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CHAPTER 6

HEALTH ASSESSMENT OF SEABIRDS ON ISLA GENOVESA, GALÁPAGOS ISLANDS

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ABSTRACT.—A multispecies colony of seabirds was studied on the island of Genovesa, in the northern part of the Galápagos archipelago, Ecuador, in 2003, to establish baseline health parameters and to test specifically for *Chlamydophila psittaci*, known to exist elsewhere in the archipelago. Twenty-three Red-footed Boobies (*Sula sula*), 24 Great Frigatebirds (*Fregata minor*), 25 Nazca Boobies (*S. granti*), and 19 Swallow-tailed Gulls (*Creagrus furcatus*) were hand-restrained for venipuncture and collection of lacrimo-choanal-cloacal combination swabs. White blood cell (WBC) counts, differentials, and packed cell volumes were obtained and plasma chemistry analyses performed on the blood samples. Presence–absence and parasitemias of circulating hemoparasites were determined by microscopic evaluation of peripheral blood smears. *Haemoproteus*-like hemoparasites were found in three of the seabird species sampled. Prevalences were 29.2% (7 of 24) in Great Frigatebirds, 15.8% (3 of 19) in Swallow-tailed Gulls, and 8.7% (2 of 23) in Red-footed Boobies; none of the Nazca Boobies were infected. Parasitemia levels were relatively low within each of the infected species. Individual Great Frigatebirds with *Haemoproteus* infections also exhibited significantly higher heterophil-to-lymphocyte concentration ratios than birds not infected with *Haemoproteus*, an indication that birds infected with *Haemoproteus* were also physiologically stressed or, alternatively, that they were actively fighting the infection. *Haemoproteus* prevalences within Great Frigatebirds on Genovesa were not significantly different from those previously reported from conspecific hosts in the Hawaiian Islands. To compare seabird hemoparasite data with those for a sympatric terrestrial species, Galapagos Doves (*Zenaida galapagoensis*) were sampled on Genovesa in 2004 and screened for *Haemoproteus* previously reported in Galapagos Doves on other islands. Prevalence in this terrestrial endemic was high (42.3%; 11 of 26), and several birds exhibited relatively high parasitemia levels. *Chlamydophila psittaci* was not found in any birds by either serology or antigen detection methods. *Received 29 August 2005, accepted 8 September 2005.*

RESUMEN.—Estudiamos una colonia multiespecífica de aves marinas en la isla de Genovesa, en la zona norte del archipiélago de las Galápagos en Ecuador, en el año 2003, para determinar parámetros de salud básicos y probar particularmente por la presencia de *Chlamydophyla psittaci*, de la cual se sabe su existencia en el resto del archipiélago. Se tomaron muestras de sangre y de exudados combinados cloacales y lagrimo-coanales de 23 *Sula sula*, 24 *Fregata minor*, 25 *Sula granti* y 19 *Creagrus furcatus*. De las muestras de sangre, se obtuvieron conteos de glóbulos blancos, diferenciales y volúmenes celulares empaquetados, además de realizar análisis químicos del plasma. La presencia-ausencia y parasitemias de hemoparásitos circulantes fueron determinadas por medio de evaluaciones microscópicas de frotis de sangre periférica. Hemoparásitos parecidos a *Haemoproteus* fueron encontrados en tres de las especies de aves marinas muestreadas. Las frecuencias de la presencia de

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hemoparásitos fue de 29.2% (7 de 24) en *Fregata minor*, 15.8% (3 de 19) en *Creagrus furcatus* y 8.7% (2 de 23) en *Sula sula;* ningún individuo de *Sula granti* estuvo infectado. Los niveles de parasitemia fueron relativamente bajos en cada una de las especies infectadas. Individuos de *Fregata minor* infectados con *Haemoproteus* también mostraron proporciones significativamente altas en concentraciones de heterofil a linfocitos en comparación con las aves no infectadas, lo que indica que las aves infectadas con *Haemoproteus* también estuvieron estresadas fisiológicamente o también pudieron estar luchando activamente la infección. Las frecuencias de *Haemoproteus* en individuos de *Fregata minor* en la isla de Genovesa no fueron significativamente diferentes de las frecuencias previamente registradas en hospederos con específicos en las islas Hawaianas. Para comparar la información de la presencia de Hemoparásitos en aves marinas con datos de especies terrestres simpáticas, se muestrearon individuos de *Zenaida galapagoensis* en la isla de Genovesa en el ano del 2004 y se determino la presencia de *Haemoproteus*, la cual había sido reportada previamente en individuos de *Zenaida galapagoensis* provenientes de otras islas. La frecuencia en esta especie terrestre endémica fue alta (42.3%; 11 de 26) y algunas aves presentaron niveles de parasitemia relativamente altos. *Chlamydophyla psittaci* no se encontró en ninguna de las aves cuando se utilizaron métodos de detección serológicos o de antígeno.

The Galápagos Islands are located on the equator in the Pacific Ocean, ~1,000 km west of mainland Ecuador in South America. They have been inhabited by humans for less than two centuries, and their biodiversity remains largely intact, with only ~5% of species having been lost (Gibbs et al. 1999); all the 28 breeding land-bird species, 26 of which are endemic, remain. High endemism extends to seabirds, despite the huge individual ranges of some members of this group (Dearborn et al. 2003). Nineteen seabird species nest in the Galápagos Islands, including four endemic species and six endemic subspecies (Harris 1984).

In 1959, 90% of the area of the archipelago was set aside as a national park. However, the resident human population has grown rapidly, and exotics are continually being introduced despite increasing efforts to exclude them. The Charles Darwin Research Station and the Galápagos National Park are concerned about the introduction of avian diseases that could result in extinctions of Galápagos avifauna comparable to extinctions in Hawaii (Wikelski et al. 2004). Disease has been implicated as a major factor in the population declines and extinctions in the endemic Hawaiian avifauna (Warner 1968; van Riper et al. 1986, 2002), which now comprises less than half the species present at Polynesian settlement. Avian pox (*Avipoxvirus* spp.) and avian malaria (*Plasmodium* spp.) have been implicated as the major pathogens contributing to this wave of Hawaiian extinctions beginning in the mid-1800s (van Riper and Scott 2001, van Riper et al. 2002).

Island populations may be particularly susceptible to new pathogens, because they have been exposed to fewer pathogens than mainland populations (Lewis 1968a, b; Dobson 1988; Goüy de Bellocq et al. 2002). Colonizers from the mainland will not represent all the parasites found in their population of origin (Dobson and May 1986). Populations may grow quickly in the absence of pathogens and selection for resistance is relaxed, facilitating the invasion of pathogens arriving later (Dobson 1988). To illustrate using the example of Hawaiian songbirds, some Hawaiian endemic birds had higher *Plasmodium* parasitemia levels, higher pox prevalence, and heavier infections than introduced birds (van Riper et al. 1986, 2002).

Concerns over the status of native avian populations in archipelagos and the effects of introduced diseases have been the impetus for an active health-surveillance project in the Galápagos Islands (Miller et al. 2002, Wikelski et al. 2004). Health surveys of Galápagos birds have found a number of pathogens in domestic chickens (Gottdenker et al. 2005). Tests have shown clearly that the *Avipoxvirus* in the chickens is a different virus than the two canarypox-like *Avipoxvirus* strains infecting the endemic landbirds (Thiel et al. 2005). Studies aimed at seeing whether other pathogens are transferring from chickens to endemics are underway. General health surveys conducted on the Waved Albatross (*Phoebastria irrorata*) established baseline values for a generally healthy population (Padilla et al. 2003). A four-island comparison of

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the introduced Rock Pigeon (*Columba livia*) and the only endemic dove, the Galapagos Dove (*Zenaida galapagoensis*), revealed no evidence of transmission of pathogens in either direction, despite the occurrence of *Trichomonas gallinae* in Rock Pigeons on San Cristobal, *Chlamydophila psittaci* in Galapagos Doves on Española, and the near-ubiquitous presence of a *Haemoproteus*-like blood parasite in the Galapagos Doves on every island sampled. Interestingly, the two single cases of interspecific parasite transmission found thus far involved prey-to-predator transmission of relatively benign chewing lice (Phthiraptera: Ischnocera). Louse species typically found only on Galapagos Doves (i.e. *Columbicola* spp., *Physconelloides* spp.) and introduced goats (i.e. *Bovicola* spp.) were found as "stragglers" on Galapagos Hawks (*Buteo galapagoensis*), to which they dispersed during predation events (Whiteman et al. 2004). However, it was the presence of *C. psittaci* on Española that prompted this survey of seabirds on Genovesa. *Chlamydophila psittaci* can spread rapidly as an epizootic agent in dense bird colonies (Franson 1999), and the colonial breeding habit is associated with higher rates of parasitism (Tella 2002). Few islands rival the number and density of seabirds nesting on the island of Genovesa, and we designed this survey specifically to test for the presence of *C. psittaci*, while gathering general health baseline data.

The colonial breeding habits and limited geographic distribution of most seabirds are believed to make them vulnerable to mass mortality events (Warham 1996), including infectious disease epizootics and environmental disasters. Health studies on free-ranging island-nesting populations of seabirds are limited, and few species-specific reference ranges are available (Work 1996, 1999). Establishing reference parameters from free-ranging animals is essential to conservation efforts, setting a baseline for recognition of health-related threats to a population (Spalding and Forrester 1993). Monitoring pelagic seabirds has also been suggested as a way of assessing the overall health of marine ecosystems (Uhart et al. 2003). The purpose of the present study was to establish species-specific baseline health parameters for a multispecies colony of seabirds from Genovesa, an island in the northeastern part of the Galapagos archipelago, and to test them specifically for *C. psittaci* and blood parasites known to exist on the archipelago and in seabirds elsewhere.

Materials and Methods

Between 11 and 15 July 2003, 93 seabirds from a multispecies colony were sampled on Darwin Bay, on the southern part of Genovesa (00°19′N, 89°57′W), Galápagos Islands, Ecuador. Procedures were conducted in accordance with Saint Louis Zoo institutional animal care and use committee standards. Individuals included 24 Great Frigatebirds (*Fregata minor*), 23 Red-footed Boobies (*Sula sula*), 25 Nazca Boobies (*S. granti*), and 20 Swallow-tailed Gulls (*Creagrus fulcatus*). In addition, we sampled one Yellow-crowned Night Heron (*Nyctanassa violacea*), two Short-eared Owls (*Asio flammeus galapagoensis*), and three Galapagos Doves, all of which were frequently observed foraging within the colony. Lava Gulls (*Larus fuliginosus*) were also seen occasionally in this colony, but were not sampled. The colony was chosen on the basis of size, species diversity, and accessibility, and deemed representative of other multispecies colonies on this island. This area is frequently monitored by Galápagos National Park personnel, and no historical mass mortalities have been documented. Seabirds were hand captured while resting or standing on the ground. Owls and doves were captured with hand-nets. Each bird was marked with a single permanent-marker dot on either the right leg or the ventral aspect of the beak to avoid repeated sampling. In 2004, 30 additional Galapagos Doves were sampled on the same site.

Blood was collected from the ulnar vein, and birds were inspected for hemostasis before being returned to the capture site. Microhematocrit tubes were filled, and fresh blood smears were prepared. The remaining blood was preserved in lithium heparin blood-collection tubes. Blood smears were fixed in ethanol. Blood was also preserved in a lysis buffer (Longmire et al. 1988) for molecular sexing (as described by Fridolfsson and Ellegren 1999) and future genetic and population studies. Microhematocrit tubes were centrifuged (using Mobilespin Model 128; Vulcon Technologies, Grandview, Missouri), and packed cell volumes (PCV) were measured. Heparinized plasma was separated into nalgene cryotubes (Nalge Nunc International, Rochester, New York) and stored in liquid nitrogen until further analysis. Blood smears were stained with a modified Wright-Giemsa stain (JorVet Dip-Quick; Jorgensen Laboratories, Loveland, Colorado) before being individually examined for presence of hemoparasites. Blood smears were assessed for hemoparasite presence by searching 200 oil-immersion fields at 100× magnification. Parasitemias (infection intensities) were recorded as the total number of infected erythrocytes observed during the search of 200 oil-immersion fields. An

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estimated leukocyte count was obtained as described by Fudge (2000). Differential white-blood-cell (WBC) counts were performed by counting 100 leukocytes under oil immersion. Within Great Frigatebirds, differential WBC counts were transformed into concentrations by multiplying the estimated total WBC values (WBC × 1,000 μL^{-1}) with the differential value, the product of which was then divided by 100. These values (in units of 1,000 μL^{-1} for heterophils and lymphocytes) were then used to calculate heterophil-to-lymphocyte ratios for each Great Frigatebird from which both estimated WBC concentrations and differential data were available ($n = 16$). Because heterophil-to-lymphocyte concentration ratios were not normally distributed (based on a one-sample Kolmogorov-Smirnov test), a Mann-Whitney U test was used to compare concentration ratios between birds infected and uninfected with *Haemoproteus*. Plasma remained frozen until shipped for *C. psittaci* serology testing by elementary body agglutination at the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas) and for plasma chemistry testing at a commercial veterinary laboratory (AVL Veterinary Clinical Laboratory, St. Louis, Missouri), using the ACE clinical chemistry analyzer system (Alfa Wasserman, West Caldwell, New Jersey).

Sterile Dacron tip applicators were used to collect combination swabs of the conjunctiva, choana, and cloaca, and were subsequently frozen in nalgene cryotubes with no preservatives. Swabs were submitted to the Infectious Diseases Laboratory, University of Georgia-College of Veterinary Medicine, Athens, Georgia, for *C. psittaci* antigen detection by polymerase chain reaction (PCR).

Results were analyzed using commercial statistical software packages (NCSS, Kaysville, Utah; SPSS, version 13.0, Chicago, Illinois). Data were tested for normality using a Shapiro-Wilk W test or a one-sample Kolmogorov-Smirnov test, and Mann-Whitney U tests were used to compare groups if the data were not normally distributed. QUANTITATIVE PARASITOLOGY, version 2.0 (Reiczigel and Rózsa 2001), which used distribution-free statistical tests specifically designed for comparative parasitology, was used to compare *Haemoproteus* prevalences and intensities among seabirds and within species, between sexes and age classes. This program was also used to calculate parasite distributions within species. Because 13 plasma-chemistry parameters and WBC count were compared for each subset of the data, a Bonferroni-corrected P value (0.05 of 14 = 0.004) was used to establish statistical significance. No Bonferroni correction was performed on the one data set (Great Frigatebird) used to compare heterophil-to-lymphocyte concentration ratios between birds infected and uninfected with *Haemoproteus*, given that the relationship between variables was hypothesized *a priori*.

Results

Screening of blood smears revealed circulating hemoparasites in three of the four seabirds examined, with Great Frigatebirds exhibiting the highest prevalence (29.2%; 7 of 24), followed by Swallow-Tailed Gulls (15.8%; 3 of 19), and Red-Footed Boobies (8.7%; 2 of 23); none were seen in Nazca Boobies (0 of 25; Table 1 summarizes the hematology values and hemoparasite distribution data). All circulating hemoparasites were morphologically consistent with *Haemoproteus*-like organisms (Fig. 1). *Haemoproteus* parasites were confirmed in one of the three Galapagos Doves tested in 2003 and in 11 of 26 (42.3%) of those examined in 2004, but not in the single Yellow-crowned Night Heron, nor in the Short-eared Owls. *Haemoproteus* prevalence was not significantly different between sexes within species or among individual species. Mean parasitemias (infection intensities) were low in all three positive seabirds, but relatively high in Galapagos Doves in both 2003 and 2004. We then compared prevalences within Great Frigatebirds sampled in the present study (29.2%; 7 of 24) to those published by Work and Rameyer (1996) from conspecific hosts sampled within another Pacific Island oceanic archipelago, Laysan and Tern Islands (35.6%; 32 of 90) in Hawaii, and found no significant differences in *Haemoproteus* prevalence between the two populations ($P > 0.05$).

Table 2 summarizes plasma-chemistry values for the four seabird species sampled. No evidence of *C. psittaci* was found by either plasma serology or PCR of swabs in any of the birds tested. Swallow-Tailed Gulls had consistently lower total WBC counts than the other species sampled. We compared the plasma-chemistry values between hemoparasitized and nonhemoparasitized birds and found no differences between them. Across seabird species, hemoparasitized birds had slightly lower WBC counts than nonhemoparasitized birds (WBC = $9.0 \pm 5.3 \times 10^3$ μL^{-1} vs. $8.2 \pm 4.1 \times 10^3$ μL^{-1}; $U = 531$, $P = 0.01$, $n = 91$), but this difference was not significant when Bonferroni correction was applied. Male and female birds did not differ in occurrence of hemoparasitism, total WBC counts, or PCV values. However, heterophil-to-lymphocyte concentration ratios were significantly higher for hemoparasite-infected than for noninfected Great Frigatebirds (Fig. 2; $U = 9$, $P =$

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TABLE 1. Hematological parameters and prevalence, intensity and aggregation level (var/mean ratio) of *Haemoproteus* spp. from four seabirds sampled in 2003 and one terrestrial species (Galápagos Dove) sampled in 2004, inhabiting a multispecies seabird colony on Isla Genovesa, Galápagos Islands.

PCV (%)	WBC × 1,000 μL^{-1}	Heterophils (%)	Lymphocytes (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)	*Haemoproteus* prevalence[a]	*Haemoproteus* mean intensity[b]	*Haemoproteus* var/mean ratio
Great Frigatebird (*Fregata minor*)									
55 ± 8	7.5 ± 2.7	39.0 ± 8.5	40.0 ± 12.0	0.6 ± 0.9	17.9 ± 2.7	2.5 ± 1.6	7 of 24	1.29	1.12
n = 23	n = 24	n = 16	n = 16	n = 16	n = 16	n = 16	(29.2%)	(1–2)	
Red Footed Booby (*Sula sula*)									
50 ± 7	10.3 ± 4.7	36.1 ± 16.7	54.8 ± 17.5	0.2 ± 0.4	5.2 ± 3.6	3.7 ± 2.6	2 of 23	1.00	0.95
n = 20	n = 23	n = 18	n = 18	n = 18	n = 18	n = 18	(8.7%)		
Nazca Booby (*S. granti*)									
51 ± 3	9.4 ± 3.5	46.7 ± 14.3	34.4 ± 14.2	0.5 ± 0.7	15.4 ± 7.9	3.8 ± 2.2	0 of 25	0.00	0
n = 23	n = 25	n = 24	n = 24	n = 24	n = 24	n = 24	(0%)		
Swallow-Tailed Gull (*Creagrus furcatus*)									
54 ± 5	4.8 ± 2.7	54.0 ± 11.1	36.2 ± 10.9	0.5 ± 0.8	4.4 ± 1.8	4.9 ± 4.2	3 of 19	1.00	0.89
n = 19	n = 20	n = 14	n = 14	n = 14	n = 14	n = 14	(15.8%)		
Galápagos Dove[c] (*Zenaida galapagoensis*)									
NA	NA	18 ± 5.7	69.06 ± 10.17	0.06 ± 0.24	8.4 ± 8.1	4.41 ± 2.28	11 of 26	29.36	225.76
		n = 18	n = 18	n = 18	n = 18	n = 18	(42.3%)	(1–270)	

[a] Prevalence established by detection of circulating *Haemoproteus*-like organisms on smear examination.
[b] Range if $x > 1$.
[c] Three doves were sampled in 2003, one of which was positive for *Haemoproteus* with an infection intensity of 8.
Abbreviations: PCV = packed erythrocyte volume, WBC = white blood cell count, NA = not available.

FIG. 1. Photomicrographs of hemoparasites in Galápagos birds. (A) Erythrocytes from a thin blood smear from Galápagos Dove. Arrow indicates a *Haemoproteus*-like parasite. (B) Erythrocytes from a thin blood smear from Great Frigatebird. Arrows indicate *Haemoproteus*-like parasites.

0.036, $n = 16$). Low prevalence in the other two infected seabird species precluded using this test to compare the heterophil-to-lymphocyte concentration ratios between infected and uninfected birds.

Plasma-chemistry values showed no sex-related differences, with the exception of plasma phosphorus (Table 3). A trend seen across species was that females had higher phosphorus levels ($U = 1{,}135$, $P = 0.04$, $n = 81$),

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TABLE 2. Plasma biochemistry values of four seabird species sampled in 2003 inhabiting a multispecies seabird colony on Isla Genovesa, Galápagos Islands.

Sample size (n)	Uric acid (mg dL^{-1})	CK (U L^{-1})	Total AST (U L^{-1})	bilirubin (mg dL^{-1})	Glucose (mg dL^{-1})	Ca^{++} (mg dL^{-1})	Phos (mg dL^{-1})	Na^{+} (mmol L^{-1})	K^{+} (mmol L^{-1})	Total Cl^{-} (mmol L^{-1})	protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})
Great Frigatebird (*Fregata minor*)													
24	7.7 ± 7.7	556 ± 421	248.1 ± 95.1	0.6 ± .5	212.1 ± 45.7	9.1 ± 0.66	4.7 ± 1.5	145.4 ± 8.2	3.0 ± 1.6	114.6 ± 4.5	3.58 ± 0.5	0.94 ± 0.1	2.6 ± 0.4
Red Footed Booby (*Sula sula*)													
21	10.9 ± 8.2	940.1 ± 371.4	465.8 ± 181.1	0.6 ± 0.2	180.5 ± 64.0	9.3 ± 0.7	10.8 ± 4.6	151.4 ± 3.7	6.6 ± 2.1	116.0 ± 4.7	3.56 ± 0.3	1.17 ± 0.1	2.0 ± 0.9
Nazca Booby (*Sula granti*)													
24	12.1 ± 7.2	871.8 ± 271.1	310.3 ± 141.7	0.47 ± 0.3	252.0 ± 34.6	9.5 ± 0.64	5.6 ± 2.6	154.5 ± 3.6	3.0 ± 1.2	117.9 ± 3.2	3.76 ± 0.4	1.22 ± 0.1	2.4 ± 0.6
Swallow-Tailed Gull (*Creagrus furcatus*)													
14	7.5 ± 5.9	263.3 ± 323.1	361.6 ± 172.2	1.2 ± 1.6	280.6 ± 31.8	11.0 ± 2.9	4.6 ± 4.9	155.0 ± 9.2	3.0 ± 0.7	122.2 ± 7.5	4.13 ± 0.8	1.57 ± 0.4	1.8 ± 1.3

and slightly higher total plasma calcium levels (U = 958.5, P = 0.01, n = 81). Within species, phosphorus values were higher in female than in male Nazca (U = 119.5, P = 0.0002, n = 23), Red-footed Boobies (U = 30, P = 0.03, n = 20), and Swallow-tailed Gulls (U = 22, P = 0.9, n = 14), but not in Great Frigatebirds (U = 71.5, P = 0.9, n = 24). This difference was considered significant in Nazca Boobies.

Discussion

We observed *Haemoproteus* parasites in peripheral blood smears from three of four species of Genovesa seabirds, as well as in the sympatric and endemic Galapagos Dove. Hemoparasites in the *Haemoproteus* genus have traditionally been considered incidental and relatively nonpathogenic parasites of birds and reptiles, though effects on host fitness components have been demonstrated (Merino et al. 2000, Marzal et al. 2005), and pathogenicity has been shown for certain hosts of certain hemoparasite species (Earlé et al. 1993, Garvin et al. 2003). However, molecular phylogenetic studies of malarial parasites has revealed considerable convergence in the morphological and life-history traits used traditionally to classify lineages (Perkins and Schall 2002). Furthermore, *Haemoproteus* parasites may not be monophyletic (Perkins and Schall 2002). Galapagos Doves exhibited the highest hemoparasite prevalence and parasitemias, though most individuals were sampled one year after the seabirds were. Thus, these differences within and among host species may be attributable to differences in exposure to vectors (Sol et al. 2000), host physiology (constrained by phylogeny or life history) or genetics, parasite factors (strain type or within-host evolutionary dynamics), environmental conditions, or a combination of these and other factors (Goater and Holmes 1997).

The pathogenicity of these parasites in the seabirds and doves sampled, or the effects on host fitness or reproductive success, are unknown. However, within Great Frigatebirds, birds infected with *Haemoproteus* parasites exhibited significantly higher heterophil-to-lymphocyte concentration ratios than uninfected birds. In chickens, this ratio increased when birds were exposed to social stress or corticosterone in feed, and it is thus considered to be a reliable indication of environmental stress (Gross and

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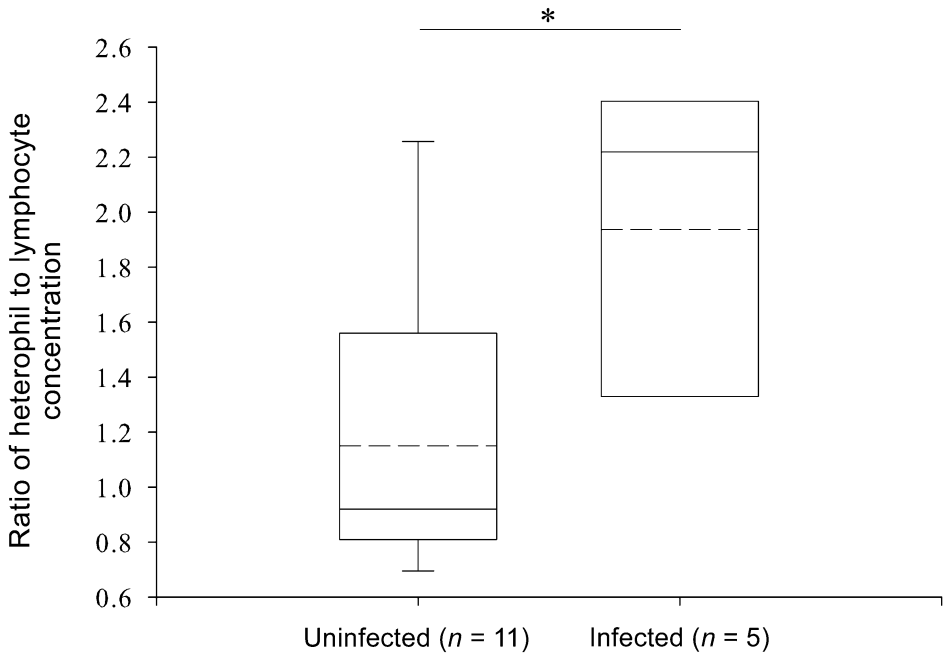

FIG. 2. Boxplot showing mean (dotted line within box), median (solid line within box), 25th and 75th percentiles (lower and upper box limits), and 5th and 95th percentiles (whiskers) for the ratio of heterophil to lymphocyte concentration from Great Frigatebirds on Isla Genovesa, Galápagos (standard differentials from thin smears were transformed into concentrations by multiplying the total white-blood-cell values (WBC × 1,000 μL^{-1}) with the differential value, divided by 100). Mean ratios of heterophils to lymphocytes were significantly higher in birds positive for *Haemoproteus* infection than in birds negative for *Haemoproteus* infection (U = 9, two-tailed P = 0.036, n = 16).

TABLE 3. Plasma phosphorus (mg dL^{-1}) values reported by sex of four seabird species sampled in 2003 inhabiting a multispecies seabird colony on Isla Genovesa, Galápagos Islands.

Male	Female	P
Great Frigatebird (*Fregata minor*)		
4.9 ± 1.69	4.5 ± 1.21	0.9
n = 14	n = 10	
Red Footed Booby (*Sula sula*)		
8.6 ± 4.12	13.3 ± 3.93	0.03
n = 10	n = 10	
Nazca Booby (*S. granti*)		
4.6 ± 0.66	6.8 ± 3.56	0.0002
n = 13	n = 10	
Swallow-Tailed Gull (*Creagrus furcatus*)		
1.7 ± 1.10	8.4 ± 5.40	0.9
n = 8	n = 6	

Siegel 1983). Alternatively, this finding could simply be a consequence of a direct immune response to malarial pathogens. Although only correlational, our finding of significantly higher heterophil-to-lymphocyte concentration ratios in infected Great Frigatebirds is notable, and the direction of causality should be investigated. Work and Rameyer (1996) did not find significant differences in blood chemistries between infected and uninfected Great Frigatebirds in Hawaii, though there were no significant differences in *Haemoproteus* infection prevalences between Hawaii and Galápagos, and parasitemias were relatively low in both studies.

In general, hemoparasites have been considered rare in wild populations of seabirds (Greiner et al. 1975, Peirce 1981, Bishop and Bennett 1992, Bennett et al. 1994, Jovani et al. 2001). More recent reports, however, show that prevalences of hemoparasites can be quite high in some wild populations of seabirds (e.g. gulls: Esparza et al. 2004, Ruiz et al. 1995, Martínez-Abraín et al. 2002; frigatebirds: Work and Rameyer 1996, 1997), whereas they are still rare in other groups (e.g. penguins: Jones and Shellam 1999a, b). A previous study of another Galápagos seabird, the Waved Albatross, on the island of Española, showed no evidence of hemoparasites

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(Padilla et al. 2003), though *Haemoproteus*-like organisms are extremely common in Galapagos Doves on the same island (Padilla et al. 2004). Relatively long embryonic development periods of seabirds (Ricklefs 1992) and the relative paucity of competent vectors in marine environments (Jovani et al. 2001) were proposed as hypotheses explaining the general absence of blood parasites in seabirds. Interestingly, Great Frigatebirds have an extremely long, 57-day incubation period and provide parental care for one year after hatching (Dearborn et al. 2001); yet *Haemoproteus* parasites have been reported from at least three populations of Great Frigatebirds, though infection intensities were relatively low in Hawaii and Galápagos (Hawaiian Islands: Work and Rameyer 1996; Galápagos Islands: present study).

Haemoproteus parasites can be vectored by hippoboscid flies and ceratopogonid midges (Atkinson 1991). Although no ceratopogonids were reported from Hawaii, several species occur within the Galápagos Islands (B. J. Sinclair pers. comm.). Given the absence of ceratopogonids in Hawaii, Work and Rameyer (1996) speculated that the *Haemoproteus* present within Great Frigatebirds was likely vectored by *Olfersia* hippoboscid species that associated with Pelicaniform birds and that they observed on Great Frigatebirds. *Olfersia* species are also present in the Galápagos, and *O. aenescens*, which is associated with Pelicaniformes, was collected in light traps on Genovesa during the 2004 sampling. Galapagos Doves also harbor a species of hippoboscid (*Microlynchia galapagoensis*), and hippoboscids are often restricted to a few host families or orders (Maa 1963). Thus, presently it is impossible to implicate either hippoboscids or ceratopogonids as vectors of *Haemoproteus* among the four bird species infected on Genovesa. Further characterization and differentiation of the Galápagos seabird hemoparasites through molecular techniques (e.g. Bensch et al. 2000, Schrenzel et al. 2003) and long-term studies will help illuminate the biology of these hemoparasites and their ecological implications at the population level and may reveal whether these birds share hemoparasite lineages and vectors. Great Frigatebirds exhibit high philopatry to nesting sites, but travel thousands of kilometers from their nesting islands (Dearborn et al. 2003), creating opportunities for transmission of vectors and *Haemoproteus* between individuals during these long-distance movements. Therefore, placing *Haemoproteus* lineages from Galápagos and Hawaiian populations of Great Frigatebirds and the *Haemoproteus* lineages from the two other Genovesa seabirds in a broader phylogenetic context is warranted.

When compared with hematologic parameters published for other adult free-ranging Red-footed Boobies and Great Frigatebirds, the ranges of total WBC counts are comparable (Work 1996, 1999). We observed slightly higher PCV values and slightly lower total WBC counts in Great Frigatebirds, but our values were comparable to previously published biological ranges for that species in the wild (Work 1996). Female gulls and the two sulid species tested had higher phosphorus and calcium levels than males, but this was only significant for phosphorus. Work (1999) observed higher phosphorus values in breeding female Brown Boobies (*S. leucogaster*) than in males of that species, which is consistent with our findings, though we did not differentiate breeding from nonbreeding females at time of sampling. Reproductive status, dietary preferences, or different activity levels can be speculated as explanations for these differences. This relationship was absent in Great Frigatebirds in both studies and has not been observed in other Pelicaniformes. In general, plasma-chemistry values were comparable to those published in the literature for other species of the same taxa.

The absence of *C. psittaci* antigen in all the birds tested is notable, and the absence of *C. psittaci* antibodies by elementary body agglutination (EBA) suggests that none of these birds had active clinical infections. In a previous study on Galapagos Doves, *C. psittaci* was present only in doves from Isla Española (Padilla et al. 2004), which, like Genovesa, contains large congregations of colonial seabirds. Charadriiforms (gulls and terns) have been reported as commonly infecting free-living birds (Brand 1989), but few studies have documented *C. psittaci* infections in wild seabirds (Franson and Pearson 1995).

The present study presents baseline health parameters for a free-ranging colony of several species of seabirds in the Galápagos Islands. The most notable finding is the presence of hemoparasites in three of the seabird species surveyed and in a sympatric terrestrial endemic bird, along with associated signs of physiological stress in *Haemoproteus*-infected Great Frigatebirds.

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Further characterization of these hemoparasites, as well as long-term population studies, are suggested to understand the implications of these findings for the protection and conservation of avifauna of the Galápagos Islands.

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