Arch. Environ. Contam. Toxicol. 37, 369–376 (1999)

Environmental Contamination and Toxicology

Effects of Mercury on Health and First-Year Survival of Free-Ranging Great Egrets (*Ardea albus*) from Southern Florida

M. S. Sepúlveda,[1,*] G. E. Williams, Jr.,[1,†] P. C. Frederick,[1] M. G. Spalding[2]

[1]Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida 32611, USA
[2]Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32611, USA

Received: 10 November 1998/Accepted: 8 April 1999

Abstract. The objectives of this study were to determine whether elevated mercury (Hg) concentrations have a negative impact on the health and survival of nestling and juvenile free-ranging great egrets (*Ardea albus*) from southern Florida. During 1994, when health and survival was monitored in a cohort of young birds with naturally variable concentrations of Hg, packed cell volume was positively correlated with blood Hg concentrations, and high Hg concentration in blood was not related to the probability of surviving during the first 10.5 months of life. During 1995, 70 first-hatched great egret chicks were included in a Hg field-dosing experiment to compare the effects of elevated Hg on health and survival. Birds were dosed while in the nest orally every 2.5 days for 15 days with 0.5 mg of methyl mercury chloride (MeHgCl) for an estimated intake of 1.54 mg MeHgCl/kg food intake. These birds were compared with controls, which received an estimated 0.41 mg MeHgCl/kg food. No differences were observed in health parameters or in the probability of surviving during the first 8 months of age between egrets that were dosed with Hg and those that were not. A likely explanation for the lack of any effects on health and survival between both groups could be that chicks at this age were eliminating most of the dietary Hg through the production of new feathers.

Methyl mercury (MeHg) can have toxic effects on wildlife (Wolfe 1998). Some of the signs of Hg intoxication in animals include weakness, numbness, impaired vision and hearing, and incoordination (Carson 1986). Mercury can also affect hematological and immunological parameters. Shaw *et al.* (1991) administered methyl mercuric chloride to mice (*Mus musculus*) and observed a decrease in hemoglobin content, number of red blood cells, and packed cell volume when compared with controls. These authors also observed marked changes in red blood cell morphology, with rupturing and disintegration of cells. Mercury can also affect the function of the immune system by damage to lymphocytes and/or macrophages (Koller 1980). In embryos and weaning mice, this heavy metal suppresses both the primary and secondary immune responses (Koller *et al.* 1977). In addition, there is evidence that Hg intoxication can increase the susceptibility of free-ranging animals to disease and/or other stresses (Scheuhammer 1987; Spalding *et al.* 1994), which could result in decreased survival. To date, there is little information on the effects of Hg on the hematological and immune systems of captive or wild birds.

In recent years, high tissue Hg concentrations in wading birds from southern Florida have been implicated as a possible cause of the decline of bird numbers in the area (Spalding *et al.* 1994; Sundlof *et al.* 1994). Since MeHg is known to accumulate in organisms and be transferred up food chains, with top-level predators generally containing high tissue Hg concentrations (Scheuhammer 1991), great egrets (*Ardea albus*) could be at risk of Hg poisoning. However, very little is known about Hg contamination in Florida great egrets.

The objectives of this study were to determine whether elevated Hg concentrations have a negative impact on the health and survival of nestling and juvenile free-ranging great egrets. During 1994, health and survival was monitored in a cohort of young birds with naturally variable concentrations of Hg. In 1995, the concentration of Hg was artificially elevated in a sample of birds using a field–Hg dosing technique, and health and survival were compared to sham-dosed controls.

Materials and Methods

Study Area and Experimental Design

This study was conducted during the great egret reproductive seasons (March to June) of 1994 and 1995. Great egret chicks were sampled from eight colonies located in Water Conservation Areas (WCAs) 3A and 3B in southern Florida (see Sepúlveda *et al.* 1999a for a map of the study area). During 1994, survival and health were monitored in 46 and 117 chicks, respectively. The age of these chicks, determined by known hatch or laying dates, ranged from 1 to 44 days (average 17.8 ± 9.2

* *Current address:* Department of Physiological Sciences, P.O. Box 100144, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32611, USA

† *Current address:* West Virginia Cooperative Research Unit, West Virginia State University, Morgantown, West Virginia 26506, USA

Correspondence to: M. S. Sepúlveda

days). In addition, each time a bird was handled, bill length was measured to the nearest mm with a plastic ruler from the base of the bill to the tip of the maxilla (average 4.2 ± 1.7 cm). During 1995, 70 first-hatched great egret chicks were included in a Hg field-dosing experiment. Birds were considered first-hatched based on information obtained from frequent visits to the nests. These birds ranged in age from 6 to 21 days (average 11.6 ± 5.2 days and 3.9 ± 0.9 cm of bill length) at the beginning of the experiment. Chicks were given either empty gelatin capsules (control group) or gelatin capsules containing 0.5 mg of methyl mercury chloride (MeHgCl) (Hg-dosed group) every 2.5 days for 15 days (total exogenous dose of 3.0 mg MeHgCl). It was estimated that chicks consumed Hg at an average daily rate of 1.54 mg MeHgCl/kg food intake (0.41 mg MeHgCl/kg food from natural food items plus 1.13 mg MeHgCl/kg food from dosing). These concentrations were calculated using food intake data from wild great egret nestlings, and Hg concentrations in great egret prey fishes from the WCAs of the Everglades (Frederick *et al.* 1999). Dosed birds received a total dose that approximately tripled the rate of Hg intake that a great egret nestling would have been expected to ingest under natural conditions in southern Florida. Survival and health parameters were compared between chicks from the two treatments (below).

Health Indicators

During 1994, the health of great egret nestlings was evaluated through the determination of packed cell volume (117 chicks; 286 samples), concentration of plasma proteins (117; 284), total number and differential counts of white blood cells (83; 177), and mean abundance of oral trematodes (117; 235) (*Clinostomum* sp. because there are two species known to infect great egrets in Florida [Sepúlveda *et al.* 1999b], and defined as the number of parasites per host divided by the number of hosts examined [Bush *et al.* 1997]). These conspicuous parasites (red-brown in color and up to 1.5 cm long) are confined to the oral cavity and are very easy to count visually in live birds. In general, health of great egrets and total Hg in blood was evaluated at least twice (range of age 5 to 38 days), at the beginning and at the end of the sampling of each nest, and from all chicks within a nest. Growth rates of these birds relative to food consumption and Hg contamination are discussed by Williams (1997). During 1995, the same blood parameters were determined in 11 great egret chicks that were dosed with MeHgCl and in 10 controls. In addition, the mean abundance of *Clinostomum* sp. was compared between 28 egrets dosed with Hg and 27 controls. The above health indicators, as well as Hg in blood, were measured once prior to the start of the dosing experiment (day 0), and at days 5, 10, and 15 after the start of the experiment.

Survival

Between April and May 1994, 46 great egret chicks were marked with colored plastic leg bands (fitted above the tarsometatarsal joint) that had radio tags (Holohil LTD, 12 g, with mortality switches; range of frequencies 165.034–165.915) attached to them. The plastic band together with the radio transmitter weighed approximately 15 g (less than 4% of the weight of the chick in all cases). Bands and radios were fitted when birds were 27 ± 11 days of age (6.4 ± 0.8 cm bill length). Usually, more than one chick from each nest was tagged. These chicks were first bled for Hg determination at approximately 5 days of age and then sampled again at the time of radio-tagging.

Between April and June 1995, 70 first-hatched great egret chicks that were included in the Hg dosing experiment were marked with aluminum leg bands (fitted above the tarsometatarsal joint) that had radio tags attached to them (American Wildlife Enterprises, 10 g, with mortality switches; range of frequencies 164.113–164.735). Of the 70 birds, 30 were dosed with MeHgCl and 40 were undosed controls. The aluminum band together with the radio transmitter weighed approximately 12 g (less than 2.3% of the weight of the chick in all cases). Bands and radios were fitted when birds were 26 ± 4 days of age (7.0 ± 0.9 cm bill length). These chicks were first bled for Hg determination at about 12 days of age and then resampled every 5 days for a total of 15 days.

For both years, prefledging survival (*i.e.*, survival between radio-tagging and departure of the young from the colony) was documented through radio signal checks on the ground every 3 days. Any bird that died during this period was collected, a complete necropsy performed to determine cause of death, and several tissues saved for Hg analysis. A bird was considered to have fledged on the midpoint date between the last day recorded in the colony and the first day it was missed from the colony. After departure from the colony area, postfledging survival was monitored through radio signals detected during aerial surveys (n = 22 in 1994; n = 31 in 1995), flown approximately twice a week during the first 3 months posttagging (June, July, and August), once a week during September, and thereafter every 40 days until the end of the study (February of 1995 and 1996, respectively). Given the 1-year life of the batteries, transmitters were considered unreliable after this date, and birds were not followed again. Aerial survey flights were performed to cover all the WCAs, but also extended north to Lake Okeechobee and south to Florida Bay. When a mortality signal was heard, efforts were made to locate the carcass on the ground as soon as possible. Birds were assumed to have died at the midpoint date between the last day recorded alive and the first day a mortality signal was heard. Birds that were not located or that had transmitters fail or fall from the bands were "censored" for the survival analysis on the day they were last known to be alive.

Collection of Samples and Mercury Analysis

Techniques for the collection of blood and for the determination of total Hg concentration are described in Sepúlveda *et al.* (1999a). Unless otherwise noted, concentrations of Hg are expressed in mg/kg on a wet weight (WW) basis.

Statistical Analysis

Health Indicators: During 1994, the relationship between blood Hg concentrations and health parameters (packed cell volume; plasma proteins; number of white blood cells; number of eosinophils, heterophils, and lymphocytes) was studied using Pearson correlation coefficients. Basophils and monocytes were found rarely in blood smears, and thus are not included in this analysis. Because parasites are generally not normally distributed in populations, Spearman's nonparametric correlation coefficients were used to assess the relationship between mean abundance of oral parasites and blood Hg concentrations. Significant correlations were identified with the test based on Fisher's Z transformation (Neter *et al.* 1983). The slope (rate of increase) and intercept (baseline level at beginning of study period) of blood Hg concentration and each of the health variables was determined separately for each bird. The averages and standard deviations of these regression slopes across all birds were calculated and used in one-sample *t* tests to determine whether the average slope differed significantly from zero. Each slope was weighted by the inverse of its variance, to compensate for the fact that some slopes were estimated with more error than other slopes. Since sampling varied in frequency and intensity between birds, the x axis for each regression was standardized so that time = 0 was the first measurement, and only chicks with four or more measurements were included in the analysis. Bill length was added as a covariate to adjust for differences in age in these analyses. During 1995, the effects of treatment (Hg and control) on blood parameters and on mean abundance of oral parasites were tested using a repeated measures ANOVA (PROC GLM; SAS Institute

1988). Since oral parasites were not normally distributed in the sample of birds studied, the mean abundance of oral parasites was log-transformed prior to its inclusion in the ANOVA. In this procedure, the means for all these variables between treatments at days 0, 5, 10, and 15 of treatment were compared.

Survival: Age at fledgling was compared between years, and between "low" and "high" Hg birds (1994) and control and Hg-dosed egrets (1995) using *t* tests (PROC TTEST; SAS Institute 1988). For the 1994 data, a log-rank test (PROC LIFETEST; SAS Institute 1988) was used to test the hypothesis that an increased Hg concentration in blood (>2.01 mg/kg) at the time of radio-tagging (mean of 27 days of age) increased the probability of dying during the first 10.5 months of age. The LIFETEST program calculates Kaplan-Meier probabilities of survival through time, incorporating censored birds. "High" blood Hg concentrations were those in the highest 10% of the values. Similarly, during 1995 a log-rank test was used to compare survival during the first 8 months of age between egrets that were dosed with Hg and between egrets that were used as controls.

Results

Health Indicators

During 1994, Hg in blood averaged 1.2 mg/kg (n = 286 samples from 117 chicks; range 0.07–3.9 mg/kg; SD = 0.7). Packed cell volume, concentration of plasma proteins, number of white blood cells, and mean abundance of oral parasites in this group of birds averaged 35.42% (n = 286; SD = 7.03), 4.9 g/dl (n = 284; SD = 0.9), 22,444 cell/µl (n = 177; SD = 12,110), and 0.02 (n = 235; SD = 0.03), respectively. The results of the weighted *t* tests indicated a significant positive correlation between blood Hg concentration and packed cell volume (n = 20; $t = 2.42$; p = 0.0258) (Figure 1). There was no significant correlation between Hg blood concentration and the remaining health parameters measured (n = 19, $t = 0.75$, p = 0.4584 for plasma proteins; n = 8, $t = -0.63$, p = 0.5436 for number of white blood cells; n = 8, $t = -0.41$, p = 0.6896 for number of heterophils; n = 8, $t = 0.97$, p = 0.3637 for number of eosinophils; n = 8, $t = -0.94$, p = 0.3775 for number of lymphocytes; and n = 23, $t = 1.18$, p = 0.2913 for mean abundance of parasites).

During 1995, control nestlings maintained a more or less constant concentration of Hg in blood throughout the experiment (mean of 0.7 mg/kg) (Figure 2). During the first 5 days of the experiment, total Hg concentration in blood of dosed birds increased from 0.7 to 3.4 mg/kg (Figure 2). Mercury concentration in blood continued increasing during days 10 and 15, although at a much lower rate (from 3.4 to 4.5 mg/kg).

The relationship between days of experiment and all of the health parameters studied are plotted by treatment (control versus Hg-dosed egrets) in Figures 3 to 6. By the end of the experiment (day 15) all parameters measured (with the exception of oral parasites) appeared lower in Hg-dosed birds compared to control egrets, but these differences were not significant (repeated measures ANOVA: F = 0.34, df = 3, p = 0.7936 plasma proteins; F = 0.34, df = 3, p = 0.7969 packed cell volume; F = 0.31, df = 3, p = 0.8201 white blood cells; F = 0.41, df = 3, p = 0.7472 heterophils; F = 0.97, df = 3, p = 0.4143 eosinophils; F = 0.72, df = 3, p = 0.5485 lymphocytes; and F = 0.55, df = 3, p = 0.5886 mean abundance of oral parasites).

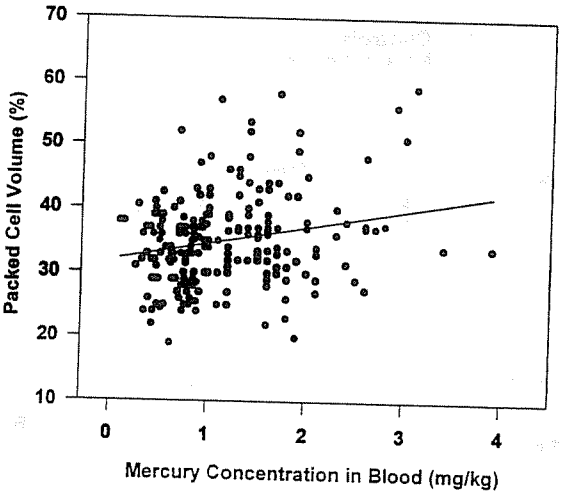

Fig. 1. Relationship between mercury in blood (mg/kg) and packed cell volume (%) in great egret nestlings during 1994

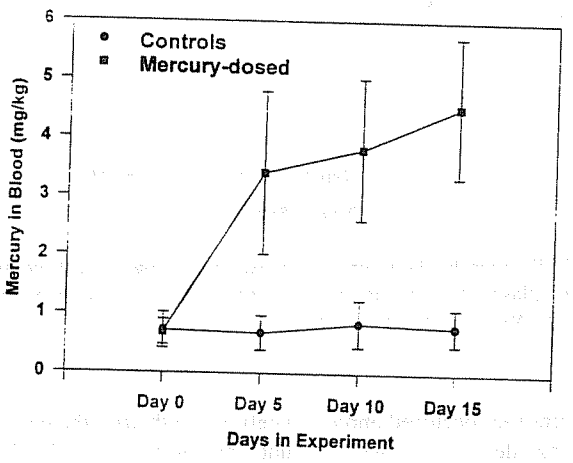

Fig. 2. Total mercury concentration in blood (mg/kg) of control and mercury-dosed great egret chicks during 1995, in relation to days in experiment. Values shown are means ± SD

Survival

Of the 46 birds radioed during 1994, 9 were never located after leaving their natal colonies; 7 birds were located once; 9 were located twice; 12 were found between three and five times; 8 were located between six and eight times; and a single egret was found on nine occasions. During the last survey (February 1995), 10 birds were still alive, 12 had died, and 24 were lost from the study because of different causes (1 had a broken radio, 4 had broken plastic bands, and signals from the remaining 19 birds could not be located during the last survey). Fledging (departure from colony) of radiotagged birds is plotted against age in Figure 7A. The overall average age at fledging during 1994 was 82 days (range 62 to 100 days). There was no difference on the average fledging age between egrets that had "low" blood Hg concentrations (average 82 days) and those that had "high" blood Hg concentrations (average 80 days) (n = 44, $t = 42$, $p > 0.05$).

Of the 12 mortalities, three occurred when birds were between 2 and 3 months of age (Figure 7B). The largest number

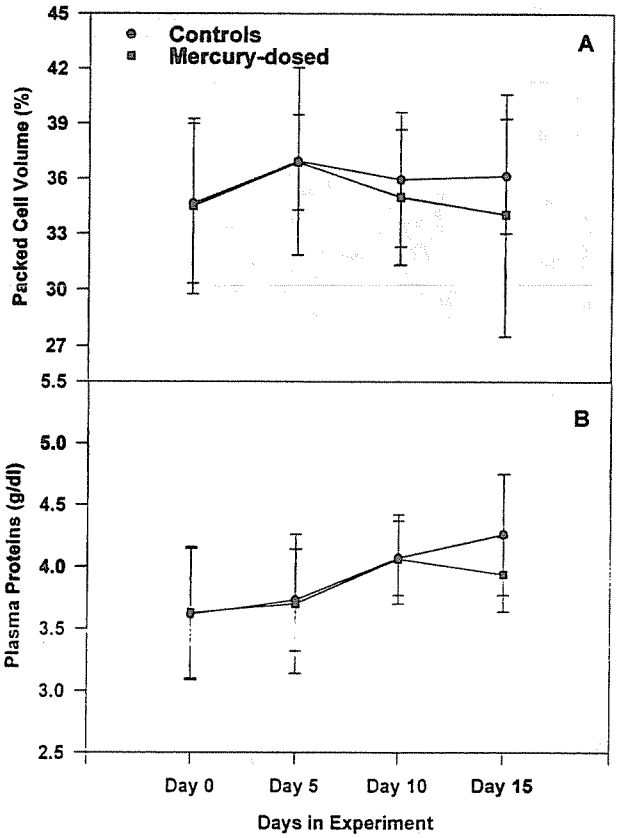

Fig. 3. Relationship between days in experiment, packed cell volume (A) and plasma proteins (B) in control and mercury-dosed great egret nestlings. Values shown are means ± SD

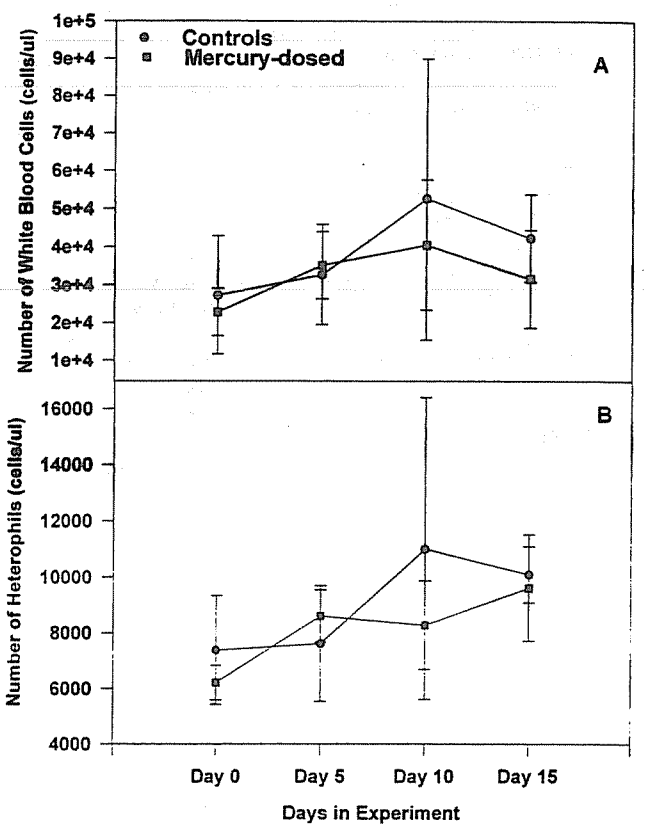

Fig. 4. Relationship between days in experiment and number of white blood cells (A) and heterophils (B) in control and mercury-dosed great egret nestlings. Values shown are means ± SD

of mortalities occurred approximately 1 month after the peak of fledging, decreasing thereafter until the birds were 320 days old. After a mortality signal was heard from the air, a search for the carcass was initiated as soon as possible. Autolysis and scavenging, however, occurred at extremely rapid rates: in all cases the cause of death could not be determined. The only remains recovered were bones, mature wing feathers, radios, and aluminum bands. On one occasion a partially scavenged carcass was recovered and a necropsy conducted. This egret was found to be infected with large numbers of feather lice (*Ciconiphilus decimfasciatus*) and with one nematode *Eustrongylides ignotus*, a parasite known to be an important cause of death of free-ranging nestlings egrets and herons (Spalding and Forrester 1993).

The effect of Hg on survival was tested using a log-rank test. From this test, there was no difference in the probability of surviving during the first 10.5 months of age between egrets that had "high" blood Hg concentrations (>2.01 mg/kg) (31.75% survival) and those that had "low" Hg concentrations (57.61% survival) (Figure 8A) ($\chi^2 = 1.4859$, df = 1, $p = 0.2229$). Overall survival for the 46 egrets radioed during 1994 was estimated to be 52.1%.

During 1995, 29 birds were never located after leaving their natal colonies (14 Hg-dosed and 15 controls); 17 birds were located between one and three times after leaving the colony (5 Hg-dosed and 12 controls); 10 egrets were located between four and seven times (3 Hg-dosed and 7 controls); and 14 birds were found between 8 and 14 times (8 Hg-dosed and 6 controls). During the last survey (February/March 1996), 5 birds were still alive, 7 had died, and 58 were lost from the study because of different causes (in 4 birds the radio fell off of the aluminum band, and signals from the remaining 54 birds could not be located during the last flight).

Of the 70 birds radioed in 1995, one died prior to leaving the colony and three lost their radios while still in the colony (Figure 7A). During 1995, birds left their natal colonies at a significantly earlier age when compared to 1994 (average fledging age of 71 days range of 51 to 88 days; $n = 111$, $t = 6.49$, $p < 0.00001$). There was no difference on the average fledging age between Hg-dosed birds (average of 73 days) and control egrets (average of 70 days) ($n = 66$, $t = -2.095$, $p > 0.05$).

Seven birds were found dead during the course of the 1995 study. One bird died prior to fledgling at an early age (19 days). At necropsy, this control female had an enlarged liver and spleen, and the glandular stomach was infected with one parasite (*E. ignotus*). The second and third mortalities occurred when birds were between 2 and 3 months of age (Figure 7B). The remaining four egrets died between 6 and 7 months of age. Of the seven birds that were found dead, six were controls and only one was a Hg-dosed bird (this latter bird died at 211 days of age) (Figure 7B). Because the only remains found from the six birds that died outside their natal colonies were a few bones and mature feathers, no definitive cause of death was established for these birds.

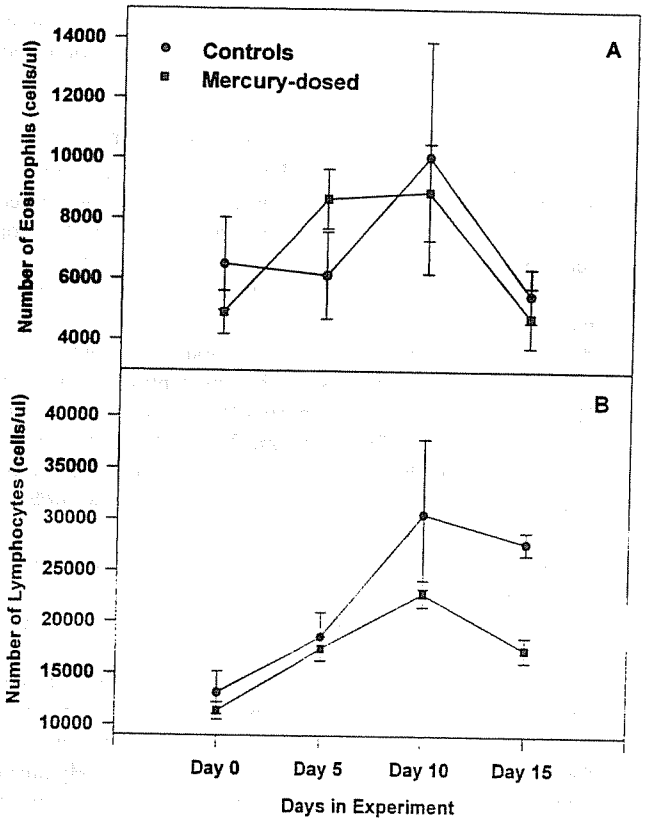

Fig. 5. Relationship between days in experiment and eosinophils (A) and lymphocytes (B) in control and mercury-dosed great egret nestlings. Values shown are means ± SD

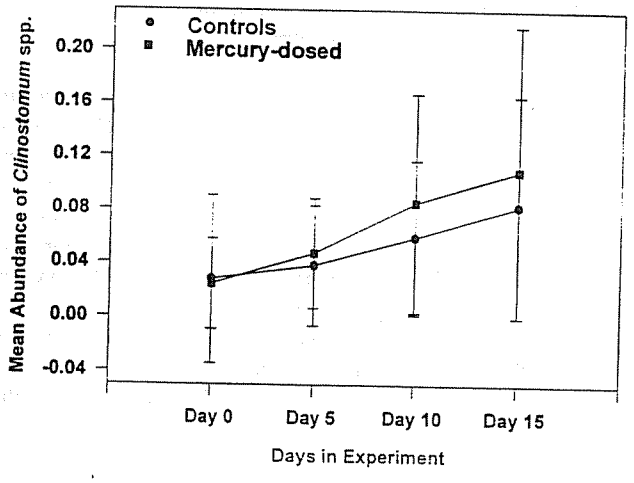

Fig. 6. Relationship between days in experiment and mean abundance of *Clinostomum* sp. parasites, in control and mercury-dosed great egret nestlings. Values shown are means ± SD

There was no difference in the probability of surviving during the first 8 months of age between egrets that were dosed with Hg (75.0% survival) and those that were not (62.7% survival) (Figure 8B) ($\chi^2 = 0.6623$, df = 1, p = 0.4158). Overall survival for the 70 egrets radioed during 1995 was estimated to be 66.0%.

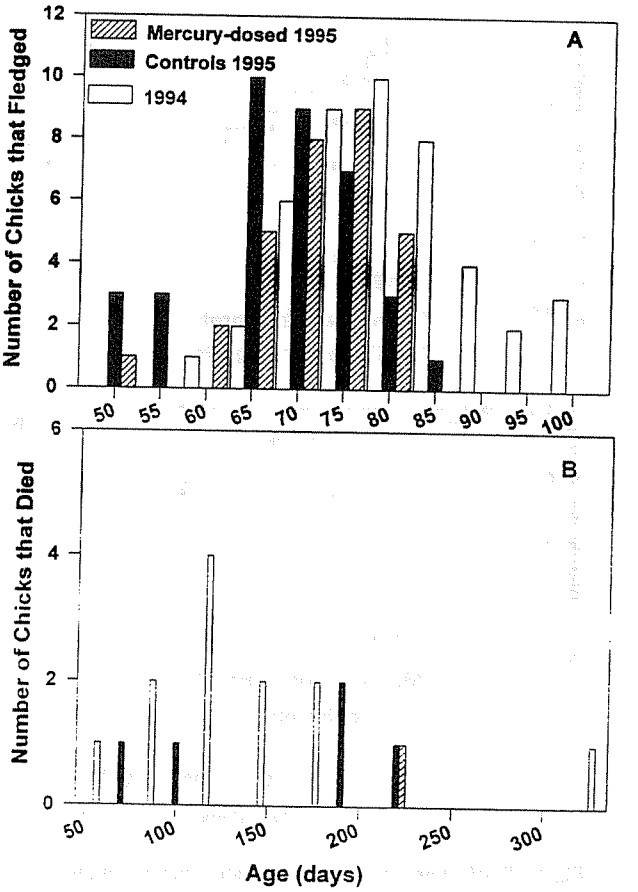

Fig. 7. Timing of fledgling (A) and of postfledgling mortality (B) during 1994 (shaded bars) and of mercury-dosed and control great egret chicks during 1995 (black and white bars) in the Everglades

Discussion

The reader should refer to Sepúlveda *et al.* (1999a) for a detailed comparison of Hg values in blood and feathers of great egret nestlings from southern Florida to Hg concentrations reported in several other nestling species sampled elsewhere.

The results obtained on the effects of Hg contamination on health parameters differed between years. During 1994 packed cell volume was positively correlated with naturally occurring blood Hg concentrations. During 1995, when the concentrations of Hg in tissues of free-ranging great egret chicks were artificially elevated through the administration of Hg, no effects of Hg contamination on health parameters were detected. However, because of differences in the methodology employed to measure the effects of Hg on health between 1994 and 1995, any comparison of the results obtained among years is probably inherently biased.

The lack of any observable effect of Hg on health parameters during 1995 emphasizes the difficulty in interpreting results obtained under field conditions without eliminating effects of possible confounding variables. The impact of other pollutant and nonpollutant environmental factors, such as inclement weather, diseases, or food shortages, are difficult to account for. In this respect, it is known that malnutrition and/or elevation of corticosteroids in response to chronic stress can cause suppres-

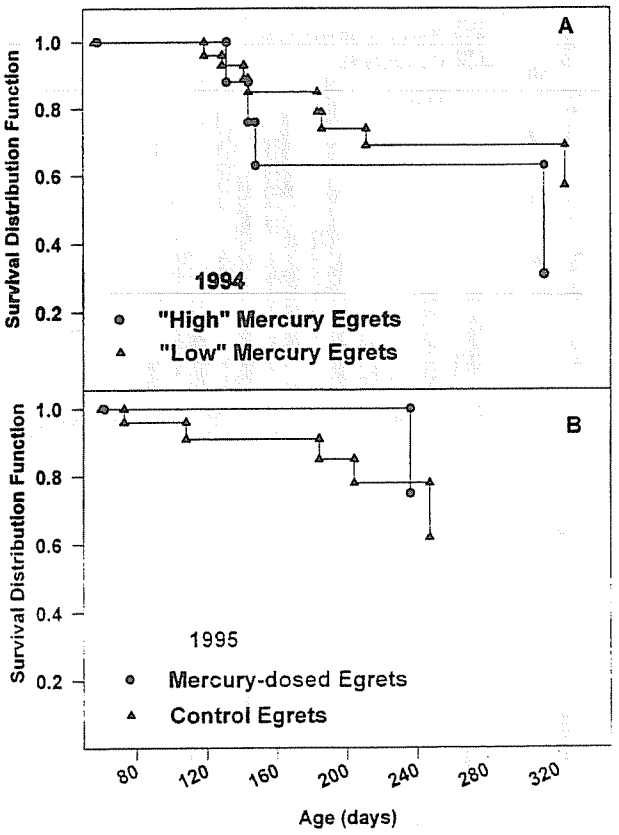

Fig. 8. Postfledging survival distribution functions of great egrets with "high" and "low" mercury concentrations during 1994 (A) and of mercury-dosed and control egrets during 1995 (B) in relation to age

sion of the immune system (Fairbrother 1994). If there was less food available in 1994 than in 1995, as suggested from nesting success records in the Everglades (Frederick 1995), then it could be hypothesized that during 1994 chicks would have been under a higher stress, which could have negatively affected several physiological parameters, including those studied here. In fact, 1995 was an exceptional breeding year for great egrets nesting in the Everglades, with reproductive parameters being the highest ever recorded in this area (Frederick 1995). Our data further support this hypothesis because egrets fledged at an earlier age and had a higher survival rate during 1995 when compared to 1994.

It is interesting to note, however, that during 1995, even though no effects of Hg on any of the health parameters studied were detected, some interesting trends were observed. The most intriguing ones had to do with the leukocyte and differential cell counts. Egrets that were dosed with Hg tended to have lower numbers of white blood cells, probably due to a decrease in the number of lymphocytes (Figures 4 and 5). In the laboratory, a suppression of humoral response in mice was noted after a relatively large dose of MeHg (17 mg/kg) was administered in a short period of time (Ohi *et al.* 1976). Rabbits administered mercuric chloride in water (10 mg/kg for 70 days) and inoculated with pseudorabies and influenza viruses had lower neutralizing antibody titers than controls (Koller 1973). In fish, there is evidence that pollutants (including heavy metals) might promote increased parasitism, by impairing the host's immune system or by favoring the survival and reproduction of the intermediate hosts (Khan and Thulin 1991). Presently, it remains unknown whether Hg in young egrets could increase their susceptibility to infection with *Clinostomum* sp. or other pathogens.

There are some indications that Hg can also affect hematological parameters in animals. Mice dosed with 24 mg/kg body weight of methyl mercuric chloride (intraperitoneal injection) for 14 days had a significant decrease inhemoglobin content, red blood cell count, and packed cell volume when compared to controls (Shaw *et al.* 1991). Rabbits given 10 mg/kg of mercuric chloride in drinking water for 70 days showed a decrease in packed cell volume compared to controls (Koller 1973). In fish dosed with sublethal concentrations of inorganic Hg, changes in erythrocyte morphology have also been reported (Chang *et al.* 1977; Panigrahi and Misra 1979). Anemia due to Hg intoxication has been documented from adult chickens (*Gallus gallus*) dosed with MeHg at a rate of 18.4 mg/kg for 42 to 87 days (Tejning 1967).

These results are in contrast to what was observed in the present study: that higher concentrations of Hg in blood were associated with higher packed cell volumes. There are two possible explanations for this outcome. The first one is that Hg could have caused a decrease in food intake and thus in the amount of water being ingested by the chicks (food is the only source of water for these birds at this age). Williams (1997) found that great egret chicks that were dosed with Hg during the 1995 field experiment decreased their food intake compared to controls. A decrease in food intake (and thus in water ingested) due to Hg could explain the increase in packed cell volume observed. The absence of a similar rise in the concentration of plasma proteins in these birds could be due to the fact that these free-ranging birds were in poor nutritional condition to start with (hypoproteinemic). This condition would have masked any increase in protein concentration due to a decrease in body water content.

Another possible explanation is that this relationship is probably an artifact. Since the concentration of Hg in blood is reported on a wet-weight basis, any change in the amount of water content in the sample is likely to alter the final Hg concentration that is reported. Thus an increase in packed cell volume (hemoconcentration probably due to dehydration) would cause an artificial increase in the concentration of Hg present in blood. This is an unlikely possibility in this study, as the average packed cell volume was very similar between birds that had "high" (over 2.01 mg/kg, packed cell volume of 39%) and "low" Hg in blood (less than 2.01 mg/kg, packed cell volume of 35%). In fact, to explain the difference in blood Hg concentration between the "low" and the "high" Hg groups (average blood Hg concentration of 1.05 mg/kg and 2.84 mg/kg, respectively) birds from the "high" Hg group would have needed a packed cell volume of almost 55%.

There are few reports on the effects of heavy metals on the immune system of free-ranging wildlife. Male mallards (*Anas platyrhynchos*) exposed to lead (through the ingestion of lead shots while foraging naturally) had significantly lower circulating numbers of white blood cells, with lower numbers of heterophils, lymphocytes, and monocytes, and lower packed cell volumes compared to controls (Rocke and Samuel 1991). Presently, there is only one report on the effects of Hg intoxication on blood parameters of free-ranging wading birds (Wolfe and Norman 1998). These authors found no correlation

between liver Hg concentrations (range of 1.39 to 1.63 mg/kg WW) in great blue heron nestlings (*Ardea herodias*) and heterophil to lymphocyte ratios. It is important to keep in mind that in our study, only a small aspect of the immune system functioning was evaluated. To adequately assess the overall immune response of an organism, it is necessary to employ a complete battery of assays, which include tests that measure both humoral immunity (*e.g.*, tests that quantify antibody production in response to specific antigens), as well as nonspecific immunity (*e.g.*, phagocytic activity of macrophages) (Fairbrother and Fowles 1990). None of these tests were performed in this study.

For both years, the concentration of Hg in tissues of great egrets was unrelated to the probability of surviving during the first 9 months of age. Most of the studies on the effects of Hg contamination on survival of nestling and juvenile birds have been conducted with non–fish-eating birds under laboratory conditions. In addition, the length, amount, and type of Hg administered, and tissues used to evaluate concentration of Hg have varied greatly between experiments. All these factors make any comparison with our results very difficult.

Mercury is known to negatively affect survival of captive birds, and also has been implicated in mortalities of free-ranging birds. In the laboratory, Heinz (1974) and Heinz and Locke (1976) observed an increase in early mortality of mallard ducklings dosed with MeHg (3 mg/kg WW). Similarly, an increase in duckling mortality was observed when adult American black ducks (*Anas rubripes*) were dosed with 3 mg/kg of MeHg (Finley and Stendell 1978). When pheasants (*Phasianus colchicus*), ducks (Rouen), and chickens were dosed at a rate of 33 mg/kg of MeHg for 35 days, it resulted in 90%, 85%, and 7.5% mortality, respectively (Gardiner 1972). Fimreite (1974) observed a 10–12% reduction in fledging rate of free-ranging common terns (*Sterna hirundo*) inhabiting a Hg-contaminated freshwater system (mean liver Hg concentration of 27 mg/kg WW). In wild grey herons (*Ardea cinerea*) (mainly first-year birds), liver Hg concentrations of over 160 mg/kg dry weight (approximately 45 mg/kg WW) coupled with cold stress and poor nutritional condition may have contributed to a massive die-off in The Netherlands (Van Der Molen *et al.* 1982). Meyer *et al.* (1998) reported a decrease in the production of Wisconsin common loon chicks (8 weeks of age) (*Gavia immer*) at lakes were blood Hg concentrations were elevated (>0.3 mg/kg WW). On the other hand, Hoffman and Curnow (1979) found no differences in liver Hg concentrations between live and dead wild great blue heron nestlings (mean Hg concentration of 0.96 mg/kg WW).

Despite the differences in the methodology employed in each of these studies, these results imply that species differences exist in sensitivity to the toxic effects of Hg. In this respect, there is some evidence that suggests that fish-eating birds have evolved different adaptations to the higher concentrations of Hg present in freshwater and marine ecosystems and thus are probably less susceptible to the effects of Hg poisoning (Smith and Armstrong 1978; Norheim *et al.* 1982; Thompson and Furness 1989). It remains unknown whether these factors, coupled with the limitations of our field dosing experiment (as discussed below), could have played a role in the results obtained.

Higher concentrations of Hg in tissues of wild birds have also been associated with an increased probability of death through chronic, debilitating diseases. Ensor *et al.* (1992) observed that juvenile common loons that died from disease had significantly higher Hg concentrations in feathers (mean of 19.8 mg/kg WW) than juveniles that died from injury (2.4 mg/kg WW). Similarly, Spalding *et al.* (1994) reported that juvenile great white herons (*A. herodias occidentalis*) that died from acute causes had lower liver Hg concentrations (1.77 mg/kg WW) compared to birds that died of chronic diseases (9.76 mg/kg WW). Gochfeld (1980) found significantly higher Hg concentrations in blood of sick common tern chicks (abnormal feather loss) (mean of 0.64 mg/kg WW), compared to normal chicks (mean of 0.37 mg/kg WW). These are interesting findings, and suggest at least a negative effect of Hg on the immune system of these species. In the present study, great egret nestlings that were dosed with Hg had considerably higher Hg concentrations in blood (average of 4.5 mg/kg WW at the end of the experiment) than those reported by Gochfeld (1980). However, because of the inability to recover fresh carcasses, the cause of death of these birds could rarely be established.

The lack of a relationship between blood Hg concentrations and first-year survival could be related to the small number of birds included in this study. Because of the high percentage of birds censored (average percentage of censored birds was 70% in 1994 and 92% in 1995) it isn't surprising that a difference of almost 26% in survival between birds that had "low" and "high" blood Hg in 1994 was detected as statistically nonsignificant. It was estimated that we would have needed to follow the survival of 175 birds in 1994 and of 157 birds in 1995 (as opposed to 46 and 70 birds, respectively) in order to achieve an α level (probability of a false positive result) of 0.05 and a β level (probability of a false negative result) of 0.2. This kind of sample size presents a big limitation for similar future studies, because of the high costs of radiotransmitters and flight time.

Finally, the absence of any significant effect of Hg on the health and survival of great egret nestlings during the course of the field dosing experiment could also be explained by the length and timing of the experiment. Because great egret chicks move out of the nest and become too mobile to catch after approximately 30 days of age, birds were dosed with Hg only for a relatively short period of time (15 days). In addition, during the time birds were being dosed, egrets were growing a complete new set of feathers. Feathers constitute an important excretion pathway of Hg in birds, and more than 50% of the body concentration of Hg in birds can be found in this tissue (Honda *et al.* 1986). At this point, it remains unclear whether a longer dosing regime, extended beyond the period of feather growing, would have negatively affected some or all the health parameters studied. Unfortunately, this approach is not possible under field conditions and would require a laboratory experiment.

Acknowledgments. This study was funded by the Florida Department of Environmental Protection (DEP) and the U.S. Fish and Wildlife Service. Our deepest thanks to Tom Atkeson from DEP for his help in designing and overseeing this project. Julio Arrecis and Tim Fitzpatrick from DEP were responsible for all the Hg analyses and their invaluable help and creative approach in solving several analytical problems is gratefully acknowledged. Katie Golden and Chris Gill provided invaluable assistance in the field. Jeff Brooks, Jeff Burm, and Joe Salatas conducted aerial surveys in the Everglades looking for great egrets with radio transmitters. Deborah Jansen (Big Cypress National

Preserve) provided help in the location of dead birds within Big Cypress National Preserve. Rob Bennetts (Florida Cooperative Fish and Wildlife Research Unit) provided field assistance and helped in the analysis of survival data. We also would like to thank Jay Harrison and Galin Jones (Department of Statistics, University of Florida) for their extensive help in the statistical analyses.

References

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

Carson BL (1986) Mercury. In: Carson BL, Elis HV III, McCann JL (eds) Toxicology and biological monitoring of metals in humans. Lewis Publishers, Ann Arbor, MI, pp 150–156

Chang LW, Dudley AW Jr, Dudley MA, Ganther HE, Sunde ML (1977) Modification of the neurotoxic effects of methylmercury by selenium. In: Rozin L, Shiraki H, Grcevic N (eds) Neurotoxicology. Raven Press, New York, NY, pp 275–282

Ensor KL, Helwig DD, Wemmer LC (1992) Mercury and lead in Minnesota common loons (*Gavia immer*). Water Quality Division, Minnesota Pollution Control Agency, St. Paul, MN, 32 pp

Fairbrother A (1994) Clinical biochemistry. In: Fossi MC, Leonzio C (eds) Nondestructive biomarkers in vertebrates. Lewis Publishers, Siena, Italy, pp 63–89

Fairbrother A, Fowles F (1990) Subchronic effects of sodium selenite and selenomethionine on several immune-functions in mallards. Arch Environ Contam Toxicol 19:836–844

Fimreite N (1974) Mercury contamination of aquatic birds in northwestern Ontario. J Wildl Manage 38:120–131

Finley MT, Stendell RC (1978) Survival and reproductive success of black ducks fed methylmercury. Environ Pollut 16:51–64

Frederick PC (1995) Wading bird nesting success studies in the Water Conservation Areas of the Everglades, 1992–1995. Final Report to the South Florida Water Management District, West Palm Beach, FL, 92 pp

Frederick PC, Spalding MG, Sepúlveda MS, Williams GE Jr, Bouton S, Lynch H, Arrecis J, Loerzel S, Hoffman D (1997) Effects of environmental mercury exposure on reproduction, health and survival of wading birds in the Florida Everglades. Final Report for the Florida Department of Environmental Protection, Tallahassee, FL, and the U.S. Fish and Wildlife Service, Atlanta, GA, 126 pp

Frederick PC, Spalding MG, Sepúlveda MS, Williams GE Jr, Nico L, Robins R (1999) Exposure of great egret (*Ardea albus*) nestlings to mercury through diet in the Everglades ecosystem. Environ Toxic Chem (in press)

Gardiner EE (1972) Differences between ducks, pheasants and chickens in tissue mercury retention, depletion, and tolerance to increasing levels of dietary mercury. Can J Anim Sci 52:419–423

Gochfeld M (1980) Tissue distribution of mercury in normal and abnormal young common terns. Mar Pollut Bull 11:362–367

Heinz GH (1974) Effects of low dietary levels of methylmercury on mallard reproduction. Bull Environ Contam Toxicol 11:386–392

Heinz GH, Locke LN (1976) Brain lesions in mallard ducklings from parents fed methylmercury. Avian Dis 20:9–17

Hoffman RD, Curnow RD (1979) Mercury in herons, egrets, and their foods. J Wildl Manage 43:85–93

Honda K, Min BY, Tatsukawa R (1986) Distribution of heavy metals and their age-related changes in the eastern great white egret, *Egretta alba modesta*, in Korea. Arch Environ Contam Toxicol 15:185–197

Khan RA, Thulin J (1991) Influence of pollution on parasites of aquatic animals. Adv Parasitol 30:201–238

Koller LD (1973) Immunosuppression produced by lead, cadmium, and mercury. Am J Vet Res 34:1457–1458

Koller LD (1980) Review/commentary. Immunotoxicology of heavy metals. J Immunopharmacol 2:269–279

Koller LD, Exon JH, Brauner JA (1977) Methylmercury: decreased antibody formation in mice. Proc Soc Exp Biol Med 155:602

Meyer MW, Evers DC, Hartigan JJ, Rasmussen PS (1998) Patterns of common loon (*Gavia immer*) mercury exposure, reproduction, and survival in Wisconsin, USA. Environ Toxic Chem 17:184–190

Neter J, Wasserman W, Kutner MH (1983) Applied regression models. Irwin, Homewood, IL, 503 pp

Norheim G, Somme L, Holt G (1982) Mercury and persistent chlorinated hydrocarbons in Antarctic birds from Bouvetoya and Dronning Maud Land. Environ Pollut (A) 28:233–240

Panigrahi AK, Misra BN (1979) Effect of mercury on the morphology of erythrocytes in *Ambas scandens*. Bull Environ Contam Toxicol 23:784–787

Rocke TE, Samuel MD (1991) Effects of lead shot ingestion on selected cells of the mallard immune system. J Wildl Dis 27:1–9

SAS Institute. Inc (1988) SAS/STAT™ User's Guide, Release 6.04 ed. SAS Institute Inc., Cary, NC, 1,028 pp

Scheuhammer AM (1987) Reproductive effects of chronic, low-level dietary metal exposure in birds. Trans North Am Wild Nat Res Conf 52:658–654

Scheuhammer AM (1991) Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. Environ Pollut 71:329–375

Sepúlveda MS, Frederick PC, Spalding MG, Williams GE Jr (1999a) Mercury contamination in free-ranging great egret nestlings (*Ardea albus*) from southern Florida. Environ Toxicol Chem (in press)

Sepúlveda MS, Spalding MG, Kinsella JM, Forrester DJ (1999b) Parasites of the great egret (*Ardea albus*) in Florida and a review of the helminths reported for the species. J Helminthol Soc Wash 66:7–13

Shaw BP, Dash S, Panigrahi AK (1991) Effect of methyl mercuric chloride treatment on hematological characteristics and erythrocyte morphology of Swiss mice. Environ Pollut 73:43–52

Smith TG, Armstrong FAJ (1978) Mercury in ringed and bearded seal tissues from Arctic Canada. Arctic 31:75–84

Spalding MG, Forrester DJ (1993) Pathogenesis of *Eustrongylides ignotus* in Ciconiforms. J Wildl Dis 29:250–260

Spalding MG, Bjork RD, Powell GVN, Sundlof SF (1994) Mercury and cause of death in great white herons. J Wildl Manage 58:735–739

Sundlof SF, Spalding MG, Wentworth JD, Steible CK (1994) Mercury in livers of wading birds (Ciconiiformes) in southern Florida. Arch Environ Contam Toxicol 27:299–305

Tejning S (1967) Biological effects of methyl mercury dicyandiamide-treated grain in the domestic fowl, *Gallus gallus* L. Oikos (Suppl) 8:7–116

Thompson DR, Furness RW (1989) The chemical form of mercury stored in South Atlantic seabirds. Environ Pollut 60:305–317

Van Der Molen EJ, Blok AA, De Graaf GJ (1982) Winter starvation and mercury intoxication in grey heron (*Ardea cinerea*) in The Netherlands. Ardea 70:173–184

Williams GE Jr (1997) The effects of methyl mercury on the growth and food consumption of great egret nestlings in the central Everglades. MS Thesis, University of Florida, Gainesville, FL, 105 pp

Wolfe M (1998) Effects of mercury on wildlife: a comprehensive review. Environ Toxic Chem 17:146–160

Wolfe M, Norman D (1998) Effects of waterborne mercury on terrestrial wildlife at Clear Lake: evaluation and testing of a predictive model. Environ Toxic Chem 17:214–227