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WEIGHTS, HEMATOLOGY, AND SERUM CHEMISTRY OF SEVEN SPECIES OF FREE-RANGING TROPICAL PELAGIC SEABIRDS

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ABSTRACT: I established reference values for weight, hematology, and serum chemistry for seven species of free-ranging Hawaiian tropical pelagic seabirds comprising three orders (Procellariiformes, Pelecaniformes, Charadriiformes) and six families (Procellariidae, Phaethontidae, Diomedeidae, Sulidae, Fregatidae, and Laridae). Species examined included 84 Hawaiian dark-rumped petrels (*Pterodoma phaeopygia*), 90 wedge-tailed shearwaters (*Puffinus pacificus*), 151 Laysan albatrosses (*Diomedea immutabilis*), 69 red-footed boobies (*Sula sula*), 154 red-tailed tropicbirds (*Phaeton rubricauda*), 90 great frigatebirds (*Fregata minor*), and 72 sooty terns (*Sterna fuscata*). Hematocrit, total plasma solids, total and differential white cell counts, serum glucose, calcium, phosphorus, uric acid, total protein, albumin, globulin, aspartate aminotransferase and creatinine phosphokinase were analyzed. Among and within species, hematology and chemistry values varied with age, sex, season, and island of collection. Despite this variation, order-wide trends were observed.

Key words: Hawaiian dark-rumped petrel, *Pterodoma phaeopygia*, wedge-tailed shearwater, *Puffinus pacificus*, Laysan albatross, *Diomedea immutabilis*, red-footed booby, *Sula sula*, red-tailed tropicbird, *Phaeton rubricauda*, great frigatebird, *Fregata minor*, sooty tern, *Sterna fuscata*, hematology, serum chemistry, body weight.

INTRODUCTION

Clinical chemistry and hematology are valuable adjuncts in the diagnosis of disease in animals (Campbell, 1994; Hochleithner, 1994). Reference values for hematology and clinical chemistry are available for a variety of species. In birds, reference hematology and blood chemistry values are most numerous for groups that are managed intensively in production and clinical settings. Examples include psittacines, raptors, and poultry (Campbell, 1994; Hochleithner, 1994). Reference values are less numerous for many captive or free-ranging species outside these groups.

Most literature on hematology and blood chemistry of seabirds covers antarctic birds (Hawkey et al., 1989; Rosa et al., 1993) or temperate coastal seabirds (Balasch et al., 1974; Wolf et al., 1985; Melrose and Nicol, 1992), and some of these involve either small (<10) sample sizes or limited information. There are metabolic studies on arctic pelagic seabirds (Myrcha and Kostelecka-Myrcha, 1980; Kostelecka-Myrcha, 1987); however, few of these present comprehensive hematology and blood chemistry values. There is a dearth of data on hematology and chemistry of tropical pelagic seabirds. Because of their gregarious breeding habits (Harrison, 1990), pelagic seabirds are susceptible to epizootics. In such cases, reference values would be a valuable aid in elucidating cause of disease. These values may also prove useful in the clinical management of captive seabirds during anthropogenic catastrophes such as oil spills.

I present reference clinical chemistry and hematology for seven species of free-ranging pelagic seabirds in Hawaii comprising three orders (Procellariiformes, Pelecaniformes. Charadriiformes) and six families (Procellariidae, Diomedeidae, Phaethontidae, Sulidae, Fregatidae, and Laridae). Where suitable, reference values were compared by season or site of collection, age, and sex.

MATERIALS AND METHODS

All seabirds were sampled from healthy colonies monitored continuously by biologists from the U. S. National Park Service, U. S. Fish and Wildlife Service, U. S. Department of Defense, or State of Hawaii Department of Fish and Wildlife. These biologists had not observed catastrophic reproductive failure at the

time of sampling or during the two previous breeding seasons. I sampled three species of procellariiforms including Hawaiian dark-rumped petrels (*Pterodoma phaeopygia*), wedge-tailed shearwaters (*Puffinus pacificus*), and Laysan albatross (*Diomedea immutabilis*); three species of pelecaniforms including red-footed boobies (*Sula sula*), red-tailed tropicbirds (*Phaeton rubricauda*), great frigatebirds (*Fregata minor*); and one charadriiform, sooty terns (*Sterna fuscata*).

Twenty-one and 35 Hawaiian dark-rumped petrels chicks were sampled from Haleakala National Park on Maui (20°43′N, 156°15′W) in October 1993 and October 1994, respectively, and 28 adults were sampled from the same site in July 1994. Forty-five wedge-tailed shearwater chicks and 45 adults were sampled on August and October 1994, respectively, from offshore islets along southeastern Oahu including west and east Makoluas and Popoia (21°23′N, 157°43′W). Twenty and 16 Laysan albatross adults were sampled in October 1993 from Laysan Island (25°46′N, 171°44′W) and Tern Island-French Frigate Shoals (23°45′N, 166°10′W), respectively, and 43 adults were sampled from Midway atoll (28°13′N, 177°22′W) in June 1994. I sampled 41 Laysan albatross chicks from Midway in June 1993, and 15 and 16 Laysan albatross chicks from Midway and Kilauea Point, Kauai (22°13′N, 159°26′W) respectively, in June 1994.

I sampled 35 and 34 red-footed booby adults and chicks, respectively, from Kanehoe Marine Corps Air Station on Oahu (21°27′N, 157°44′W) in August 1994. Fifty-three red-tailed tropicbirds adults were sampled from Tern Island in March 1994, and 36 adults and 65 chicks from Johnston Atoll (16°45′N, 169°31′W) in April 1995. I sampled 60 and 30 great frigatebirds from Laysan Island and Tern Island, respectively, in March 1994. Thirty-five sooty tern chicks and 37 adults were sampled from Johnston Atoll in July 1995.

Birds were classified as adults or chicks and adult frigatebirds as male or female based on plumage (Pratt et al., 1987). Lack of sexual dimorphism precluded classification by sex of tropicbirds, boobies, terns, albatrosses, shearwaters, and petrels. Petrels were classified as chicks or adults based on date of collection and presence or absence of juvenile plumage (Simons, 1985).

All birds were captured manually or with large hand-held hoop nets. Petrels were captured with double-door wire box traps (Simons, 1985). Petrels and tropicbirds were weighed to the nearest 10 g with a 1 kg spring scale, frigatebirds and albatrosses to the nearest 100 g with a 5 kg spring scale, boobies to the nearest 50 g with a 2.5 kg spring scale, and terns and shearwaters to the nearest 1 g with a 500 g spring scale. I assessed all birds visually and chose only healthy animals, defined as those individuals that were in good flesh, bright, alert, resistive to restraint, and with no detectable morphologic or behavioral abnormalities.

Five ml of blood was procured from the cutaneous ulnar vein in frigatebirds, albatross, boobies, and tropicbirds using 5 ml syringes and 20 gauge 26 mm needles. Three ml of blood were obtained from similar sites in petrels, terns, and shearwaters using 3 ml syringes and 22 gauge 26 mm needles. One half ml of blood was placed in 500 μl ethylenediaminetetraacetate (EDTA) tubes and the remainder in 5-ml clotting tubes. Whole blood in EDTA was stored at 4 C for up to 8 hr prior to processing for hematology. The remaining blood was allowed to clot for 12 hr at 27 C, centrifuged, serum decanted into 1.5 ml cryovials (Corning, Corning, New York, USA), and frozen at −20 C.

For albatrosses from Midway, an extra 1 ml of blood was stored in EDTA and analyzed for lead (Fernandez and Hilligoss, 1982) at the National Wildlife Health Center, Madison, Wisconsin (USA). Only healthy birds with blood lead levels at or below the limit of detection (0.02 μg/ml) were included in the study.

Combined heterophil and eosinophil counts were done in the field with a Neubauer hemocytometer, eosinophil unopettes no. 5877 (Becton Dickinson, Rutherford, New Jersey, USA) and a microscope. Total white cell counts ($10^3/\mu l$) were then calculated (Campbell, 1994). I obtained hematocrit values by spinning whole blood in heparinized capillary pipettes in a microhematocrit centrifuge for 5 min (Campbell, 1994). Plasma total solids were estimated using a temperature-adjusted refractometer (Schuco, American Caduceus Industries, Carle Place, New York) (Campbell, 1994). Blood smears from EDTA blood were made in duplicate, allowed to air dry, stored in slide boxes and returned to the laboratory for staining and microscopic examination.

Prior to conducting differentials, blood smears for each species were pooled, randomized, and read blind. For differential counts, I stained smears using Difquick (Fisher Scientific, Pittsburgh, Pennsylvania, USA) and counted 200 white cells under oil immersion (1,000×). Cells were classified as heterophils, eosinophils, lymphocytes, monocytes, or basophils based on morphology and staining characteristics (Campbell, 1994). Percentages were calculated and multiplied by the total white count to obtain absolute counts of each white cell type

(Campbell, 1994). I recorded presence or absence of hemoparasites.

Serum chemistry values including total protein (g/dl), albumin (g/dl), uric acid (mg/dl), calcium (mg/dl), phosphorus (mg/dl), aspartate aminotransferase (AST) (IU/L), creatinine phosphokinase (CPK) (IU/L), and glucose (mg/dl), were determined at the University of Wisconsin School of Veterinary Medicine in Madison, Wisconsin, using a Kodak Ektachem 500 analyzer (Eastman Kodak, Rochester, New York). This system is a dry analyzer that uses slides on which the sample is placed. The slides consist of a dry multilayer analytic element on a polyester support containing specific reagents for each analyte. Globulin was calculated by subtracting albumin from total protein. Serum chemistries were analyzed blind.

Hematology and blood chemistry values were summarized using means, standard deviations, medians, and ranges. Chemistry values from hemolyzed serum or analytes which were below the limit of detection were excluded from analysis. I compared summarized hematology and serum chemistry parameters using one way analyses of variance (ANOVA) for groups of three or more, or *t*-tests for pairs. To adjust for test-wise error and maintain an experiment-wise error rate of 0.05, I used a sequential Bonferroni adjustment (Rice, 1989) for α; $\alpha = 0.003$ for $n = 18$, the total number of parameters compared including weight, hematology and chemistry. In cases where assumption of normality or equal variance were violated, a Kruskall-Wallis ANOVA or the Mann-Whitney U test for multiple or pair-wise comparisons, respectively, were used. In cases of significant differences with ANOVA, multiple pair-wise comparisons were done using Student-Newman-Keuls and Dunn's test for parametric and non-parametric procedures, respectively (Daniel, 1987). Statistical tests were done using Sigmastat (Jandel Scientific Software, San Rafael, California, USA).

To determine age differences, I compared chicks versus adults for petrels, shearwaters, terns, boobies, and tropicbirds collected from Johnston in 1995, and albatrosses collected from Midway in 1994. Group-wise comparisons for great frigatebird males, females and chicks were done separately for Tern Island and Laysan Island. I also compared individuals from the following groups to see if they could be pooled: petrel chicks collected in 1993 and 1994; albatross chicks from Midway in 1993, 1994 and from Kauai in 1994; albatross adults from Midway in 1994, Laysan Island, and Tern Island in 1993; red-tailed tropicbird adults from Tern Island in 1993 and Johnston Atoll in 1995. Great frigatebird males, females, and chicks were compared separately between Tern Island and Laysan Island.

RESULTS

In all species, heterophils were larger than red cells, had a dense homogenous, dark, blue-purple segmented nucleus, and a clear cytoplasm packed with masses of brick-red fusiform granules that partially obscured the nucleus. Basophils were smaller than heterophils or eosinophils, had a round, dense, homogenous, unsegmented, purple-blue nucleus, and clear cytoplasm with many coarse to fine purple round granules. Lymphocytes were variably sized with a round purple-blue coarsely textured nucleus surrounded by homogenous blue cytoplasm with well-defined borders. Monocytes were the largest white cell with an ameboid, finely granular blue-purple nucleus surrounded by a pale ground-glass blue wispy cytoplasm with ill-defined borders and occasional variably-sized vacuoles.

Eosinophils were similar in size to heterophils with a segmented nucleus; however, granule morphology varied among species. Eosinophil cytoplasm of shearwaters, terns, and petrels contained densely packed, orange, round, plump granules. Albatross eosinophils contained loosely packed tiny round bright red-orange granules while granules of booby eosinophils were somewhat larger and brighter orange. Great frigatebird eosinophils contained loosely packed, plump, elliptical, orange granules distributed among variably sized clear vacuoles. Tropicbird eosinophils had a clear to blue cytoplasm containing sparse numbers of amorphous to round, large, dull-orange granules interspersed with numerous small, clear, well defined vacuoles. Thrombocytes were about 75% as large as red cells, ovoid, with a uniformly dense purple-blue nucleus and a clear to light blue cytoplasm.

In all species, red cell morphology was uniform and polychromasia variable. The only hemoparasite seen was an intracellular organism in frigatebird red cells with

TABLE 1. Reference weights, hematology and serum chemistry of Hawaiian dark rumped petrels chicks and adults from Maui.

	Chicks				Adults			
	Mean	SD	Range	*n*	Mean	SD	Range	*n*
Weight (g)	495[a]	62	390–640	53	432	32	380–500	28
Hematology								
Hematocrit (%)	49	5	34–58	56	49	4	42–59	28
Total solids (g/dl)	3.6[a]	0.4	2.8–4.6	56	4.1	0.6	3.1–5.4	28
Lymphocyte ($10^3/\mu l$)	13.90[a]	10.64	1.28–58.61	55	4.36	2.59	1.12–11.94	27
Heterophil ($10^3/\mu l$)	2.31[a]	1.26	0.21–6.02	55	3.39	1.50	1.00–6.98	27
Monocyte ($10^3/\mu l$)	0.09	0.16	0.00–0.77	55	0.10	0.11	0.00–0.46	27
Eosinophil ($10^3/\mu l$)	0.68[a]	0.58	0.09–2.73	55	1.99	1.24	0.50–5.45	27
Basophil ($10^3/\mu l$)	1.16	0.68	0.18–3.26	55	1.11	0.53	0.22–2.06	27
Total white cells ($10^3/\mu l$)	18.14[a]	11.09	5.16–65.12	55	10.94	3.46	5.03–19.13	27
Serum chemistry								
Glucose (mg/dl)	316	35	222–375	28	329	43	256-405	21
Calcium (mg/dl)	10.1[a]	0.8	8.5–12.2	28	7.0	1.6	3.1–9.4	21
Phosphorus (mg/dl)	3.6[a]	1.3	1.8–8.3	28	0.8	0.3	0.5–1.3	14
Uric acid (mg/dl)	2.2[a]	0.6	1.2–3.5	28	7.3	4.4	1.9–14.1	21
Protein (g/dl)	2.7[a]	0.9	1.7–6.5	28	3.1	0.5	2.4–4.5	21
Albumin (g/dl)	1.3[a]	0.4	1.0–3.3	28	1.5	0.2	1.2–1.8	21
Globulin (g/dl)	1.3[a]	0.5	0.7–3.2	28	1.7	0.4	1.1–2.7	21
Aspartate amino-transferase (IU/L)	106[a]	28	71–169	28	212	116	104–667	21
Creatinine phosphokinase (IU/L)	114[a]	90	23–344	23	46	16	23–72	10

[a] Value significantly different than value for same parameter among adults ($P < 0.003$).

morphology compatible to that of *Haemoproteus* spp.

There were no significant differences between petrel chicks collected in 1993 and 1994, so their values were pooled. Compared to chicks, adult petrels had significantly lower weight, lymphocyte and total white cell count, calcium, phosphorus, and creatinine phosphokinase (CPK) concentrations, but significantly greater total solids, heterophil and eosinophil counts, uric acid, total protein, globulin, albumin, and aspartate amino-transferase (AST) concentrations (Table 1).

Shearwater adults had significantly lower weight, lymphocyte and total white cell count, calcium, phosphorus, albumin, and CPK concentrations, but significantly greater hematocrit, heterophil and monocyte count, glucose, uric acid, globulin, and AST concentrations than chicks (Table 2).

Adult albatrosses from Midway in 1994 had significantly lower total solids, lymphocyte and total white counts, calcium, phosphorus, and globulin concentrations, but significantly greater hematocrits, eosinophil counts, and glucose, uric acid, and AST concentrations than chicks from Midway in 1994 (Tables 3, 4).

Adult boobies had significantly lower lymphocyte and total white cell counts, but significantly greater weight, hematocrit, heterophil and eosinophil counts, and AST concentrations than chicks (Table 5).

Compared to chicks, adult tropicbirds from Johnston had significantly lower lymphocyte, eosinophil, and total white counts, and calcium and phosphorus concentrations, but significantly greater hematocrits and total solids (Table 6).

On Tern Island and Laysan Island, great frigatebird adult males and females had significantly greater hematocrits than chicks. On Tern Island only, females weighed significantly more than chicks or

TABLE 2. Reference weights, hematology and serum chemistry of wedge tailed shearwater chicks and adults from Oahu.

	Chicks				Adults			
	Mean	SD	Range	*n*	Mean	SD	Range	*n*
Weight (g)	467[a]	50	365–590	45	390	35	315–450	45
Hematology								
Hematocrit (%)	37[a]	3	30–43	45	48	3	39–53	45
Total solids (g/dl)	3.7	0.3	3.0–4.4	45	3.9	0.4	3.0–4.8	45
Lymphocyte ($10^3/\mu l$)	26.04[a]	13.49	7.61–70.05	45	12.93	6.21	3.49–27.42	45
Heterophil ($10^3/\mu l$)	2.49[a]	0.95	0.91–5.05	45	4.00	2.09	0.57–10.47	45
Monocyte ($10^3/\mu l$)	0.15[a]	0.19	0.00–0.77	45	0.45	0.48	0.00–2.05	45
Eosinophil ($10^3/\mu l$)	0.74	2.21	0.00–14.81	45	0.48	0.51	0.00–1.95	45
Basophil ($10^3/\mu l$)	0.34	0.44	0.00–2.68	45	0.31	0.31	0.00–1.05	45
Total white cells ($10^3/\mu l$)	29.75[a]	13.93	9.45–76.56	45	18.18	7.17	5.03–34.32	45
Serum chemistry								
Glucose (mg/dl)	202[a]	23	145–255	45	248	28	202–328	42
Calcium (mg/dl)	11.8[a]	1.2	7.8–13.8	45	8.8	1.4	3.8–10.5	42
Phosphorus (mg/dl)	8.8[a]	2.0	5.2–13.9	44	1.6	0.9	0.5–3.6	35
Uric acid (mg/dl)	6.2[a]	4.3	1.2–15.8	45	10.2	5.1	2.7–24.5	42
Protein (g/dl)	3.2	0.4	2.6–4.2	45	3.4	0.4	2.7–4.2	42
Albumin (g/dl)	1.7[a]	0.2	1.4–2.2	45	1.5	0.1	1.2–1.8	42
Globulin (g/dl)	1.5[a]	0.2	1.2–2.0	45	1.8	0.3	1.4–2.5	42
Aspartate amino-transferase (IU/L)	153[a]	41	95–274	45	258	93	121–544	42
Creatinine phosphokinase (IU/L)	899[a]	899	147–4,473	45	86	69	25–306	35

[a] Value significantly different than value for same parameter among adults ($P < 0.003$).

males. Females had significantly greater calcium concentrations than males which, in turn, had significantly greater concentrations than chicks. On Laysan Island only, adults had significantly greater weights, and total protein, albumin and globulin concentrations, but significantly lower uric acid concentrations than chicks. Female adults had significantly greater total solids, and calcium and phosphorus concentrations, than either males or chicks. Male adults had significantly lower total solids and phosphorus concentrations than chicks (Tables 7, 8).

Sooty tern adults had significantly lower weights, lymphocyte and total white counts, and calcium, phosphorus, and CPK concentrations, but significantly greater hematocrits and AST concentrations than chicks (Table 9).

Great frigatebirds of all age and sex groups from Laysan Island had significantly greater glucose concentrations than corresponding groups on Tern Island. Great frigatebird adult males and chicks from Laysan had significantly lower phosphorus concentrations than corresponding groups on Tern (Tables 7, 8). Laysan albatross adults from Laysan Island had significantly greater lymphocyte counts, glucose and phosphorus concentrations, but significantly lower CPK concentrations than those from Tern Island (Table 3). Albatross chicks from Kauai had significantly lower total solids, phosphorus, total protein, and globulin concentrations than those from Midway in 1994 (Table 4).

Prenesting Laysan albatross adults from Tern and Laysan collected in October 1993 had significantly greater weight, total solids, and albumin concentrations, but significantly lower uric acid concentrations than post-incubation adults from Midway collected in 1994 (Table 3). Albatross chicks from Midway in 1993 could not be pooled with those collected from Midway

TABLE 3. Reference weights, hematology and serum chemistry of Laysan albatross adults from Laysan Island, Tern Island (n = 16) and Midway Atoll 1994 (n = 43).

	Laysan Island				Tern Island			Midway Atoll 1994		
	Mean	SD	Range	n	Mean	SD	Range	Mean	SD	Range
Weight (kg)	3.0[a]	0.2	2.6–3.5	20	2.9[a]	0.3	2.0–3.2	2.8[b]	0.3	2.3–3.5
Hematology										
Hematocrit (%)	39	3	30–44	20	39	4	28–44	37	6	22–47
Total solids (g/dl)	5.7[a]	0.7	4.8–7.6	20	5.3[a]	0.9	4.2–8.3	4.4[b]	0.6	3.2–6.0
Lymphocyte ($10^3/\mu l$)	3.80[a]	1.19	1.66–6.01	20	6.54[b]	2.36	2.84–10.34	4.61[a]	3.19	1.37–19.36
Heterophil ($10^3/\mu l$)	10.21	3.31	6.55–19.79	20	12.61	4.11	7.20–19.34	9.35	3.74	4.31–24.24
Monocyte ($10^3/\mu l$)	0.02	0.04	0.00–0.13	20	0.07	0.16	0.00–0.41	0.00	0.00	0.00–0.00
Eosinophil ($10^3/\mu l$)	4.40	2.28	1.33–10.71	20	4.98	2.26	0.83–8.50	7.34	4.19	0.96–20.53
Basophil ($10^3/\mu l$)	1.09	0.46	0.26–2.16	20	1.39[a]	0.55	0.41–2.20	0.89[b]	0.42	0.12–1.79
Total white cells ($10^3/\mu l$)	19.52	4.49	12.69–29.53	20	25.59	6.03	13.84–33.91	22.19	7.31	9.82–42.55
Serum chemistry										
Glucose (mg/dl)	162[a]	34	67–219	17	61[b]	24	35–99	153[a]	46	77–254
Calcium (mg/dl)	11.5	4.0	9.0–24.3	17	11.0	3.9	7.0–23.0	10.2	1.4	7.6–13.9
Phosphorus (mg/dl)	4.3[a]	1.7	2.3–8.5	17	9.2[b]	3.1	6.5–19.2	4.3[a]	2.0	1.1–14.7
Uric acid (mg/dl)	2.5[a]	0.7	1.7–4.1	17	2.1[a]	0.7	1.0–3.5	7.1[b]	4.3	1.3–19.9
Protein (g/dl)	4.9	0.4	4.3–5.7	17	5.0	0.5	4.0–6.4	4.5	0.7	2.9–6.1
Albumin (g/dl)	1.9[a]	0.2	1.6–2.2	17	1.9[a]	0.2	1.5–2.5	1.6[b]	0.3	1.1–2.3
Globulin (g/dl)	3.1	0.3	2.7–3.5	17	3.1	0.3	2.5–3.9	2.9	0.5	1.8–3.8
Aspartate amino-transferase (IU/L)	139	18	114–172	17	147	24	111–197	171	59	105–354
Creatinine phosphokinase (IU/L)	907[a]	495	278–2,151	17	1,051[b]	751	324–2,278	354[b]	271	52–1,365

[a,b] Values across a row with different letters are significantly different ($P < 0.003$).

TABLE 4. Reference weights, hematology and serum chemistry of Laysan albatross chicks from Kauai and Midway Atoll in 1993 and 1994.

	Kauai 1994				Midway Atoll 1994 (n = 16)			Midway Atoll 1993			
	Mean	SD	Range	n	Mean	SD	Range	Mean	SD	Range	n
Weight (kg)	2.8[a]	0.6	1.7–3.9	15	2.9[a]	0.6	1.8–4.0	2.4[b]	0.4	1.6–3.4	40
Hematology											
Hematocrit (%)	32	5	25–38	15	30	6	22–48	30	5	18–38	41
Total solids (g/dl)	4.1[a]	0.7	3.0–5.2	15	5.7[b]	1.2	4.4–8.5	5.2[c]	0.9	3.5–7.2	41
Lymphocyte ($10^3/\mu l$)	25.20	9.77	14.99–42.85	15	30.57[a]	12.31	14.98–60.95	19.86[b]	9.50	2.65–43.22	41
Heterophil ($10^3/\mu l$)	6.68[a]	2.05	3.81–9.98	15	8.55[a]	2.54	4.20–15.13	5.95[b]	4.80	0.92–22.25	41
Monocyte ($10^3/\mu l$)	0.00	0.00	0.00–0.00	15	0.00	0.00	0.00–0.00	0.02	0.09	0.00–0.56	41
Eosinophil ($10^3/\mu l$)	1.24	1.21	0.00–4.49	15	1.71	1.53	0.00–4.53	1.30	1.05	0.00–5.70	41
Basophil ($10^3/\mu l$)	0.77	0.50	0.13–1.74	15	0.61	0.53	0.00–1.66	1.07	1.26	0.00–5.68	41
Total white cells ($10^3/\mu l$)	33.89	10.44	21.26–55.29	15	41.44[a]	12.36	26.10–68.87	28.19[b]	12.52	7.46–58.55	41
Serum chemistry											
Glucose (mg/dl)	136[a]	23	83–175	14	68[b]	45	27–170	116[a]	32	35–168	37
Calcium (mg/dl)	12.2[a]	0.7	11.3–13.5	14	11.5[a]	1.1	10.0–13.3	10.1[b]	1.3	7.2–12.3	37
Phosphorus (mg/dl)	10.7[a]	2.7	7.3–16.5	14	13.8[b]	2.7	9.4–19.1	8.4[c]	3.0	3.2–15.4	37
Uric acid (mg/dl)	3.5	1.7	1.4–7.6	14	3.6	2.0	1.9–8.6	3.4	1.1	1.3–6.7	37
Protein (g/dl)	3.9[a]	0.8	2.7–5.0	14	5.3[b]	1.0	3.9–7.1	4.3[a]	0.8	2.8–5.9	37
Albumin (g/dl)	1.5	0.3	1.1–1.9	14	1.8	0.3	1.4–2.4	1.6	0.3	1.1–2.1	37
Globulin (g/dl)	2.4[a]	0.5	1.5–3.2	14	3.5[b]	0.7	2.5–4.7	2.7[a]	0.6	1.7–4.0	37
Aspartate amino-transferase (IU/L)	98	21	57–133	14	97	21	67–140	105	31	59–191	37
Creatinine phosphokinase (IU/L)	534	266	194–1,146	14	541	387	189–1,689	655	765	76–4,446	36

[a,b,c] Values across a row with different letters are significantly different ($P < 0.003$).

TABLE 5. Referent weight, hematology and serum chemistry of red footed booby adults and chicks from Oahu.

	Chicks				Adults			
	Mean	SD	Range	*n*	Mean	SD	Range	*n*
Weight (kg)	1.0[a]	0.1	0.9–1.2	34	1.1	0.1	0.9–1.4	35
Hematology								
Hematocrit (%)	44[a]	3	37–48	34	48	3	41–54	35
Total solids (g/dl)	3.5	0.4	2.8–4.4	34	3.8	0.5	3.0–4.6	35
Lymphocyte ($10^3/\mu l$)	11.40[a]	8.21	2.32–44.35	34	3.26	1.41	1.11–8.26	35
Heterophil ($10^3/\mu l$)	3.39[a]	1.66	0.26–6.85	34	5.73	2.18	2.52–11.97	35
Monocyte ($10^3/\mu l$)	0.10	0.12	0.00–0.50	34	0.19	0.23	0.00–0.79	35
Eosinophil ($10^3/\mu l$)	0.28[a]	0.29	0.00–0.97	34	0.57	0.40	0.03–1.79	35
Basophil ($10^3/\mu l$)	0.14	0.14	0.00–0.59	34	0.16	0.12	0.00–0.47	35
Total white cells ($10^3/\mu l$)	15.32[a]	8.44	4.40–49.28	34	9.91	2.69	5.27–15.22	35
Serum chemistry								
Glucose (mg/dl)	179	35	94–237	32	196	43	113–293	32
Calcium (mg/dl)	8.2	2.8	3.4–13.2	32	8.5	1.9	4.5–10.9	32
Phosphorus (mg/dl)	7.1	4.5	2.1–20.9	32	5.9	3.5	2.1–12.9	32
Uric acid (mg/dl)	16.2	8.8	3.0–32.7	32	13.8	7.1	3.3–27.8	32
Protein (g/dl)	2.7	0.3	2.1–3.4	32	3.0	0.4	2.3–4.5	32
Albumin (g/dl)	1.2	0.1	1.0–1.5	31	1.3	0.2	1.0–2.0	32
Globulin (g/dl)	1.6	0.2	1.2–1.9	31	1.7	0.3	1.3–2.5	32
Aspartate aminotransferase (IU/L)	302[a]	150	134–922	32	502	163	279–831	32
Creatinine phosphokinase (IU/L)	341	210	22–937	32	256	220	40–1,067	28

[a] Value significantly different than value for same parameter among adults ($P < 0.003$).

in 1994 (Table 4). Similarly, tropicbird adults from Tern collected in 1993 could not be pooled with those collected from Johnston in 1995 (Table 6).

DISCUSSION

Body weights of seabirds in this study were within ranges observed by others (Frings and Frings, 1961; Fleet, 1974; Nelson, 1975, 1978; Flint and Nagy, 1984; Pettit et al., 1984; Simons, 1985). The staining method used for differential counts was adequate to allow distinction of white cell types; for all species, morphology of heterophils, monocytes and lymphocytes was similar to that of other avian species (Campbell, 1994). Variation in morphology of eosinophils has been noted in other species (Hawkey and Dennett, 1989). Often, basophil granules were bleached and looked like those of chickens (Hodges, 1974). Description of the parasite identified as *Haemoproteus* spp., its parasitemia, and prevalence are described by Work and Rameyer (1996).

There was marked variability in hematology and chemistry parameters even when I controlled for age, sex, location, and time of collection. All serum chemistry samples were analyzed at the same laboratory by the same technician, and I read all the differential counts in attempts to eliminate human variation. However, these reference values do encompass several confounders. Although population history was known, individual history was not. Hence, things like inapparent pathologic processes, diurnal variation (Rehder et al., 1982), and sex differences in species lacking sexual dimorphism could contribute to the variability observed here.

Had I not used the sequential Bonferroni adjustment to analyze these data, many more significant differences would have been noted. Rice (1989) pointed out that there was a 95% chance of finding a

TABLE 6. Reference weights, hematology and serum chemistry of red tailed tropicbird adults and chicks from Johnston Atoll and Tern Island in 1994 and 1995.

	Chicks—Johnston Atoll 1995				Adults—Johnston Atoll 1995				Adults—Tern Island 1994			
	Mean	SD	Range	*n*	Mean	SD	Range	*n*	Mean	SD	Range	*n*
Weight (g)	673	87	440–840	65	643	70	540–790	36	677	50	580–785	53
Hematology												
Hematocrit (%)	38[a]	6	30–55	64	51	4	41–60	35	50	4	41–60	52
Total solids (g/dl)	3.6[a]	0.5	2.6–5.2	64	4.0	0.5	3.2–4.8	35	4.0	0.5	2.4–5.0	52
Lymphocyte ($10^3/\mu l$)	6.67[a]	3.07	1.30–15.9	64	2.25	0.93	0.94–5.37	35	1.91	1.02	0.67–5.21	52
Heterophil ($10^3/\mu l$)	3.55	1.44	1.29–7.69	64	2.95	1.11	1.34–5.66	35	3.77	1.40	1.46–10.1	52
Monocyte ($10^3/\mu l$)	0.22	0.22	0.00–1.07	64	0.22[b]	0.17	0.00–0.61	35	0.02	0.05	0.00–0.24	52
Eosinophil ($10^3/\mu l$)	3.45[a]	2.57	0.16–14.8	64	1.06	0.61	0.08–2.98	35	1.29	0.77	0.20–3.55	52
Basophil ($10^3/\mu l$)	0.18	0.15	0.00–0.84	64	0.07	0.09	0.00–0.37	35	0.07	0.07	0.00–0.26	52
Total white cells ($10^3/\mu l$)	14.07[a]	5.86	5.26–34.5	64	6.55	1.81	3.75–12.0	35	7.06	2.47	2.80–17.4	52
Serum chemistry												
Glucose (mg/dl)	233	31	140–294	59	227	30	199–259	3	235	43	117–336	23
Calcium (mg/dl)	8.1[a]	2.8	2.6–14.6	59	5.6	3.0	2.1–7.4	3	7.2	2.3	3.4–10.8	23
Phosphorus (mg/dl)	6.8[a]	2.8	1.6–17.3	59	2.9	1.4	1.3–4.1	3	5.8	3.8	1.4–15.3	23
Uric acid (mg/dl)	16.1	10.1	2.2–37.3	59	6.8	9.9	1.1–18.2	3	4.4	3.6	1.0–13.4	23
Protein (g/dl)	2.4	0.3	2.0–3.1	45	2.9	0.1	2.8–2.9	2	3.3	0.5	2.2–4.4	23
Albumin (g/dl)	1.2	0.2	1.0–1.6	44	1.3	0.0	1.3–1.3	2	1.4	0.2	1.0–2.0	23
Globulin (g/dl)	1.2	0.2	0.9–2.0	45	1.6	0.1	1.5–1.6	2	1.8	0.3	1.2–2.5	23
Aspartate amino-transferase (IU/L)	103	42	50–219	59	119	67	75–196	3	158	109	39–435	23
Creatinine phosphokinase (IU/L)	103	64	28–203	12	NA[c]	NA	NA	0	29	0	0–0	1

[a] Value significantly different than value for same parameter among Johnston Atoll adults ($P < 0.003$).
[b] Value significantly different than value for same parameter among Tern Island adults ($P < 0.003$).
[c] NA, not applicable.

TABLE 7. Reference weights, hematology and serum chemistry of great frigatebird adult females (n = 10), adult males (n = 10) and chicks (n = 10) from Tern Island.

	Adult females			Adult males			Chicks		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Weight (kg)	1.6[a]	0.1	1.3–1.8	1.2[b]	0.1	1.1–1.3	1.3[b]	0.2	1.1–1.6
Hematology									
Hematocrit (%)	52[a]	2	49–55	49[b]	3	43–53	45[c]	4	38–52
Total solids (g/dl)	5.6	2.0	3.6–8.6	3.8	0.5	3.4–4.6	4.1	0.3	3.6–4.6
Lymphocyte ($10^3/\mu l$)	2.81	3.48	0.47–11.82	1.03	0.36	0.66–1.88	1.88	0.91	0.69–3.32
Heterophil ($10^3/\mu l$)	8.01	4.19	3.87–16.18	6.11	2.24	3.96–11.64	6.30	1.93	3.38–9.47
Monocyte ($10^3/\mu l$)	0.07	0.20	0.00–0.63	0.02	0.03	0.00–0.07	0.03	0.05	0.00–0.15
Eosinophil ($10^3/\mu l$)	1.28	0.96	0.52–3.79	1.45	0.77	0.65–2.96	2.47	1.36	0.89–5.43
Basophil ($10^3/\mu l$)	0.09	0.14	0.00–0.37	0.02	0.04	0.00–0.11	0.04	0.06	0.00–0.21
Total white cells ($10^3/\mu l$)	12.25	5.24	4.96–20.23	8.64	2.58	5.74–13.77	10.72	3.39	5.98–16.20
Serum chemistry									
Glucose (mg/dl)	128	29	73–162	118	27	76–167	94	37	36–151
Calcium (mg/dl)	17.7[a]	7.0	9.5–28.8	9.2[b]	1.0	8.1–11.3	8.9[a]	1.1	6.6–10.2
Phosphorus (mg/dl)	13.7	4.9	7.7–23.3	10.5	2.5	6.1–13.5	12.6	2.7	7.6–17.2
Uric acid (mg/dl)	4.5	2.6	1.7–9.7	5.1	2.8	3.0–11.2	13.9	10.1	4.6–26.7
Protein (g/dl)	4.4	0.9	3.4–5.9	3.5	0.5	3.0–4.5	3.6	0.3	3.2–4.0
Albumin (g/dl)	1.8	0.3	1.4–2.2	1.5	0.2	1.3–1.8	1.5	0.1	1.3–1.7
Globulin (g/dl)	2.6	0.6	2.0–3.7	2.1	0.3	1.7–2.7	2.1	0.2	1.8–2.4
Aspartate amino-transferase (IU/L)	268	72	153–399	381	106	247–528	261	50	185–371
Creatinine phosphokinase (IU/L)	233	170	30–612	364	485	10–1,589	184	120	82–424

[a,b,c] Values across a row with different letters are significantly different ($P < 0.003$).

TABLE 8. Reference weights, hematology and serum chemistry of great frigatebird adult females (n = 20), adult males (n = 20) and chicks (n = 20) from Laysan Island.

	Adult females			Adult males			Chicks		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Weight (kg)	1.6[a]	0.2	1.3–2.0	1.3[b]	0.1	1.0–1.5	1.2[c]	0.1	0.9–1.4
Hematology									
Hematocrit (%)	52[a]	4	45–57	51[a]	4	43–57	44[b]	6	28–54
Total solids (g/dl)	6.6[a]	1.6	4.0–10.0	4.3[b]	0.5	3.2–5.2	4.6[c]	0.8	3.2–5.8
Lymphocyte ($10^3/\mu l$)	2.28	1.19	0.33–4.15	1.43	0.63	0.50–2.90	1.91	1.09	0.25–4.45
Heterophil ($10^3/\mu l$)	6.86	2.72	3.04–14.65	6.11	2.36	2.02–12.39	7.06	3.18	1.02–16.40
Monocyte ($10^3/\mu l$)	0.00	0.01	0.00–0.03	0.05	0.09	0.00–0.35	0.02	0.06	0.00–0.23
Eosinophil ($10^3/\mu l$)	1.28	0.73	0.21–2.46	1.41	0.91	0.05–4.04	1.45	0.95	0.36–4.14
Basophil ($10^3/\mu l$)	0.03	0.08	0.00–0.32	0.03	0.04	0.00–0.10	0.05	0.08	0.00–0.23
Total white cells ($10^3/\mu l$)	10.45	4.04	4.73–21.38	9.03	3.35	3.52–17.58	10.49	4.61	1.65–23.42
Serum chemistry									
Glucose (mg/dl)	181	24	134–228	211	28	164–298	208	41	97–274
Calcium (mg/dl)	17.6[a]	6.8	4.3–27.5	8.9[b]	2.2	5.0–15.6	8.0[b]	1.6	4.5–11.1
Phosphorus (mg/dl)	8.7[a]	3.4	3.5–14.6	4.6[b]	1.3	2.8–8.3	5.9[c]	1.6	2.6–8.4
Uric acid (mg/dl)	5.2[a]	6.1	1.8–29.5	4.3[a]	1.4	2.3–8.5	18.7[b]	12.4	3.0–42.3
Protein (g/dl)	4.5[a]	0.6	3.6–6.2	3.7[a]	0.4	3.0–4.4	3.6[b]	0.6	2.2–4.3
Albumin (g/dl)	1.8[a]	0.2	1.5–2.1	1.5[a]	0.1	1.3–1.8	1.4[b]	0.2	1.0–1.7
Globulin (g/dl)	2.7[a]	0.5	2.1–4.2	2.2[a]	0.3	1.7–2.6	2.2[b]	0.4	1.2–2.7
Aspartate amino-transferase (IU/L)	267[a]	97	144–531	328[a]	127	154–624	315[b]	111	166–589
Creatinine phosphokinase (IU/L)	369[a]	199	44–815	235[a]	234	44–905	346[b]	225	44–976

[a,b,c] Values across a row with different letters are significantly different ($P < 0.003$).

TABLE 9. Reference weights, hematology and serum chemistry of sooty tern adults and chicks from Johnston Atoll.

	Chicks				Adults			
	Mean	SD	Range	*n*	Mean	SD	Range	*n*
Weight (g)	212[a]	14	170–245	35	199	12	170–225	37
Hematology								
Hematocrit (%)	40[a]	3	35–50	35	47	3	41–55	36
Total solids (g/dl)	4.1	0.8	2.8–7.4	35	3.9	0.5	2.8–5.0	36
Lymphocyte ($10^3/\mu l$)	23.12[a]	12.08	5.80–58.10	35	14.29	8.13	4.34–41.40	34
Heterophil ($10^3/\mu l$)	7.54	4.32	0.97–21.64	35	5.25	2.65	2.20–14.71	34
Monocyte ($10^3/\mu l$)	0.40	0.56	0.00–2.42	35	0.17	0.17	0.00–0.72	34
Eosinophil ($10^3/\mu l$)	1.67	1.36	0.05–6.50	35	1.23	0.93	0.10–3.94	34
Basophil ($10^3/\mu l$)	0.26	0.23	0.00–0.80	35	0.27	0.21	0.00–0.79	34
Total white cells ($10^3/\mu l$)	32.99[a]	15.58	7.48–80.82	35	21.22	8.99	6.78–49.28	34
Chemistry								
Glucose (mg/dl)	224	44	114–292	24	267	54	201–387	11
Calcium (mg/dl)	12.1[a]	1.5	7.1–14.2	24	9.4	1.1	7.7–11.1	12
Phosphorus (mg/dl)	15.6[a]	4.5	9.3–25.2	24	3.9	1.7	1.6–6.5	12
Uric acid (mg/dl)	10.7	7.9	3.9–29.6	24	12.3	7.3	4.1–28.3	12
Protein (g/dl)	3.6	0.5	2.5–4.5	24	3.3	0.3	2.9–3.8	11
Albumin (g/dl)	1.7	0.2	1.2–2.0	24	1.5	0.2	1.4–1.8	11
Globulin (g/dl)	1.9	0.3	1.3–2.5	24	1.8	0.2	1.5–2.2	11
Aspartate amino-transferase (IU/L)	239[a]	55	125–333	24	522	171	315–928	12
Creatinine phosphokinase (IU/L)	487[a]	237	178–1,317	24	90	65	22–239	9

[a] Value significantly different than value for same parameter among adults ($P < 0.003$).

significant difference between one of 12 pairs of parameters when analyzing each at the 0.05 level of significance. Rice (1989) concluded that using a sequential Bonferroni adjustment to adjust for experiment-wide error of 0.05 was more appropriate when analyzing tables of statistical tests. Also, many investigators (including many cited here), failed to test hematologic or serum chemistry data for normality and adjust their use of parametric or nonparametric statistical tests accordingly. Yet, others have noted that such data do not always follow a normal distribution (Kirkwood et al., 1995).

In spite of inherent analytic and biologic variation, some trends could be discerned across species and orders. Except for petrels, adults of all species studied here had greater hematocrits than chicks. Aside from great frigatebirds, adults of all species tended towards lower lymphocyte and white cell counts and phosphorus concentrations and greater AST concentrations than chicks. Such age differences are not seen in all avian species (Wolf et al., 1985; Abelenda et al., 1993).

For procellariiform birds, juvenile weights were not consistently higher than adults but this may have been due to time of sampling. Procellariiforms chicks gain weight as they age and begin losing it shortly before fledging (Pettit et al., 1984). Laysan albatross chicks in this study were sampled near fledging while shearwaters and petrels were sampled at least 1 mo prior to fledging. Although differences were not always significant, heterophil counts, uric acid, and glucose concentrations appeared to increase with age while calcium concentrations decreased. Hematocrits of Laysan albatross chicks were similar to those reported by Sileo and Fefer (1987). Hematocrits in albatross and wedge-tailed shearwater chicks were lower than those of Manx shearwater chicks

(*Puffinus puffinus*) (Kirkwood et al., 1995). Hematocrits of petrel and shearwater adults were similar to those of Wilson's stormy petrel adults (*Oceanites oceanicus*) (Myrcha and Kostelecka-Myrcha, 1980). When compared to Manx shearwaters chicks (Kirkwood et al., 1995), tropical procellariiform chicks had higher total white, lymphocyte, eosinophil, and basophil counts.

The only trend observed in pelecaniform birds was an increase in hematocrit, total protein, albumin, and globulin concentrations with age. Wolf et al. (1985) found that in brown pelicans (*Pelecanus occidentalis*), hematocrit, globulin, and protein concentration increased with age but albumin concentration decreased. Compared to black-faced cormorant (*Leucocarbo fuscescens*) fledglings (Melrose and Nicol, 1992), tropical pelecaniform chicks had lower monocyte, white cell count, and protein concentration, but greater basophil counts and calcium and phosphorus concentrations. Compared to brown pelican fledglings (Wolf et al., 1985), tropical pelecaniform chicks had lower glucose, protein, albumin, globulin, and AST concentrations, but greater uric acid concentrations. Adult red-footed boobies had lower glucose, protein, and albumin concentrations, but greater hematocrit values compared to captive adult north Atlantic gannets (*Sula bassana*) (Balasch et al., 1974). Compared to captive pelecaniform adults (Balasch et al., 1974; Wolf et al., 1985), tropicbird and frigatebird adults had higher hematocrits.

For unknown reasons, many of the tropicbird adult blood samples from Johnston atoll in 1995 underwent delayed hemolysis while clotting, thus accounting for the low sample size of serum chemistry results in this group. This was not encountered in samples from adults at Tern Island and in other species. I was unable to trace any difference in handling of blood between the two sites. Creatinine phosphokinase values below the limit of detection account for the low samples size of this analyte in several species.

Sooty tern chicks had lower hematocrits, and glucose and uric acid concentrations than other charadriiform chicks such as little auks (*Plautus alle*) (Kostelecka-Myrcha, 1987) and herring gulls (*Larus argentatus*) (Jeffrey et al., 1985). Compared to charadriiform adult terns, skuas and gulls, sooty tern adults had higher hematocrits except for little auks (Balasch et al., 1974; Myrcha and Kostelecka-Myrcha, 1980; Kostelecka-Myrcha, 1987; Rosa et al., 1993). Sooty tern adults had lower protein and albumin concentrations than either adult common black-headed gulls (*Larus ridibundus*) or herring gulls (Balasch et al., 1974). Adult sooty terns also had lower phosphorus concentrations and greater calcium, uric acid, albumin, protein, and AST concentrations than adult MacCormick's skuas (*Catharacta maccormicki*) (Rosa et al., 1993).

Hematology and chemistry values of frigatebirds varied significantly with sex. Most notably, high calcium in adult breeding female frigatebirds has been noted in breeding females of other pelecaniform birds (Wolf et al., 1985). Compared to adult males or female great cormorants (*Phalacrocorax carbo*) (Balasch et al., 1974), adult male or female frigatebirds had higher protein and albumin concentrations and lower glucose concentrations.

Geographic variation also existed and was evidenced by my inability to pool values from frigatebirds or Laysan albatrosses sampled 2 wk apart on Tern and Laysan Islands in 1993, or albatross chicks sampled from Midway and Kauai 2 wk apart in 1994. The short sampling interval between the two islands would presumably rule out seasonal variation (Abelenda et al., 1993). We sampled birds during daylight hours and samples were processed in a similar fashion on all islands. Hence, diurnal variation (Rehder et al., 1982) or variation in sample storage (Hochleithner, 1994) would not be a likely cause. Foraging location or food sources were possible

causes of different blood values between islands. Interestingly, differences between islands were consistent for both frigatebirds and albatrosses in that both species from Laysan island had greater total solids and glucose concentrations than those from Tern island.

In the case of albatrosses with undetermined sex, interisland variation may have been caused by sample bias toward one sex. Also, lead exposure has historically been a problem in albatross at Midway (Sileo and Fefer, 1987) and the contaminant can affect hematologic values in birds (Lumeij, 1985). However, my including only samples from healthy bird with undetectable blood lead should have eliminated this contaminant as a source of variability in hematology and chemistry values of albatrosses.

Seasonal variation could also account for many of the differences seen between prenesting adult albatross on Tern Island and Laysan Island, versus post-incubating adults on Midway. Wolf et al. (1985) noted that in adult brown pelicans, uric acid, total protein, albumin, albumin/globulin ratio, hematocrit, calcium, and phosphorus all increased from the period of June through November, to December through May. They attributed this to a combination of weather, reproductive stage, and molting physiology. Adults foraging to feed chicks on Midway would be more likely to have a full proventriculus and increased serum uric acid (Hochleithner, 1994). It was fairly common to see adults regurgitate when apprehended on Midway whereas this was a rare event on Laysan and Tern. High albumin in adults on Tern island and Laysan island versus Midway could be explained by pre-laying adults (Hochleithner, 1994) and could also explain the higher total solids in the former two groups. Seasonal variation could account for the lower weight of albatross adults on Midway in 1994. Fisher (1967) noted a 24% weight loss in Laysan albatross adults from start of incubation in February until mid-May; this was attributed, in part, to the rigors of parental care. In contrast, heavier adults sampled on Tern and Laysan in October had just returned from the open sea and had not undergone the prolonged stress of incubation and foraging to feed chicks.

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