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Environmental Toxicology and Chemistry, Vol. 26, No. 9, pp. 1896–1903, 2007

Printed in the USA
0730-7268/07 $12.00 + .00

CONTAMINANT-ASSOCIATED ALTERATION OF IMMUNE FUNCTION IN BLACK-FOOTED ALBATROSS (*PHOEBASTRIA NIGRIPES*), A NORTH PACIFIC PREDATOR

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(*Received* 6 *October* 2006; *Accepted* 29 *March* 2007)

Abstract—Environmental pollution is ubiquitous and can pose a significant threat to wild populations through declines in fitness and population numbers. To elucidate the impact of marine pollution on a pelagic species, we assessed whether toxic contaminants accumulated in black-footed albatross (*Phoebastria nigripes*), a wide-ranging North Pacific predator, are correlated with altered physiological function. Blood samples from adult black-footed albatrosses on Midway Atoll, part of the Hawaiian (USA) archipelago, were analyzed for organochlorines (e.g., polychlorinated biphenyls [PCBs] and chlorinated pesticides), trace metals (silver, cadmium, tin, lead, chromium, nickel, copper, zinc, arsenic, selenium, and total mercury), and a sensitive physiological marker, peripheral white blood cell immune function (mitogen-induced lymphocyte proliferation and macrophage phagocytosis). We found a positive significant relationship between organochlorines, which were highly correlated within individual birds ($p < 0.001$, $r > 0.80$, Spearman correlation for all comparisons; PCBs, 160 ± 60 ng/ml plasma [mean ± standard deviation]; DDTs, 140 ± 180 ng/ml plasma; chlordanes, 7.0 ± 3.6 ng/ml plasma; hexachlorobenzene, 2.4 ± 1.5 ng/ml plasma; $n = 15$) and increased lymphocyte proliferation ($p = 0.020$) as well as percentage lymphocytes ($p = 0.033$). Mercury was elevated in black-footed albatrosses (4,500 ± 870 ng/ml whole blood, $n = 15$), and high mercury levels appeared to be associated ($p = 0.017$) with impaired macrophage phagocytosis. The associations we documented between multiple contaminant concentrations and immune function in endangered black-footed albatrosses provide some of the first evidence that albatrosses in the North Pacific may be affected by environmental contamination. Our results raise concern regarding detrimental health effects in pelagic predators exposed to persistent marine pollutants.

Keywords—Black-footed albatross Immune Phagocytosis Lymphocyte proliferation Pollutants

INTRODUCTION

Long-lived, upper-trophic-level species, such as seabirds, marine mammals, and humans, accumulate elevated concentrations of organochlorines and mercury through a marine diet [1–4], but adverse impacts from these nonpoint-source pollutants are rarely documented. Studies that simultaneously measure contaminant exposure and physiological effects in free-ranging wild populations are needed to better understand the extent to which current levels of environmental contamination may be reducing fitness and/or survival of individuals. We examined contaminant body burden concentrations of free-ranging, adult black-footed albatrosses (*Phoebastria nigripes*) and measured selected immune function parameters to investigate if nonpoint-source marine contamination is associated with adverse health effects in a top marine predator.

Albatrosses were chosen to evaluate contaminant effects because they are long-lived, upper-trophic-level seabirds that travel and forage over vast ranges [5] and, thus, likely integrate contaminants distributed across large oceanographic regions. Black-footed albatrosses breed mainly on the Hawaiian archipelago and forage throughout the waters of the North Pacific [6]. Several studies have described two- to fivefold higher organochlorine concentrations in black-footed albatrosses compared to Laysan albatrosses (*Phoebastria immutabilis*) [4,7,8], a closely related North Pacific albatross with similar ecology [9,10]. Finkelstein et al. [7] recently determined that the elevated organochlorine and mercury body burdens in black-footed albatrosses result primarily from regional segregation of their North Pacific foraging areas and that their most probable route of contaminant exposure was via global (i.e., nonpoint source) contamination, a finding supported by Jones et al. [4]. Notably, contaminant concentrations measured in black-footed albatrosses were of comparable magnitude [4] to concentrations associated with reproductive deformities and impaired immune function in birds from the Great Lakes [11,12]. However, the impact of contaminants on black-footed albatross physiological function and, indirectly, potential population dysfunction is unknown.

Wildlife population health is dictated, in part, by a current population's reproductive output, because this output will affect the growth rate of future populations [13]. A correlative relationship between contaminant body burden and lifetime reproductive output can help to indicate if marine contamination is affecting the population growth rate of black-footed albatrosses. However, for species such as black-footed albatross, with a reproductive life span of up to half a century [6],

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measuring an individual's lifetime reproductive output is not logistically feasible. Thus, the first step to determine the potential of marine contamination to affect the population stability of long-lived pelagic species is to determine if physiological processes are affected by contaminant exposure. Unfortunately, measuring physiological changes and relating these changes to contaminant exposure in wild populations is challenging [14]. Wild species are exposed to multiple stressors (e.g., food limitation and predation pressure) and an unknown number of environmental contaminant mixtures that can confound the ability to understand contaminant effects on growth, reproduction, and survival [15–17]. Field studies that measure biological markers of physiological change are thus an important step in elucidating contaminant-induced effects in wild populations [18–20].

Immune function is recognized as a sensitive biological marker to examine the impacts of contaminant exposure in wildlife, because multiple contaminants alter immune function [21], immune dysfunction may be observed in the absence of clinical signs of toxicity [22], and immune dysfunction has detrimental health implications for individuals [14,23,24]. Immune function has thus been used to assess contaminant-induced effects in wild birds [20,25–27], and inappropriate immune responses have been shown to compromise avian fitness [28] as well as reduce long-term survival [29]. Laboratory studies have demonstrated that altered in vitro immune function is indicative of impaired immune ability [23,30,31], and in vitro assays have been used for decades to evaluate avian immune function [32,33].

Our overall objective was to investigate the associations between contaminant exposure and immune function in an upper-trophic-level marine predator, black-footed albatross. Contaminant-induced physiological changes in black-footed albatrosses were examined by measuring in vitro immune function (mitogen-induced lymphocyte proliferation and macrophage phagocytosis) on cryopreserved peripheral white blood cells (WBCs) [34]. Because multiple contaminants have been shown to alter immune function [21], concentrations of 11 trace metals (silver, cadmium, tin, lead, chromium, nickel, copper, zinc, arsenic, selenium, and total mercury), polychlorinated biphenyls (PCBs), and chlorinated pesticides (DDTs, chlordanes, and hexachlorobenzene) were measured in each bird for which immune function was assessed.

MATERIALS AND METHODS

Study area and sample collection

All samples were collected on Midway Atoll (28°13′N, 177°13′W; 1,800 km northwest of Honolulu, HI, USA). Blood samples were collected from 15 banded, adult black-footed albatrosses hand-caught from several locations on the island during the breeding season in May 2001. Seven milliliters of blood were collected from the cutaneous ulnar vein with a 21-gauge, winged collection kit attached to a preheparinized syringe. Following blood sampling, all birds were immediately released and showed no visible signs of distress or harm.

Sample preparation

Immediately after collection, blood was transferred to two heparinized, evacuated glass tubes (Vacutainer®; Fisher Scientific, Pittsburgh, PA, USA) as follows: 1 to 2 ml for metal analysis, and 5 to 6 ml for separation of plasma and WBCs. A small aliquot of blood (~0.25 ml) was placed in a heparinized tube for hematocrit determination. Whole blood for metal analyses was frozen at −20°C until analysis. Blood was kept cool until plasma was separated by centrifugation at 1,000 *g* for 10 min (within ~3 h of collection). Plasma was placed in kilned, glass vials and frozen at −20°C for organochlorine analyses. White blood cells were isolated from the remaining cell fraction on a density gradient and cryopreserved for evaluation of immune function [34].

Contaminant analysis

Plasma organochlorine concentrations were determined using a modification of methods reported by Jarman et al. [35] and Newman et al. [36]. Approximately 1 ml of plasma was spiked with PCB 207 (as a recovery standard), extracted, and fractionated by Florisil® (U.S. Silica Company, Berkeley Springs, WV, USA) chromatography. Both fractions were spiked with an internal instrument standard (to account for volume differences) and analyzed on a Hewlett-Packard 6890 Series II capillary gas chromatograph (Agilent Technologies, Wilmington, DE, USA) utilizing electron-capture detectors. Two columns (length, 60 m; inner diameter, 0.25 mm; film thickness, 0.25 μm; DB-5 and DB-17) provided dual-column confirmation (Agilent Technologies). National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) solutions assured accuracy of the calibration curves. The PCB 207 recoveries were 88% ± 8% (mean ± standard deviation), and all samples were recovery corrected. Polychlorinated biphenyls are reported as the sum of 60 PCB congeners measured. Chlorinated pesticide compounds were measured including DDT and its metabolites, *o,p′*-dichlorodiphenyldichloroethane (DDD), *o,p′*-dichlorodiphenyldichloroethylene (DDE), *o,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE, 1,1-*bis*-(4-chlorophenyl)-2-chloroethylene (*p,p′*-DDMU), *p,p′*-DDT, chlordanes (heptachlor epoxide, oxychlordane, methoxychlor, *cis*-nonachlor, *trans*-nonachlor, *cis*-chlordane, and *trans*-chlordane), and hexachlorobenzene. The DDTs are reported as the sum of DDT compounds measured and chlordanes as the sum of chlordane compounds measured.

Because copper, zinc, nickel, and selenium levels are essential elements, toxicity can occur either from deficiency or excess exposure. Chromium is an essential element as well but is considered to be highly toxic [37]. We assessed total tin concentrations because organic tin compounds (tributyltin and triphenyltin) can bioaccumulate in squid [38], a main component of the black-footed albatross diet [39]. The remaining metals measured (silver, cadmium, lead, arsenic, and mercury) are nonessential and cause toxic effects in animals [40]. Whole-blood concentrations of silver, cadmium, tin, lead, chromium, nickel, copper, zinc, arsenic, and selenium were determined under trace metal–clean, high-efficiency particulate air (HEPA)–filtered air (Class 100; Air Purification Technologies, La Jolla, CA, USA) laboratory conditions using previously published methods [41,42] with the following changes: ^{103}Rh and ^{209}Bi were added to all external standards and samples as internal standards before analyses. Samples were analyzed for multiple elements on a Finnigan MAT-Element 1 double-focusing magnetic sector inductively coupled plasma mass spectrometer (ThermoQuest, San Jose, CA, USA) in low (silver, cadmium, tin, and lead), medium (chromium, nickel, copper, and zinc), and high (arsenic and selenium) resolutions. External standardization for these elements was via certified standards (Spex Industries, Edison, NJ, USA). The analytical detection limits for the above elements were as follows: ^{107}Ag,

Table 1. Black-footed albatross (*Phoebastria nigripes*) contaminant concentrations used to evaluate exposure effects[a]

	Geometric mean	Mean	Standard deviation	Range
Arsenic (ng/ml whole blood)	520	620	400	220–1,600
Cadmium (ng/ml whole blood)	12	13	7.3	8.1–31
Copper (ng/ml whole blood)	260	270	70	180–430
Lead (ng/ml whole blood)	11	13	6.9	5.1–26
Mercury (ng/ml whole blood)	4,400	4,500	870	3,400–6,400
Selenium (ng/ml whole blood)	27,000	29,000	9,700	16,000–49,000
Tin (ng/ml whole blood)	6.0	6.1	1.2	4.0–8.5
Zinc (ng/ml whole blood)	4,100	4,200	290	3,800–4,800
Polychlorinated biphenyls (ng/ml plasma)	150	160	60	100–280
DDTs (ng/ml plasma)	100	140	180	40–780
Chlordanes (ng/ml plasma)	6.3	7.0	3.6	3.1–14
Hexachlorobenzene (ng/ml plasma)	2.1	2.4	1.5	1.2–6.1

[a] $n = 15$.

0.01 ng/ml; ^{110}Cd, 0.08 ng/ml; ^{118}Sn, 0.02 ng/ml; ^{208}Pb, 0.08 ng/ml; ^{52}Cr, 0.24 ng/ml; ^{60}Ni, 0.36 ng/ml; ^{63}Cu, 0.37 ng/ml; ^{66}Zn, 212 ng/ml; ^{75}As, 0.77 ng/ml; and ^{78}Se, 78.5 ng/ml. The NIST Standard Reference Material (SRM) 955 (lead in blood) and SRM 1577 (bovine liver), as well as sample aliquots spiked with known quantities of each element, were used for quality control. Recoveries of all elements were between 90 and 120%, and recoveries from samples run in replicate were within 10% of each other.

Whole-blood samples were analyzed for total mercury concentrations by En Chem (Green Bay, WI, USA). Sample preparation and analyses followed U.S. Environmental Protection Agency SW-846 Method 7471 (Revision 1, 1994) on a PerkinElmer Flow Injection Mercury System 100 (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA). Standard Reference Material (dog-fish liver [DOLT-3]; National Research Council, Ottawa, ON, Canada) and laboratory biological control sample spike recoveries were between 88 and 94%. Sample (albatross blood) spike recoveries were between 104 and 105%, and the difference in recoveries between blind duplicate samples was 1 to 4%.

Contaminants evaluated for relationships with immunophysiology (Table 1) had geometric mean concentrations greater than 1 ng/ml. Concentrations below this value were believed to have little, if any, toxicological importance.

Hematology (hematocrit, WBC counts and differentials)

Hematology variables were assessed to identify diseased individuals and provided important baseline data regarding the health status of each bird sampled. Hematocrit was determined by the microhematocrit method [43], and total WBC counts were determined with the Unopette® (Becton Dickinson, Franklin Lakes, NJ, USA) prepared diluent [44] within 3 h of sample collection. Thin blood smears were prepared within 5 min of sample collection [43]. Slides were sent to a commercial diagnostic laboratory (Antech Diagnostics, Irvine, CA, USA) where they were stained with Wright/Giemsa and evaluated for red blood cell morphology and for the presence of thrombocytes and hemoparasites. A WBC differential [43] also was performed on 100 WBCs. The six measured hematology variables (Table 2) were examined for relationships with contaminant exposure.

Immune function assays

We examined two independent measures of in vitro immune function, lymphocyte proliferation and macrophage phagocytosis. Cryopreserved WBCs were evaluated for mitogen-induced T-lymphocyte proliferation and macrophage phagocytosis according to the methods described by Finkelstein et al. [34]. Cryopreserved WBCs had sufficient viability (74% $\pm$ 6%) and cell numbers ($15 \times 10^6 \pm 6 \times 10^6$ live lymphocytes/ml) to perform immune function tests. Briefly, for mitogen-induced T-lymphocyte proliferation, 100 μl of 3.5×10^6 live lymphocytes/ml were distributed per well in triplicate wells in 96-well plates with either concanavalin A mitogen (Con A; 2.5 and 5.0 μg/well; Sigma, St. Louis, MO, USA) or no mitogen (nonstimulated). Plates were incubated for 48 h (41°C, 5% CO_2), after which the 5-bromo-2′-deoxyuridine (BrdU; Boehringer Manneheim, Indianapolis, IN, USA) labeling agent was added to each well and the plates incubated for an additional 22 h. Cells were harvested, and BrdU label absorbance was measured on an enzyme-linked immunosorbent assay plate reader (model 3550-Ultraviolet; Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The arithmetic mean absorbance (from triplicate wells) percentage of nonstimulated cells from each sample was used for statistical analysis. Macrophage phagocytic capacity was evaluated by adding 50 μl of 1×10^7 opsonized fluorescein isothiocyanate–labeled yeast particles to plated macrophages to obtain an approximately 1:100 ratio of macrophage to yeast

Table 2. Black-footed albatross (*Phoebastria nigripes*) hematology values[a]

	Mean	Standard deviation	Range
Total white blood cells/μl	18,000	3,600	12,000–24,000
% Lymphocytes	30	7	17–44
% Heterophils	65	8	47–75
% Monocytes	3	4	0–15
Heterophil to lymphocyte ratio	2.4	0.9	1.2–4.2
% Hematocrit	40	3	34–44

[a] $n = 15$.

Table 3. Description of variables and principle components (PCs) used in multiple linear regression (MLR) models to assess impacts from contaminant exposure on hematology, phagocytosis, and lymphocyte proliferation in black-footed albatross (*Phoebastria nigripes*)[a]

Category	PC	% Variance explained by PC	Representative variables[b]
Dependent variables			
Hematology	Hematology PC1	53	% Lymphocytes
	Hematology PC2	21	Total white blood cells[c]
Phagocytosis	Phagocytosis PC1	60	1–2, 5–10, >10 yeast
	Phagocytosis PC2	30	3–4 yeast[c]
Proliferation	NA	NA	NA
Independent variables			
Contaminant concentrations	Contaminant PC1	41	Organochlorines
	Contaminant PC2	18	Copper, tin
	Contaminant PC3	13	Mercury[c]
	Contaminant PC4	11	Zinc[c]

[a] Hematology variables are listed in Table 2 and contaminant variables in Table 1. NA = not applicable.
[b] Important in MLR models as defined by a loading score greater than 0.71 or less than −0.71, correlation coefficients between variables, and scatter plots of dependent versus independent variables.
[c] This was the only variable represented by the respective PC; thus, the actual data from this variable, not the PC score, were used in regression models.

cells. Plates were incubated for 15 min at 41°C and 5% CO_2, washed three times with phosphate-buffered saline, placed on ice to stop phagocytosis, and examined under an inverted fluorescent microscope (Axiovert 200; Carl Zeiss MicroImaging, Thornwood, NY, USA) at ×320 magnification. Phagocytosis was quantified by counting the number of yeast cells ingested by all macrophages within predefined portions of a grid with at least 100 macrophages counted per well.

Multiple phagocytic measurements (% macrophages that ingested 1–2, 3–4, 5–10, and >10 yeast) were recorded to investigate subtle differences in phagocytic performance between samples as established by previous studies evaluating in vitro macrophage phagocytosis [34,45,46]. Lymphocyte proliferation responses to either 2.5 or 5 μg/well of Con A were recorded within the same experiment to determine which concentration of Con A achieved a higher assay sensitivity. The proliferation responses to 2.5 and 5 μg/well of Con A were similar and highly correlated ($r = 0.95$, $n = 15$), and the 2.5 μg/well response was used for evaluation of associations with contaminant exposure.

Data analysis

The relationship between contaminants and immunophysiology was examined using principal component multiple regression analysis (PCA) to avoid the confounding effects of colinearity among independent variables (contaminants) [47]. The PCA reduced the number of independent variables to principal components (PCs) (Table 3), which represents a synthetic aggregate of colinear variables. Principal components were used in analyses if their eigenvalues were greater than one [47]. Variables with a PC loading of more than 0.71 or less than −0.71 (the correlation coefficient of the relationship between the component and original variable) were determined to be excellent and exceptionally well represented by the PC [48]. Stepwise multiple-linear-regression (MLR) and nonlinear-regression models were then used to examine the relationships between variables and PC scores. Principal components represented by one variable were replaced by that variable's actual values (i.e., contaminant concentrations) in regression analyses. Interpretation of relationships from MLR model results were guided by each variable's degree of loading on a PC and the correlation coefficients between variables, then confirmed using scatter plots. Data were log transformed when necessary to fit parametric model assumptions. All statistical tests were performed using Systat® (10th ed, 2000; SPSS, Chicago, IL, USA) and an α level of 0.05. Adjustments of p values for multiple comparison tests in ecology and toxicology is debated, because this procedure can inappropriately invalidate relationships that are important and warrant further research [49,50]. For this reason, recent studies that examined relationships between contaminant exposures and biological effects in seabirds did not perform multiple-comparison adjustments [19,51], and we also did not adjust p values for interpretations of significant relationships in the present study.

RESULTS

Contaminant exposure

Black-footed albatross contaminant concentrations varied from two- to sevenfold among individuals with the exception of DDTs, for which a 19.5-fold difference was found between the lowest and highest individual sampled (Table 1). Polychlorinated biphenyls and DDTs comprised 96% (PCBs, 56%; DDTs, 40%) of the organochlorines measured. Oxychlordane and *trans*-nonachlor comprised the majority of the chlordane compounds detected (55 and 39%, respectively). All organochlorine compounds evaluated were highly correlated within individual birds ($p < 0.001$, $r > 0.80$, Spearman correlation, for all comparisons; PCBs, 160 ± 60 ng/ml plasma; DDTs, 140 ± 180 ng/ml plasma; chlordanes, 7.0 ± 3.6 ng/ml plasma; hexachlorobenzene, 2.4 ± 1.5 ng/ml plasma; $n = 15$). Total mercury (4,500 ± 870 ng/ml whole blood), however, was not correlated with the organochlorines measured ($p > 0.20$, $r < 0.29$, Spearman correlation, for all comparisons, $n = 15$), suggesting mercury and organochlorines have different bioaccumulation pathways, toxicokinetic dynamics, and/or sources.

Contaminant associations with immunophysiology

Principal components analysis. The PCA reduced 12 contaminants (Table 1) to four PCs that together explained 83% of the variation of the original 12 variables. Contaminant PC1 was represented by organochlorines, PC2 by copper and tin, and PC3 and PC4 by mercury and zinc, respectively (Table 3). The PCA also reduced six hematology measurements (Table

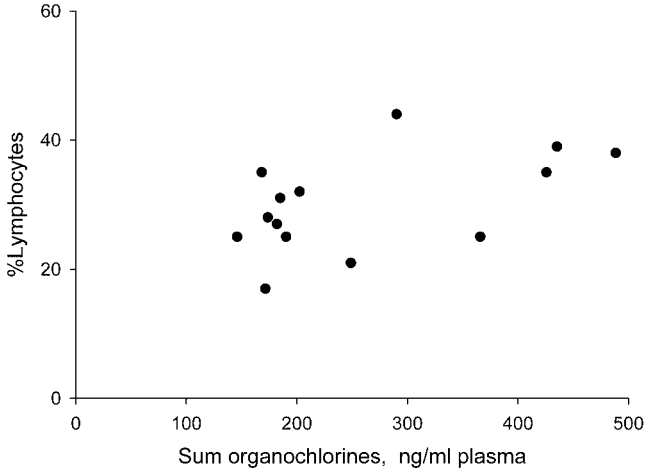

Fig. 1. Black-footed albatross (*Phoebastria nigripes*) organochlorine (polychlorinated biphenyls, DDTs, chlordanes, hexachlorobenzene) concentrations (contaminant principle component [PC] 1) had a significant relationship with percentage lymphocytes (hematology PC2, $t = -2.38$, $r^2 = 0.303$, $p = 0.033$, $n = 15$). A data point was removed from the graph for clarity.

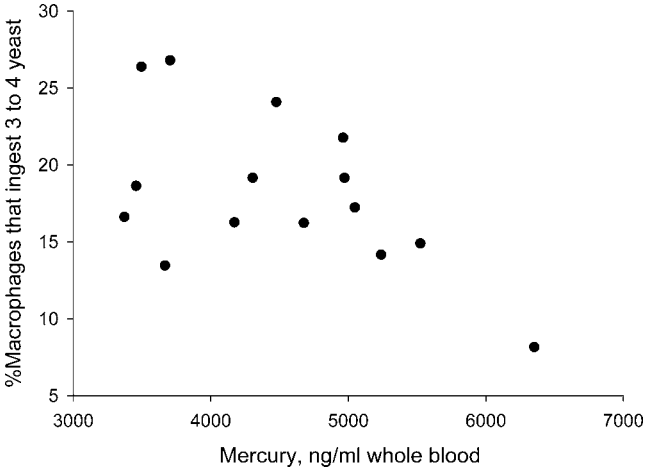

Fig. 2. Blood mercury concentrations had a significant negative association ($t = -2.77$, $p = 0.017$, $n = 15$) with black-footed albatross (*Phoebastria nigripes*) phagocytosis (% macrophages that ingested three to four yeast).

2) to two PCs that together explained 74% of the variation of the original variables. Hematology PC1 represented several colinear (nonindependent) hematological values (% lymphocytes, % heterophils, and heterophil to lymphocyte ratio), whereas PC2 only represented total WBCs. The PCA reduced four phagocytic measurements to two PCs that together explained 90% of the variation of the original variables. Phagocytosis measurements of 1 to 2, 5 to 10, and more than 10 yeast were explained by phagocytosis PC1, whereas measurements of 3 to 4 yeast were represented by phagocytosis PC2.

Principal components represented by one variable were replaced by that variable's actual value in regression analyses. Thus, physiological function (dependent variables: hematology PC1, total WBCs, phagocytosis PC1, three to four yeast category, and lymphocyte proliferation) were evaluated for relationships in stepwise MLR models with contaminant exposure (independent variables: contaminant PC1, contaminant PC2, mercury, and zinc) (Table 3).

Hematology. A stepwise MLR model indicated that contaminant PC1 (organochlorines) had a significant relationship ($t = -2.38$, $r^2 = 0.30$, $p = 0.033$, $n = 15$) with hematology PC1 (% lymphocytes) (Fig. 1). We found no relationship between total WBCs and contaminant exposure.

Phagocytosis. A MLR model containing contaminant PC2 (copper and tin, $t = 2.62$, $p = 0.022$) as well as mercury ($t = -2.77$, $p = 0.017$) explained a significant amount of the variability for the percentage of macrophages that ingested yeast (% macrophages that ingested 3 to 4 yeast, $F_{2,12} = 7.36$, $r^2 = 0.55$, $p = 0.008$). The observed copper and tin concentrations (contaminant PC2) in black-footed albatrosses most likely are too low to be of toxicological concern, but their significant association with mercury and phagocytosis highlights the complexity of understanding the effect of multiple contaminant mixtures on physiological function. The observed negative relationship between mercury and phagocytosis (Fig. 2) may suggest that mercury is associated with immunological changes in black-footed albatrosses. We found no relationship between contaminant exposure and phagocytosis PC1 (% macrophages that ingested 1–2, 5–10, and >10 yeast).

Lymphocyte proliferation. A MLR model determined that contaminant PC1 (organochlorines) had a significant positive relationship with mitogen-induced lymphocyte proliferation ($t = 2.38$, $r^2 = 0.30$, $p = 0.033$, $n = 15$). A graphical representation of the relationship between contaminant PC1 and lymphocyte proliferation indicated that this relationship was best explained by a nonlinear model with the following power equation: $y = a(x^b)$ (95% confidence intervals: $a = 5.65$–6.58, $b = 0.020$–0.21; $r^2 = 0.35$, $p = 0.02$, $n = 15$) (Fig. 3, a constant (2) was added to each data point to ease curve-fitting routines). The asymptotic nonlinearity is plausible given the physiological constraints of the lymphocyte proliferation response.

Health status, age, and gender. Health status, age, and gender were examined for confounding effects on the observed relationships between contaminant exposure and immunophys-

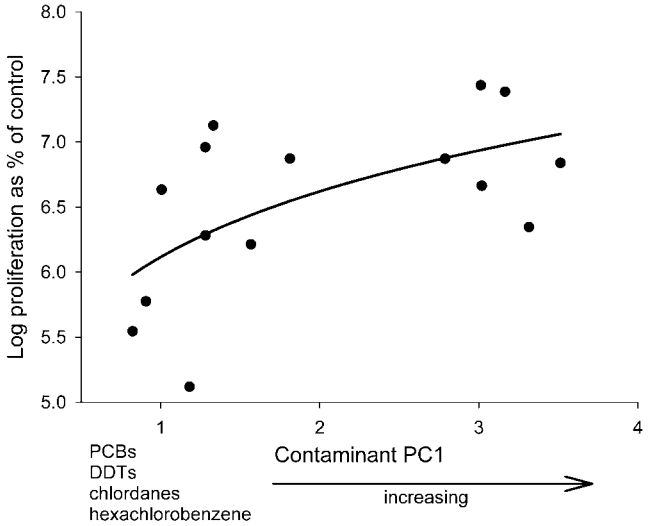

Fig. 3. The significant positive relationship between contaminant principle component (PC) 1 (organochlorines) and lymphocyte proliferation in black-footed albatross (*Phoebastria nigripes*) was best explained by a nonlinear model with the following power equation: $y = a(x^b)$ (95% confidence intervals: $a = 5.65$–6.58, $b = 0.020$–0.21; $r^2 = 0.35$, $p = 0.02$, $n = 15$; a constant (2) was added to each data point to ease curve-fitting routines). PCBs = polychlorinated biphenyls.

iology. All sampled black-footed albatrosses appeared to be in good health and showed no overt signs of sickness or starvation. None of the birds showed evidence of hemoparasites or abnormal red blood cell morphology, and WBC measurements did not indicate acute infection or disease (Table 2). Thus, no evidence was found that health status influenced our results. Of the 15 birds sampled, minimum ages could be assigned to 13 birds using banding data. Birds were divided into two categories of maturity: Breeders (age, six years or older; $n = 9$), and prebreeders (age, three to five years; $n = 4$) [6]. We found no evidence that maturity influenced our findings (analysis of covariance, $p > 0.26$ for all interactions). The sex of all birds was determined using DNA from whole blood (Zoogen Services, Davis, CA, USA). Fourteen birds were male, and one bird was female. The results for the female bird fell in the middle range of all data analyses, suggesting no confounding affect of gender on our results.

DISCUSSION

Our data show that altered lymphocyte proliferation was significantly associated with elevated blood levels of organochlorines. Higher organochlorine concentrations were significantly correlated with increased lymphocyte proliferation and an increased proportion of lymphocytes. The immune and hematological assays both independently demonstrated that organochlorine exposures may be altering physiological processes in black-footed albatrosses. We also found evidence suggesting an association between blood mercury concentrations and decreased macrophage phagocytosis. Our work suggests that contaminant exposure may be affecting the immunological status of black-footed albatross.

Hematology

Organochlorine concentrations were positively associated with a PC representing several related (nonindependent) hematological values (% lymphocytes, % heterophils, heterophil to lymphocyte ratio). Changes in WBC counts with respect to contaminant exposure are not indicative of impaired immune function per se, but combined with other immune measures, such changes provide information regarding an individual's health status. For example, increased ratios of heterophils to lymphocytes are used as a measure of stress in chickens [52], and altered WBC counts have been associated with suppressed avian immune function [26,45].

Macrophage phagocytosis

Macrophage phagocytosis is one of the first lines of defense against pathogens, and it also is important for antigen presentation and subsequent antibody production. Impaired phagocytic ability increases susceptibility to bacterial and parasitic infection [30], and macrophage phagocytosis has been used to assess immunological response to contaminant exposure in wildlife populations [14,53]. Albatross macrophages from cryopreserved peripheral WBCs responded well in our phagocytosis measurements; 76% of macrophages ingested yeast, with the number of yeast particles that macrophages ingested varying across the categories recorded (% macrophages that ingested 1–2 yeast, 33% ± 5%; 3–4 yeast, 18% ± 5%; 5–10 yeast, 20% ± 7%; >10 yeast, 5% ± 4%).

We found that total mercury levels in black-footed albatrosses were negatively associated with phagocytosis, measured as the proportion of macrophages that ingested three to four yeast. However, these results were strongly influenced by one bird (Fig. 2). We cannot explain why this relationship was observed only for the three to four yeast category, and more research is needed to confirm or contradict these initial findings. Associations between mercury and altered immune function may be difficult to evaluate in wild birds; recent studies have found no effect of mercury on immune function in common eiders [54–56]. Nonetheless, mercury is considered to be highly toxic, and both organic and inorganic mercury have been shown to suppress vertebrate immune responses [3,21,57]. Black-footed albatrosses have fourfold higher mercury body burden concentrations than Laysan albatrosses [7], and mercury contamination is believed to be increasing in the marine environment [58].

Lymphocyte proliferation

Lymphocyte stimulation and subsequent proliferation in response to antigens is an important first step in antibody production. As such, mitogen-induced lymphocyte proliferation has become a well-established in vitro measure of avian immune function [24,33,59]. We found that organochlorine concentrations were positively associated with increased proliferation of cryopreserved lymphocytes stimulated with Con A, a T-cell mitogen (Fig. 3). Because plasma organochlorines were highly correlated with one another, we did not attempt to isolate the potential effects of individual organochlorine compounds on black-footed albatross immune function.

Organochlorine exposure was found to be positively associated with avian immune responses in previous studies [20,26,60]. For example, at concentrations similar to those we measured in black-footed albatrosses, Caspian terns in the Great Lakes showed a positive relationship between plasma organochlorines and antibody production [20]. Organochlorines are well-known endocrine disruptors [61], and hormonal imbalance can lead to altered (either suppressed or enhanced) immune function [23,62]. The observed positive relationship between organochlorine concentrations and lymphocyte proliferation reported in the present study may result from a direct effect on lymphocyte function or from an indirect effect of hormonal regulation on lymphocyte response. Individuals with increased lymphocyte proliferation may suggest misregulation of immune function, possibly in the direction of hypersensitivity. The specific health consequences of increased proliferation are not known, but a recent study by Møller and Saino [29] suggests that launching an inappropriate immune response leads to reduced long-term survival in birds.

Conservation implications for black-footed albatrosses

Populations of black-footed albatrosses are declining in many of their important breeding colonies [63], with current negative trends likely to continue over the next 60 years (three generations) [64]. Resulting in part from these assessments, black-footed albatrosses have been designated as endangered by the World Conservation Union. Bycatch mortality from fishing operations [64,65] and exposure to high concentrations of organochlorine contaminants [4,65] have been recognized as possible contributors to these population declines.

To our knowledge, the data presented here are the first to show a significant association between high contaminant loads and altered immune function in black-footed albatrosses. Contaminant-induced immune alteration may contribute to disease outbreaks similar to those observed in marine mammals [66,67] and albatrosses, because they nest in close proximity to each other in large colonies [5], are at increased risk for

disease transmission [68]. Black-footed albatross is a long-lived species [6], and immune impairment also may reduce long-term survival [29], which could ultimately contribute to population-level declines.

CONCLUSION

We report significant associations between altered immune function (lymphocyte proliferation and macrophage phagocytosis) and elevated blood levels of nonpoint-source persistent pollutants (organochlorines and mercury) in an upper-trophic-level marine predator, the black-footed albatross. Documenting negative effects from any source of pollution is challenging, yet for local pollution, ample evidence exists regarding impacts to marine wildlife. For example, local pollution sources in the Great Lakes and St. Lawrence River Estuary have been linked to reduced reproductive success in seabirds [12,69] and increased tumors and infection in beluga whales [70].

The impact of nonpoint-source pollution on the marine environment is more difficult to understand, because global pollutants are ubiquitous [71] and found at elevated concentrations in marine predators with large foraging ranges [72,73]. Nonpoint-source pollution is of growing international concern, especially for high-latitude regions such as the Arctic [74], where polar bears, seabirds, and humans may be at risk [2,19,75]. Our findings in the present study are important, because they suggest that nonpoint-source pollution in the North Pacific, an area of high productivity and biological importance, is associated with altered immune function in a top marine predator.

Acknowledgement—We thank the U.S. Fish and Wildlife Service, Midway Atoll National Wildlife Refuge, A. Keitt, R. Franks, N. Hoffman, and L.A. Woodward for research support; P.T. Raimondi, M.T. Tinker, and L. Hernandez Cadena for statistical advice; and S. Rodriguez-Pastor, D. Austin, and two anonymous reviewers for editorial comments. This research was funded by the U.S. Environmental Protection Agency (Science to Achieve Results [STAR] Graduate Fellowship), the University of California Toxic Substances Research and Teaching Program, and the Switzer Environmental Fellowship Program.

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