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IMMUNOLOGICAL AND REPRODUCTIVE HEALTH ASSESSMENT IN HERRING GULLS AND BLACK-CROWNED NIGHT HERONS IN THE HUDSON–RARITAN ESTUARY

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Abstract—Previous studies have shown inexplicable declines in breeding waterbirds within western New York/New Jersey Harbor between 1996 and 2002 and elevated polychlorinated dibenzo-*p*-dioxins and polychlorinated biphenyls (PCBs) in double-crested cormorant (*Phalacrocorax auritus*) eggs. The present study assessed associations between immune function, prefledgling survival, and selected organochlorine compounds and metals in herring gulls (*Larus argentatus*) and black-crowned night herons (*Nycticorax nycticorax*) in lower New York Harbor during 2003. In pipping gull embryos, lymphoid cells were counted in the thymus and bursa of Fabricius (sites of T and B lymphocyte maturation, respectively). The phytohemagglutinin (PHA) skin response assessed T cell function in gull and heron chicks. Lymphocyte proliferation was measured in vitro in adult and prefledgling gulls. Reference data came from the Great Lakes and Bay of Fundy. Survival of prefledgling gulls was poor, with only 0.68 and 0.5 chicks per nest surviving to three and four weeks after hatch, respectively. Developing lymphoid cells were reduced 51% in the thymus and 42% in the bursa of gull embryos from New York Harbor. In vitro lymphocyte assays demonstrated reduced spontaneous proliferation, reduced T cell mitogen-induced proliferation, and increased B cell mitogen-induced proliferation in gull chicks from New York Harbor. The PHA skin response was suppressed 70 to 80% in gull and heron chicks. Strong negative correlations ($r = -0.95$ to -0.98) between the PHA response and dioxins and PCBs in gull livers was strong evidence suggesting that these chemicals contribute significantly to immunosuppression in New York Harbor waterbirds. Environ. Toxicol. Chem. 2013;32:548–561. © 2012 SETAC

Keywords—New York Harbor Fish-eating birds Immunotoxicology Dioxins Polychlorinated biphenyls

INTRODUCTION

The numbers of breeding colonial waterbirds declined significantly within western New York/New Jersey Harbor from 1996 to 2002, despite apparently abundant food and nesting habitat [1]. Specifically, breeding herring gulls (*Larus argentatus*), black-crowned night herons (*Nycticorax nycticorax*), great and snowy egrets (*Casmerodius albus* and *Egretta thula*), and glossy ibis (*Plegadis falcinellus*) essentially have disappeared from Shooters, Pralls, and Isle of Meadows Islands, except for approximately a dozen heron nests on Pralls. Double-crested cormorant nests decreased by approximately 25% on Shooters Island and nearby navigational aid structures. The eggs of double-crested cormorants nesting in the Hudson Raritan Estuary contain significant concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and planar polychlorinated biphenyls (PCBs) [2,3]. Multiple sources of contaminants in the Hudson Raritan Estuary might affect the health, reproduction, and populations of aquatic wildlife, including historic releases of a 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (TCDD) from a former 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) manufacturing facility along the Passaic River in New Jersey [4,5]. To date, however, no published studies have investigated the potential links between exposure to these chemicals and ecological health metrics in birds such as immunological status or the possible geographic extent of these effects. In other ecosystems, fish-eating birds such as gulls, terns, herons, and cormorants have been shown to be excellent sentinel species to assess and monitor the impacts of persistent contaminants. Detailed reproductive and health effect studies of contaminants have been conducted in these species, especially the herring gull, for more than three decades in the Great Lakes [6–9].

The present study emphasized immune function, because PCDDs, PCBs, and other organochlorines have been shown to be associated with immunological effects in many laboratory experiments [10,11] and in wild birds, including colonial waterbirds [11–16]. Immunological effects of these organochlorines, particularly the dioxin-like chemicals, include atrophy of the primary lymphoid organs (thymus and, in birds, bursa of Fabricius), suppressed T cell function, suppressed antibody production, and increased susceptibility to infectious diseases. Immunological assays (cellularity and masses of lymphoid organs, the phytohemagglutinin [PHA] skin response, and lymphoproliferation assays) were chosen for the present study based on their general utility as indicators of immune status in birds [11,17] or previously demonstrated associations with organochlorines. Embryonic exposure to PCBs or dioxins is associated with decreases in the number of developing lymphocytes in the thymus and bursa of Fabricius in chickens [18–20] and herring gulls (C.J. Kelly, 2003, Master's thesis, Wright State University, Dayton, OH, USA). The PHA skin response for T cell–mediated immunity is one of the most common immune function assays in ecological and toxicological studies in birds [11,17]. Several studies have shown associations between exposure to PCBs and PCDDs and suppression of

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the PHA response in fish-eating birds [12–14]. Phytohemagglutinin injected intradermally in a chick's wing stimulated T cells to release chemical messengers (cytokines), causing a localized inflammatory influx of white blood cells and fluid. These studies found that the greater the stimulation index (change in skin thickness 24 h after injection), the stronger the immune response. Lymphoproliferation assays, especially for T cells, are good indicators of immunotoxicity in laboratory rodents [21–23], and methods have been developed to employ these laboratory tests following cryopreservation of avian lymphocytes in the field [24].

The present study assessed reproduction, immune function, and contaminant exposure in waterbirds on breeding colonies on Swinburne and Hoffman Islands in lower New York Harbor during 2003 in parallel to similar studies in the Great Lakes [12,13]. The present study's specific objectives were to assess the immune function of herring gulls and black-crowned night herons in lower New York Harbor; to investigate the prefledgling survival of herring gulls in lower New York Harbor; and to explore the potential associations between environmental contaminants and impaired immune function or reproduction.

MATERIALS AND METHODS

Study design

Biological assessment by researchers at Wright State University and chemical assessment by staff at the Columbia Environmental Research Center of the U.S. Geological Survey were performed independently; that is, each laboratory was blind to the data generated by the other group until the statistical analysis was conducted.

The only herring gull and black-crowned night heron colonies in the Hudson Raritan Estuary suitable for study (based on colony size and accessibility and proximity to the Passaic River, Newark Bay, Kill van Kull, and Arthur Kill) were in lower New York Harbor east of Staten Island. Herring gulls were studied on Swinburne Island and black-crowned night herons on nearby Hoffman Island from May 28 to July 9, 2003. Adult herring gulls were trapped over their nests at mid-incubation for blood sampling using a walk-in automatic drop trap (May 30). Pipping herring gull embryos were collected around the median hatch date for the colony (June 3–5). Enclosures (~80 cm high with 1.7×2.5 cm plastic mesh supported by metal poles) were erected around individual or groups of herring gull nests (which were otherwise unmanipulated by egg and embryo sampling) at mid-incubation to contain chicks for immune function assays and to calculate prefledgling survival. Enclosures could not exclude avian or mammalian predators, although no such predation was observed. Immune function tests were conducted on three- to four-week old herring gull chicks (July 1, 2, and 7). Age was determined by observed hatch dates and body size measurements [12]. Chicks were weighed using a spring scale. Body size was assessed by measuring the wing chord with a ruler, and the head-to-bill, tarsus, and keel lengths were measured with calipers. The numbers of surviving chicks in the enclosures were counted at median ages of three and four weeks posthatch and divided by the number of nests to determine chick productivity. Four-week-old herring gull chicks (July 8–9) were re-measured, euthanized by decapitation, and necropsied. Various organs (including the spleens) were removed, weighed, and frozen for chemical and biochemical analyses [6,25]. Immune function tests were conducted on black-crowned night heron chicks in tree nests at median ages of approximately two weeks posthatch. For both gulls and herons, nest mates of appropriate age were sampled to maximize sample sizes. All collections were carried out under federal and state migratory bird permits and with the approval of the Laboratory Animal Care and Use Committee at Wright State University.

No suitable reference sites with clearly documented low contamination were identified within or near the Hudson-Raritan Estuary. As such, reference sites outside this ecosystem were chosen based on previous studies and included Chantry Island in northeastern Lake Huron and Kent Island in the Bay of Fundy, Atlantic Ocean [7,8,12,25]. Chantry Island (2001–2002) was a reference site for both herring gulls and black-crowned night herons, and Kent Island in the Bay of Fundy, Atlantic Ocean (2001), was an additional reference site for herring gulls.

Lymphoid organ development in herring gull embryos

Pipping herring gull embryos (one per nest) were collected from Swinburne Island in lower New York Harbor during early June 2003 and from Chantry Island in Lake Huron during 2002 (C.J. Kelly, 2003, Master's thesis, Wright State University, Dayton, OH, USA). Embryos were kept alive in a cooler with hot water bottles until necropsy in a portable laboratory, which occurred later during the collection day. The thymus gland and bursa of Fabricius were removed, weighed, and homogenized, and lymphoid cells numbers and viability were counted at 400× on a hemacytometer using Trypan blue dye [18,19; C.J. Kelly, 2003, Master's thesis, Wright State University, Dayton, OH, USA]. Thymus, bursa, and spleen mass indices were calculated as percent of body mass.

T cell–mediated immune response in herring gulls and black-crowned night herons

Herring gull and black-crowned night heron chicks were assessed for immunosuppression using an in vivo PHA skin test [12]. At approximately three weeks of age for the gulls and two weeks of age for the herons, the skin thickness in each patagial wing web was measured to the nearest 0.05 mm using low tension, pressure-sensitive calipers (Dyer Company). One wing web was injected with 0.1 ml of 1 mg PHA-P/ml (Sigma-Aldrich) in sterile phosphate buffered saline (PBS) without calcium or magnesium (Sigma-Aldrich), whereas the other received 0.1 ml of PBS. Wing webs were re-measured 24 ± 3 h later. A stimulation index was calculated as the change in thickness of the PHA-injected wing web minus the change in thickness of the PBS-injected wing web. The same source and formulation of PHA were used for all birds, including those from the Chantry and Kent Island reference sites.

Lymphoproliferation responses in herring gulls

Approximately 4 to 7 ml of blood was collected into heparinized vacutainer tubes (Becton Dickinson) from the brachial vein of adult, three-week old (on the second day of the PHA test) and four-week old herring gulls and stored on ice for less than 6 h. Lymphocytes were isolated by slow spin centrifugation at 120 *g* for 20 to 25 min and then another 5 min [24]. Following each centrifugation period, the buffy coat was removed with approximately half of the plasma by swirling a sterile, fine-tipped, disposable pipette to create a vortex that lifted the lymphocytes into the plasma. Both aliquots of lymphocytes and plasma were combined. Lymphocytes were then separated from plasma by centrifugation at 600 *g* for 10 min, resuspended in Origen freezing medium (10% dimethyl sulfoxide [DMSO], Fisher Scientific), cryopreserved by rate-controlled freezing in a Nalgene freezing container on dry ice, and stored long-term in liquid nitrogen.

In the laboratory, cells were thawed, washed with RPMI-BSA, and incubated for 2 to 3 h at 37°C [24]. Following resuspension in Weber medium, cells were counted for viability using Trypan Blue exclusion and cell type by Natt and Herrick solution. Cells (3×10^5) in Weber medium were added to each well of a 96-well plate. Mitogen treatments included concanavalin A (1 μg Con A) and phytohemagglutinin + phorbol myristate acetate (2 ng PHA-P + 4 μg PMA) for T cells and lipopolysaccharide (2 μg LPS) for B cells. If cell numbers were sufficient, additional wells were treated with 0.5 μg Con A. Lymphocytes were cultured at 40°C and 5% CO_2 for 48 h, after which 20 μl of bromodeoxyuridine (BrdU) labeling reagent was added for another 18 h. Cells were then fixed and treated with nucleases and an anti-BrdU monoclonal antibody conjugated with peroxidase. After the peroxidase substrate was added, plates were read using an ELISA reader at 405 nm. Stimulation indices (SI) were calculated as mean optical density in mitogen-stimulated wells divided by mean optical density in nonstimulated wells.

Contaminant concentrations in avian liver tissues

Livers were removed and weighed immediately after gull chicks were decapitated and allowed to bleed out. The left lobe of each liver was placed in an acetone-hexane rinsed amber jar and stored on wet ice for transport to Wright State University by University personnel, where they were stored at –20°C. These samples were later transferred to the Columbia Environmental Research Center following standard documentation and chain of custody procedure with sample logs as specified by the Research Center and the U.S. Geological Survey. Contaminant analyses were conducted on all nine chicks that were assessed for the PHA and SRBC tests that also survived to 4 weeks of age, and on three other randomly selected chicks that were not part of these other tests (i.e., from nests outside the enclosures).

Sample prep and instrumental analysis—Organics. Chemical analyses were conducted by staff at the Columbia Environmental Research Center. Percent lipid and moisture were determined for each of the tissue samples. Thirty organochlorine pesticides and 141 individual PCB congeners were assayed using gas chromatography with electron capture detection [26,27]. Only the pesticides and PCB congeners that were found to be consistently above detection limits were reported, as was total PCB concentrations as a sum of all congeners measured (Table 1). Non-ortho-PCB congeners and PCDD and PCDF congeners were isolated from the gull liver extracts with a series of chromatographic cleanup procedures—reactive cleanup, high-performance gel permeation, porous graphitic carbon, and alumina—and were then quantified using isotope dilution gas chromatography with high resolution mass spectrometry (GC/HRMS) [28,29]. Tetra-octa-polychlorinated dibenzothiophenes (PCDTs) were monitored by GC/HRMS [30]. Dioxin-like compound Toxic Equivalents (TEQs) were calculated using World Health Organization avian toxic equivalency factors [31].

Sample prep and instrumental analysis—Inorganics. Heavy metals and trace elements in livers were measured by inductively coupled mass spectrometry (ICP-MS) using the TotalQuant method that sums multiple isotopes of targeted elements after suitable acid digestion to a final digestate of 6% nitric acid [32]. Arsenic and Se were determined by flow injection hydride generation atomic absorption spectroscopy (FIHGAAS) [32] after suitable acid digestion (10% hydrochloric acid for final digestate), whereas Hg was determined directly by thermal combustion-gold amalgamation atomic absorption spectrometry (TCGAAAS) with a direct mercury analyzer [32].

Table 1. Detected organochlorine concentrations in prefledgling herring gull livers collected from Swinburne Island in lower New York Harbor during 2003[a]

	Concentration (ng/g wet wt)				
	Mean	SE	Median	Minimum	Maximum
Pentachlorobenzene	0.55	0.23	0.31	0.14	3.0
Pentachloro anisole	0.46	0.080	0.39	0.19	1.2
alpha-BHC (a-HCH)	0.23	0.020	0.25	0.13	0.38
delta-BHC (d-HCH)	0.17	0.030	0.14	0.070	0.31
Heptachlor epoxide	3.7	0.76	3.7	0.010[b]	7.6
Oxychlordane	9.9	1.1	10	3.3	14
cis-Chlordane	0.55	0.10	0.43	0.15	1.3
cis-Nonachlor	2.3	0.30	2.2	0.81	4.7
trans-Nonachlor	13	2.2	12	3.6	32
p,p'-DDE	34	6.6	29	7.9	91
p,p'-DDD	1.7	0.25	1.5	0.81	3.4
Mirex	0.86	0.11	0.84	0.35	1.8
Total PCBs	380	76	320	150	1,100
% Lipid	3.4	0.31	3.1	2.7	6.7

[a] $N = 12$ livers for all compounds.
[b] 0.010 ng/g (detection limit) was for one bird in which heptachlor epoxide was not detected.
SE = standard error; BHC = benzenehexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; PCBs = polychlorinated biphenyls.

Chemistry quality assurance and quality control—Organics

Reference fish material positive control sample had a total-PCB concentration of 7,000 ng/g, close to our historical average result for that sample (6,700 ng/g). Triplicates of the positive control carp showed excellent precision for non-ortho PCBs and PCDD/PCDFs; that is, <4% and <20% relative standard deviation (RSD), respectively. Data are also consistent with our historical non-ortho PCB, PCDD, and PCDF QC data (<20% RSD of historic values, except for the 1,2,3,7,8,9-Hexachloro- and 1,2,3,4,7,8,9-Heptachlorofurans, which have shown greater variability over time). The PCB congener, total PCB, and organochlorine pesticide concentrations (ng/g wet wt) for the herring gull livers and the associated QC samples were corrected for analytical recovery of the surrogates; all values were reported at three significant figures. Liver concentrations below a PCB congener or a pesticide's method detection limit were censored at that level. Surrogate recoveries for PCBs and pesticides averaged 77 to 83%, within QC limits (50–125%). Organochlorine pesticides and toxaphene recoveries for matrix spikes were also within QC limits. Matrix spike recoveries of PCB congeners were in the acceptable range (50–125%) with a few exceptions for congeners near the method detection limit or with partial interferences. Isotopically labeled non-ortho PCB, PCDD, and PCDF surrogate recoveries were typically above 50%, within our quality assurance range (25–125%).

Chemistry quality assurance and quality control—Inorganics

Reference fish tissue material (NRCC DORM-2) and whole egg powder (NIST SRM 8415) analyzed by ICP-MS semi-quantitative scan exhibited recoveries ranging from 79 to 113%, with the exception of one low Fe recovery (65%) and one high Cr recovery (200%). Recoveries of elements from two liver reference materials (dogfish liver, NRCC DOLT-2, and bovine liver NIST 1577b) ranged from 74 to 131%, with the exception of one high Cr recovery (251%). Recoveries of elements from various reference/research materials digested and analyzed in

conjunction with the determination of As and Se by quantitative FIHGASS and Hg by TCGAAAS were within specified limits for all elements where limits exceeded the method detection limits. In one exceptional case for As, the measured value was 2% below the lower limit value. Triplicate digestion and analysis of all other metals in one bird liver sample by ICP-MS semiquantitative scan exhibited percent RSDs $\leq$ 22%, except for two cases of Al (49 and 61%) and one case of Fe (35%). Replicate digestion or combustion and analysis of samples for As, Se, and Hg determination produced percent RSDs < 9.

Recoveries of elements spiked into tissue samples prepared for the semiquantitative scan ranged from 88 to 133%, with an average recovery of 108%. Liver spike recoveries ranged from 88 to 128% and averaged 105%. Samples of bird egg and liver spiked with As, Se, and Hg exhibited recoveries ranging from 93 to 114% and averaged 103%. Recoveries of As and Se in analysis (postdigestion) spikes ranged from 94 to 101%.

Statistical analyses

Wilcoxon's rank sum test was used to compare the following endpoints between birds from lower New York Harbor and Chantry Island: the reference was lymphocyte proliferation, thymocyte numbers, and bursal lymphoid cell numbers in gulls and phytohemagglutinin skin responses in herons. The Kruskal-Wallis test was used to compare phytohemmagglutinin skin responses among lower New York Harbor and marine and freshwater reference sites (Kent and Chantry Islands, respectively). In herring gull chicks from lower New York Harbor, associations between immunological parameters and contaminant concentrations, body size, growth, and condition were assessed using Pearson's correlation procedure. Correlations were reported only if significant ($p < 0.05$) (Table 2).

RESULTS

Herring gull chick liver chemistry

The presence of 2,3,7,8-TCDD and related dioxin-like compounds and other organic and inorganic residues was confirmed in the herring gull livers from the present harbor study area. Organochlorine pesticides and total PCBs were consistently detected in herring gull livers ($n = 12$; Table 1). Tables 3 and 4 summarize the congener concentrations of chlorinated- dioxins, furans, and dioxin-like PCBs, including avian-based 2,3,7,8-TCDD EQs (TEQs). Dioxins, furans, and PCBs accounted for 13.2, 20.7, and 66.2% of total TEQs, respectively. No PCDTs were detected except for a tetra-PCDT (TCDT) of < 1 pg/g. Table 5 summarizes the metals that were found consistently at reportable concentrations in these samples ($n = 12$).

Atrophy of lymphoid organs in herring gull embryos

The number of developing lymphoid cells in both the thymus and bursa were greatly reduced in pipping herring gull embryos in lower New York Harbor (Fig. 1). The mean number of live thymocytes in the thymi of gull embryos from Swinburne Island ($2.7 \pm 0.4 \times 10^7$ cells; mean $\pm$ se) was 51% lower than at Chantry Island, the reference site ($5.5 \pm 1.0 \times 10^7$ cells) ($p < 0.0010$). The thymocyte number at Swinburne was slightly lower than at the two most contaminated Great Lakes sites, West Sister Island in western Lake Erie ($3.5 \pm 0.5 \times 10^7$ cells) and Saginaw Bay ($3.2 \pm 0.7 \times 10^7$ cells) (C.J. Kelly, 2003, Master's thesis, Wright State University, Dayton, OH, USA). Likewise, the mean number of live bursal lymphoid cells was 42% lower at Swinburne ($6.4 \pm 0.6 \times 10^6$ cells) compared to Chantry ($1.1 \pm 0.1 \times 10^7$ cells) ($p < 0.0012$). The number of live bursal lymphoid cells was $5.0 \pm 0.1 \times 10^6$ at both West

Table 2. Statistically significant[a] correlations between immune variables and contaminants, body size, growth, and liver composition in prefledgling herring gulls from Swinburne Island in lower New York Harbor during 2003

			Pearson's	
Immune Variable	Exposure, size, growth, or liver variable	*n*	*r*	*p*
PHA Stimulation Index	% lipid in liver	9	–0.86	0.0027
	% moisture in liver	9	0.77	0.016
	Na	9	0.81	0.0085
	Ti	9	0.69	0.041
	Rb	9	0.67	0.047
	Se	9	–0.82	0.0065
	Pentachlorobenzene	9	–0.74	0.021
	p,p′-DDE	9	–0.87	0.0023
	Mirex	9	–0.80	0.0091
	Total PCBs	9	–0.95	0.0001
	2,3,7,8-TCDD	9	–0.98	<0.0001
	TCDF	9	–0.83	0.0055
	PCB 126	9	–0.82	0.0063
	Total TEQs	9	–0.89	0.0012
	Sum PCB TEQs	9	–0.88	0.0018
	Sum m-PCB TEQs	9	–0.96	<0.0001
	Sum n-PCB TEQs	9	–0.87	0.0022
	PCDD TEQs	9	–0.94	0.0002
	PCDF TEQs	9	–0.88	0.002
Bursal index	Thymic index	20	0.70	0.0006
Spleen index	Body mass 4 weeks	14	–0.54	0.048
	% lipid in liver	12	–0.60	0.037
	% moisture in liver	12	0.72	0.0078
	Na	12	0.61	0.037

[a] Table lists only those variables with $p < 0.05$ for Pearson's correlation.
PHA = phytohemagglutinin; DDE = 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene; PCBs = polychlorinated biphenyls; TCDD = 2,3,7,8-tetrachlorinated dibenzo-p-dioxin; TCDF = 2,3,7,8-tetrachlorinated dibenzo-p-furan; TEQ = dioxin toxic equivalents; PCDDs = polychlorinated dibenzodioxins; PCDFs = polychlorinated dibenzofurans.

Table 3. Concentrations of PCDDs, PCDFs, and planar PCBs in prefledgling herring gull livers collected from Swinburne Island in lower New York Harbor during 2003[a]

	Concentration (pg/g wet wt)				
Compound	Mean	Standard error	Median	Minimum	Maximum
Dioxins					
2,3,7,8-tetrachloro	4.6	2.4	2.0	1.1	31.0
1,2,3,7,8-pentachloro	1.3	0.2	1.0	0.3	2.4
1,2,3,4,7,8-hexachloro	1.3	0.1	1.4	0.5	2.0
1,2,3,6,7,8-hexachloro	6.4	1.1	5.6	2.1	13.6
1,2,3,7,8,9-hexachloro	1.1	0.1	1.1	0.6	1.9
1,2,3,4,6,7,8-heptachloro	28.6	5.7	22.7	5.5	64.8
Octachloro	99.3	36.3	60.4	11.8	482.0
Sum dioxins	142.6	41.1	103.6	23.2	558.2
Furans					
2,3,7,8-tetrachloro	1.0	0.3	0.7	0.3	4.3
1,2,3,7,8-pentachloro	0.5	0.1	0.3	0.2	2.0
2,3,4,7,8-pentachloro	6.3	1.3	5.3	2.1	18.9
1,2,3,4,7,8-hexachloro	13.7	9.0	5.2	1.8	113
1,2,3,6,7,8-hexachloro	5.2	1.7	3.6	0.9	22.9
2,3,4,6,7,8-hexachloro	0.4	0.0	0.4	0.2	0.4
1,2,3,7,8,9-hexachloro	2.3	0.4	1.9	0.9	4.8
1,2,3,4,6,7,8-heptachloro	19.1	4.1	16.1	2.2	53.4
1,2,3,4,7,8,9-heptachloro	0.8	0.1	0.8	0.4	1.7
Octachloro	34.6	7.6	28.0	3.7	90.2
Sum furans	83.7	16.6	72.3	15.2	211.1
PCBs					
PCB 81	23.2	5.3	18.4	8.7	78.0
PCB 77	138	23.3	112	39.9	317
PCB 126	203	54.7	136	62.2	699
PCB 169	41.4	9.4	30.9	10.4	107
Sum n-PCBs	406	80.5	317	171.8	1059
PCB 105	6,800	1,300	5,100	2,500	17,000
PCB 114	470	110	300	150	1400
PCB 118	24,000	5,100	17,000	8,700	69,000
PCB 123	490	110	420	1,450	1,400
PCB 156	2,100	480	1,300	650	6300
PCB 157	670	170	480	0.0	2200
PCB 167	1,100	270	700	370	3,700
PCB 189	270	56	170	66	740
Sum m-PCBs	36,360	7,540	26,800	12,670	102,300

[a] $N = 12$ livers for all compounds.
PCDDs = polychlorinated dibenzodioxins; PCDFs = polychlorinated dibenzofurans; PCBs = polychlorinated biphenyls.

Sister and Saginaw Bay (C.J. Kelly, 2003, Master's thesis, Wright State University, Dayton, OH, USA).

In vivo T cell–mediated immune response in herring gulls and black-crowned night herons

Both herring gull and black-crowned night heron chicks in lower New York Harbor had some of the lowest mean PHA responses ever recorded by the senior author (including multiple years of replication of response levels at reference and contaminated sites), indicating severe suppression of T cell–mediated immunity (Fig. 2). Specifically, PHA responses in herring gulls varied significantly among the three sites ($p < 0.0001$). Gulls on Swinburne Island ($n = 13$) had a mean stimulation index of only 0.16 mm. Mean responses were 0.85 mm at the marine reference and 0.6 to 0.8 mm at Great Lakes reference sites (the present study and Grasman et al. [12]). Gulls at polluted Great Lakes sites typically have mean responses of 0.3 to 0.4 mm [12]. Black-crowned night herons on Hoffman Island ($n = 16$) had a mean stimulation index of 0.18 mm compared to 0.5 mm at the Chantry Island reference site (the present study; $p < 0.0001$) and 0.25 to 0.3 mm at contaminated Great Lakes sites in western Lake Erie and Saginaw Bay (K. Grasman, Calvin College, unpublished data).

The suppressed PHA response in lower New York Harbor gulls was strongly correlated with several organochlorine contaminants (Table 2 and Fig. 3). Strong linear correlations ($n = 9$) were evident for 2,3,7,8-TCDD (Pearson's $r = –0.98$), total PCBs ($r = –0.95$), sum m-PCBs ($r = –0.96$), PCDD TEQs ($r = –0.94$), total TEQs ($r = –0.89$), and DDE ($r = –0.87$). Other measures of exposure to dioxin-like chemicals and PCBs also showed strong negative correlations with the PHA response (Table 2). Only one metal or trace element, Se, was negatively correlated with the PHA response, although Na, Ti, and Rb were positively correlated. Overall, these correlation analyses suggest that dioxins or PCBs make a strong contribution to the suppressed T cell function observed in herring gull chicks in lower New York Harbor.

Lymphoproliferation responses in herring gulls

Herring gulls from lower New York Harbor exhibited altered lymphocyte proliferation, most notably reduced spontaneous proliferation and enhanced LPS-induced proliferation in three- and four-week-old chicks (Table 6 and Fig. 4). Spontaneous proliferation in the absence of a mitogen is presumably associated with lymphocyte proliferation in vivo at the time of blood collection. Mean spontaneous proliferation in New York Harbor gulls was suppressed approximately 40% in all three ages,

Table 4. Dioxin toxic equivalent concentrations for PCDDs, PCDFs, and planar PCBs in prefledgling herring gull livers from Swinburne Island in lower New York Harbor during 2003[a]

	Dioxin toxic equivalent concentration (pg/g wet wt)				
Compound	Mean	Standard error	Median	Minimum	Maximum
Dioxins					
2,3,7,8-tetrachloro	4.57	2.42	1.95	1.10	31.00
1,2,3,7,8-pentachloro	1.29	0.21	1.00	0.30	2.40
1,2,3,4,7,8-hexachloro	0.07	0.01	0.07	0.03	0.10
1,2,3,6,7,8-hexachloro	0.06	0.01	0.06	0.02	0.14
1,2,3,7,8,9-hexachloro	0.11	0.01	0.11	0.06	0.19
1,2,3,4,6,7,8-heptachloro	0.03	0.01	0.02	0.01	0.06
Octachloro	0.01	0.00	0.01	0.00	0.05
Sum dioxins	6.14	2.55	3.15	1.65	33.6
Furans					
2,3,7,8-tetrachloro	0.98	0.32	0.65	0.30	4.30
1,2,3,7,8-pentachloro	0.05	0.01	0.03	0.02	0.20
2,3,4,7,8-pentachloro	6.28	1.32	5.30	2.10	18.9
1,2,3,4,7,8-hexachloro	1.37	0.90	0.52	0.18	11.3
1,2,3,6,7,8-hexachloro	0.52	0.17	0.36	0.09	2.29
2,3,4,6,7,8-hexachloro	0.04	0.00	0.04	0.02	0.04
1,2,3,7,8,9-hexachloro	0.23	0.04	0.19	0.09	0.48
1,2,3,4,6,7,8-heptachloro	0.19	0.04	0.16	0.02	0.53
1,2,3,4,7,8,9-heptachloro	0.01	0.00	0.01	0.00	0.02
Octachloro	0.00	0.00	0.00	0.00	0.01
Sum furans	9.65	2.42	7.18	3.13	33.9
PCBs					
PCB 81	2.32	0.53	1.84	0.87	7.80
PCB 77	6.92	1.17	5.61	1.99	15.83
PCB 126	20.34	5.47	13.62	6.22	69.93
PCB 169	0.04	0.01	0.03	0.01	0.11
Sum n-PCBs	29.6	6.57	21.9	11.2	87.5
PCB 105	0.68	0.13	0.51	0.25	1.7
PCB 114	0.05	0.01	0.03	0.01	0.14
PCB 118	0.24	0.05	0.17	0.09	0.69
PCB 123	0.00	0.00	0.00	0.00	0.01
PCB 156	0.21	0.05	0.13	0.06	0.63
PCB 157	0.07	0.02	0.05	0.00	0.22
PCB 167	0.01	0.00	0.01	0.00	0.04
PCB 189	0.00	0.00	0.00	0.00	0.01
Sum m-PCBs	1.27	0.26	0.90	0.42	3.47
Sum PCBs	30.9	6.8	22.4	11.8	89.3
Total	46.7	11.4	31.6	19.2	156.8

[a] $N = 12$ livers for all compounds.
PCDDs = polychlorinated dibenzodioxins; PCDFs = polychlorinated dibenzofurans; PCBs = polychlorinated biphenyls.

although statistically significant only in three- ($p < 0.0043$) and four- ($p < 0.036$) week-old chicks but not adults ($p < 0.42$; Table 6 and Fig. 4A). Total B lymphocyte proliferation stimulated by LPS was significantly increased by 63% in three-week-old chicks ($p < 0.0044$) and four-week-old chicks ($p < 0.033$) but not in adults ($p < 0.63$; Table 6 and Fig. 4b). T cell proliferation stimulated by PHA + PMA or Con A generally was not significantly different between sites, except for a 15% decrease in the PHA + PMA treatment for three-week-old gulls from New York Harbor ($p < 0.051$). Likewise, four-week-old gulls from New York Harbor had a marginally significant ($p < 0.13$) 17% decrease in PHA + PMA stimulated proliferation compared to the Chantry Island reference site. No statistically significant correlations were found between proliferation variables and contaminants in chicks that were consistent between three and four weeks of age.

Prefledgling survival of herring gulls

Prefledgling survival in herring gulls was measured by monitoring survival of chicks within enclosures on Swinburne Island in lower New York Harbor ($n = 23$ total nests in three enclosures). Chick productivity was calculated at three weeks posthatch (the standard time for this calculation in herring gulls) was 0.68 chicks per nest. This is below the level necessary to maintain a stable population (0.8 chicks per nest) [33]. Furthermore, more than 25% of the chicks that survived to three weeks of age died in the next week; at four weeks post-hatch, there were only 0.5 chicks per nest. Although not evaluated quantitatively, survival of herring gull chicks on nearby Hoffman Island and great black-backed gull chicks on Swinburne Island also was poor—very few surviving three- to four-week-old chicks were observed despite a large number of nests.

DISCUSSION

Associations of immunotoxicity with dioxins, PCBs, and other organochlorines

Although the immune assays in this study were not chemical specific, dioxins and PCBs clearly cause immunosuppresssion in laboratory animals [10], and environmental exposures have been associated with similar immunosuppression or increased infections in wildlife. Polychlorinated biphenyls and DDE were negatively associated with proliferation of T cells in male bottlenose dolphins (*Tursiops truncatus*) from the west coast of Florida [34]. Young harbor seals (*Phoca vitulina*) fed PCB-

Table 5. Detected heavy metal and trace metal concentrations in prefledgling herring gull livers collected from Swinburne Island in lower New York Harbor during 2003[a]

	Concentration (ug/g dry wt)				
	Mean	Standard error	Median	Minimum	Maximum
Na	3,058	90.8	3100	2,400	3,500
Mg	1,180	13.1	1,200	1,100	1.200
Al	1.2	0.1	1.0	0.7	2.3
K	9,800	237	9,600	8,600	11,000
Ti	7.0	0.4	7.0	4.3	9.2
Mn	23.5	0.9	23.0	20.0	29.0
Fe	764	122	635	310	1,800
Co	0.3	0.0	0.3	0.2	0.4
Cu	173	23.4	175	69.0	340
Zn	120	5.2	120	95.0	150
As[b]	4.6	0.7	3.8	2.0	8.6
Se[b]	3.4	0.2	3.1	2.6	4.7
Rb	10.3	0.6	9.7	7.3	14.0
Mo	2.6	0.2	2.5	1.9	3.5
Ag	3.9	0.5	3.2	1.8	6.4
Cd	1.2	0.4	0.8	0.2	4.3
Hg[c]	0.7	0.2	0.5	0.3	2.2
% moisture	69.1	0.4	68.8	66.5	70.9

[a] $N = 12$ livers for all compounds.
[b] As and Se determined by flow injection hydride generation atomic absorption spectroscopy.
[c] Hg determined by thermal decomposition of liver sample, gold amalgamation, and detection by atomic absorption spectroscopy.

contaminated fish from the Baltic Sea had reduced delayed-type hypersensitivity responses (T cell–mediated skin responses) and reduced T cell proliferation in vitro, supporting the role of contaminant-induced immunosuppression in an outbreak of phocine distemper virus in wild seals [35]. Stranded harbor porpoises (*Phocoena phocoena*) dying from infections had higher tissue PCB concentrations than those dying of acute trauma [36]. In glaucous gulls (*Larus hyperboreus*) of the Svalbard archipelago in the Barents Sea, total organochlorine exposure was positively correlated with gastrointestinal parasite load [15].

Pipping herring gull embryos from lower New York Harbor had greatly reduced numbers of developing lymphocytes both in the thymi and bursa of Fabricius, the sites of T and B cell maturation, respectively. The magnitude of lymphoid atrophy ($\sim 40-50\%$) was similar to that observed in embryos from contaminated Great Lakes areas (Saginaw Bay and Lake Erie) (C.J. Kelly, 2003, Master's thesis, Wright State University, Dayton, OH, USA) [21]. In a previous study, thymic atrophy was negatively correlated with PCB concentrations in the yolk sacs of pipping herring gull embryos (PCDDs and PCDFs were not assessed; K. Grasman, Calvin College, unpublished data). In herring gull chicks of the Great Lakes, thymic atrophy was associated with liver ethoxyresorufin-O-deethylase (EROD) activity, an indicator of exposure to PCBs and dioxins [17]. In chickens, PCBs and dioxins decreased the mass and lymphoid cellularity of the thymus and bursa of Fabricius [18–20]. In chicken embryos, the dioxin-like PCBs 126 and 77 and Aroclor 1254 increased apoptosis in thymocytes 1 d before hatch [20]. In pipping herring gull embryos in the Great Lakes, thymocyte apoptosis was altered at sites with PCB-associated thymic atrophy (C.J. Kelly, 2003, Master's thesis, Wright State University, Dayton, OH, USA). Lymphocyte proliferation assays in New York Harbor gulls gave further evidence of disrupted immune function (Table 6 and Fig. 4). As cited earlier, altered lymphocyte proliferation has been observed in wildlife and laboratory species exposed to dioxins and PCBs.

The magnitude of suppression of the PHA skin response in New York Harbor herring gulls and black-crowned night heron chicks was among the greatest ever observed by the senior author's research team. Dioxins and PCBs exhibited strong negative correlations ($r = -0.89$ to -0.98) with the PHA skin response in herring gull chicks (Fig. 3). The PHA skin test integrates many important T cell functions and is recognized as one of the most sensitive assays employed by avian immunotoxicologists and ecologists [11,17]. In avian laboratory studies, elimination of T cell function by irradiation or immunosuppressive drugs reduces the PHA response by 50 to 60% (reviewed in [37]). The magnitude of suppression in herring gulls and black-crowned night herons in lower New York Harbor was approximately 70 to 80% lower than at reference sites. For comparison, 30 to 50% suppression was observed in herring gull and Caspian tern chicks at contaminated Great Lakes sites [12]. In Caspian tern chicks, the PHA response showed a strong negative association with total PCBs and DDE in plasma of individual birds [13].

Two related Great Lakes studies allow comparison of the PHA skin response to liver organochlorine concentrations in herring gull chicks. Livers were collected for contaminant analysis (pooled by site) from herring gull chicks at 11 colonies, including Lake Winnipeg (reference colony), Hamilton Harbour, and Saginaw Bay during 1991 and western Lake Erie during 1992 [25]. The same team conducted the PHA skin test on herring gull chicks at all four colonies during 1992 and additionally in 1993 and 1994 for Saginaw Bay [12]. The PHA skin response was suppressed at contaminated sites (Lake Erie, Hamilton Harbour, and Saginaw Bay) with liver concentrations of 75 to 267 pg/g TEQs, 1.3 to 8.9 pg/g 2,3,7,8-TCDD, and 0.792 to 1.191 ug/g PCBs, compared to the reference site (Winnipeg) with 16 pg/g TEQs, 0.85 pg/g 2,3,7,8-TCDD, and 0.132 ug/g PCBs. (PCB concentrations are reported directly from Fox et al. [25]; TEQs were recalculated to include congeners not used originally in Fox et al. [25]; 2,3,7,8-TCDD is reported as unpublished data from the same data set.) Mean concentrations in New York Harbor gull chicks in the present study were 47 pg/g TEQs, 4.6 pg/g 2,3,7,8-TCDD, and 0.382 ug/g PCBs, although maximal concentrations reached 157 pg/g TEQs, 31 pg/g 2,3,7,8-TCDD, and 1.100 ug/g PCBs (Tables 2

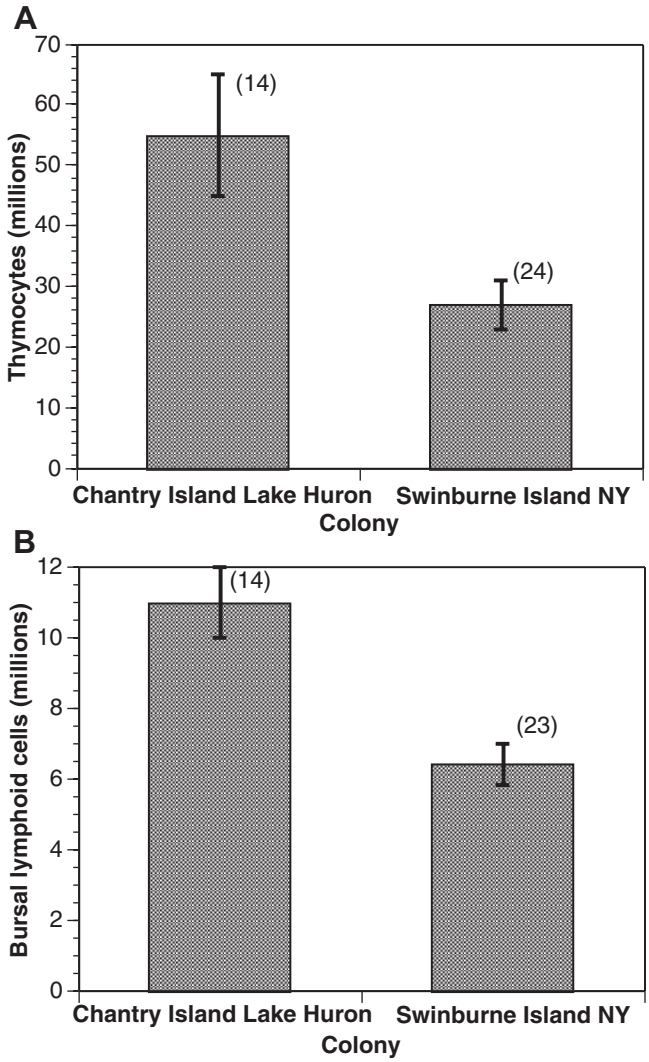

Fig. 1. Number of (**A**) viable thymocytes and (**B**) lymphoid cells in the bursa of Fabricius in pipping herring gull embryos collected from Swinburne Island in lower New York Harbor during 2003 and Chantry Island in Lake Huron in 2002. Means for both immune cell types were significantly lower on Swinburne Island compared with the reference site (Wilcoxon $p < 0.01$). Numbers in parentheses indicate sample sizes of chicks. Error bars indicate $\pm$ 1 standard error of the mean.

and 3). (TEQs for the Great Lakes and New York Harbor included the same set of PCDD and PCDF congeners. TEQs for New York reported above included seven PCB congeners with relatively low contributions to TEQs that were not available for the Great Lakes dataset. If these congeners are eliminated, mean and maximal TEQs in New York were slightly lower at 44 and 148 pg/g, respectively.) Mean DDE concentrations in New York gulls (0.033 ug/g) were much lower than pooled DDE concentrations at the Great Lakes sites (0.158 – 0.505 ug/g) and marginally lower than at the reference site (0.059 ug/g) in Fox et al. [25], suggesting that the negative correlation between DDE and PHA response in New York birds may not be causal but instead the result of co-correlation between DDE and immunotoxic dioxins and PCBs. Although no tissues were collected for contaminant analysis in black-crowned night herons in the present study, significant concentrations of dioxins and PCBs have been reported previously in the eggs of this species nesting in and near the Hudson Raritan Estuary [38].

Laboratory studies with chickens have shown that birds can be susceptible to immunotoxic effects at exposures similar to those observed in herring gulls from lower New York Harbor. In chicken embryos exposed from the beginning to the end of incubation (as in the present field study), thymic and bursal atrophy have been observed over a dose range of approximately 13 to 80 pg TEQs/g egg (converting from PCB concentrations to TEQs using World Health Organization toxic equivalency factors) [18–20]. As a species, herring gulls are approximately 33 to 50 times less sensitive to 2,3,7,8-TCDD compared to chickens, and 7- to 200-fold less sensitive to other planar PCDDs, PCDFs, and PCBs [39,40]. Still, some individual gulls are as sensitive as the average chicken [39].

Studies spanning a diverse phylogeny of organisms support the biological plausibility of these low exposure effects in herring gulls of New York Harbor. Adductor muscle concentrations as low as 2.0 pg 2,3,7,8-TCDD/g altered gonadal development, egg fertilization, and embryonic development in eastern oysters (*Crassostrea virginica*) in both controlled dosing studies in the laboratory and field studies, where oysters from uncontaminated areas were transplanted to contaminated areas (Newark Bay and Arthur Kill) [41]. Female rainbow trout (*Oncorrhynkis mykiss*) fed 1.8 ng 2,3,7,8-TCDD/kg food for 300 d experienced significantly reduced survival, as did their offspring [42]. These effects occurred at liver concentrations

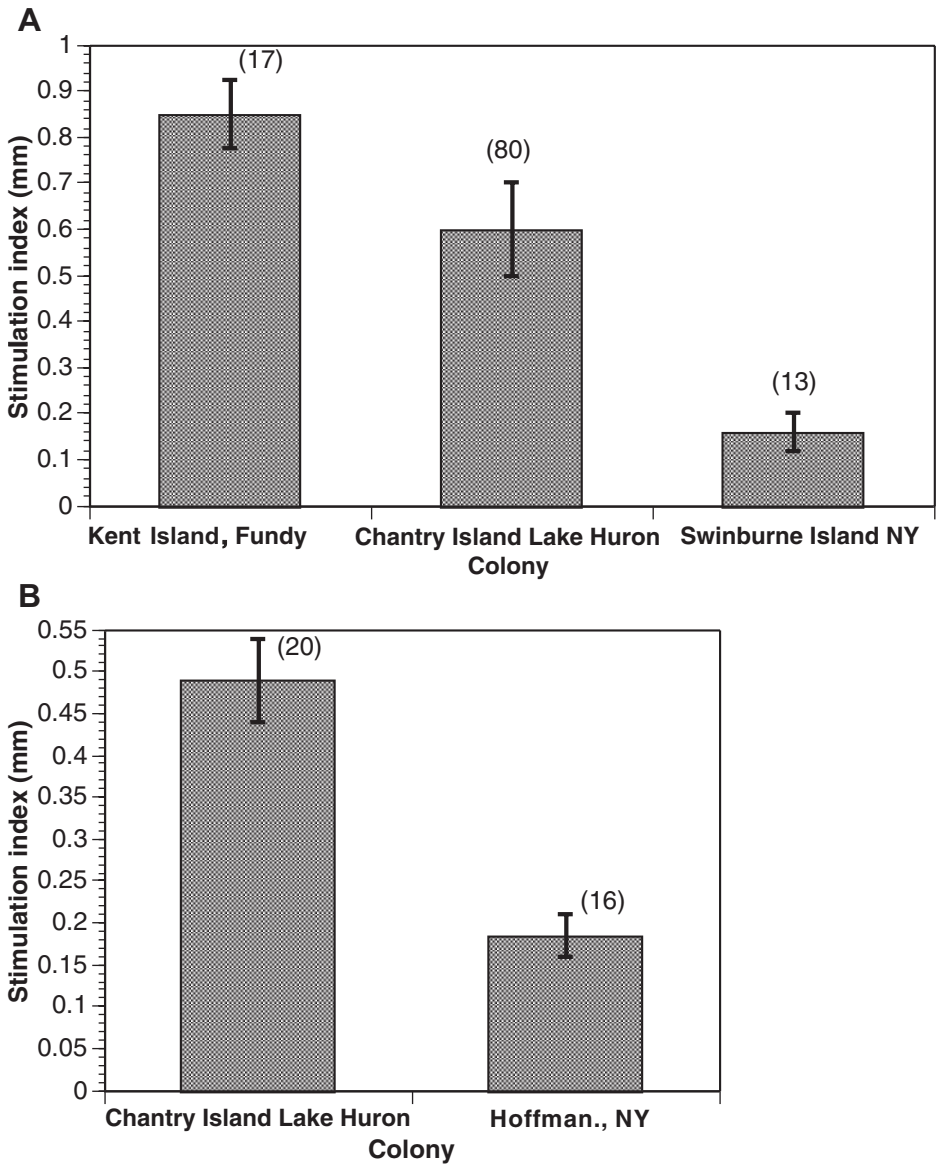

Fig. 2. Phytohemagglutinin skin response for T lymphocyte-mediated immunity in prefledgling (**A**) herring gull chicks and (**B**) black-crowned night heron chicks in lower New York Harbor. Mean responses differed significantly between sites for both herring gulls (Kruskal-Wallis $p < 0.00010$) and herons (Wilcoxon $p < 0.0001$). Herring gulls were sampled on Swinburne Island in lower New York Harbor during 2003, Chantry Island in Lake Huron from 2001 to 2002, and Kent Island in the Bay of Fundy during 2001. Black-crowned night herons were sampled on Hoffman Island in lower New York Harbor during 2003 and Chantry Island in Lake Huron from 2001 to 2002. Numbers in parentheses indicate sample sizes of chicks. Error bars indicate ± 1 standard error of the mean.

less than 1 ng/kg. Following a spill of PCBs into Saglek Bay, Labrador, Canada, the PHA response in prefledgling black guillemots (*Cyphus grille*) was suppressed significantly, both statistically and biologically [14]. The PHA response was suppressed 50% in a group with mean liver PCBs of only 111 ng/g total PCBs and 70% in a group with mean liver PCBs of 1,928 ng/g. The mean PCB concentration in reference birds was 46 ng/g. Mean liver PCBs in the New York Harbor herring gulls were 3.4 times higher than the intermediate group of guillemots that had a 50% suppressed response.

Potential associations of immunotoxicity with other contaminants

Several classes of chemicals of emerging concern, particularly polybrominated diphenyl ethers (PBDEs) and brominated dioxins and furans were not measured in the present study but likely were present in bird tissues. A survey of halogenated organic contaminants in and around New York Harbor before and after the World Trade Center (WTC) disaster showed that brominated chemicals were often found in higher concentrations than similar chlorinated chemicals [43]. Concentrations of PBDEs exceeded those of PCBs in post-WTC sewage sludge, and in some pre- and post-WTC water samples and post-WTC sediment samples. World Health Organization dioxin toxic equivalents for polybrominated dibenzodioxins and furans (PBDDs and PBDFs) were higher than those of PCDDs and PCDFs in WTC runoff and many water and sediment samples. In Japan, livers and eggs of common cormorants (*Phalacrocorax carbo*) contained significant concentrations of PBDEs, PBDDs, PBDFs, and polybrominated biphenyls (PBBs), showing biomagnification to levels of potential concern [44]. The PBDDs and PBDFs were lower than PCDDs and PCDFs. Elevated concentrations of PBDEs were found in herring gull eggs from the Great Lakes [45]. The presence of 2,4,6,8-tetrachlorodibenzothiophene (TCDT) in aquatic biota in

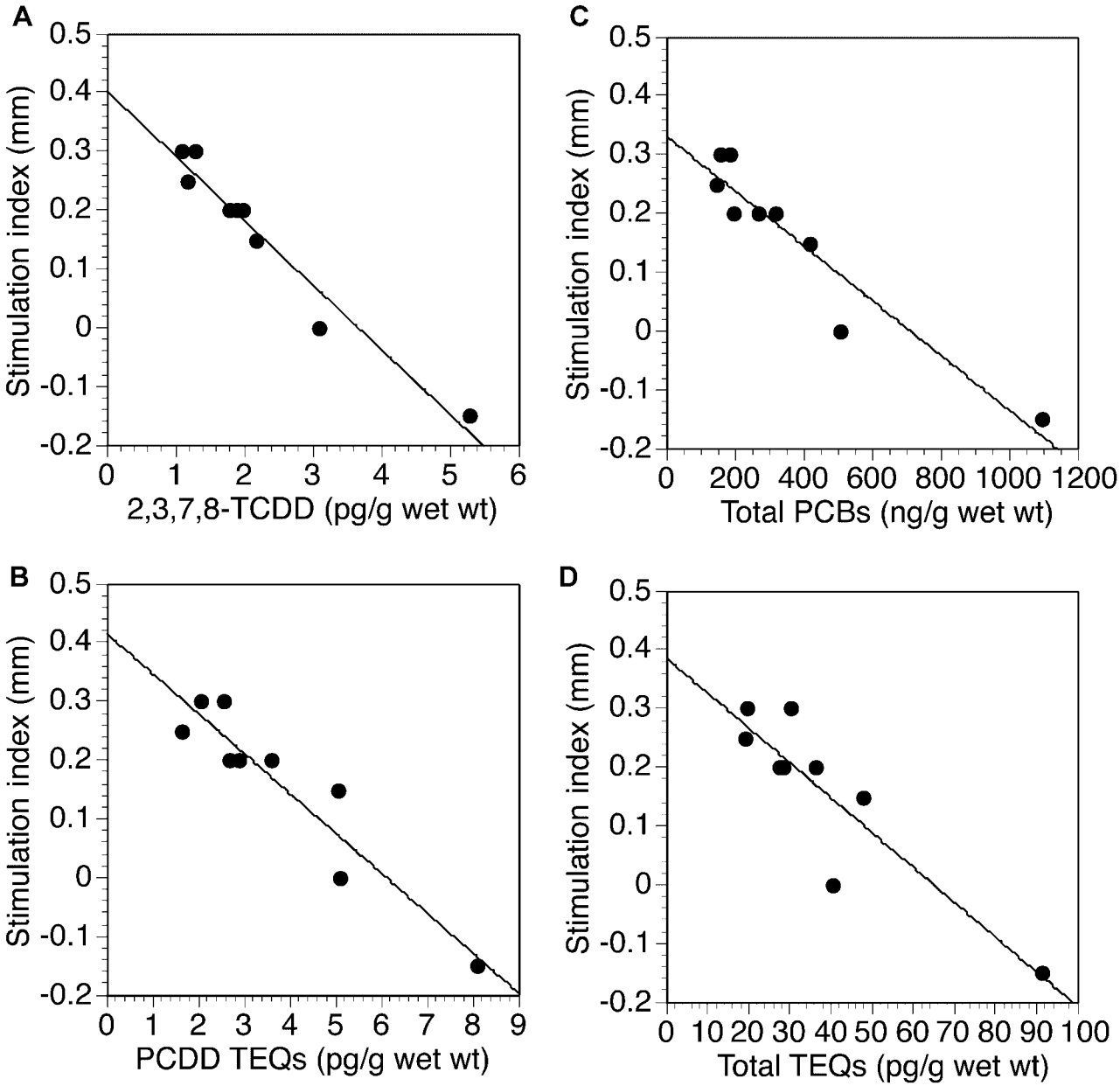

Fig. 3. Associations between selected contaminants and the phytohemagglutinin skin response for T lymphocyte-mediated immunity in prefledgling herring gull chicks collected from Swinburne Island in lower New York Harbor during 2003. Contaminants measured in livers included (**a**) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), (**b**) dioxin toxic equivalents attributable to polychlorinated dibenzodioxins (PCDD TEQs), (**c**) total polychlorinated biphenyls (total PCBs), and (**d**) total dioxin toxic equivalents attributable to polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and planar PCBs (total TEQs). For all correlations, $p < 0.05$.

the Hudson Raritan Estuary [46–48] also suggests the need to quantify polychlorinated dibenzothiophenes (PCDTs) in birds.

The PHA skin response was negatively correlated with liver Se concentrations, but correlations with most dioxins and PCBs were stronger (Table 2). Liver concentrations of Se in herring gull chicks in New York Harbor (mean of 3.4 ug/g dry wt, range 2.6 – 4.7; Table 5) appeared to be below concentrations associated with immunotoxicity, reproductive impairment, and growth retardation. Normal concentrations of Se are generally 12 to 16 ug/g dry weight in bird livers [49] and 7.86 ug/g for herring gulls in the United Kingdom ([50] as described by Ohlendorf et al. [51]). New York Harbor concentrations were similar to control Se concentrations in laboratory studies. In mallard drakes (*Anas platyrhynchos*) exposed to sodium selenite in drinking water for 12 weeks, no immunotoxicity was found at exposures that produced mean liver concentrations of 5 to 6 ug Se/g dry weight [52]. Controls had liver concentrations of 4 ug/g. Selenomethionine in drinking water increased liver Se to 15 ug/g and significantly reduced T cell function (delayed-type hypersensitivity response to tuberculin antigen) [52]. In young mallards fed 10 ug Se/g as sodium selenite or selenomethionine, minimal or no effects on growth were observed [53]. Livers of these birds accumulated 12 ug Se/g dry weight (3.5 ug/g wet wt) in one week of exposure and 20 ug/g dry weight (6 ug/g wet wt) after six weeks. Liver Se concentrations in controls were 0.7 to 1.3 ug/g dry weight (0.2 – 0.4 ug/g wet wt).

Contaminants and disease susceptibility. The relationship among pollutant exposure, immune status, and susceptibility to pathogenic diseases or parasitic infestation is a significant

Table 6. Lymphocyte proliferation in herring gulls collected from Hoffman Island in lower New York Harbor and Chantry Island in Lake Huron during 2003[a]

		Age[b]		
Proliferation variable	Site/*p* value	Adult	3-week chick	4-week chick
Spontaneous proliferation (absorbance)	Chantry	0.294 (0.052)	0.400 (0.039)	0.508 (0.058)
	NY Harbor	0.180 (0.025)	0.231 (0.029)	0.314 (0.022)
	Wilcoxon p	0.42	0.0043	0.036
LPS Stimulation Index	Chantry	4.37 (0.40)	3.08 (0.22)	3.09 (0.32)
	NY Harbor	4.17 (0.37)	5.18 (0.61)	4.42 (0.54)
	Wilcoxon p	0.63	0.0044	0.033
PHA + PMA Stimulation Index	Chantry	3.22 (0.22)	3.86 (0.24)	3.54 (0.28)
	NY Harbor	3.19 (0.39)	3.34 (0.59)	3.01 (0.30)
	Wilcoxon p	0.46	0.051	0.13
0.5 ug Con A Stimulation Index	Chantry	2.71 (0.22)	3.22 (0.23)	2.97 (0.23)
	NY Harbor	3.18 (0.43)	3.11 (0.53)	2.38 (0.17)
	Wilcoxon p	0.83	0.27	0.098
1.0 ug Con A Stimulation Index	Chantry	4.55 (0.51)	3.40 (0.20)	3.96 (0.46)
	NY Harbor	3.49 (0.30)	4.87 (0.88)	3.84 (0.50)
	Wilcoxon p	0.15	0.48	0.95

[a] N = 16–30 for Chantry and 12–24 for New York Harbor.
[b] Numbers indicate mean (standard error).
LPS = lipopolysaccharide; PHA = phytohemagglutinin; PMA = phorbol myristate acetate; Con A = concanavalin A.

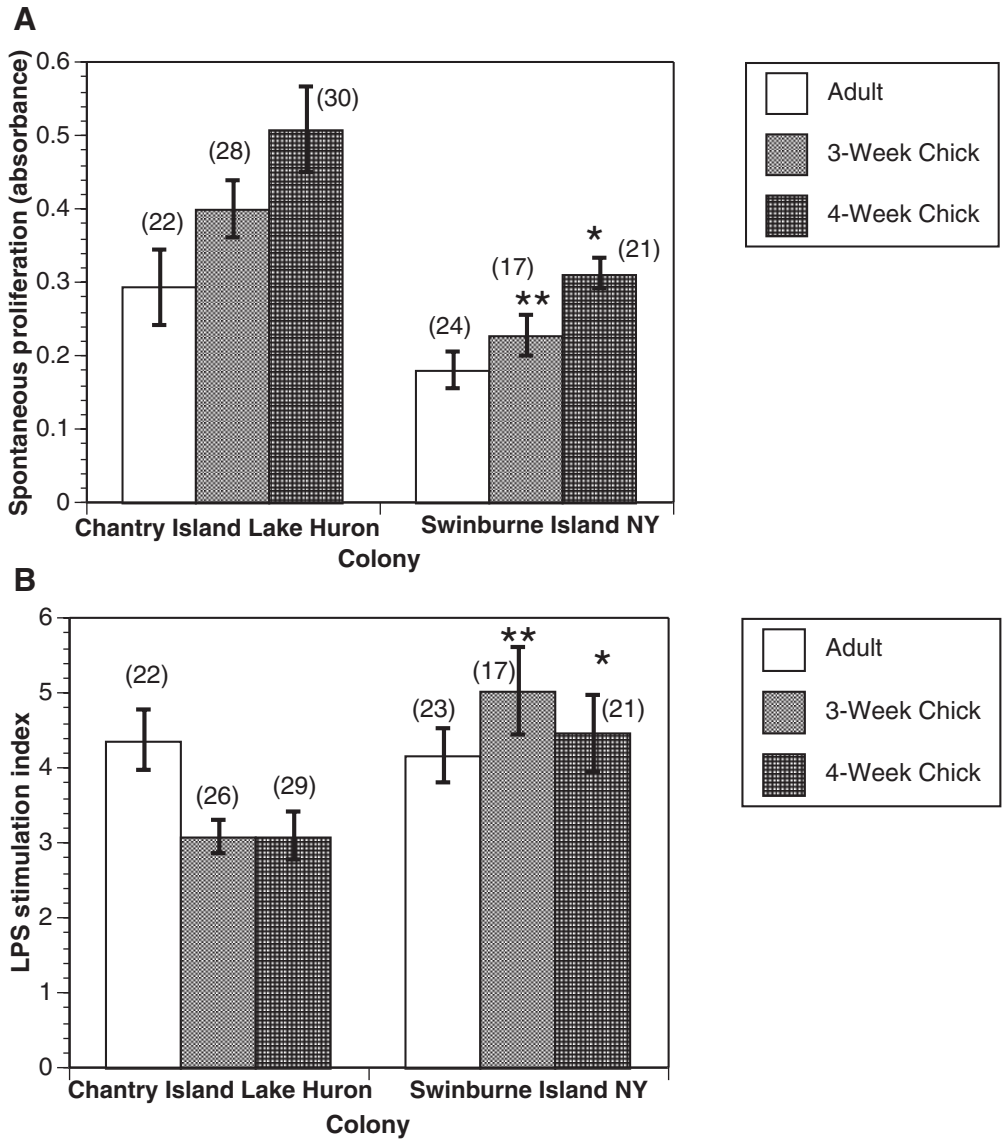

Fig. 4. Lymphocyte proliferation in herring gulls collected from Swinburne Island in lower New York Harbor and Chantry Island in Lake Huron during 2003. (**A**) Spontaneous or control proliferation in the absence of any mitogen. (**B**) Lipopolysaccharide (LPS)-stimulated proliferation. Numbers in parentheses indicate sample sizes. Error bars indicate ± 1 standard error of the mean. * indicates $p < 0.05$ and ** indicates $p < 0.001$ compared to Chantry using the Wilcoxon test.

concern. In laboratory animals contaminant-induced immunosuppression defined by immune function assays is usually associated with increased morbidity and mortality caused by challenge infections [22]. Similarly, field studies have demonstrated associations between contaminants and increased infections in free-living wildlife. More specifically, the PHA skin test results from the present study indicate a strong inverse relationship of T cell function with dioxin-like and total PCB as well as p,p′-DDE residues in prefledgling herring gull chicks. Mice exposed to 2,3,7,8-TCDD exhibited a dose-responsive increase in mortality following an otherwise nonlethal influenza virus infection [54]. Low pathogenic avian influenza has been positively reported in many bird species, with low global prevalence in herring gulls (1.4%) [55]. No herring gulls tested in New Jersey have been reported positive for low pathogenic avian influenza ([55]; supporting online material at: www.sciencemag.org/cgi/content/full/312/5772/384/DC1). To date, several reports have found highly pathogenic avian influenza, specifically the H5N1 subtype, in herring gulls from Denmark (U.S. Geological Survey, http://www.nwhc.usgs.gov/disease_information/avian_influenza/affected_species_chart.jsp). Virus-host challenge protocols are available for use in gulls, including H5N1 [56,57], providing a potential avenue for future assessments. Other potential studies might investigate interactions between contaminant-induced immunosuppression and health consequences of dermestid beetles, ectoparasites, whose presence has been identified in black-crowned night heron nests in New York Harbor and other colonies on the northeastern Atlantic coast [58].

Summary of major findings and ecological significance

The present study has demonstrated poor prefledgling survival in herring gulls and suppressed immune function in herring gull and black-crowned night heron chicks on islands in lower New York Harbor. Altered immunological endpoints included severely suppressed T lymphocyte function (PHA skin response) in both species, significantly reduced numbers of developing lymphocytes in the thymus and bursa of Fabricius of herring gull embryos, and altered in vitro lymphoproliferation responses in herring gull chicks. The observed immunosuppression was consistent with the immunological effects of dioxins and PCBs, although exposure was generally lower than in Great Lakes gulls that were also immunosuppressed [12]. In herring gull chicks, however, measures of dioxin and PCB exposure exhibited strong negative correlations ($r = -0.89$ to -0.98) with the PHA skin response (Fig. 3), suggesting that these chemicals contributed to the immunosuppression in New York Harbor birds. Immunological impairments and low prefledgling survival were consistent with the previously reported reduced breeding population numbers in the lower Newark Bay – Arthur Kill area [1]. The impaired T and B cell development and function observed in fish-eating birds of lower New York Harbor is consistent with exposure to organochlorines, especially PCDDs and PCBs, although definitive causal associations cannot be made without further investigation.

The biological and ecological relevance of suppressed immune function responses is a significant consideration. Immunotoxicity screening studies in laboratory rodents have shown that immune function endpoints are excellent indicators of immunotoxicity and decreased host resistance in challenge infection experiments [21–23,59]. The present field study of colonial waterbirds from New York Harbor demonstrated alterations in lymphoproliferation responses as well as suppression of the PHA skin response, which is one of the most sensitive integrative tests of T cell function in wild birds [11,17]. In a review of 12 immune function studies, nine of which employed the PHA test, immune responses were the most significant predictors of subsequent survival of young wild birds [60]. In an assessment of 280 introduction attempts in 38 avian species, immunocompetence as assessed by the PHA skin response was an important positive predictor of the ability of birds to colonize new areas (i.e., found new local populations) [61]. Hence, the immunological endpoints measured in the present field study are some of the best available for detecting immunotoxicity, and the observed changes in these endpoints are likely to have significant effects on disease resistance, survival, and other measures of ecological fitness.

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