116–121.

FRIESEN, V. L., D. J. ANDERSON, T. E. STEEVES, H. JONES, AND E. A. SCHREIBER. 2002. Molecular support for species status of the Nazca Booby. Auk 119:820–826.

GAILLARD, J.-M., D. PONTIER, D. ALLAIN, J. D. LEBRETON, J. TROUVILLIEZ, AND J. CLOBERT. 1989. An analysis of demographic tactics in birds and mammals. Oikos 56:59–76.

GASPARINI, J., A. ROULIN, V. GILL, S. A. HATCH, AND T. BOULINIER. 2006. In kittiwakes food availability partially explains the seasonal decline in humoral immunity. Functional Ecology 20:457–463.

GOLET, G. H., D. B. IRONS, AND J. A. ESTES. 1998. Survival costs of chick rearing in Black-legged Kittiwakes. Journal of Animal Ecology 67:827–841.

GOLET, G. H., J. A. SCHMUTZ, D. B. IRONS, AND J. A. ESTES. 2004. Determinants of reproductive costs in the long-lived Black-legged Kittiwake: A multiyear experiment. Ecological Monographs 74:353–372.

GOODMAN, D. 1974. Natural selection and a cost ceiling on reproductive effort. American Naturalist 108:247–268.

GRAY, C. M., M. D. L. BROOKE, AND K. C. HAMER. 2005. Repeatability of chick growth and food provisioning in Manx Shearwaters *Puffinus puffinus*. Journal of Avian Biology 36:374–379.

GREEN, A. J. 2001. Mass/length residuals: Measures of body condition or generators of spurious results? Ecology 82:1473–1483.

GREIVES, T. J., J. W. MCGLOTHLIN, J. M. JAWOR, G. E. DEMAS, AND E. D. KETTERSON. 2006. Testosterone and innate immune function inversely covary in a wild population of breeding Dark-eyed Juncos (*Junco hyemalis*). Functional Ecology 20:812–818.

GUERRA, M., AND H. DRUMMOND. 1995. Reversed sexual size dimorphism and parental care: Minimal division of labour in the Blue-footed Booby. Behaviour 132:479–496.

HAMER, K. C., A. S. LYNNES, AND J. K. HILL. 1999. Parent-offspring interactions in food provisioning of Manx Shearwaters: Implications for nestling obesity. Animal Behaviour 57:627–631.

HAMER, K. C., R. A. PHILLIPS, J. K. HILL, S. WANLESS, AND A. G. WOOD. 2001. Contrasting foraging strategies of gannets *Morus bassanus* at two North Atlantic colonies: Foraging trip duration and foraging site fidelity. Marine Ecology Progress Series 224:283–290.

HANSON, L. 1979. Immunoglobulins. II. Immunological mechanisms. Pages 127–151 *in* Plasma Proteins (B. Blombäck and L. A. Hanson, Eds.). Wiley, New York.

HANSSEN, S. A., I. FOLSTAD, AND K. E. ERIKSTAD. 2003. Reduced immunocompetence and cost of reproduction in Common Eiders. Oecologia 136:457–464.

HANSSEN, S. A., D. HASSELQUIST, I. FOLSTAD, AND K. E. ERIKSTAD. 2004. Costs of immunity: Immune responsiveness reduces survival in a vertebrate. Proceedings of the Royal Society of London, Series B 271:925–930.

HANSSEN, S. A., D. HASSELQUIST, I. FOLSTAD, AND K. E. ERIKSTAD. 2005. Cost of reproduction in a long-lived bird: Incubation effort reduces immune function and future reproduction. Proceedings of the Royal Society of London, Series B 272:1039–1046.

HARRIS, E. K., B. K. COOIL, G. SHAKARJI, AND G. Z. WILLIAMS. 1980. On the use of statistical models of within-person variation in long-term studies of healthy individuals. Clinical Chemistry 26:383–391.

HASSELQUIST, D., M. F. WASSON, AND D. W. WINKLER. 2001. Humoral immunocompetence correlates with date of egg-laying and reflects work load in female Tree Swallows. Behavioral Ecology 12:93–97.

HOLLMÉN, T., J. C. FRANSON, M. HARIO, S. SANKARI, M. KILPI, AND K. LINDSTRÖM. 2001. Use of serum biochemistry to evaluate nutritional status and health of incubating Common Eiders (*Somateria mollissima*) in Finland. Physiological and Biochemical Zoology 74:333–342.

HÕRAK, P., S. JENNI-EIERMANN, I. OTS, AND L. TEGELMANN. 1998. Health and reproduction: The sex-specific clinical profile of Great Tits (*Parus major*) in relation to breeding. Canadian Journal of Zoology 76:2235–2244.

HÕRAK, P., L. TEGELMANN, I. OTS, AND A. P. MØLLER. 1999. Immune function and survival of Great Tit nestlings in relation to growth conditions. Oecologia 121:316–322.

HOUSTON, A. I., AND N. B. DAVIES. 1985. The evolution of cooperation in life history in the Dunnock, *Prunella modularis*. Pages 471–487 *in* Behavioural Ecology: Ecological Consequences of Adaptive Behaviour (R. M. Sibly and R. H. Smith, Eds.). Blackwell Scientific, Oxford, United Kingdom.

HUMPHRIES, C. A., V. D. AREVALO, K. N. FISCHER, AND D. J. ANDERSON. 2006. Contributions of marginal offspring to reproductive success of Nazca Booby (*Sula granti*) parents: Tests of multiple hypotheses. Oecologia 147:379–390.

HUYVAERT, K. P., AND D. J. ANDERSON. 2004. Limited dispersal by Nazca Boobies *Sula granti*. Journal of Avian Biology 35:46–53.

ILMONEN, P., T. TAARNA, AND D. HASSELQUIST. 2000. Experimentally activated immune defence in female Pied Flycatchers results in reduced breeding success. Proceedings of the Royal Society of London, Series B 267:665–670.

ILMONEN, P., T. TAARNA, AND D. HASSELQUIST. 2002. Are incubation costs in female Pied Flycatchers expressed in humoral immune responsiveness or breeding success? Oecologia 130:199–204.

JACOBSEN, K.-O., K. E. ERIKSTAD, AND B.-E. SÆTHER. 1995. An experimental study of the costs of reproduction in the kittiwake *Rissa tridactyla*. Ecology 76:1636–1642.

JONES, I. L. 1994. Mass changes of Least Auklets *Aethia pusilla* during the breeding season: Evidence for programmed loss of mass. Journal of Animal Ecology 63:71–78.

KALMBACH, E., R. G. NAGER, R. GRIFFITHS, AND R. W. FURNESS. 2004. Increased reproductive effort results in male-biased offspring sex ratio: An experimental study in a species with reversed sexual size dimorphism. Proceedings of the Royal Society of London, Series B 268:2175–2179.

KENNEDY, M. W., AND R. G. NAGER. 2006. The perils and prospects of using phytohaemagglutinin in evolutionary ecology. Trends in Ecology and Evolution 21:653–655.

KITAYSKY, A. S. 1999. Metabolic and developmental responses of alcid chicks to experimental variation in food intake. Physiological and Biochemical Zoology 72:462–473.

KLASING, K. C. 2004. The costs of immunity. Acta Zoologica Sinica 50:961–969.

LACK, D. 1968. Ecological Adaptations for Breeding in Birds. Methuen, London.

LEMKE, H., A. COUTINHO, AND H. LANGE. 2004. Lamarckian inheritance of somatically acquired maternal IgG phenotypes. Trends in Immunology 25:180–186.

LESSELLS, C. M., AND P. T. BOAG. 1987. Unrepeatable repeatabilities: A common mistake. Auk 104:116–121.

LEWIS, S., E. A. SCHREIBER, F. DAUNT, G. A. SCHENK, K. ORR, A. ADAMS, S. WANLESS, AND K. C. HAMER. 2005. Sex-specific foraging behaviour in tropical boobies: Does size matter? Ibis 147:408–414.

LEWIS, S., S. WANLESS, D. A. ELSTON, M. DUHR SCHULTZ, E. MACKLEY, M. DU TOIT, J. G. UNDERHILL, AND M. P. HARRIS. 2006. Determinants of quality in a long-lived colonial species. Journal of Animal Ecology 75:1304–1312.

LIFJELD, J. T., P. O. DUNN, AND L. A. WHITTINGHAM. 2002. Short-term fluctuations in cellular immunity of Tree Swallows feeding nestlings. Oecologia 130:185–190.

LITTELL, R. C., G. A. MILLIKEN, W. W. STROUP, AND R. D. WOLFINGER. 1996. SAS System for Mixed Models. SAS Institute, Cary, North Carolina.

LITTELL, R. C., J. PENDERGAST, AND R. NATARAJAN. 2000. Modelling covariance structure in the analysis of repeated measures data. Statistics in Medicine 19:1793–1819.

LONGMIRE, J. L., G. F. GEE, C. L. HARDEKOPF, AND G. A. MARK. 1992. Establishing paternity in Whooping Cranes (*Grus americana*) by DNA analysis. Auk 109:522–529.

LORENTSEN, S.-H. 1996. Regulation of food provisioning in the Antarctic Petrel *Thalassoica antarctica*. Journal of Animal Ecology 65:381–388.

LORMEE, H., C. BARBRAUD, AND O. CHASTEL. 2005. Reversed sexual size dimorphism and parental care in the Red-footed Booby *Sula sula*. Ibis 147:307–315.

MANESS, T. J., AND D. J. ANDERSON. 2007. Serial monogamy and sex ratio bias in Nazca Boobies. Proceedings of the Royal Society of London Series B 274:2047–2054.

MANESS, T. J., M. A. WESTBROCK, AND D. J. ANDERSON. 2007. Ontogenic sex ratio variation in Nazca Boobies ends in male-biased adult sex ratio. Waterbirds 30:10–16.

MARTIN, L. B., II, P. HAN, J. LEWITTES, J. R. KUHLMAN, K. C. KLASING, AND M. WIKELSKI. 2006. Phytohemagglutinin-induced skin swelling in birds: Histological support for a classic immunoecological technique. Functional Ecology 20:290–299.

MARTIN, T. E., A. P. MØLLER, S. MERINO, AND J. CLOBERT. 2001. Does clutch size evolve in response to parasites and immunocompetence? Proceedings of the National Academy of Sciences USA 98:2071–2076.

MAUCK, R. A., AND T. C. GRUBB, JR. 1995. Petrel parents shunt all experimentally increased reproductive costs to their offspring. Animal Behaviour 49:999–1008.

MCFARLANE, H. 1973. Immunoglobulins in populations of subtropical and tropical countries. Advances in Clinical Chemistry 16:153–238.

MCNAMARA J. M., C. E. GASSON, AND A. I. HOUSTON, 1999. Incorporating rules for responding into evolutionary games. Nature 401:368–371.

MEDAWAR, P. B. 1952. An Unsolved Problem of Biology. H.K. Lewis, London.

MERILÄ, J., AND C. HEMBORG. 2000. Fitness and feather wear in the Collared Flycatcher *Ficedula albicollis*. Journal of Avian Biology 31:504–510.

MERINO, S., J. MARTÍNEZ, A. P. MØLLER, L. SANABRIA, F. DE LOPE, J. PÉREZ, AND F. RODRÍGUEZ-CAABEIRO. 1999. Phytohaemagglutinin injection assay and physiological stress in nestling House Martins. Animal Behaviour 58:219–222.

MERINO, S., J. MORENO, G. TOMÁS, J. MARTÍNEZ, J. MORALES, J. MARTÍNEZ-DE LA PUENTE, AND J. L. OSORNO. 2006. Effects of parental effort on blood stress protein HSP60 and immunoglobulins in female Blue Tits: A brood size manipulation experiment. Journal of Animal Ecology 75:1147–1153.

MORENO, J. 2003. Lifetime reproductive success in seabirds: Interindividual differences and implications for conservation. Scientia Marina 67 (Supplement 2):7–12.

MORENO, J., J. J. SANZ, S. MERINO, AND E. ARRIERO. 2001. Daily energy expenditure and cell-mediated immunity in Pied Flycatchers while feeding nestlings: Interaction with moult. Oecologia 129:492–497.

NAVARRO, J., AND J. GONZÁLEZ-SOLÍS. 2007. Experimental increase of flying costs in a pelagic seabird: Effects on foraging strategies, nutritional state and chick condition. Oecologia 151:150–160.

NELSON, J. B. 1978. The Sulidae. Oxford University Press, Oxford, United Kingdom.

NISBET, I. C. T. 2002. Common Tern (*Sterna hirundo*). *In* The Birds of North America, no. 618 (A. Poole and F. Gill, Eds.). Birds of North America, Philadelphia.

NOLAN, V., JR., E. D. KETTERSON, D. A. CRISTOL, C. M. ROGERS, E. D. CLOTFELTER, R. TITUS, S. J. SCHOECH, AND E. SNAJDR. 2002. Dark-eyed Junco (*Junco hyemalis*). *In* The Birds of North America, no. 716 (A. Poole and F. Gill, Eds.). Birds of North America, Philadelphia.

NORDLING, D., M. ANDERSSON, S. ZOHARI, AND L. GUSTAFSSON. 1998. Reproductive effort reduces specific immune response and parasite resistance. Proceedings of the Royal Society of London, Series B 265:1291–1298.

NORRIS, K., AND M. R. EVANS. 2000. Ecological immunology: Life history tradeoffs and immune defense in birds. Behavioral Ecology 11:19–26.

OTS, I., AND P. HÕRAK. 1996. Great Tits *Parus major* trade health for reproduction. Proceedings of the Royal Society of London, Series B 263:1443–1447.

OWENS, I. P. F., AND K. WILSON. 1999. Immunocompetence: A neglected life history trait or conspicuous red herring? Trends in Ecology and Evolution 14:170–172.

PAP, P. L., AND R. MARKUS. 2003. Cost of reproduction, T-lymphocyte mediated immunocompetence and health status in female and nestling Barn Swallows *Hirundo rustica*. Journal of Avian Biology 34:428–434.

PENNYCUICK, C. J. 1989. Bird Flight Performance. Oxford University Press, Oxford, United Kingdom.

PITMAN, R. L., AND J. R. JEHL, JR. 1998. Geographic variation and reassessment of species limits in the "masked" boobies of the eastern Pacific Ocean. Wilson Bulletin 110:155–170.

PYLE, P., N. NUR, W. J. SYDEMAN, AND S. D. EMSLIE. 1997. Cost of reproduction and the evolution of deferred breeding in the Western Gull. Behavioral Ecology 8:140–147.

QUERALTÓ, J. 2004. Intraindividual reference values. Clinical Chemistry and Laboratory Medicine 42:765–777.

RÅBERG, L., M. GRAHN, D. HASSELQUIST, AND E. SVENSSON. 1998. On the adaptive significance of stress-induced immunosuppression. Proceedings of the Royal Society of London, Series B 265:1637–1641.

RÅBERG, L., J. A. NILSSON, P. ILMONEN, M. STJERNMAN, AND D. HASSELQUIST. 2000. The cost of an immune response: Vaccination reduces parental effort. Ecology Letters 3:382–386.

RANDS, S. A., I. C. CUTHILL, AND A. I. HOUSTON. 2006. Explaining individual variation in patterns of mass loss in breeding birds. Theoretical Biology and Medical Modelling 3:1–24.

REES, M. J., AND A. W. NORDSKOG. 1981. Genetic control of serum immunoglobulin G levels in the chicken. Journal of Immunogenetics 8:425–431.

REID, W. V. 1987. The cost of reproduction in the Glaucous-winged Gull. Oecologia 74:458–467.

RICKLEFS, R. E. 1982. Some considerations on sibling competition and avian growth rates. Auk 99:141–147.

RICKLEFS, R. E. 1984. The optimization of growth rate in altricial birds. Ecology 65:1602–1616.

RICKLEFS, R. E. 1987. Response of adult Leach's Storm-Petrels to increased food demand at the nest. Auk 104:750–756.

RICKLEFS, R. E. 1992. The roles of parent and chick in determining feeding rates in Leach's Storm-Petrel. Animal Behaviour 43:895–906.

RICKLEFS, R. E., AND M. WIKELSKI. 2002. The physiology/life-history nexus. Trends in Ecology and Evolution 17:462–468.

RITZ, M. S. 2007. Sex-specific mass loss in chick-rearing South Polar Skuas *Stercorarius maccormicki*—Stress induced or adaptive? Ibis 149:156–165.

ROBERTSON, R., B. STUTCHBURY, AND R. COHEN. 1992. Tree Swallow (*Tachycineta bicolor*).*In* The Birds of North America, no. 11 (A. Poole, P. Stettenheim, and F. Gill, Eds.). Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, D.C.

ROBINSON, J., K. C. HAMER, AND L. CHIVERS. 2002. Developmental plasticity in Arctic Terns *Sterna paradisaea* and Common Terns *S. hirundo* in response to a period of extremely bad weather. Ibis 144:344–346.

SÆTHER, B. E., R. ANDERSEN, AND H. C. PEDERSEN. 1993. Regulation of parental effort in a long-lived seabird—An experimental manipulation of the cost of reproduction in the Antarctic Petrel, *Thalassoica antarctica*. Behavioral Ecology and Sociobiology 33:147–150.

SAINO, N., A. M. BOLZERN, AND A. P. MØLLER. 1997a. Immunocompetence, ornamentation, and viability of male Barn Swallows (*Hirundo rustica*). Proceedings of the National Academy of Sciences USA 94:549–552.

SAINO, N., S. CALZA, AND A. P. MØLLER. 1997b. Immunocompetence of nestling Barn Swallows in relation to brood size and parental effort. Journal of Animal Ecology 66:827–836.

SAINO, N., M. INCAGLI, R. MARTINELLI, R. AMBROSINI, AND A. P. MØLLER. 2001. Immunity, growth and begging behaviour of nestling Barn Swallows *Hirundo rustica* in relation to hatching order. Journal of Avian Biology 32:263–270.

SAINO, N., M. INCAGLI, R. MARTINELLI, AND A. P. MØLLER. 2002. Immune response of male Barn Swallows in relation to parental effort, corticosterone plasma levels, and sexual ornamentation. Behavioral Ecology 13:169–174.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

SANZ, J. J. 2001. Latitudinal variation in female local return rate in the philopatric Pied Flycatcher (*Ficedula hypoleuca*). Auk 118:539–543.

SARKER, N., M. TSUDZUKI, M. NISHIBORI, AND Y. YAMAMOTO. 1999. Direct and correlated response to divergent selection for serum immunoglobulin M and G levels in chickens. Poultry Science 78:1–7.

SARKER, N., M. TSUDZUKI, M. NISHIBORI, H. YASUE, AND Y. YAMAMOTO. 2000. Cell-mediated and humoral immunity and phagocyctic ability in chicken lines divergently selected for serum immunoglobulin M and G levels. Poultry Science 79:1705–1709.

SHELDON, B. C., AND S. VERHULST. 1996. Ecological immunology, costly parasite defences and tradeoffs in evolutionary ecology. Trends in Ecology and Evolution 11:317–321.

SHUTLER, D., A. MULLIE, AND R. G. CLARK. 2004. Tree Swallow reproductive investment, stress, and parasites. Canadian Journal of Zoology 82:442–448.

SNOEIJS, T., R. PINXTEN, AND M. EENS. 2005. Experimental removal of the male parent negatively affects growth and immunocompetence in nestling Great Tits. Oecologia 145:165–173.

SOLER, J. J., L. DE NEVE, T. PÉREZ-CONTRERAS, M. SOLER, AND G. SORCI. 2002. Tradeoff between immunocompetence and growth in Magpies: An experimental study. Proceedings of the Royal Society of London, Series B 270:241–248.

STASZEWSKI, V., AND T. BOULINIER. 2004. Vaccination: A way to address questions in behavioral and population ecology? Trends in Parasitology 20:17–22.

SZÉP, T., AND A. P. MØLLER. 1999. Cost of parasitism and host immune defence in the Sand Martin *Riparia riparia*: A role for parent–offspring conflict? Oecologia 119:9–15.

TELLA, J. L., G. R. BORTOLOTTI, R. D. DAWSON, AND M. G. FORERO. 2000. The T-cell-mediated immune response and return rate of fledgling American Kestrels are positively correlated with parental clutch size. Proceedings of the Royal Society of London, Series B 266:891–895.

TERSHY, B. R., AND D. A. CROLL. 2000. Parental investment, adult sex ratios, and sexual selection in a socially monogamous seabird. Behavioral Ecology and Sociobiology 48:52–60.

TOWNSEND, H. M. 2004. Evolutionary ecology of reproduction in Nazca Boobies (*Sula granti*). Ph.D. dissertation, Wake Forest University, Winston-Salem, North Carolina.

TOWNSEND, H. M., AND D. J. ANDERSON. 2007a. Assessment of costs of reproduction in a pelagic seabird using multi-state mark–recapture models. Evolution 61:1956–1968.

TOWNSEND, H. M., AND D. J. ANDERSON. 2007b. Production of insurance eggs in Nazca Boobies: Costs, benefits, and variable parental quality. Behavioral Ecology 18:841–848.

TOWNSEND, H. M., K. P. HUYVAERT, P. J. HODUM, AND D. J. ANDERSON. 2002. Nesting distributions of Galápagos Boobies (Aves: Sulidae): An apparent case of amensalism. Oecologia 132:419–427.

TOWNSEND, H. M., T. J. MANESS, AND D. J. ANDERSON. 2007. Offspring growth and parental care in sexually dimorphic Nazca Boobies (*Sula granti*). Canadian Journal of Zoology 85:686–694.

VERBEKE, G., AND G. MOLENBERGHS. 2000. Linear Mixed Models for Longitudinal Data. Springer, New York.

VINEY, M., E. M. RILEY, AND K. L. BUCHANON. 2005. Optimal immune responses: Immunocompetence revisited. Trends in Ecology and Evolution 20:665–669.

VISSER, G. H. 2002. Chick growth and development in seabirds. Pages 439–466 *in* Biology of Marine Birds (E. A. Schreiber and J. Burger, Eds.). CRC Press, Boca Raton, Florida.

WALDMANN, T. A., R. M. BLAESE, AND W. STROBER. 1970. Physiological factors controlling immunoglobulin metabolism. Pages 269–286 *in* Plasma Protein Metabolism: Regulation of Synthesis, Distribution, and Degradation (M. A. Rothschild and T. A. Waldmann, Eds.). Academic Press, New York.

WARR, G., K. MAGOR, AND D. HIGGINS. 1995. IgY: Clues to the origins of modern antibodies. Immunology Today 16:392–398.

WEIMERSKIRCH, H. 2002. Seabird demography and its relationship with the marine environment. Pages 115–135 *in* Biology of Marine Birds (E. A. Schreiber and J. Burger, Eds.). CRC Press, Boca Raton, Florida.

WEIMERSKIRCH, H., O. CHASTEL, AND L. ACKERMANN. 1995. Adjustment of parental effort to manipulated foraging ability in a pelagic seabird, the Thin-billed Prion *Pachyptila belcheri*. Behavioral Ecology and Sociobiology 36:11–16.

WEIMERSKIRCH, H., G. FRADET, AND Y. CHEREL. 1999. Natural and experimental changes in chick provisioning in a long-lived seabird, the Antarctic Prion. Journal of Avian Biology 30:165–174.

WEIMERSKIRCH, H., M. LE CORRE, Y. ROPERT-COUDERT, A. KATO, AND F. MARSAC. 2006. Sex-specific foraging behaviour in a seabird with reversed sexual dimorphism: The Red-footed Booby. Oecologia 146:681–691.

WEIMERSKIRCH, H., P. A. PRINCE, AND L. ZIMMERMANN. 2000. Chick provisioning by the Yellow-nosed Albatross *Diomedea chlororhynchos*: Response of foraging effort to experimentally increased costs and demands. Ibis 142:103–110.

WENDELN, H., AND P. H. BECKER. 1999. Effects of parental quality and effort on the reproduction of Common Terns. Journal of Animal Ecology 68:205–214.

WERSCHKUL, D. F., AND J. A. JACKSON. 1979. Sibling competition and avian growth rates. Ibis 121:97–102.

WESTBROCK, M. A. 2005. Offspring sex ratio variation in two seabird species. M.S. thesis, Wake Forest University, Winston-Salem, North Carolina.

WESTFALL, P., R. TOBIAS, D. ROM, R. D. WOLFINGER, AND Y. HOCHBERG. 1999. Multiple Comparisons and Multiple Tests Using the SAS System. SAS Institute, Cary, North Carolina.

WESTNEAT, D. F., J. WEISKITTLE, R. EDENFIELD, T. B. KINNARD, AND J. P. POSTON. 2004. Correlates of cell-mediated immunity in nestling House Sparrows. Oecologia 141:17–23.

WIERSMA, P., C. SELMAN, J. R. SPEAKMAN, AND S. VERHULST. 2004. Birds sacrifice oxidative protection for reproduction. Proceedings of the Royal Society of London, Series B (Supplement) 271:S360–S363.

WILLIAMS, T. D., J. K. CHRISTIANS, J. J. AIKEN, AND M. EVANSON. 1999. Enhanced immune function does not depress reproductive output. Proceedings of the Royal Society of London, Series B 266:753–757.

YOCCOZ, N. G., K. E. ERIKSTAD, J. O. BUSTNES, S. A. HANSSEN, AND T. TVERAA. 2002. Costs of reproduction in Common Eiders (*Somateria mollissima*): An assessment of relationships between reproductive effort and future survival and reproduction based on observational and experimental studies. Journal of Applied Statistics 29:57–64.

ZUK, M., AND A. M. STOEHR. 2002. Immune defense and host life history. American Naturalist 160 (Supplement):S9–S22.

## APPENDIX 1: DETAILS OF STATISTICAL ANALYSIS

### LINEAR MIXED MODELS

The measurements of the $i$th individual ($\mathbf{y}_i$) are modeled by the combination of fixed effects ($\boldsymbol{\beta}\mathbf{X}_i$) affecting all individuals, individual-specific effects ($\mathbf{Zb}_i$), and an error term ($\boldsymbol{\epsilon}_i$) that allows serial correlation of measurements from the same individual. Thus, $\mathbf{y}_i = \boldsymbol{\beta}\mathbf{X}_i + \mathbf{Zb}_i + \boldsymbol{\epsilon}_i$, where $\boldsymbol{\beta}$ is the vector of fixed effects parameters, $\mathbf{X}_i$ is the design matrix of the fixed effects, $\mathbf{Z}_i$ is the design matrix for random effects, $\mathbf{b}_i$ is the vector of random effects, and $\boldsymbol{\epsilon}_i$ is the vector of random errors of the $i$th individual. The between-individual variation is assumed to be normally distributed and independent, hence $\mathbf{b}_i \sim N(0,\mathbf{G})$. The within-individual variation includes normally distributed autocorrelated errors, hence $\boldsymbol{\epsilon}_i \sim N(0,\mathbf{R}_i)$. Assuming the independence and normality of $\mathbf{b}_i$ and $\boldsymbol{\epsilon}_i$, then the repeated measurements ($\mathbf{y}_i$) are independent and normally distributed with a mean of $\boldsymbol{\beta}\mathbf{X}_i$, and covariance of $\mathbf{R}_i + \mathbf{Z}_i\mathbf{G}\mathbf{Z}_i'$ (Verbeke and Molenberghs 2000).

Linear mixed models were fitted using the iterative approach (Verbeke and Molenberghs 2000, Fitzmaurice et al. 2004). First, the mean structure ($\boldsymbol{\beta}\mathbf{X}_i$) was modeled with a saturated fixed-effects model, including offspring age-class, offspring sex, parent sex, nestling period, and all interaction terms. Then, the residual variance was partitioned into (1) the between-individual component ($\mathbf{G}$), (2) the within-individual autocorrelation component ($\mathbf{R}_i$), and possibly (3) a time-dependent component that represents temporal heterogeneity. We tested the fit of five specific covariance structures that have a plausible biological interpretation.

### COVARIANCE STRUCTURES

The simple or variance components (VC) covariance structure has only a single

(error) variance parameter, which indicates that the between-individual ($var_b$) variance and autocorrelation are both effectively zero (Littell et al. 2000). The VC structure implies that a trait is regulated within the species-specific range but not around individual-specific set-points (Queraltó 2004).

The compound symmetric (CS) covariance structure estimates the between-individual variance ($var_b$) in comparison with the sum of the residual variance (Littell et al. 2000), $\rho = var_b/(var_b + var_e)$ is the intraclass correlation coefficient, and $var_e$ is the error variance. For the CS covariance structure, $\rho$ is the repeatability of a trait (Lessells and Boag 1987) and implies the presence of individual-specific (homeostatic) set-points. However, the correlation between measurements at two time-points is constant regardless of the length of the time between measurements and repeated measurements within individuals appear to follow a random walk. This implies weak regulation of the trait or considerable measurement error (Harris et al. 1980).

The first-order autoregressive with random effects (AR1+RE) covariance structure has a matrix of random intercepts (effects) that account for the between-individual variation ($var_b$) and also estimates the serial correlation (autocorrelation) between measurements within individuals (Littell et al. 2000). The correlation between measurements decreases exponentially with time between measurements so that the correlation between measurements of the same individuals is $r = \rho^t$, where $t$ is the number of equally spaced time intervals between measurements. The magnitude of $\rho$ implies the degree of homeostatic regulation of that trait, with higher values implying more stringent control (Harris et al. 1980, Queraltó 2004).

The CS and AR1 + RE covariance structures can have additional covariance parameters that represent time-dependent heterogeneity. These CSH and ARH1 + RE covariance structures require estimation of additional covariance parameters that are age-specific (e.g., $cov_{10}$, $cov_{20}$, $cov_{30}$, etc. for covariance parameters at days 10, 20, 30, etc.). Variance and covariance parameters for these five covariance structures are shown in table 8.1 of Verbeke and Molenberghs (2000).

To test the goodness-of-fit of alternative covariance structures, we used Akaike's Information Criteria (AIC) and Schwartz's Bayesian Information Criterion (BIC). The BIC is penalized more strongly by inclusion of additional parameters than the AIC and, therefore, more strongly favors the most parsimonious model (Littell et al. 2000). The restricted log-likelihood ratio test was used to assess significance of competing models (Fitzmaurice et al. 2004). These were calculated from the difference in $-2\ln(\ell)$ scores of nested models, which follow a chi-square distribution with degrees of freedom equal to the difference in the number of model parameters, assuming asymptotic normality (Fitzmaurice et al. 2004). Because the test that a covariance parameter equals zero is at the boundary of the parameter space, the likelihood ratio test is conservative (Fitzmaurice et al. 2004). We present $-2\ln(\ell)$ scores and use conventional chi-square distributions here. A table of exact critical values of mixed chi-square distributions is available (Fitzmaurice et al. 2004, table C1). The models are presented in the tables in order of increasing complexity. Our use of the BIC favors the most parsimonious model, and the conventional chi-square value makes the significance tests conservative. Scaled residuals based on Cholesky decomposition were used for diagnostic residual analysis (Fitzmaurice et al. 2000), especially when evaluating the use of log transformations.

After fitting the covariance structure, we tested whether the covariance parameters of a particular structure differed by offspring sex, parent sex, or fledging age-class. The $-2\ln(\ell)$, AIC, and BIC of models with these additional covariance parameters are shown in the tables under the best-fit covariance structure, and the model fit was tested as above.

### Missing Data

Ten families hatched their egg(s) late enough to prevent measurement and blood collection after offspring day 70. This could bias our results if hatch date interacted with the variables of interest, because we lack some samples from these nestlings and their parents. Therefore, we analyzed data from all families between the initial measurement and day 70 for a hatch-date main effect and interaction in a balanced design. In another balanced design, we excluded the 10 late families and repeated the analysis. Concordant results of these analyses suggested that hatch-date interactions were uninformative. All other missing observations are presumed to be missing at random (Fitzmaurice et al. 2004) and should not bias our analyses.

## APPENDIX 2: TABLES OF STATISTICAL ANALYSES

TABLE 1. Linear-mixed-model analysis of $\log_{10}$-transformed offspring body mass between days 0 and 120 as a function of offspring age, sex, and nestling period. Fixed effects were tested using the best-fit covariance structure (bold). Covariance structures are defined and discussed in Appendix 1. Goodness-of-fit of alternative covariance structures was based on the smallest absolute value of Akaike's Information Criterion (AIC) and Schwartz's Bayesian Information Criterion (BIC). For the best-fit covariance structure, we tested the fit of models with separate covariance parameter estimates for offspring sex (O) or nestling period (N).

| Fixed effects | df | $F$ | $P$ |
|---|---|---|---|
| Age-class (A) | 12 and 404 | 3, 211.10 | <0.0001 |
| Offspring sex (O) | 1 and 35 | 75.11 | <0.0001 |
| A * O | 12 and 404 | 1.62 | 0.082 |
| Nestling period (N) | 1 and 35 | 29.25 | <0.0001 |
| A * N | 12 and 404 | 0.52 | 0.91 |
| O * N | 1 and 35 | 3.19 | 0.083 |
| A * O * N | 12 and 404 | 0.81 | 0.64 |

| Covariance structure | Number of parameters | $-2\ln(\ell)$ | AIC | BIC |
|---|---|---|---|---|
| **VC** | **1** | **–1,252.8** | **–1,250.8** | **–1,249.2** |
| O | 2 | –1,258.6 | –1,254.6 | –1,251.3 |
| N | 2 | –1,268.8 | –1,264.8 | –1,261.5 |
| CS | 2 | –1,294.4 | –1,290.4 | –1,287.2 |
| AR1 + RE | 3 | –1,343.9 | –1,337.9 | –1,333.0 |
| CSH | 14 | –1,390.9 | –1,362.9 | –1,340.0 |
| ARH1 + RE | 15 | –1,433.8 | –1,403.8 | –1,379.3 |

TABLE 2. Linear-mixed-model analysis of offspring culmen length between days 0 and 120 as a function of offspring age, sex, and nestling period. Fixed effects were tested using the best-fit covariance structure (bold). Covariance structures are defined and discussed in Appendix 1. Goodness-of-fit of alternative covariance structures was based on the smallest absolute value of Akaike's Information Criterion (AIC) and Schwartz's Bayesian Information Criterion (BIC). For the best-fit covariance structure, we tested the fit of models with separate parameter estimates for offspring sex (O) or nestling period (N). Competing models, those with the absolute value $-2\log(\ell)$ scores closer to zero but with more parameters, were tested using the likelihood ratio test as indicated by superscript letters. Notation: $var_b$ = between-individual variance; $var_{10}$ = within-individual variance on day 10, $var_{20}$ = within-individual variance on day 20, $var_{30}$ = within-individual variance on day 30, etc.; and $\rho$ = autocorrelation coefficient.

| Fixed effects | df | $F$ | $P$ |
|---|---|---|---|
| Age-class (A) | 12 and 404 | 17, 016.91 | <0.0001 |
| Offspring sex (O) | 1 and 35 | 15.11 | 0.0004 |
| A * O | 12 and 404 | 9.46 | <0.0001 |
| Nestling period (N) | 1 and 35 | 1.21 | 0.28 |
| A * N | 12 and 404 | 1.43 | 0.15 |
| O * N | 1 and 35 | 0.68 | 0.42 |
| A * O * N | 12 and 404 | 0.53 | 0.90 |

| Covariance structure | Number of parameters | $-2\ln(\ell)$ | AIC | BIC |
|---|---|---|---|---|
| VC | 1 | 2,068.8 | 2,070.8 | 2,072.4 |
| CS | 2 | 1,863.7 | 1,867.7 | 1,871.0 |
| AR1 + RE | 3 | 1,525.4 | 1,529.2 | 1,532.7 |
| CSH | 14 | 1,691.8 | 1,719.8 | 1,742.7 |
| **ARH1 + RE** | **15** | **1,360.6**[a,b] | **1,355.7** | **1,411.5** |
| O | 30 | 1,332.8[a] | 1,359.0 | 1,434.7 |
| N | 30 | 1,352.1[b] | 1,375.1 | 1,453.3 |

[a] $G^2 = 27.8$, df = 15, $P = 0.022$.
[b] $G^2 = 8.5$, df = 15, $P = 0.90$.

| | Daughter | | Son | |
|---|---|---|---|---|
| Parameter | Estimate | 95% CI | Estimate | 95% CI |
| $Var_b$ | 0.48 | 0.27–1.09 | 0.22 | 0.07–0.36 |
| $Var_0$ | 0.10 | 0.02–17.56 | 0.01 | 0.00–0.09 |
| $Var_{10}$ | 4.31 | 2.50–9.17 | 8.05 | 4.59–17.61 |
| $Var_{20}$ | 9.74 | 5.86–19.34 | 12.94 | 7.43–28.02 |
| $Var_{30}$ | 8.51 | 5.08–17.13 | 20.71 | 11.83–45.27 |
| $Var_{40}$ | 7.24 | 4.30–14.64 | 17.08 | 9.71–37.66 |
| $Var_{50}$ | 3.85 | 2.26–7.96 | 10.24 | 5.79–22.85 |
| $Var_{60}$ | 2.94 | 1.73–6.05 | 9.67 | 5.44–21.75 |
| $Var_{70}$ | 2.94 | 1.75–5.93 | 7.51 | 4.26–16.64 |
| $Var_{80}$ | 1.72 | 1.02–3.47 | 3.29 | 1.91–7.01 |
| $Var_{90}$ | 1.62 | 0.98–3.19 | 2.36 | 1.40–4.85 |
| $Var_{100}$ | 1.28 | 0.78–2.48 | 2.16 | 1.30–4.30 |
| $Var_{110}$ | 1.43 | 0.89–2.70 | 2.08 | 1.26–4.07 |
| $Var_{120}$ | 1.82 | 1.12–3.46 | 2.54 | 1.55–4.92 |
| $\rho$ | 0.902 | 0.853–0.951 | 0.925 | 0.883–0.971 |

TABLE 3. Linear-mixed-model analysis of offspring wing length between days 0 and 120 as a function of offspring age, sex, and nestling period. Fixed effects were tested using the best-fit covariance structure (bold). Covariance structures are defined and discussed in Appendix 1. Goodness-of-fit of alternative covariance structures was based on the smallest absolute value of Akaike's Information Criterion (AIC) and Schwartz's Bayesian Information Criterion (BIC). For the best-fit covariance structure, we tested the fit of models with separate parameter estimates for offspring sex (O) or nestling period (N). Competing models, those with the absolute value $-2\log(\ell)$ scores closer to zero but with more parameters, were tested using the likelihood ratio test as indicated by superscript letters. Notation: $var_b$ = between-individual variance; $var_{10}$ = within-individual variance on day 10; $var_{20}$ = within-individual variance on day 20; $var_{30}$ = within-individual variance on day 30, etc.; and $\rho$ = autocorrelation coefficient.

| Fixed effects | df | $F$ | $P$ |
|---|---|---|---|
| Age-class (A) | 12 and 404 | 13, 425.21 | <0.0001 |
| Offspring sex (O) | 1 and 35 | 8.66 | 0.0057 |
| A * S | 12 and 404 | 14.04 | <0.0001 |
| Nestling period (N) | 1 and 35 | 8.42 | 0.0064 |
| A * N | 12 and 404 | 1.96 | 0.026 |
| O * N | 1 and 35 | 2.59 | 0.12 |
| A * O * N | 12 and 404 | 0.57 | 0.87 |

| Covariance structure | Number of parameters | $-2\ln(\ell)$ | AIC | BIC |
|---|---|---|---|---|
| VC | 1 | 3,389.6 | 3,391.6 | 3,393.3 |
| CS | 2 | 3,103.0 | 3,107.0 | 3,110.3 |
| AR1 + RE | 3 | 2,731.4 | 2,735.4 | 2,838.7 |
| CSH | 14 | 2,811.7 | 2,839.7 | 2,862.6 |
| **ARH1 + RE** | **15** | **2,492.0**[a] | **2,520.0** | **2,542.9** |
| O | 30 | 2,481.7 | 2,537.7 | 2,583.5 |
| N | 30 | 2,472.5[a] | 2,528.5 | 2,574.4 |

[a] $G^2 = 19.5$, df = 15, $P = 0.19$.

| Parameter | Estimate | 95% CI |
|---|---|---|
| $Var_b$ | 0.41 | 0.27–0.69 |
| $Var_0$ | 0.02 | 0.01–0.15 |
| $Var_{10}$ | 8.98 | 6.03–14.78) |
| $Var_{20}$ | 35.08 | 24.11–55.72 |
| $Var_{30}$ | 66.57 | 45.86–105.40 |
| $Var_{40}$ | 190.92 | 131.86–301.07 |
| $Var_{50}$ | 149.57 | 103.54–235.06 |
| $Var_{60}$ | 201.30 | 139.68–315.22 |
| $Var_{70}$ | 178.88 | 124.46–278.98 |
| $Var_{80}$ | 137.89 | 96.22–214.11 |
| $Var_{90}$ | 112.40 | 78.59–174.00 |
| $Var_{100}$ | 85.84 | 60.09–132.65 |
| $Var_{110}$ | 66.33 | 46.40–102.62 |
| $Var_{120}$ | 60.70 | 42.30–94.42 |
| $\rho$ | 0.910 | 0.886–0.944 |

TABLE 4. Linear-mixed-model analysis of offspring [IgG] between days 10 and 110 as a function of offspring age, sex, and nestling period. Fixed effects were tested using the best-fit covariance structure (bold). Covariance structures are defined and discussed in Appendix 1. Goodness-of-fit of alternative covariance structures was based on the smallest absolute value of Akaike's Information Criterion (AIC) and Schwartz's Bayesian Information Criterion (BIC). For the best-fit covariance structure, we tested the fit of models with separate parameter estimates for offspring sex (O) or nestling period (N). Competing models, those with the absolute value $-2\log(\ell)$ scores closer to zero but with more parameters, were tested using the likelihood ratio test as indicated by superscript letters. Notation: $\text{var}_b$ = between-individual variance, $\text{var}_w$ = within-individual variance, and $\rho$ = autocorrelation coefficient.

| Fixed effects | df | $F$ | $P$ |
|---|---|---|---|
| Age-class (A) | 5 and 142 | 91.57 | <0.0001 |
| Offspring sex (O) | 1 and 34 | 0.16 | 0.69 |
| A * S | 5 and 142 | 1.39 | 0.23 |
| Nestling period (N) | 1 and 34 | 0.03 | 0.86 |
| A * N | 5 and 142 | 3.12 | 0.011 |
| O * N | 1 and 34 | 0.14 | 0.71 |
| A * O * N | 5 and 142 | 0.16 | 0.93 |

| Covariance structure | Number of parameters | $-2\ln(\ell)$ | AIC | BIC |
|---|---|---|---|---|
| VC | 1 | 815.5 | 817.4 | 814.4 |
| CS | 2 | 676.2 | 680.2 | 683.4 |
| **AR1 + RE** | **3** | **666.3**[a,b,c] | **672.0** | **676.9** |
| O | 5 | 661.5[c] | 671.5 | 679.7 |
| N | 5 | 665.2 | 675.2 | 683.3 |
| CSH | 7 | 661.7[b] | 675.1 | 686.5 |
| ARH1 + RE | 8 | 657.8[a] | 673.8 | 686.9 |

[a] $G^2 = 8.5$, df = 5, $P = 0.13$.
[b] $G^2 = 4.6$, df = 4, $P = 0.33$.
[c] $G^2 = 4.8$, df = 2, $P = 0.091$.

| Parameter | Estimate | 95% CI |
|---|---|---|
| $\text{var}_b$ | 2.79 | 1.58–6.20 |
| $\text{var}_w$ | 1.71 | 1.07–3.17 |
| $\rho$ | 0.461 | 0.153–0.768 |

TABLE 5. Linear-mixed-model analysis of offspring growth as a function of offspring [IgG] for three phases of the nestling period. In the first phase (days 10 and 30), [IgG] was stable and the mean value of [IgG] was entered into the analysis. In the middle phase (days 30 and 50), [IgG] was increasing and the mean value and difference ($\Delta$ = final-initial) of [IgG] were analyzed. In the final phase (days 70–110), [IgG] was again stable and the mean value of [IgG] was used. Because of the short intervals being analyzed, the CS covariance structure was used to control for inter-individual variation.

| | | Log(body mass) | | | Culmen length | | | Wing length | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Phase | Source | df | $F$ | $P$ | df | $F$ | $P$ | df | $F$ | $P$ |
| Days 10 and 30 | Age-class (A) | 1 and 36 | 6.57 | <0.0001 | 1 and 36 | 1, 398.75 | <0.0001 | 1 and 36 | 599.09 | <0.0001 |
| | Mean [IgG] (ml) | 1 and 36 | 0.01 | 0.91 | 1 and 36 | 0.04 | 0.85 | 1 and 36 | 0.11 | 0.74 |
| | A * ml | 1 and 36 | 0.04 | 0.84 | 1 and 36 | 0.06 | 0.81 | 1 and 36 | 0.72 | 0.40 |
| Days 30 and 50 | Age-class (A) | 1 and 36 | 70.89 | <0.0001 | 1 and 36 | 889.47 | <0.0001 | 1 and 36 | 1, 354.86 | <0.0001 |
| | mean [IgG] (ml) | 1 and 36 | 0.16 | 0.70 | 1 and 36 | 0.20 | 0.66 | 1 and 36 | 0.00 | 0.99 |
| | A * ml | 1 and 36 | 0.04 | 0.85 | 1 and 36 | 0.51 | 0.48 | 1 and 36 | 0.03 | 0.87 |
| Days 30 and 50 | Age-class (A) | 1 and 34 | 121.84 | <0.0001 | 1 and 34 | 1, 450.44 | <0.0001 | 1 and 34 | 2, 226.64 | <0.0001 |
| | $\Delta$ [IgG] ($\Delta$I) | 1 and 34 | 0.31 | 0.58 | 1 and 34 | 1.07 | 0.31 | 1 and 34 | 1.30 | 0.26 |
| | A * $\Delta$I | 1 and 34 | 0.00 | 0.96 | 1 and 34 | 0.72 | 0.40 | 1 and 34 | 0.18 | 0.68 |
| Days 70–110 | Age-class (A) | 3 and 106 | 7.96 | <0.0001 | 3 and 106 | 101.02 | <0.000 | 3 and 106 | 965.02 | <0.0001 |
| | Mean [IgG] (ml) | 1 and 36 | 0.17 | 0.68 | 1 and 36 | 0.52 | 0.48 | 1 and 36 | 0.92 | 0.34 |
| | A * ml | 3 and 106 | 1.41 | 0.24 | 3 and 106 | 1.15 | 0.33 | 3 and 106 | 0.20 | 0.89 |

TABLE 6. Linear-mixed-model analysis of parental foraging effort between days 50 and 110 as a function of parent sex and offspring age-class, sex, and nestling period. Fixed effects were tested using the best-fit covariance structure (bold). Covariance structures are defined and discussed in Appendix 1. Goodness-of-fit of alternative covariance structures was based on the smallest absolute value of Akaike's Information Criterion (AIC) and Schwartz's Bayesian Information Criterion (BIC). For the best-fit covariance structure, we tested the fit of models with separate parameter estimates for parent sex (P), offspring sex (O), or nestling period (N). Competing models, those with the absolute value $-2\log(\ell)$ scores closer to zero but with more parameters, were tested using the likelihood ratio test as indicated by superscript letters. Notation: $\text{var}_f$ = between-family variance, $\text{var}_w$ = within-individual variance, and $\rho$ = autocorrelation coefficient.

| Fixed effects | df | $F$ | $P$ |
|---|---|---|---|
| Age-class (A) | 3 and 204 | 204.99 | <0.0001 |
| Parent sex (P) | 1 and 68 | 56.00 | <0.0001 |
| A * P | 3 and 204 | 1.46 | 0.23 |
| Offspring-sex (O) | 1 and 68 | 1.35 | 0.25 |
| A * O | 3 and 204 | 3.03 | 0.031 |
| P * O | 1 and 68 | 0.16 | 0.69 |
| A * P * O | 3 and 204 | 0.18 | 0.91 |
| Nestling period (N) | 1 and 68 | 1.89 | 0.17 |
| P * N | 1 and 68 | 1.48 | 0.23 |
| A * N | 3 and 204 | 0.65 | 0.58 |
| A * P * N | 3 and 204 | 0.49 | 0.69 |
| O * N | 1 and 68 | 0.02 | 0.89 |
| P * O * N | 1 and 68 | 0.16 | 0.69 |
| A * O * N | 3 and 204 | 0.44 | 0.72 |
| A * P * O * N | 3 and 204 | 0.72 | 0.54 |

| Covariance structure | Number of parameters | $-2\ln(\ell)$ | AIC | BIC |
|---|---|---|---|---|
| VC | 1 | 2,545.2 | 2,549.2 | 2,552.5 |
| CS | 2 | 2,539.8 | 2,545.8 | 2,550.8 |
| **AR1 + RE** | **3** | **2,527.8**[a,b] | **2,533.8** | **2,538.8** |
| P | 5 | 2,525.6 | 2,535.6 | 2,543.8 |
| O | 5 | 2,527.4 | 2,537.4 | 2,545.5 |
| N | 5 | 2,524.8[b] | 2,534.8 | 2,543.0 |
| CSH | 5 | 2,534.5 | 2,546.5 | 2,556.3 |
| ARH1 + RE | 6 | 2,522.4[a] | 2,534.4 | 2,647.6 |

[a] $G^2 = 5.4$, df = 3, $P = 0.14$.
[b] $G^2 = 3.0$, df = 2, $P = 0.22$.

| Parameter | Estimate | 95% CI |
|---|---|---|
| $\text{var}_f$ | 105.8 | 44.6–489.3 |
| $\text{var}_w$ | 500.5 | 397.8–649.3 |
| $\rho$ | 0.367 | 0.200–0.534 |

TABLE 7. Linear-mixed-model analysis of parental body mass between days 10 and 110 as a function of parent sex, offspring age-class and sex, and nestling period. Fixed effects were tested using the best-fit covariance structure (bold). Covariance structures are defined and discussed in Appendix 1. Goodness-of-fit of alternative covariance structures was based on the smallest absolute value of Akaike's Information Criterion (AIC) and Schwartz's Bayesian Information Criterion (BIC). For the best-fit covariance structure, we tested the fit of models with separate parameter estimates for parent sex (P), offspring sex (O), or nestling period (N). Competing models, those with the absolute value $-2\log(\ell)$ scores closer to zero but with more parameters, were tested using the likelihood ratio test as indicated by superscript letters. Notation: $\text{var}_b$ = between-individual variance, and $\text{var}_w$ = within-individual variance.

| Fixed effects | df | $F$ | $P$ |
|---|---|---|---|
| Parent sex (P) | 1 and 68 | 165.12 | <0.0001 |
| Age-class (A) | 5 and 259 | 29.43 | <0.0001 |
| P * A | 5 and 259 | 1.62 | 0.15 |
| Nestling period (N) | 1 and 68 | 8.33 | 0.0052 |
| P * N | 1 and 68 | 0.03 | 0.86 |
| A * N | 5 and 259 | 0.72 | 0.61 |
| P * A * N | 5 and 259 | 1.68 | 0.14 |
| Offspring sex (O) | 1 and 68 | 0.33 | 0.57 |
| P * O | 1 and 68 | 0.11 | 0.74 |
| A * O | 5 and 259 | 2.98 | 0.012 |
| P * A * O | 4 and 259 | 1.00 | 0.41 |
| N * O | 1 and 68 | 0.77 | 0.38 |
| P * N * O | 1 and 68 | 3.54 | 0.064 |
| A * N * O | 4 and 259 | 3.00 | 0.019 |
| P * A * N * O | 4 and 259 | 0.75 | 0.56 |

| Covariance structure | Number of parameters | $-2\ln(\ell)$ | AIC | BIC |
|---|---|---|---|---|
| VC | 1 | 4,079.3 | 4,081.3 | 4,083.6 |
| **CS** | **2** | **3,998.6**[a,b,c,d] | **4,002.6** | **4,007.3** |
| P | 4 | 3,995.2 | 4,003.2 | 4,012.5 |
| O | 4 | 3,995.5 | 4,003.5 | 4,012.8 |
| N | 4 | 3,994.8[d] | 4,002.8 | 4,012.2 |
| AR1 + RE | 3 | 3,995.7[a] | 4,001.7 | 4,008.7 |
| CSH | 7 | 3,990.7[b] | 4,004.7 | 4,021.0 |
| ARH1 + RE | 8 | 3,985.9[c] | 4,001.9 | 4,020.6 |

[a] $G^2 = 2.9$, df = 1, $P = 0.089$.
[b] $G^2 = 7.9$, df = 5, $P = 0.16$.
[c] $G^2 = 12.7$, df = 6, $P = 0.050$.
[d] $G^2 = 3.8$, df = 2, $P = 0.15$.

| Parameter | Estimate | 95% CI |
|---|---|---|
| $\text{var}_b$ | 4,926 | 3,333–8,015 |
| $\text{var}_w$ | 6,627 | 5,619–7,934 |

TABLE 8. Linear-mixed-model analysis of parent [IgG] between days 10 and 110 as a function of parent sex, offspring age-class and sex, and nestling period. Fixed effects were tested using the best-fit covariance structure (bold). Covariance structures are defined and discussed in Appendix 1. Goodness-of-fit of alternative covariance structures was based on the smallest absolute value of Akaike's Information Criterion (AIC) and Schwartz's Bayesian Information Criterion (BIC). For the best-fit covariance structure, we tested the fit of models with separate parameter estimates for parent sex (P), offspring sex (O), or nestling period (N). Competing models, those with the absolute value –2log($\ell$) scores closer to zero but with more parameters, were tested using the likelihood ratio test as indicated by superscript letters. Notation: $var_f$ = between-family variance, $var_b$ = between-individual variance, $var_{w-M}$ = within-individual variance for mothers, $var_{w-F}$ = within-individual variance for fathers, $\rho_M$ = autocorrelation coefficient for mothers, $\rho_F$ = autocorrelation coefficient for fathers.

| Fixed effects | df | $F$ | $P$ |
|---|---|---|---|
| Age-class (A) | 5 and 141 | 1.38 | 0.24 |
| Parent sex (P) | 1 and 34 | 0.05 | 0.82 |
| A * P | 5 and 123 | 1.22 | 0.30 |
| Offspring sex (O) | 1 and 34 | 1.67 | 0.20 |
| A * O | 5 and 141 | 0.78 | 0.56 |
| P * O | 1 and 34 | 3.07 | 0.089 |
| A * P * O | 5 and 123 | 0.34 | 0.88 |
| Nestling period (N) | 1 and 34 | 0.03 | 0.87 |
| A * N | 5 and 141 | 0.81 | 0.54 |
| P * N | 1 and 34 | 0.00 | 0.97 |
| O * N | 1 and 34 | 0.76 | 0.39 |
| A * P * N | 5 and 123 | 0.51 | 0.77 |
| A * O * N | 5 and 141 | 2.16 | 0.062 |
| P * O * N | 1 and 34 | 0.00 | 0.97 |
| A * P * O * N | 5 and 123 | 0.54 | 0.74 |

| Covariance structure | Number of parameters | $-2\ln(\ell)$ | AIC | BIC |
|---|---|---|---|---|
| VC | 2 | 1,478.9 | 1,482.9 | 1,486.1 |
| CS | 3 | 1,296.5[a] | 1,302.5 | 1,307.4 |
| **AR1 + RE** | **4** | **1,286.3**[a,b,c] | **1,294.3** | **1,309.2** |
| P | 6 | 1,278.7[c] | 1,305.4 | 1,295.4 |
| O | 5 | 1,282.2 | 1,294.2 | 1,304.0 |
| N | 5 | 1,284.3 | 1,296.3 | 1,306.1 |
| CSH | 8 | 1,294.9 | 1,310.9 | 1,324.0 |
| ARH1 + RE | 9 | 1,284.6[b] | 1,302.6 | 1,317.3 |

[a] $G^2 = 10.2$, df = 1, $P = 0.0014$.
[b] $G^2 = 1.7$, df = 5, $P = 0.89$.
[c] $G^2 = 7.6$, df = 2, $P = 0.022$

| Parameter | Estimate | 95% CI |
|---|---|---|
| $var_f$ | 3.87 | 2.13 to 9.10 |
| $var_b$ | 2.99 | 1.46 to 4.53 |
| $var_{w-M}$ | 1.27 | 0.98 to 1.73 |
| $var_{w-F}$ | 1.64 | 0.97 to 3.36 |
| $\rho_M$ | 0.105 | −0.142 to 0.351 |
| $\rho_F$ | 0.552 | 0.268 to 0.837 |