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Effects of Elevated Mercury on Reproductive Success of Long-legged

Wading Birds in the Everglades

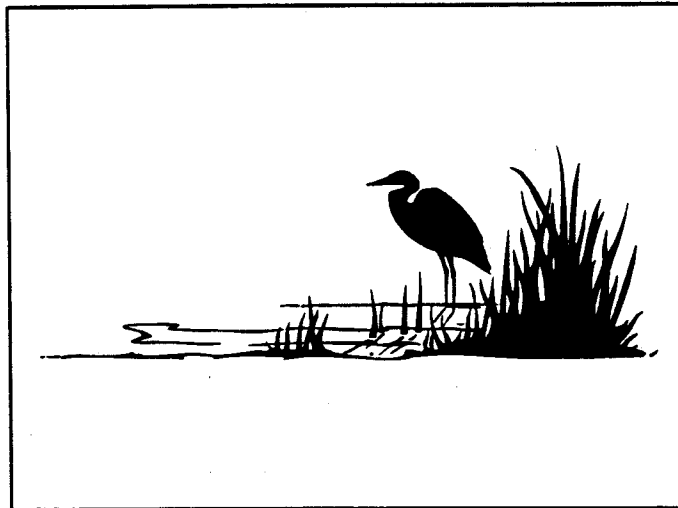
Annual Report

September 1995

for the

Florida Department of Environmental Protection

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Executive Summary

This report summarizes work performed on this project between August 1994 and July 1995, and includes new analyses of the 1994 field season.

During 1994 and 1995 we collected a total of 537 blood and 210 growing feather samples from 252 great egret nestlings (190 nests) from eight colonies located in Water Conservation Areas 3A and 3B in the Everglades. All samples for 1995 are not yet analyzed. For the 1994 data, mercury concentrations in blood increased significantly during the first month of age, and concentrations in growing feathers were not significantly affected by size of chicks. Geographic location (colony site) had a significant effect on both blood and feather values. The pattern of "high" and "low" mercury colonies does not seem to follow any obvious ecological gradient, but intra-colony differences among nests were insignificant contributors to variance in blood and feather mercury values.

Blood parameters (white blood cell count and packed-cell volume) and number of parasites measured in individual chicks were used to define a health criteria that divided chicks into "sick" and "healthy" categories. Mercury concentrations in growing feathers were significantly associated with indicators of sickness among the older of the chicks (bill length > 4.2 cm).

During 1994 and 1995, we monitored the post-fledging survival of a total of 46 great egret chicks (30 nests) with radio transmitters. We estimated survival at 73% during the first 10.5 months of age, and found that mercury concentrations in blood and feathers were not associated with survival. Juvenile great egrets tended to disperse from natal colonies to the north and west of the Everglades, and tended to settle in shallowly flooded agricultural land for extended periods.

We measured great egret chick food intake rates in order to estimate mercury exposure in wild birds, and in order to examine the relationship between mercury load and chick appetite.

Using an isotopically or radio-labeled water method, we obtained food intake measurements from 24 great egret chicks. When the effect of chick age was controlled for, these data revealed significant differences in food intake between chicks in the Hidden and L-67 colonies. There was no significant correlation between food intake and mercury in growing feathers, and a nearly significant correlation between food intake and blood mercury concentrations.

We collected breeding plumes shed by adults at solitary nests of 16 great blue herons by repeatedly searching nest sites during the post-nuptial molt. The mercury values of these plumes will be compared with feather values from 40 great blue heron carcasses representing the population at large, in order to assess the effect of mercury on the ability of herons to come into reproductive condition and breed successfully.

We analyzed associations among mercury values of different tissues from 187 great egrets and 51 great blue herons. Preliminary results indicate that mercury concentrations in powder down and growing feathers are strongly predictive of concentrations in liver, brain, muscle, and kidney of both great egrets and great blue herons. Mercury was distributed unevenly among different tissues, with highest values in feathers, and lowest in brain. Age and location had a significant influence on mercury concentrations in blood, liver, brain, muscle, kidney, powder down, and growing feathers. Birds collected from Broward county had significantly higher mercury values than those from Dade, Collier, and Monroe counties. Sex had no significant influence on any of the tissue mercury values, and mercury concentrations increased with age of bird.

The sublethal effects of methylmercury chloride were studied in four captive adult great egrets by dosing with methylmercury (two dosed, two control). Dosing was accomplished with implanted osmotic pumps for a period of 28 d, with a target intake rate of 5 mg/kg in food. Three of the birds died, at least two of which were likely the victims of severe weather during the experiment. By comparing the remaining bird in its undosed and dosed

state, we found measurable changes in the electroretinogram (ERG), with a decrease in the scotopic amplitude and latency. This response is consistent with previous reports on effects of mercury on vision. In addition, there were ocular histological differences between the singled dosed bird that survived until completion of the experiment and the control birds.

During 1994 and 1995, regurgitations obtained from 75 great egret chicks were examined for prey composition. Fifteen different species of fresh-water fishes were identified, in addition to insects and crustaceans. These data will be coupled with fish species-specific mercury determinations from other studies, to hone the estimate of mercury exposure to young birds in the Everglades.

During 1995, we performed a field experiment to compare the effects of elevated mercury on appetite and survival of great egret chicks. We monitored food intake (42 chicks in 42 nests) and survival (72 birds in 72 nests) that were divided into dosed ($n = 33$) and placebo ($n = 31$) treatments. Survival is currently being monitored, and both mercury and food intake samples are in the process of being analyzed.

In summary, our work to date suggests that mercury values that are in the high end of the range encountered by wading birds in the Everglades may reduce the appetite of adult great egrets, and may result in alteration of eye function and pathology in adult great egrets. In addition, we have found significant differences in naturally occurring mercury in great egret chicks that appears to be attributable to geographic location within the Everglades. Our work has also suggested that mercury may be nondestructively sampled in herons and egrets through the use of growing feathers and powderdown, and that samples from these tissues are predictive of mercury in other key body tissues.

Acknowledgments

This study is funded by the Florida Department of Environmental Protection (DEP), by the United States Fish and Wildlife Service (USFWS), and further supported by DEP through analyses of all mercury samples. We wish to thank Dr. Tom Atkeson, of the DEP, for his help in designing and overseeing this project. Julio Arrecis and Tim Fitzpatrick from DEP were responsible for all the mercury analyses, and their invaluable help and creative approach in solving several analytical problems is gratefully acknowledged. We especially wish to thank Chris Gill and Katie Golden for their assistance in the field. Hugo Ochoa provided assistance in the field, and helped in statistical analyses and construction of figures. Laura Quinn of the Florida Keys Wild Bird Rehabilitation Center provided captive birds for the experimental study as well as numerous carcasses from several species of wading birds. Deborah Jansen (Big Cypress National Preserve) and Dianne Singler (Nature Conservancy) also provided carcasses of egrets and herons; we also thank Deb Jansen for helping with transportation to locate dead birds within Big Cypress National Preserve. We are indebted to Ken Nagy (University of California at Los Angeles) and Leo Stenberg (University of Miami) for extensive help in analyzing the deuterium samples, and for helping us to design a vacuum line for blood-water distillations. We thank Dr. Donald J. Forrester (University of Florida, Department of Pathobiology, College of Veterinary Medicine) for providing materials and a space in his laboratory. Dr. Stephen M. Roberts (University of Florida, Department of Physiological Sciences, College of Veterinary Medicine) helped in the design of the dosing experiments. Henry Gholtz, John Johnson, and Ken Clark (University of Florida, School of Forest Resources and Conservation) generously allowed us the use of laboratory space, and equipment necessary for processing deuterium samples. Rob Bennetts (Florida Cooperative Research Unit) provided field assistance as well as help in analyzing survival data. Bill Loftus (National Biological Survey) and Ted Lange (Florida Game and Freshwater Fish Commission) provided useful information on mercury concentration

in fish from the Everglades. George Burgess (Florida Museum of Natural History) and Leo Nicco and Rob Robins (National Biological Service, Gainesville) helped us in the identification of fishes. Finally, we wish to thank biologists with the Florida Game and Freshwater Fish Commission for assistance in collecting carcasses and incapacitated birds.

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Introduction

In recent years, high concentrations of mercury have been found in several taxa of Everglades animals, including fish, Florida Panthers (*Felis concolor coryi*), and wading birds (Spalding and Forrester 1991). Concentrations are high enough in fish to have prompted warnings against human consumption, and could have been the causative agent in the death of one Florida panther.

The finding of mercury in Everglades animals has led to studies of the location of mercury hotspots within the Everglades, contributors to mercury deposition, and research into how mercury becomes concentrated in Everglades food webs. However, an understanding of the actual effect of mercury contamination on wild populations of vertebrate species affected is almost completely lacking. As a vital link in this chain of research, we therefore have proposed work which describes the sublethal effects of mercury contamination on a vertebrate group that may be particularly susceptible, the long-legged wading birds.

Breeding populations of wading birds (herons, egrets, ibises, storks, and spoonbills) have declined by over 90% in the Everglades ecosystem since the 1940's (Ogden 1994). The declines are apparently due to a combination of effects which include reductions of total habitat of at least 30%, salinization of the estuarine feeding zone, decreases in the amount of short hydroperiod marsh, compartmentalization and isolation of large sections of the marsh by dikes and canals, and unpredictable hydropatterns due to water management (Bancroft et al. 1994; Ogden 1994; Frederick and Spalding 1994). These effects have greatly shortened the annual time window available for birds to breed, and generally reduced feeding options. In addition, the Everglades populations seem to be at risk from a nematode parasitic infection related to human land use (Spalding et al. 1993). Currently, wading birds breed in small numbers, in small numbers relative to nonbreeders, and abandon breeding attempts quite easily (Frederick and Collopy 1989; Hoffman et al. 1991).

It is likely that wading bird declines in the Everglades began well before mercury concentrations increased. Although mercury may not have caused the initial reductions in the breeding population, mercury contamination could be a prime contributor to the effect of these ecological stresses, since (as below) mercury is likely to affect courtship, chick rearing behaviors, the ability to gather food during reproductive attempts, and the ability of fledgling birds to learn foraging techniques. Thus mercury could have significant effects upon the ability of many birds to come into reproductive condition, successfully attract a mate, rear young, and have those young survive to a breeding age. For these reasons, mercury loading could hold a keystone position among the factors affecting Everglades wading birds.

It should be recognized that the Everglades mercury problem is not limited to resident birds, and also has the potential to affect wading birds from the entire Southeast region. Hundreds of thousands of wading birds congregate in the Everglades to overwinter each year (Bancroft et al. 1992), and disperse northward each spring. This makes the Everglades mercury problem a regional concern as well.

Relatively little is known about sublethal effects of mercury on long-legged wading birds. Mercury concentrations in livers of ciconiiforms measured in southern Florida from 1988 to 1991 ranged to 18.84 mg/kg (wet weight) in nestlings and 74.54 mg/kg in adults (Spalding and Forrester 1991). Significantly higher values were found in both nestlings and adult birds in the central Everglades and eastern Florida Bay region when compared to the Lake Okeechobee/northern Everglades and the western mangrove/western Florida Bay regions. Spalding and Forrester (1991) also found that juvenile great white herons that died of disease and starvation in Florida had significantly higher mercury concentrations (> 5 mg/kg) in their livers than did those which died of acute, traumatic causes.

Mercury concentrations higher than 100 mg/kg wet weight have been reported in livers of birds of the genus *Ardea* (Van der Molen et al. 1982; Fimreite 1974; Dustman et al., 1972). Van der Molen et al. (1982) found that high mercury concentrations also reduced the survival

of gray herons (*A. cinerea*) during stressful winter weather conditions. Van der Molen et al. also found that oral administration of methylmercury to gray herons resulted in lethal concentrations in the livers between 415 and 752 mg/kg dry weight (using their 28% conversion this is equivalent to 116 - 210 mg/kg wet weight). The age of the birds dosed, and the dose given was not stated. In other studies of non-ciconiiform birds, experimental dosing with mercury resulted in brain lesions, decreased reproduction, incoordination, paralysis, brain lesions in offspring, behavioral changes in offspring, and reduced survival in offspring (Borg 1969; Fimreite and Karstad 1971; Spann et al. 1972; Pass 1975; Heinz and Locke 1976; Finley and Stendell 1978; Heinz 1975, 1979). The lowest concentration of mercury in liver in mallard ducks with (*Anas platyrhynchos*) brain lesions consistent with methylmercury poisoning was 5 mg/kg wet weight (Pass 1975).

The highest mercury concentrations in ciconiiform eggs so far measured in Florida were in great egret (*Casmerodius albus*) nests from central Shark Slough in 1972 (up to 0.91 mg/kg, wet weight, Ogden et al. 1974). It is unclear what effects these concentrations may have on ciconiiform young. Fimreite (1974) found reduced hatching in common tern (*Sterna hirundo*) eggs that had a mean concentration of 3.65 mg/kg (wet weight) when compared to eggs from another colony that had a mean concentration of 1.00 mg/kg wet weight. However, he also found considerable variation between species in the threshold concentrations at which eggs fail to hatch. Sooty tern (*Sterna fuscata*) eggs from the Dry Tortugas, Florida, collected from a colony with no apparent hatching problem contained a mean of 7.93 mg/kg mercury wet weight (Stoneburner et al. 1980). Behavioral differences were noted between mallard ducklings hatched from eggs containing 1 mg/kg (wet weight) and controls (Heinz 1975, 1979). Black duck (*Anas rubripes*) eggs that failed to hatch contained 6.08 to 9.62 mg/kg (wet weight, Finley and Stendell 1978). Brain lesions have been described in nestling mallard ducklings hatched from eggs with 5.46 to 7.18 mg/kg (wet weight) (Heinz and Locke 1976).

The observed effects of mercury on motor skills (above) in the laboratory could easily

be manifested in free-ranging adult wading birds in a number of ways that could strongly affect reproduction, such as lowered foraging efficiency, and altered courtship behavior. Decreased feeding and courtship abilities could strongly affect the ability to come into reproductive condition, the ability to obtain a mate, and the ability to feed and care for young. In addition, it is likely that mercury-contaminated young might also have trouble developing motor skills, especially foraging skills. Coupled with a reduction in overwinter survival of adults and, particularly, juveniles the sublethal effects of mercury could severely reduce recruitment to populations of ciconiiform birds. Since there is evidence that substantial proportions of the wading birds in the Everglades are not coming into reproductive condition (Hoffman et al. 1991), that reproduction is relatively easily interrupted, and that many birds have elevated mercury concentrations, it seems of interest to establish the role of mercury in influencing reproduction of these birds in the Everglades.

The concentrations of mercury so far recorded in adults and young from the Everglades are certainly in the range in which they could be affecting behavior and survival. However, this conclusion is quite tenuous, given the paucity of work on effects of mercury in ciconiiform birds. The ability to extrapolate effects observed in the laboratory is further complicated by the other environmental stresses, such as reduced food resources experienced by wading birds in the Everglades ecosystem (Loftus and Eklund 1994).

The overall purpose of this study is to determine whether or not the presently observed mercury concentrations in wading birds in southern Florida are detrimental to the reproductive success of adults and survival of young wading birds. Where possible, the project will also search for evidence which sheds light on the more difficult question to answer; whether or not mercury concentrations affect the ability of adults to initiate breeding.

Summary of Progress in 1994/5

This report covers work done between August of 1994 and August of 1995, and is not intended to include work reported in the 1994 annual report. In many cases, however, sample analysis (particularly mercury determination and food intake estimation) were not completed from the 1994 field season at the time of the 1994 report. This report therefore includes considerable new information and analyses from the 1994 field season. The reader should bear in mind that a complete picture of the entire research project to date must include both 1994 and 1995 reports.

I. MERCURY CONCENTRATIONS IN BLOOD AND GROWING FEATHERS FROM WILD GREAT EGRET NESTLINGS

Introduction

One of the initial objectives of this study has been to determine a tissue or tissues, and a sampling method, that can be used to accurately monitor mercury burdens in wild populations of herons and egrets. The achievement of this goal requires, of course, that several tissues be sampled simultaneously, that relative mercury deposition rates in these tissues be understood, and particularly for young birds, that the chronology and dynamics of mercury transport to these tissues is known. In this section, we report on the results of a large sampling of blood and feathers from great egret nestlings in the Everglades. In addition, we include analyses of the effect of colony location on mercury concentrations, and an assessment of the relationship between health indicators and mercury concentrations across individuals.

Table 1.1 Summary of mercury sampling of great egret nestlings during 1994, by colony.

Colony *	Chicks Sampled	Nests Sampled	Average Frequency of Sampling	Collection Events [^]	Blood Samples Collected	Feather Samples Collected **
Frog City South	2	1	Every 7 days	4	8	1
Hidden/L-28	29	14	Every 6 days	22	91	26
Deer Island	4	2	Every 5 days	5	16	3
L-67	43	22	Every 13 days	12	84	27
Mud Canal	13	6	Every 6 days	5	36	8
JW-1	17	8	Every 13 days	5	21	9
Alley North	14	7	Every 12 days	4	32	10
Total	122	60		57	288	84

Table 1.2 Summary of mercury sampling of great egret nestlings during 1995, by colony.

Colony *	Chicks Sampled***	Nests Sampled	Average Frequency of Sampling	Collection Events	Blood Samples Collected	Feather Samples Collected **
Tamiami East	32	32	Every 2.5 days	19	105	32
Hidden/L-28	45	45	Every 2.5 days	20	144	41
L-67	14	14	-	1	0	14
Mud Canal	11	11	-	1	0	11
JW-1	14	14	-	1	0	14
Alley North	14	14	-	1	0	14
Total	130	130		43	249	126

* Locations of colonies can be found in Figure 1.

**Growing scapular feathers were collected usually during the last visit to the colony.

***Only largest chick of nest sampled.

[^] Number of times a colony was visited for sampling of chicks.

Materials and Methods

With the objective of determining the variability in concentrations of mercury in the nestlings of free-ranging great egrets, a total of 122 great egret chicks (60 nests) from seven colonies located in Water Conservation Areas 3A and 3B were sampled for mercury from late March to mid-May 1994 (Figure 1.1 and Table 1.1). In general, all chicks within a nest were sampled for mercury. Chicks were sampled for blood and growing scapular feathers at least twice between approximately 5 days to 30 days of age.

From mid April to early June 1995, a total of 249 blood samples and 126 growing scapular feathers were collected from 130 great egret chicks (130 nests) (Table 1.2). Only the largest chick in each nest was sampled for blood and feathers during 1995. In order to examine possible differences in mercury concentrations between years, an effort was made to sample the same colonies both years. With the exception of Hidden and Tamiami East colonies, only growing scapular feathers were collected for mercury analysis during 1995. Great egret chicks from these latter two colonies were those used in a field mercury dosing experiment during 1995, (see Chapter VIII), and were sampled repeatedly for both blood and feathers (Table 1.2).

Blood was drawn from the jugular vein using 1 ml insulin syringes and 27 gauge needles, and stored in 3 ml heparinized vacutainers. Generally, the amount of blood collected did not exceed 0.6 ml. During 1994, blood was kept refrigerated until it was sent to the DEP laboratory for analysis. The time span between collection and submission of samples did not exceed two weeks. A different technique for the storage and analysis of blood samples was utilized during 1995. After collection, the volume and weight of each blood sample was measured using a micropipet and an analytical balance, respectively. In general, volumes

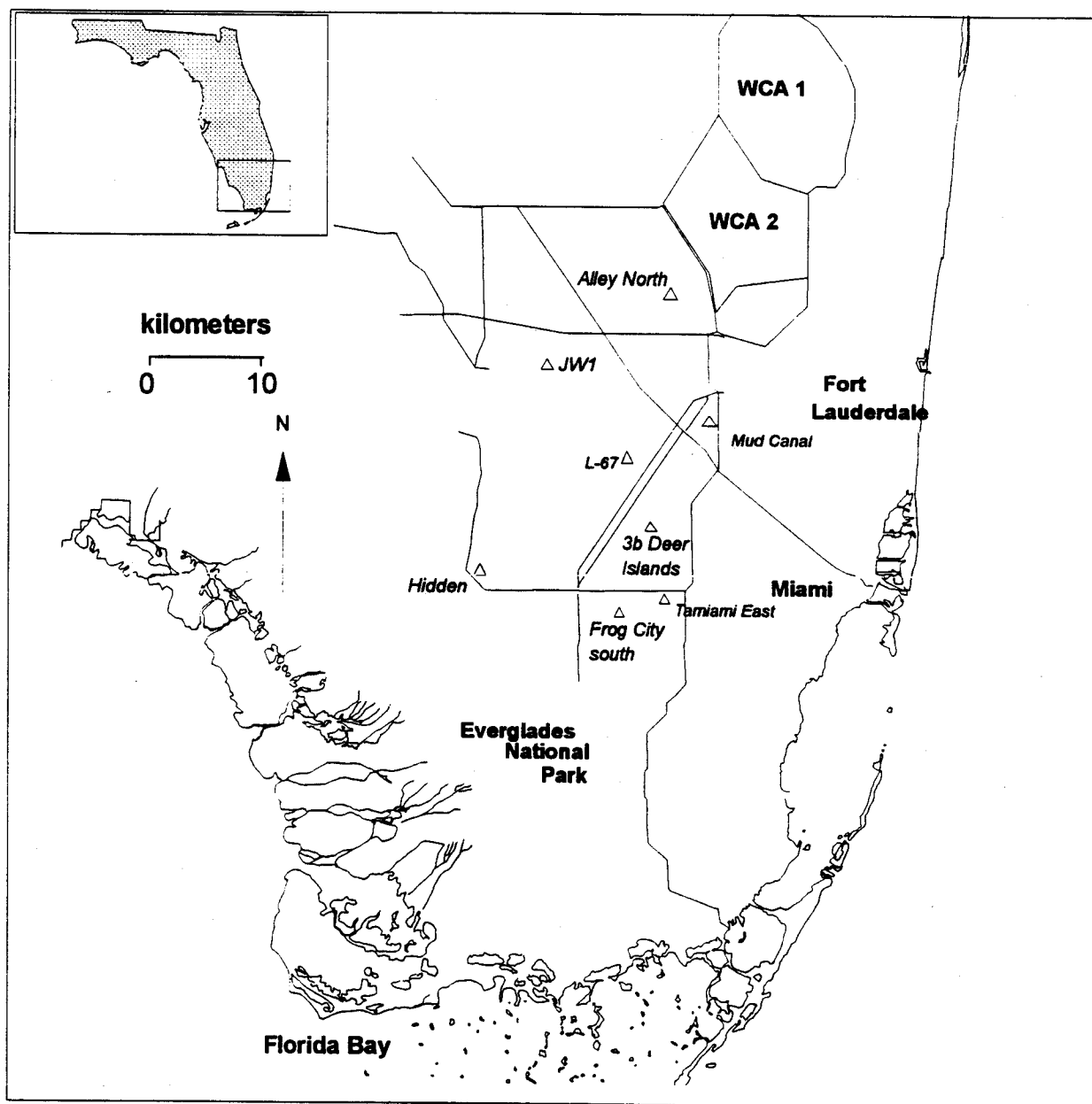


Figure 1.1. Map of the study area in southern Florida, showing locations of colonies (triangles) in Water Conservation Area 3, in relation to access roads and major habitation.

between 0.3 to 0.4 ml (approximately 0.3 to 0.4 g) of blood was saved for mercury analysis. Blood was then pipetted into Teflon-sealed glass vials and digested with 1 ml of nitric acid. Samples were then stored at room temperature until submitted for analysis.

Chicks were first bled when they were approximately 5 days old, and at various intervals until they were 25 to 30 days of age. Early attempts showed that before 5 days of age, even the largest veins were difficult to collect blood from. However, total amounts of food ingested during this period are extremely small, and it is likely that chicks up to 5 days of age receive almost all of their mercury burden from the egg rather than from food. The frequency of bleeding varied depending on the colony (Tables 1.1 and 1.2), and ranged from every 2.5 to every 13 days. These variable collection times were the result of a desire to record both short term variation in blood mercury concentrations in some colonies, as well as less frequent, more synoptic collections to measure geographic variation among colonies. Growing scapular feathers were collected only once from any chick, usually during the last visit to the nest, when chicks were between 20 and 30 days of age.

Total mercury concentrations in blood and feathers were determined at the Department of Environmental Protection laboratory. The presence of mercury was determined using a flame atomic absorption spectrophotometer, after digestion of the samples with sulfuric and nitric acids. The digested tissues were then heated with potassium permanganate and potassium persulfate.

When chicks were bled they also were weighed, measured (culmen length), and checked for the presence of parasites (*Eustrongylides ignotus*, see Spalding 1990 and *Clinostomum* sp.). During 1994, packed cell volumes, plasma proteins, and white blood cell counts were determined almost every time a blood sample was collected, and were used to evaluate the general health of the birds and to study possible changes in blood parameters due to mercury. With similar objectives, during 1995, packed cell volumes, plasma proteins, and white blood cell counts were performed only in a subset of chicks ($n = 20$ chicks).

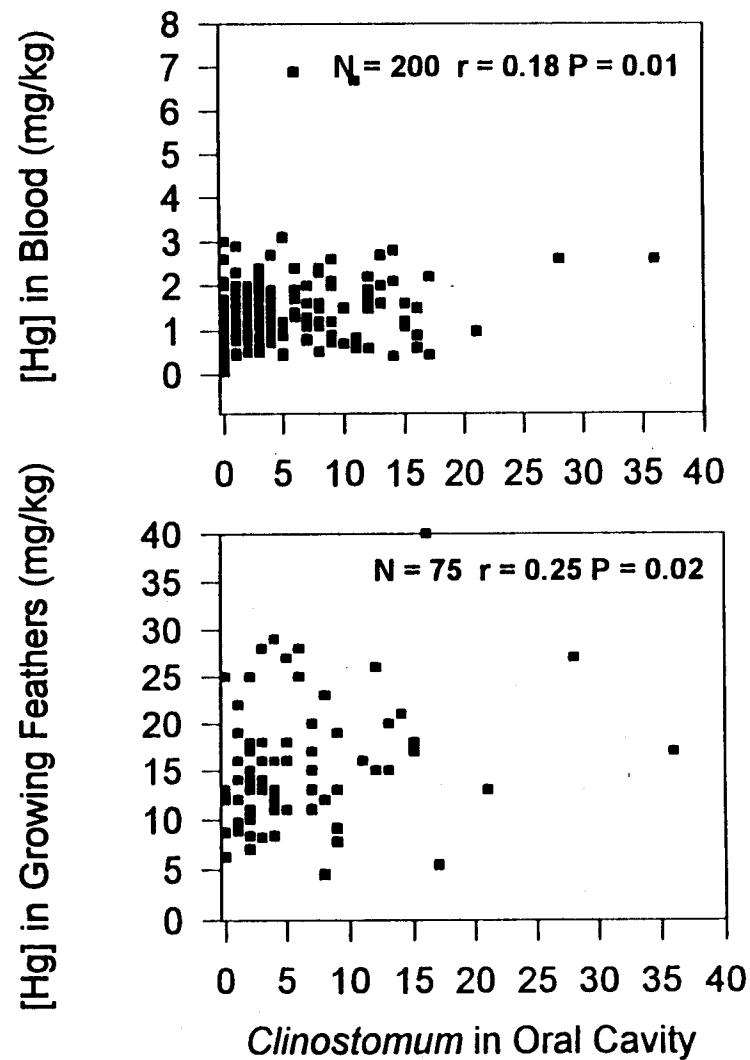
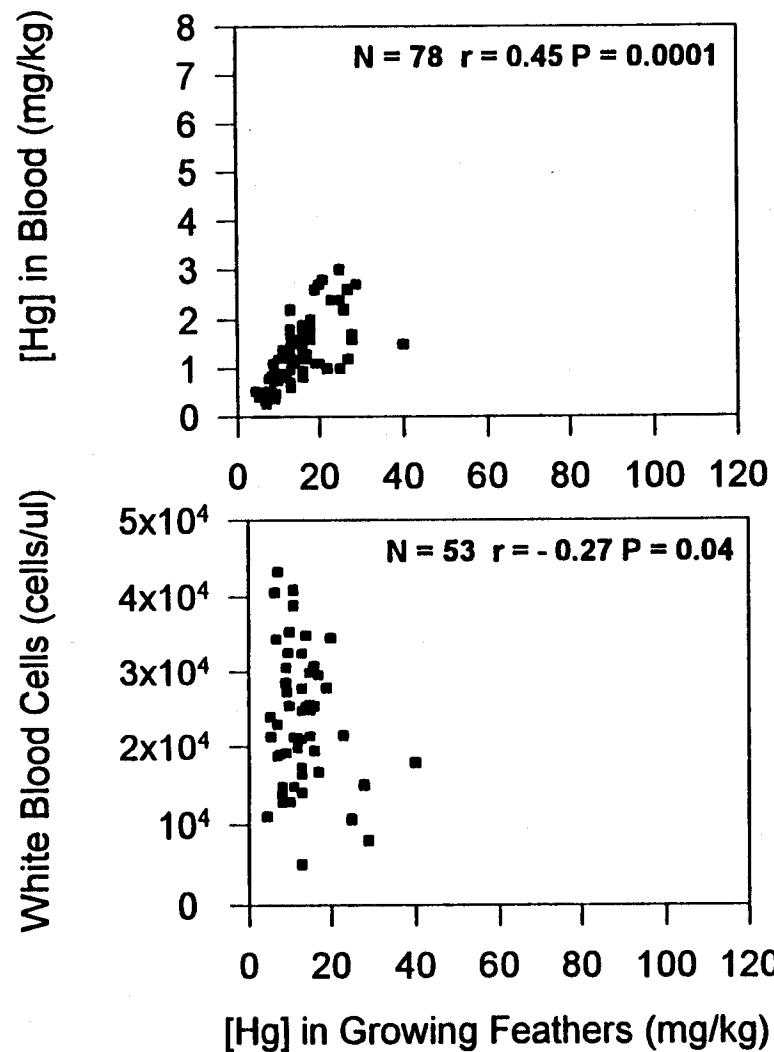


Figure 1.2 Correlations between mercury concentration in blood and feathers (mg/kg) and number of white blood cells and oral parasites.

A Pearson Correlation Analysis (PROC CORR, SAS Institute 1988) was used to test the null hypothesis that chick variables (bill length, body weight, concentration of mercury in blood and in growing feathers, number of oral parasites (*Clinostomum* sp.), packed cell volume, and number of white blood cells) were not associated. The effect of chick size on blood mercury concentration was calculated using a two-sided sign test. Correlation coefficients between date of collection and blood mercury concentrations were calculated for each bird sampled, and the proportion of chicks that had positive correlation coefficients was then calculated and tested for significance. Chicks that were sampled only once were not included in the analysis. Since growing feathers were sampled only once, correlation coefficients were calculated for blood mercury concentrations only. This analysis was performed both with birds from the same colony, and by combining chicks sampled from all colonies. Analysis of Covariance (ANCOVA) using linear models (PROC GLM, SAS Institute 1988) was used to assess the effects of different factors (colony and nest) on the dependent variables (mercury concentration in blood and in feathers). With the objective of describing differences in mercury concentrations between colonies, while accounting for differences in sample sizes, ages of chicks, and packed cell volume and number of oral parasites (these two latter variables were significantly correlated with mercury concentrations) the average mercury concentration for each location was predicted at prespecified values (means) of packed cell volume and number of oral parasites through the use of Least-square means (LSM).

Since mercury values from blood and feathers collected during 1995 are all pending lab analysis, we present only the results of analyses of 1994 data.

Results and Discussion

a. Mercury concentration in blood and growing feathers

We examined the relationship between mercury concentrations in nestling feathers and

blood through a correlation analysis. Mercury concentrations in feathers were more variable than those in blood, a significant correlation was found between concentrations of mercury in growing feathers and blood ($n = 78$, $r = 0.46$, $P = 0.0001$) (Figure 1.2).

When great egret chicks from all colonies were included in the analysis, the correlation coefficient between blood mercury concentration and age was positive ($n = 81$, $Z = 3.4$). Correlation coefficients between mercury concentration in blood and age within chicks of the same colony were significant only for birds from Alley North ($n = 12$, $Z = 2.3$) and L-67 ($n = 26$, $Z = 3.5$). With the exception of Hidden colony ($n = 24$ chicks with more than one blood sample), the absence of an increase of blood mercury concentration with age in other colonies could probably be explained by the small number of birds sampled more than once (Frog City $n = 2$; JW1 $n = 3$; Deer Island $n = 4$; and Mud Canal $n = 10$).

These results suggest that, during the first month of age, there is an increase of mercury concentration in blood with age. Interestingly, this increase of mercury in blood with age still seems to occur despite the fact that in these birds large amounts of mercury are being excreted through growing feathers. In fact, the average blood mercury concentration in great egret chicks (all ages combined) was approximately 13 times lower than that in growing feathers (1.23 mg/kg vs 16.08 mg/kg). The absence of a pattern in blood mercury concentration with age in birds from Hidden colony remains unclear, and deserves further analysis.

b. Differences in mercury concentrations between colonies and between nests

We found an effect of colony on blood ($F = 4.56$, $P = 0.0003$, $DF = 6$) and growing feather mercury concentrations ($F = 8.56$, $P = 0.0001$, $DF = 5$), when the effects of age, packed cell volume, and number of oral parasites was controlled for. No differences were found when comparing nests within colonies.

For both blood and growing feathers, chicks from JW1 had the highest concentrations

Table 1.3 Contingency table of chicks considered "healthy" and "sick", and between chicks that had "normal" and "high" concentrations of mercury in blood and growing feathers, all ages combined.

Health Status	[Hg] in Blood		Total
	"Normal"	"High"	
"Healthy"	208	18	226
"Sick"	55	7	62
Total	263	25	288

Chi-Square = 0.67, P = 0.41.

Health Status	[Hg] in Feathers		Total
	"Normal"	"High"	
"Healthy"	62	6	68
"Sick"	11	5	16
Total	73	11	84

Chi-Square = 5.72, P = 0.17.

Table 1.4 Contingency tables of chicks considered "healthy" and "sick", and between chicks that had "normal" and "high" concentrations of mercury in blood and growing feathers, by age classes ("young" < 4.2 cm bill length; "old" > 4.2 cm).

"YOUNG" CHICKS			
Health Status	[Hg] in Blood		Total
	"Normal"	"High"	
"Healthy"	90	7	97
"Sick"	31	2	33
Total	121	9	130

Chi-Square = 0.05, P = 0.82.

"OLD" CHICKS			
Health Status	[Hg] in Blood		Total
	"Normal"	"High"	
"Healthy"	118	11	129
"Sick"	24	5	29
Total	142	16	158

Chi-Square = 1.97, P = 0.16.

Health Status	[Hg] in Feathers		Total
	"Normal"	"High"	
"Healthy"	8	1	9
"Sick"	1	1	2
Total	9	2	11

Chi-Square = 1.66, P = 0.19.

Health Status	[Hg] in Feathers		Total
	"Normal"	"High"	
"Healthy"	54	5	59
"Sick"	10	4	14
Total	64	9	73

Chi-Square = 4.22, P = 0.04.

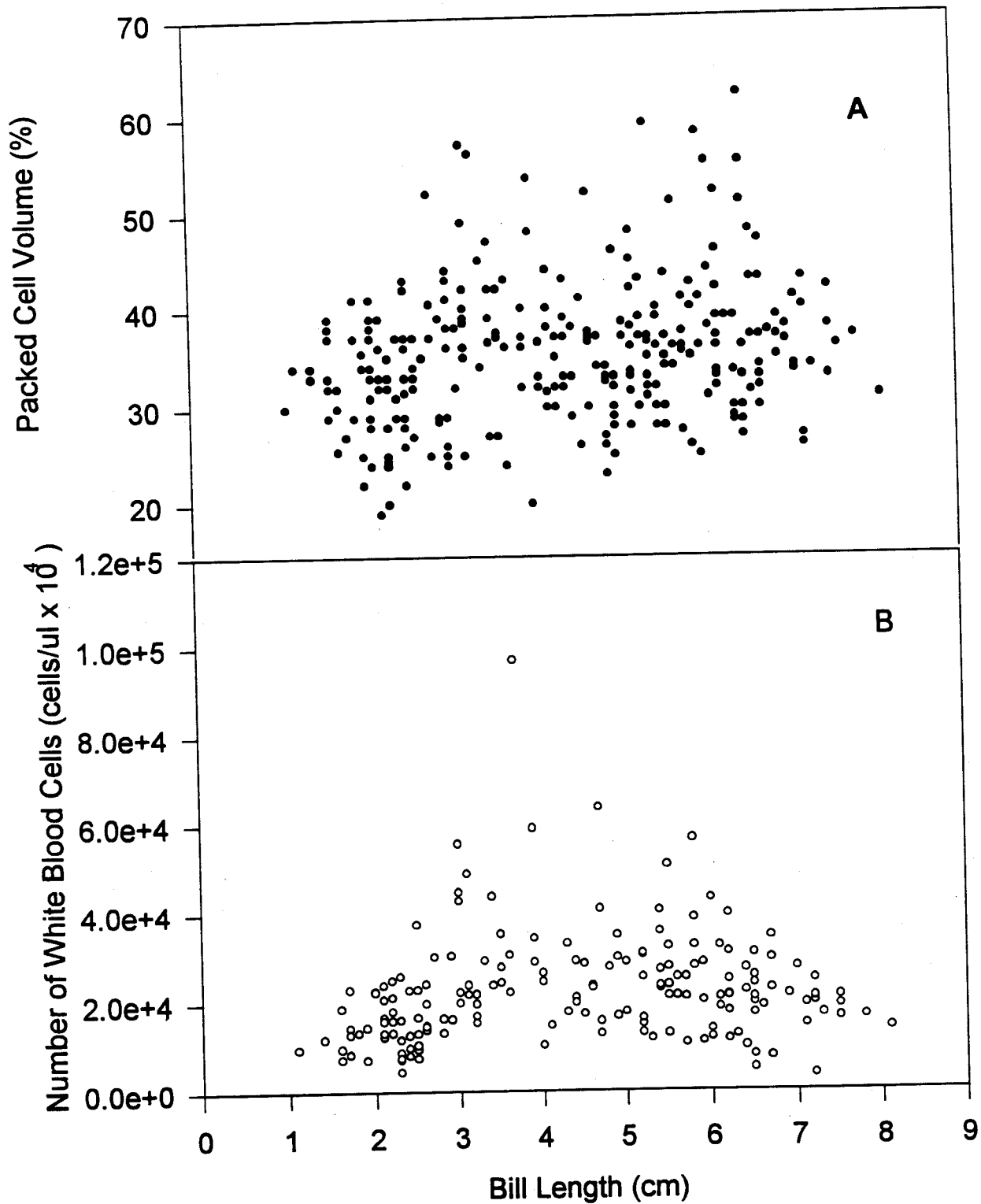


Figure 1.3 Relationship between bill length and packed cell volume (A) and number of white blood cells (B), in great egret nestlings.

"healthy" birds. A great egret nestling was considered "sick" if it fell in at least one of the following categories. Those birds with a packed cell volume of more than 50% were considered dehydrated, while those with a packed cell volume of less than 27% were considered anemic. On the other hand, it was assumed that chicks that had more than 40,000 white cells/ul were experiencing an immunological response, while those that had less than 7,000 white cells/ul were compromised. Percentages of packed cell volume and number of white blood cells from great egret nestlings are presented in Figure 1.3. Parasitic loads of over 30 Clinostomum were considered unusually high and probably associated with disease and other stresses. Birds that did not fall into any of these categories were considered "healthy". Mercury concentrations in blood and growing feathers of great egret nestlings were divided into two categories: "high" (> 2.1 mg/kg and > 25 mg/kg, respectively) and "normal" (< 2.1 mg/kg and < 25 mg/kg, respectively). These groups were defined so that "high" corresponded to the highest 10% of mercury concentrations and "normal" corresponded to the remaining 90%. Since some of the variables used in this analysis are known to be affected by the age of the chicks (number of oral parasites and packed cell volume), birds were divided in two age classes based on bill length and body weight. Great egret chicks were considered "young" if their bill length was less than 4.1 cm, and "old" if their bill length was equal or higher than 4.1 cm. Tables with the groups "sick", "healthy", "high", and "normal" were then constructed and the resulting associations compared using chi-squares (PROC FREQ, CHISQ, SAS Institute 1988). This analysis was also performed with all the ages combined.

Contingency analysis between chicks considered "healthy" and "sick", and between chicks that had "high" and "normal" mercury concentrations in blood and growing feathers (all ages combined), showed that there was no association between health status and "high" mercury concentrations in blood ($n = 288$, $X^2 = 0.679$, $P = 0.41$) (Table 1.3). However, "high" mercury concentrations in growing feathers were associated with the poor health status of the chicks ($n = 84$, $X^2 = 5.72$, $P = 0.017$) (Table 1.3). When chicks were divided into

two age classes, there was no association between health status and mercury concentrations in blood of either "young" ($n = 130$, $X^2 = 0.05$, $P = 0.82$) nor "old" chicks ($n = 158$, $X^2 = 1.97$, $P = 0.16$). Similarly, there was no association between mercury concentration in growing feathers and health status of "young" great egrets ($n = 11$, $X^2 = 1.66$, $P = 0.19$). However, "high" mercury concentrations in feathers of "old" great egret chicks was associated with "sickness" ($n = 73$, $X^2 = 4.22$, $P = 0.04$) (Table 1.4).

At this point, the mechanism that explains these relationships is not fully understood. It should be noted, however, that during 1995 we conducted a field mercury dosing experiment that will allow us to further explore these relationships through antibody quantification of both chicks that were dosed with mercury and of controls (see Chapter VIII).

Table 2.1 Summary of 1994 radio-telemetry data.

Colony	Nest N°	Chick Body Mass(g)	Chick Size	Weight of Transmitters as % of body mass	Date of Deployment	Current Status
Hidden/L-28	25	473	Largest	2.54%	04/24/94	Last located on 07/26/94 in impounded pasture (26 29.04N, 81 33.425W).
	25	473	Smallest	2.54%	04/24/94	Last located on 08/28/94 (approximate location 25 49.47N, 80 45.67W).
	26	568	Largest	2.11%	05/08/94	Found dead on 08/15/94 (25 48 580N, 80 50.081W).
	26	465	Smallest	2.58%	05/08/94	Heard mortality signal on 08/28/94 (25 48 500N, 80 50.251W) Found nothing to indicate death of bird.
	27	533	Largest	2.25%	04/24/94	Last located on 02/18/95 (26 00 81N, 81 03.24W).
	27	508	Smallest	2.36%	04/24/94	Last located on 08/11/94 in wet prairie (25 37.13N, 80 54.73W).
	114	638	Only	1.88%	05/06/94	Heard mortality signal on 08/11/94 in orange grove, north of Imokalle. Found nothing to indicate death of bird
	128	670	Largest	1.79%	05/21/94	Last located on 10/29/95 in Big Cypress (26 13.23N, 81 03.97W).
	132	593	Largest	2.02%	05/06/94	Heard mortality signal on 08/15/94. Found nothing to indicate death of bird.
	132	450	Smallest	2.87%	05/06/94	Found dead on 07/22/94 (25 57.847N, 80 55.278W).
	215	480	Largest	2.50%	05/06/94	Last noted at colony on 08/27/94.
	215	460	Smallest	2.61%	05/06/94	Found dead on 07/26/94 in Big Cypress (25 59.445N, 81 59.843W)
	221	595	Largest	2.02%	05/21/94	Found dead on 08/11/94. Transmitter with strong indentation possibly from bill.
	221	555	Smallest	2.16%	05/21/94	Heard mortality signal on 06/29/94.
	972	555	Largest	2.16%	04/11/94	Found dead on 08/14/94 in Big Cypress (26 04.18N, 81 11.55 W).
Alley North	51	780	Largest	1.54%	05/02/94	Last located on 08/22/94 in sugarcane fields (26 33.75N, 80 48.92).
	51	700	Smallest	1.71%	05/02/94	Last noted at colony on 06/14/94.
	55	540	Smallest	2.22%	05/02/94	Last located on 02/18/95 (26 27.54N, 81 03.10W).
	59	770	Largest	1.56%	05/02/94	Last noted at colony on 06/20/94.
	59	620	Smallest	1.94%	05/02/94	Found dead on 02/18/95 in a cypress swamp close to an orange grove (26 58.713N, 80 27.522W).
	64	695	Largest	1.73%	05/02/94	Last located on 02/18/95 (26 49.67N, 80 28.67W).
	65	765	Largest	1.57%	05/02/94	Heard mortality signal on 10/29/94. Found nothing to indicate death of bird.
	66	494	Largest	2.43%	05/08/94	Found dead on 07/06/94.
	66	493	Medium	2.43%	05/08/94	Last located on 10/29/94 in canal between sugarcane fields (26 23.85N, 80 37.76W).
	66	492	Smallest	2.44%	05/08/94	Last located on 07/15/94 in ditch between sugarcane fields (26 42.00N, 81 38.086W).
	70	710	Largest	1.69%	05/02/94	Last located on 02/18/95 (26 13.60N, 81 00.33W).
	70	695	Smallest	1.73%	05/02/94	Last located on 02/18/95 (26 12.07N, 80 38.38W).
Deer Island L-67	116	498	Largest	2.41%	04/18/94	Found dead on 08/29/94 approximately 150m from colony.
	180	478	Largest	2.51%	05/10/94	Last located on 02/18/95 (26 28.13N, 81 02.51W).
	185	540	Largest	2.22%	05/21/94	Last located on 07/08/94 in colony
	186	740	Largest	1.62%	05/10/94	Last located on 08/22/94 in flooded area between cypress and palmettos (26 30.02N, 81 14.84W).
	190	619	Largest	1.94%	05/10/94	Found dead on 09/14/94 in Big Cypress (25 59.950N, 80 59.064W).
	190	306	Smallest	3.92%	05/10/94	Last located at colony on 07/22/94.
	191	568	Largest	2.11%	05/10/94	Last located on 02/18/95 (26 34.05N, 81 02.61W).
	191	316	Smallest	3.80%	05/10/94	Seen alive at colony on 05/16/94, but signal was very weak. Never heard signal again
	234	598	Largest	2.01%	05/21/94	Found dead on 07/22/94 by I-75.
	248	580	Largest	2.07%	05/21/94	Last located on 07/13/94 in a pasture agricultural area (approx. 26 35.00N, 81 03.00W).
	248	548	Smallest	2.19%	05/21/94	Last located at colony on 07/26/94.
	323	520	Largest	2.31%	05/13/94	Found dead on 09/14/94 (25 37.802N, 80 36.486W).
	325	540	Largest	2.22%	05/13/94	Last located on 02/18/95 (26 12.77N, 81 24.47W).
	336	500	Only	2.40%	05/13/94	Last located on 02/18/95 (25 35.45N, 80 35.94W).
	336	558	Largest	2.15%	05/13/94	Last located at colony on 06/29/94.
	338	414	Smallest	2.90%	05/13/94	Found dead on 10/29/94 in a cypress forest (26 28.24N, 81 08.62W).
	341	602	Smallest	1.99%	05/13/94	Last located on 07/22/94 in a wet prairie (25 22.00N, 80 35.00W).
Frog City South	312	720	Largest	1.67%	05/14/94	Last located on 02/18/95 (26 32.82N, 80 43.64W).
	312	540	Smallest	2.22%	05/14/94	Last located on 10/29/94 in cypress forest with some pastures (26 48.77N, 81 42.20W).

Table 2.2 Summary of 1995 radio-telemetry data.

Colony	Nest N°	Chick Body Mass(g)	Chick Size	Weight of Transmitters as % of body mass	Date of Deployment	Current Status
Hidden	60	630	Largest	1.59%	04/30/95	Last located on 06/22/95 (26 16.34N, 80 46.29W).
	61	571	Largest	1.75%	04/30/95	Last noted at colony on 06/23/95.
	62	540	Largest	1.85%	05/18/95	Last located on 07/20/95 (25 48.52N, 80 52.49W).
	65	730	Largest	1.37%	04/30/95	Last noted at colony on 06/25/95.
	66	810	Largest	1.23%	06/03/95	Heard mortality signal on 06/23/95. Collected transmitter but found nothing to indicate death of bird.
	69	740	Largest	1.35%	05/23/95	Heard mortality signal on 08/08/95. Collected transmitter but found nothing to indicate death of bird.
	70	650	Largest	1.54%	05/23/95	Last noted at colony on 07/06/95.
	71	590	Largest	1.69%	05/23/95	Last located on 08/08/95 (25 44.20N, 81 04.54W).
	74	600	Largest	1.67%	05/23/95	Last located on 08/08/95 (25 43.47N, 81 51.25W).
	75	695	Largest	1.44%	05/18/95	Last noted at colony on 07/06/95.
	76	610	Largest	1.64%	05/28/95	Last located on 08/08/95 (25 43.68N, 81 05.87W).
	100	760	Largest	1.32%	05/20/95	Last noted at colony on 07/06/95.
	101	630	Largest	1.59%	05/20/95	Last located at colony on 07/06/95.
	104	660	Largest	1.52%	05/25/95	Last located at colony on 07/06/95.
	105	630	Largest	1.59%	05/20/95	Last located on 08/08/95 (25 53.85N, 81 57.81W).
	109	740	Largest	1.35%	05/20/95	Last located on 08/08/95 (25 57.71N, 81 00.00W).
	110	820	Largest	1.22%	05/20/95	Last located at colony on 07/06/95.
	111	745	Largest	1.34%	06/03/95	Heard mortality signal on 06/23/95. Collected transmitter but found nothing to indicate death of bird.
	112	800	Largest	1.25%	05/20/95	Last located on 08/08/95 (25 45.54N, 81 55.20W).
	113	990	Largest	1.01%	05/20/95	Last located on 08/08/95 (25 46.38N, 80 39.91W).
	114	650	Largest	1.54%	05/20/95	Last located on 07/26/95 (25 42.85N, 80 51.28W).
	117	790	Largest	1.27%	05/30/95	Last located on 08/08/95 (25 51.95N, 81 11.63W).
	126	580	Largest	1.72%	05/20/95	Last located at colony on 06/29/95.
	132	600	Largest	1.67%	05/20/95	Last located on 08/08/95 (25 53.85N, 80 57.92W).
	441	705	Largest	1.42%	05/28/95	Last located at colony on 07/03/95.
	442	740	Largest	1.35%	05/23/95	Heard mortality signal on 06/05/95. Found nothing to indicate death of bird.
	443	650	Largest	1.54%	05/18/95	Heard mortality signal on 08/08/95. Collected several mature feathers and powder down.
	447	690	Largest	1.45%	05/28/95	Last located on 08/08/95 (25 43.65N, 80 53.50W).
	454	720	Largest	1.39%	05/20/95	Last located on 08/08/95 (25 44.65N, 80 53.28W).
	488	930	Largest	1.08%	06/03/95	Last located at colony on 07/14/95.
	494	660	Largest	1.52%	05/30/95	Last located on 08/09/95 (25 48.41N, 80 53.19W).
	769	820	Largest	1.22%	05/08/95	Last located at colony on 06/12/95.
	770	690	Largest	1.45%	05/23/95	Last located on 08/08/95 (25 46.11N, 81 02.92W).
	772	720	Largest	1.39%	06/03/95	Last located at colony on 07/06/95.
	776	590	Largest	1.69%	05/25/95	Last located on 08/09/95 (25 43.70N, 80 53.42W).
	798	570	Largest	1.75%	05/18/95	Last located on 08/09/95 (25 49.78N, 80 52.14W).
	UM1	1000	Largest	1.00%	06/03/95	Last located on 08/08/95 (25 42.55N, 80 55.50W).
	UM2	935	Largest	1.07%	06/09/95	Heard mortality signal on 07/20/95. Collected several mature feathers.
	UM3	875	Largest	1.14%	06/03/95	Last located on 08/09/95 (25 50.90N, 81 13.79W).
	UM4	880	Largest	1.14%	06/03/95	Last located on 08/09/95 (25 45.00N, 80 56.22W).
Tamiami East	450	600	Largest	1.67%	05/24/95	Last located at colony on 07/06/95.
	497	440	Largest	2.27%	05/11/95	Last located at colony on 07/03/95.
	498	510	Largest	1.96%	05/21/95	Last located on 07/21/95 (26 41.83N, 80 33.47W).
	709	740	Largest	1.35%	05/19/95	Last located at colony on 07/06/95.
	710	630	Largest	1.59%	05/24/95	Last located at colony on 07/06/95.
	711	620	Largest	1.61%	05/11/95	Last located on 07/21/95 (25 34.57N, 80 34.15W).

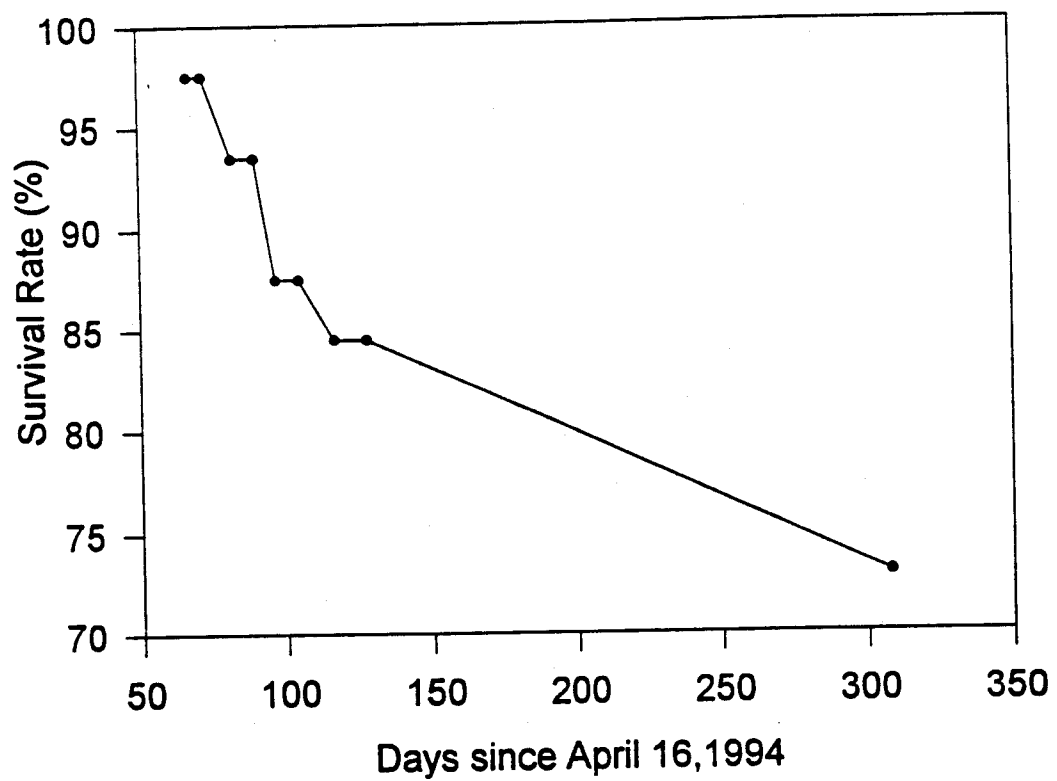


Figure 2.1 Cumulative survival rate of great egret chicks over time, since deployment of first radiotransmitter (April 16, 1994).

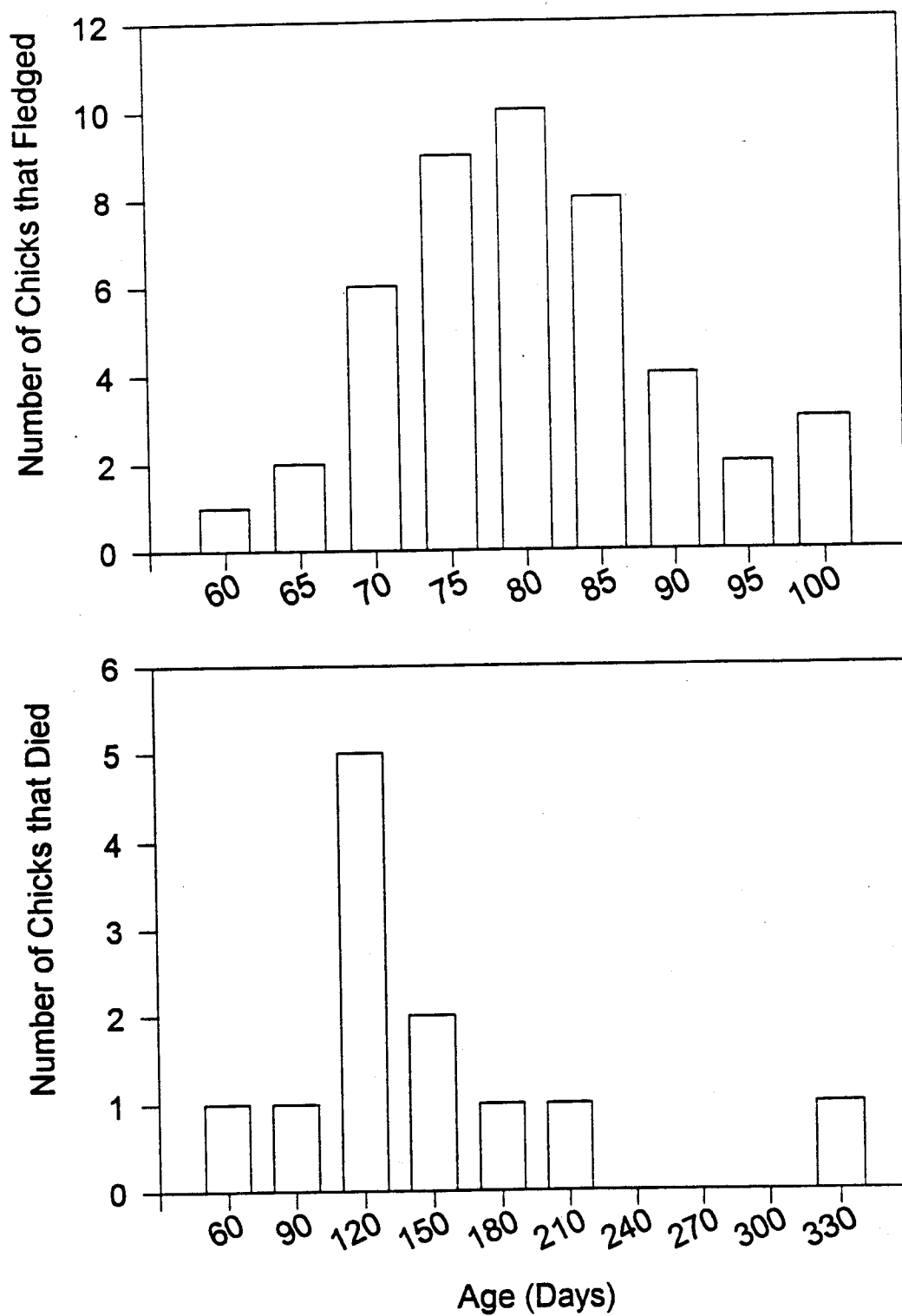


Figure 2.3 Timing of fledging and of post-fledging mortality of great egret chicks from colonies studied in the Everglades.

Table 2.3 Mercury concentrations (mg/kg; wet weight) in tissues from six radioed great egret juveniles.

Colony of Origin	Date last Sampled	[Hg] in Blood(mg/kg)	[Hg] in Growing Feathers(mg/kg)	Date found Dead	Age in Days	[Hg] in Mature Feather(mg/kg)	[Hg] in Powder Down(mg/kg)	[Hg] in Liver(mg/kg)	[Hg] in Brain(mg/kg)	[Hg] in Muscle(mg/kg)
Deer Island	04/16/94	0.85	8.3	06/29/94	100	9.6	-	12.0	0.28	2.0
Hidden/L-28	05/06/94	0.83	18	06/15/94	62	19.0	39	-	-	-
Hidden/L-28	05/21/94	2.2	13	08/11/94	110	14.0	3.2	-	-	-
L-67	05/13/94	-*	9.7	10/29/94	195	11.0	-	-	-	-
L-67	05/10/94	1.2	15	09/14/94	136	26.0	-	-	-	-
L-67	05/13/94	1.5	13	09/14/94	149	22.0	-	-	-	-
L-67	05/21/94	-	18	07/22/94	97	26.0	-	-	-	-

* No data available.

II. EFFECTS OF MERCURY ON SURVIVAL OF JUVENILE GREAT EGRETS

Introduction

Our objective in this part of the study was to determine whether elevated mercury concentrations have an effect on the survival of free-ranging postfledging and juvenile great egrets. In both years of study, the approach has been to monitor survival of chicks with known mercury loads, either by regular nest and colony checks (prefledging) or by the use of radio transmitters with mortality sensors (postfledging). In 1994, we monitored survival of a cohort of young birds with naturally variable mercury values. In 1995, we elevated the mercury of a sample of chicks using a field-dosing technique (Chapter VIII), and will compare their survival to sham-dosed controls.

Materials and Methods

Between mid April and mid May 1994, we marked 46 great egret chicks (30 nests) (Table 2.1) with individually identifiable colored plastic leg bands (fitted above the tarsometatarsal joint on the right leg) that had radio tags (Holohil LTD, 12 g, with mortality switches) attached to them. The criteria used for determining which chicks were fitted with a radio transmitter included: a) having at least two previous samples of blood for mercury analysis (all the chicks); b) having information on mercury concentration from one of the parents (three chicks: one from Deer Island, and two from Frog City South); and c) having information on chick food intake (19 chicks, see chapter III on food intake). All chicks were also fitted with U. S. Fish and Wildlife Service numbered aluminum bands below the tarsometatarsal joint on the left leg at approximately 15 days of age.

The nestlings were marked in five different colonies: Hidden ($n = 15$), Alley North ($n =$

12), Deer Island (n = 1), L-67 (n = 16), and Frog City South (n = 2, see Table 2.1). Usually, more than one bird from each nest was tagged. The plastic band together with the radio transmitter weighed approximately 15 g (less than 5% of the weight of the chick in all cases) (Table 2.1). These chicks were first bled for mercury at approximately 5 days of age, and then sampled for mercury again (blood and growing scapular feathers) at the time of radio-tagging. Between radio-tagging and the departure of the young from the colony, survival was documented through signal checks on the ground every three days. After departure from the colony area, survival was monitored through aerial surveys (n = 22), flown approximately twice a week during the first two months post-tagging (June and July), once a week during August, once a month during September-November, and once in February 1995. Not all birds were located on every survey flight, however, and most individuals were rarely located more than once every 14 days, even during the period of most intensive tracking. Birds were last monitored in February 1995, given the one year life of the batteries, and transmitters were considered unreliable after this point. When a bird was located both the location (in coordinates, using a GPS unit) as well as a general description of the area were recorded. When a mortality signal was heard, efforts were made to locate the carcass on the ground as soon as possible.

Survival during the first 10.5 months of age was estimated using the staggered Kaplan-Meier procedure (Pollock et al., 1989). This technique allows gradual (or staggered) entry of birds into the study, and accounts for birds being lost (or censored) due to failure or loss of radios, or emigration.

A maximum likelihood analysis of the logistic regression (PROC CATMOD, SAS Institute, 1988) was used to test the hypothesis that an increased average mercury concentration in blood at the time of radio-tagging (23 - 30 d of age) increased the probability of dying during the first 10.5 months of age. Since mercury concentrations differed significantly between colonies (Chapter I), colony was added as a cofactor in this analysis.

Censored birds (n = 23) lost from the study due to radio failure, and/or emigration were excluded from this analysis.

During 1995, the effects of mercury on the survival of great egret fledglings and juveniles was studied using a slightly different approach. A total of 70 first-hatched great egret nestlings from 70 different nests were involved in a mercury dosing experiment carried out in the field. In this experiment, 30 of the birds were dosed with methylmercury chloride at an estimated rate of 1.13 mg/kg of food ingested for a period of 15 days, while the remaining 40 birds were used as controls (see Chapter VIII for more details). Between late April and early June 1995, all 70 great egret chicks were fitted with radio transmitters (American Wildlife Enterprises, 10 g) this time attached to aluminum leg bands. The nestlings used in this experiment were raised either in Hidden colony (n = 40) or in Tamiami East colony (n = 30) (Table 2.2). These chicks were first bled for mercury at an approximate age of 10 days, and then sampled for mercury concentrations every five days (blood) and at the time of radio tagging (blood and growing scapular feathers, at 23 - 30 d of age). Survival of the young is presently being monitored through biweekly aerial surveys.

Results and Discussion

A summary of the radio-telemetry data for 1994 is presented in Table 2.1. As of February 18, 1995, 11 birds were still alive, 12 had died, and 23 were lost from the study because of different causes (1 had a broken radio, 4 had broken plastic bands, and 17 were never located after their departure from the colony).

The survival rate of great egrets was estimated as 73% for the first 10.5 months of age (Figure 2.1). Using band recovery data, Kahl (1963) estimated that 24% of great egret chicks fledged in Mississippi survived for their first year. Our results indicate a much higher survival rate for juvenile great egrets in the Everglades.

A map with the movements of the radio-tagged birds is presented in Figure 2.2. In general, dispersal was to the north of natal colonies and towards shallowly flooded agricultural lands. Several birds were sighted near farm crops and sugarcane fields. This pattern was consistent among birds from different colonies. Many birds made only local movements once they had completed an initial post-breeding dispersal.

Mortality of radiotagged great egrets is plotted against age in Figure 2.3. Of the twelve birds that died, two (17%) died between the ages of 60 and 90 days. The peak of fledgling events occurred when birds were between 75 and 85 days old (Figure 2.3). The largest number of mortalities (33%) occurred approximately one month after the peak of fledging, decreasing thereafter until the birds were 210 days old (50%). In almost all cases, the only remains found were radios, bones, and feathers (mature primaries and powder down). On one occasion a partially scavenged carcass was recovered and liver, brain, and muscle analyzed for mercury concentrations. Mercury concentrations in feathers from six of these birds are presented in Table 2.3. Generally, mercury concentration increased from the period when the birds were last handled until the time of their death (Table 2.3). The single juvenile bird for which we had organ values for mercury had high concentrations (12 mg/kg).

After including colony as a factor when analyzing the relationship between mercury concentration and survivorship, concentrations of mercury in blood and feathers did not have an effect on the survival of great egret chicks ($n = 23$, $X^2 = 24.6$, $P = 0.06$ and $n = 17$, $X^2 = 4.99$, $P = 0.06$, respectively). It is important to note that this aspect of the project is still ongoing, and that effects of mercury on the survival of juvenile great egrets is presently being studied using a larger sample size ($n = 70$) and larger differences in prefledging mercury concentrations (see Chapter VIII).

III. USE OF ISOTOPICALLY-LABELED WATER IN GREAT EGRET CHICKS AS A METHOD TO DETERMINE FOOD INTAKE

Introduction

Preliminary work with captive adult great egrets dosed with mercury in 1994 suggested that high dietary mercury intake can suppress appetite. The nestling stage is a time of extremely rapid development, requiring that chicks consume large quantities of food. If mercury concentrations in naturally occurring food items were high enough to suppress the appetite of great egret nestlings, it is possible that growth and development would be retarded, resulting in fewer chicks surviving to become breeding adults. Our approach has been to measure food intake of nestling great egrets while simultaneously monitoring the mercury content of their blood and growing feathers. This information both aids our understanding of the mechanism by which mercury can affect recruitment into the breeding population and provides concrete information on mercury intake for modeling of mercury dynamics and effects.

Our original plan for measuring food intake of great egret chicks involved the use of electronic scales, placed underneath nests to enable measurement of the mass of food delivered. Nest-scales require direct observation of parental food deliveries and sample size is therefore tightly constrained by the number of hours available for observation. In addition, we found in early 1994, that some adult great egrets respond to nest scales by rebuilding their nest and reconnecting it to surrounding vegetation, making it impossible to accurately weigh food deliveries. After encountering these difficulties, we decided instead to use an isotopically-labeled water method because it has several important advantages over nest-scales in the determination of food intake. First, food intake can be monitored continuously using labeled water, rather than periodically, as is the case with nest-scales. Second, since the use of

labeled water does not require observer attendance, a much larger sample can be achieved with the same amount of effort. Third, labeled water can be used to measure individual food intake, while, at present, nest-scales are poor at discerning deliveries to individual young. Finally, the labeled water method is an extremely accurate way to measure food intake, with values from other studies deviating 6.1 % or less from actual mass of food intake (7 different species, Nagy 1989). The accuracy of the nest-scale method is not known.

Labeled water, which is injected into an organism, serves as a marker of the water contained within the organism. By measuring the change in concentration of such a marker, it is possible to calculate the amount of water turnover that has occurred within the organism. Nestling ciconiiform birds obtain moisture only through water contained in food, metabolic water produced during digestion, and by respiration (absorption through skin is negligible). They cannot obtain water through drinking until they are mobile. Metabolic water and water intake through respiration can be calculated and accounted for and the remaining water intake can attributed to water contained in food items. Since the water content in food items (predominantly fish) for great egrets is known (Kushlan et. al, 1986), food intake can be calculated with considerable accuracy.

During the 1994 field season, we used the hydrogen isotope, deuterium (DHO, deuterated water), as the marker in our food intake observations. Deuterium samples are very time-consuming to process and its measurement requires the use of a special mass spectrometer equipped with a tube-cracking apparatus, which are both rare and expensive to use. The advantage of using deuterium as a marker is that its use requires no special permits. The other commonly used marker, tritium (THO, Tritiated water), is easier to use and less expensive to measure than deuterium. Tritium, however, is radioactive and requires several special permits for use in field studies. Due to time constraints, we were forced to use deuterium as our marker for 1994 studies. In 1995, however, there was ample time to go through the permitting process, so we switched to tritium (see Chapter XIII).

Materials and Methods

a. Field Procedures.

During 1994 great egret chicks in Hidden and L-67 colonies were first injected with DHO at approximately 8 days of age. The chicks were subsequently resampled for DHO dilution through blood samples, and reinjected with DHO every 3 days until they were impossible to recapture (ca 25 days old). We entered colonies every third day just after daylight in order to minimize heat stress on chicks and eggs. Because the DHO method assumes that an organism's mass is entirely composed of body components, we also needed to ensure that chick's stomachs were empty. All chicks in each study nest were included in food intake measurements. We weighed each chick to the nearest g using a 300 g or 1000 g Pesola scale, depending on size. Measurements of culmen (bill) and tarso-metatarsis were taken to the nearest mm using rulers. A brief physical exam noting relative amount of body fat, presence/absence of *Eustrongilides ignotus* (Spalding, 1990) and *Clinostomum* sp. parasites, presence of food in the stomach, and any obvious external illness was conducted.

On the initial visit to a nest, we injected chicks intramuscularly with a 3% or 10% solution of DHO, depending on size of chick, at the rate of .09 g DHO/kg body weight. After allowing one hour for isotope equilibration, we took a 0.3 ml blood sample from the jugular vein for later use as a measure of initial DHO concentration. While at the nest, blood samples were placed in capillary tubes, flame-sealed using a miniature torch, and labeled according to colony, date, nest, chick, and sample number. On each subsequent visit to a nest, a blood sample was taken pre-injection, for use as a measure of final DHO concentration during the previous three day interval. The chick was then reinjected with DHO and re-bled according to the procedure described above. Chicks were reinjected and bled every three days until they died or proved impossible to recapture (ca 25 d old).

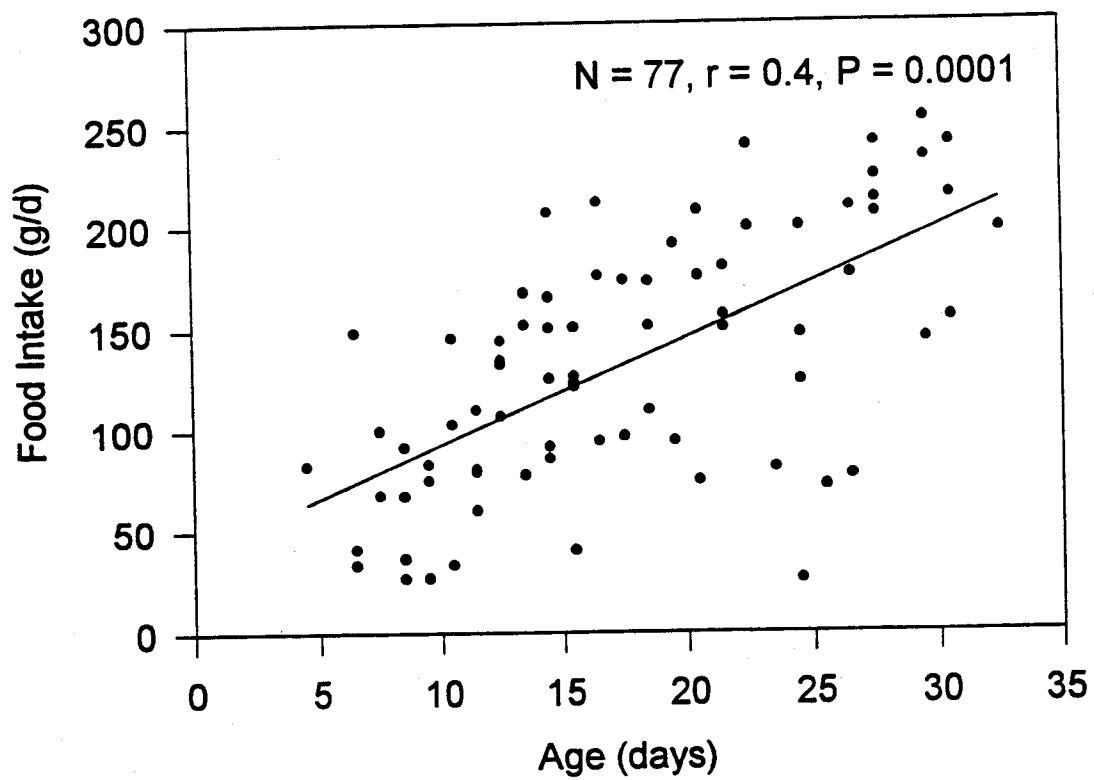


Figure 3.1 Food Intake in great egret chicks in relation to age.

This same technique was used for tritiated water (THO) in 1995, except that chicks were dosed with THO at a rate of 1 mCi/kg body weight and were revisited every five days (see Chapter VIII for details of sample sizes).

b. Laboratory Procedures.

The measurement of deuterium with a mass spectrometer requires hydrogen/deuterium gas derived from a pure water sample. We extracted water from samples by vacuum distillation of the blood. Within a vacuum line (2.5×10^{-2} Torr), the capillary tube containing a blood sample was cracked and heated, causing the water contained in the sample to vaporize. The vapor was then condensed into an ampule immersed in a cold trap cooled by liquid nitrogen. The ampule containing the frozen water sample was then flame-sealed and removed from the vacuum manifold.

After the distillation procedure, 4 μ l of the sample water was combined with 400 mg of zinc reagent (Biogeochemical Laboratories, Indiana University) inside a new ampule, frozen, and once again placed under vacuum and flame-sealed. Samples were then placed in a furnace and baked at 500° C for 30 minutes, causing the sample water to react with the zinc reagent to produce hydrogen gas and zinc oxide. Deuterium/hydrogen ratios of the hydrogen gas were measured with a VG Isotech mass spectrometer equipped with a special tube-cracking apparatus, located at University of Miami. The D/H ratios were then converted to atom excess percentages.

c. Calculations.

Water turnover was calculated using the following equation from Nagy (1983):

$$TO = (2000 * (BW_f - BW_i) * \ln((H_i * BW_i)/(H_f * BW_f)) / ((M_i + M_f) * \ln(BW_f/BW_i) * t) + (((2000 * (BW_f - BW_i))/(t * (M_i + M_f))) \text{ where}$$

TO = water turnover (ml/kg/d)

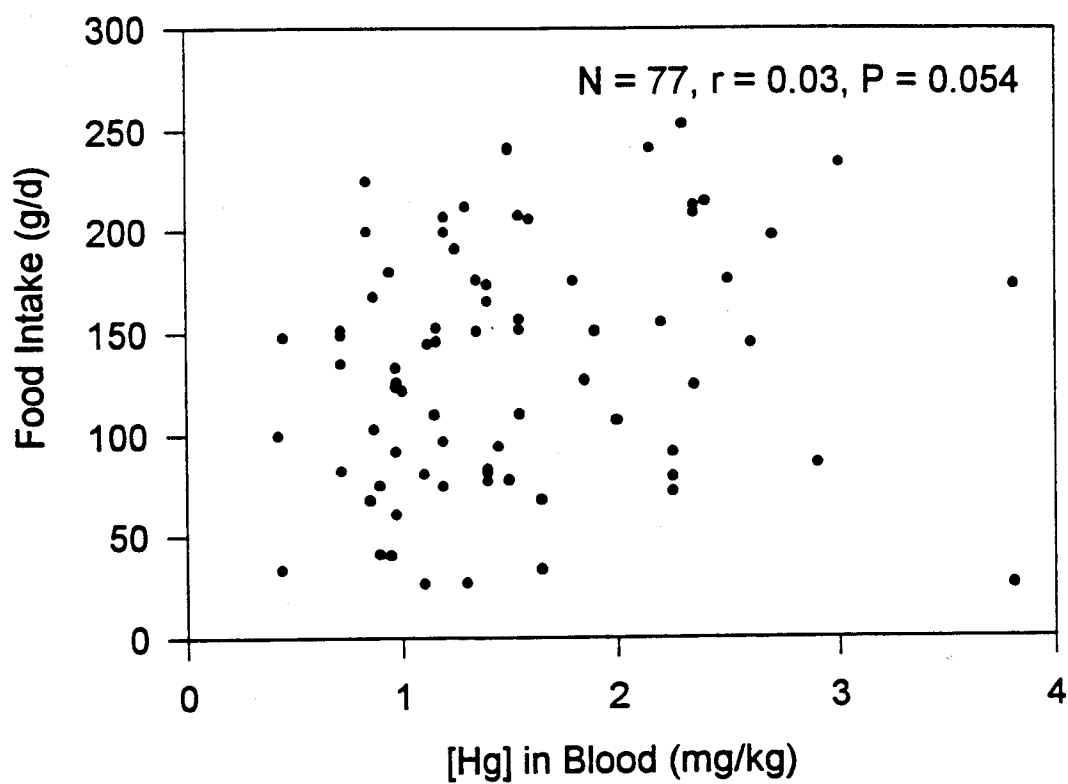


Figure 3.2 Variability of great egret chick food intake in relation to mercury concentration in blood.

BWi = initial body water of chick (ml)

BWf = final body water of chick (ml)

Hi = initial atom excess percent of deuterium in chick

Hf = final atom excess percent of deuterium in chick

Mi = initial mass of chick (g)

Mf = final mass of chick (g)

t = number of days in interval

Turnover was then converted to food intake using the following equation (Nagy, pers. comm.;

Dunn, 1975):

Food Intake (g/d) = $(TO/.87) * (((0.001 * Mi) + (0.001 * Mf)/2)$, where

TO = water turnover (ml/kg/d)

0.87 = water content of fish (72 %) + metabolic water (7.5 %) + respiration water (7.5 %)

Mi = initial mass of chick (g)

Mf = final mass of chick (g)

We performed an analysis of covariance (ANCOVA) to test if food intake was affected by colony site, or concentrations of mercury in both blood and growing scapular feathers. Age of chick was controlled for in the analysis of variance.

Results and Discussion

We analyzed a total of 154 blood samples, representing 77 three-day food intake intervals. The samples were taken from a total of 24 chicks (13 from Hidden and 11 in the L-67 colonies). When the effect of age of the chicks was controlled for, mean food intake was different between colonies (ANCOVA, $F = 6.57$, $P = 0.0124$). Food intake also varied with chick age (ANCOVA, $F = 60.75$, $P = 0.0001$), with individual intakes ranging from 26.5 g/d

for an 8 day old chick to 240.1 g/d for a 22 day old chick (Figure 3.1).

We compared food intake with blood and feather mercury concentrations. Mercury concentrations for both blood and growing feathers were available for most chicks used in the food intake study ($n = 21$ for blood, $n = 20$ for growing feathers). Whenever possible, blood mercury values were calculated as the mean of blood mercury samples taken at the beginning and end of the three-day food intake interval. When samples were unavailable from the beginning and ending days of a food intake interval, means were calculated using blood mercury values from the dates closest to the beginning and ending days of the food intake interval. Mercury values for growing scapular feathers came from samples taken during the last handling of any chick (ca 25-30 d old).

There was a marginally significant negative association between food intake and blood mercury concentrations (Figure 3.2, ANCOVA, $F = 4.40$, $P = 0.0532$). No association was found between food intake and mercury concentrations in growing feathers (Figure 3.3, ANCOVA, $F = 0.51$, $P = 0.4864$). Two reasons for the difference in strength of association of blood and feather mercury concentrations with food intake are: 1. Sample size ($n = 154$ for blood, $n = 20$ for feathers); the much smaller sample of feather values would make an analysis for differences in food intake less sensitive. 2. Blood samples are representative of mercury intake for a particular three-day interval (or nearly so), while feather samples are representative of mercury accumulated over the entire observational period. One would expect that a measure of mercury more closely associated with the food intake interval in question would more closely represent the effects of mercury on food intake during that interval.

Our food intake data suggest geographic differences in food intake among colonies in the WCA's. It is not clear whether this difference is related to differing mercury concentrations in chicks between colonies. The most likely explanation for differing food intake between colonies is probably due to differing food availability, since foraging areas used by birds from the two colonies were quite different (Frederick, 1994).

The possibility remains that the concentrations of mercury found in naturally occurring prey items in the WCA's and surrounding areas may affect the appetite of great egret chicks. Our work in 1995 specifically seeks to answer this question by artificially elevating the mercury intake of great egret chicks and comparing their food intake against that of controls (see section VIII).

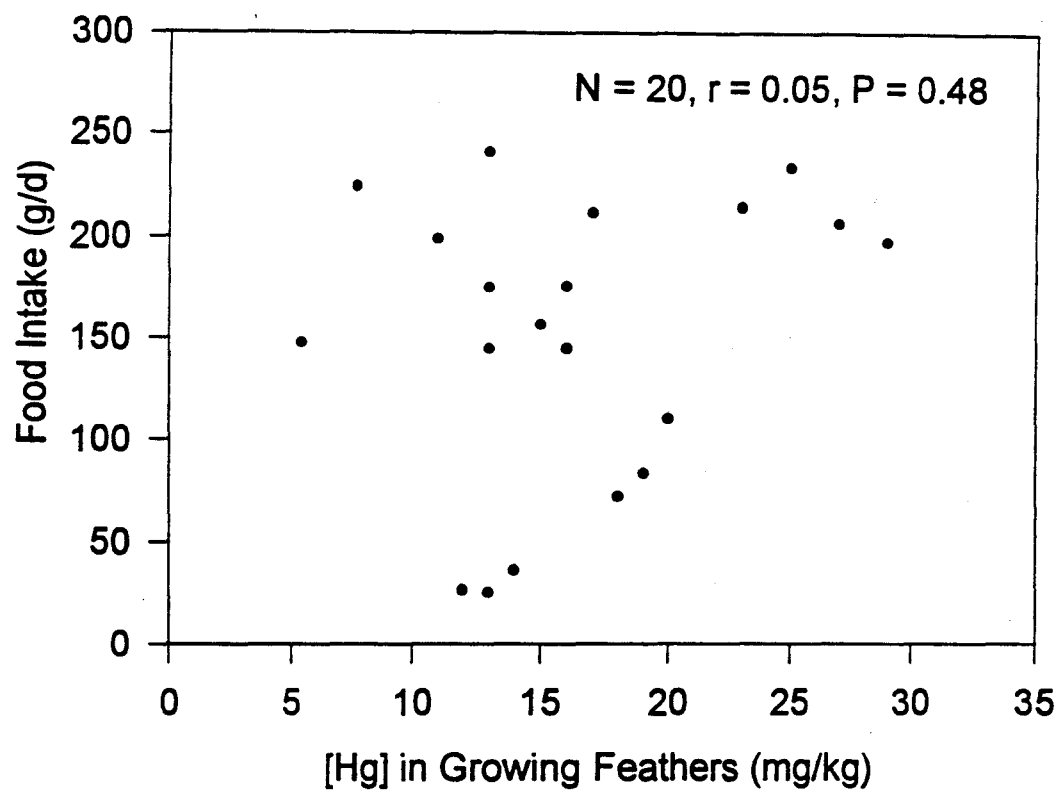


Figure 3.3 Food Intake of great egret chicks in relation to mercury concentrations in growing feathers.

IV. COMPARISON OF MERCURY CONCENTRATIONS IN BREEDING AND NONBREEDING ADULT GREAT BLUE HERONS (Ardea herodias).

Introduction

Since mercury is suspected to affect appetite and motor function in adult birds, it is possible that mercury contamination might interfere with several aspects of breeding. Mechanisms for such an affect could include 1) a failure of the bird to achieve adequate nutritional and hormonal physiological condition for the production of quality eggs or sperm, 2) failure to exhibit or perform the quality of appropriate behavioral signals and responses that are needed for courtship, copulation, nestbuilding, and incubation of eggs, and 3) failure to capture sufficient food to successfully rear young.

Great egrets were the focus of this effort in 1994, but too few breeding adults were trapped ($n = 5$), and too few roadkill carcasses were available for a comparison with non-breeding birds. In addition, the breeding plumes we collected in colonies could not be unambiguously attributed to breeding birds.

Great blue herons became the focus of this investigation in 1995 because there were indications that this species would be more susceptible to trapping, because the species nests solitarily in the Everglades, and because a large sample of traumatically injured birds from rehabilitation centers and roadkill collections in Florida was available.

In March and April of 1995 we located solitary nests of great blue herons via airboat survey, and used a motorized, radio controlled syringe to immobilize adult birds while sitting on their eggs. Despite excellent reported success with this device with seabirds (Wilson and Wilson, 1989) and considerable effort (27 attempts involving approximately 400 man-hours), only two adults were immobilized using this method. Both birds immediately abandoned their nests. The main problem with the method was the positioning of the syringe in the nest cup so

that it would hit the pectoral mass of the incubating bird. Using video recordings at a nest, it became clear that the syringe must be placed at the appropriate side of the nest to avoid intra-abdominal injection. Because the positioning of the adult bird was impossible to control, and because nest abandonment was unacceptable, this method was abandoned.

During the course of repeated visits to great blue heron nests in 1995, we discovered that adults begin to shed breeding-plumage feathers during the postnuptial molt while the chicks are still in the nest. Since great blue herons often nest solitarily in the Everglades (Frederick 1994), it is therefore possible to collect breeding plumes from these breeding adults, and be sure that there would be no confusion with nestling feathers or with feathers from nonbreeding birds (a potential problem in colonies). Published molt chronologies suggest that any plumes shed at this time would have been grown during the few months or weeks preceding courtship. Breeding plumes collected at nests with large young would also ensure that samples would only be collected from birds that had successfully accomplished all three of the above mentioned phases of the breeding cycle. If elevated mercury concentrations at the time of courtship and nest initiation prevent successful breeding then successfully breeding birds should have feather concentrations below a threshold concentration when compared to non-breeding adult birds, or when compared with the population of adults as a whole.

Materials and Methods

Solitary great blue heron nests in small tree islands were located by airboat in Conservation Areas 2 and 3. Locations with more than two nests were excluded as these might also serve as roost sites for non-breeding birds. Nest location was recorded using a Global Positioning System. Nests were visited early in the morning when chicks were half to full-sized and the area around the nest was searched for feathers. Feathers from the head, neck, and scapular area (identified by comparison with carcasses) that had grown during the

pre-nuptial period were collected, and will be analyzed for mercury.

Results and Discussion

Nuptial feathers from 16 great blue heron nests (2 from birds captured on the nest and 14 from nests with chicks) were collected during the 1995 breeding season. Mercury analysis is pending. These results will be compared either to feather or liver concentrations (converted using a feather/liver regression, see Chapter V) from our library of necropsied birds that meet the following criteria: 1) adult birds captured or found dead in south Florida during the peak of the breeding season (March through May) with no evidence of gonadal enlargement, and 2) all adult birds captured or found dead. The former sample of birds is more likely to include true nonbreeders - those that are adults at the correct time of year for breeding but that are not breeding. The latter sample is a sample of adult birds at large. With these comparisons, we hope to test the null hypothesis that there are no differences in mercury concentrations between successfully breeding birds, presumed non-breeding birds, and adult birds in general.

V. MERCURY CONCENTRATIONS IN TISSUES FROM WADING BIRDS

Introduction

Although mercury concentrations in livers of wading birds have been documented to some extent (Spalding et al., 1994; Sundlof et al., 1994), the relationship between hepatic mercury concentration and the concentration of mercury in tissues that can be readily and non-destructively sampled from live birds (e. g. blood and feathers) remains poorly known. In addition, very little is known about the distribution of mercury among tissues of wading birds. For these reasons, it is important to determine the relationship between mercury concentrations of different tissues, particularly between liver, feathers, and blood of egrets and herons. These analyses also will help us understand where mercury is deposited in the body of this group of birds.

Materials and Methods

Tissues from a total of 187 great egrets and 51 great blue herons (30 blue and 21 white color morphs) were collected from carcasses or nondestructively sampled from live birds in several south Florida counties (Broward = 100 birds, Dade = 67, Monroe = 52, Collier = 15) from June 1991 through February 1995. Information on the location for four of the birds was not recorded. In the case of the great egrets, the sample included a total of 140 nestlings, 6 fledglings, 14 juveniles, 22 adults, and 5 birds of unknown age. For the great blue herons, tissues from a total of 20 juveniles, 29 adults, and 2 birds of unknown age were examined for mercury concentrations (Table 5.1). Birds were assigned to the different age classes based on bill length and plumage.

A large proportion of the samples from nestling great egrets (blood and feathers) were collected

Table 5.1 Number of individuals sampled, by species and age.

Category	Blood	Liver	Brain	Muscle	Kidney	Ovary	Testes	Feces	Nails	Pancreas	Plumes	Powder Down	Growing Feathers	Mature Feathers
Nestling GE*	291	24	23	16	23	0	0	0	0	2	0	0	95	0
Fledgling GE	1	1	1	1	1	0	0	0	0	0	0	1	1	5
Juvenile GE	1	7	5	5	5	2	1	1	1	3	0	5	3	7
Adult GE	4	10	10	8	10	1	7	2	2	4	0	11	7	7
Ad. Breeding GE	6	2	2	2	1	0	0	0	0	0	12	1	1	0
Juvenile GBH**	2	7	14	1	4	0	1	0	0	0	0	12	2	2
Adult GBH	6	11	12	9	7	1	3	1	0	0	0	7	7	3
Ad. Breeding GBH	4	3	7	2	2	0	2	1	0	0	1	10	1	1

* GE = great egret

** GBH = great blue and great white heron.

Table 5.2 Summary of correlation analysis of mercury concentrations (wet weight; mg/kg) in tissues of great egrets (GE), great blue herons (GB) of both species combined (All) *. Numbers in parentheses indicate sample size.

											Growing Feather	Mature Feather
Blood	Liver	Brain	Muscle	Kidney	Ovary	Testes	Feces	Pancreas	Plumes	P. Down		
Blood	All (7)GBH(4)	All(7)GBH(4)	GBH(4)	NS(7)*	NS(1)	NS(2)	NS(1)	NS(2)	All(7)GE(6)	GBH(4)	All(84), GE(81)	NS(5)
	Liver	All(70)GBH(26)GE(44)	All(55)GBH(18)GE(38)	All(63)GBH(18)GE(44)	All(8)	NS(18)	All(5)	NS(10)	NS(9)	All(26)GBH(14)GE(12)	All(44)GBH(13)GE(31)	GE(11)GBH(12)
		Brain	All(53)GBH(18)GE(34)	All(61)GBH(20)GE(41)	NS(5)	All(18)GE(8)	NS(5)	All(8)GE(8)	NS(9)	All(40)GBH(20)GE(11)	All(42)GBH(13)GE(29)	NS(18)
			Muscle	All(48)GBH(15)GE(34)	NS(8)	All(18)GBH(8)GE(7)	NS(5)	All(8)GE(8)	NS(7)	All(21)GBH(12)GE(8)	All(35)GBH(12)GE(23)	NS(18)
				Kidney	All(6)GBH(3)	All(17)GE(8)	NS(5)	All(10)GE(10)	NS(8)	All(24)GBH(12)GE(12)	All(40)GE(30)	NS(17)
					Ovary	#	NS(1)	NS(2)	#	NS(4)	NS(5)	NS(2)
						Testes	NS(3)	NS(3)	NS(8)	All(13)GE(7)	All(8)GBH(4)	NS(8)
							Feces	NS(3)	#	#	NS(4)	NS(2)
								Pancreas	NS(1)	All(4)GE(4)	NS(3)	NS(7)
									Plumes	NS(11)	NS(6)	NS(2)
										P. Down	All(18)GBH(8)GE(10)	NS(16)
											Growing Feather	NS(9)
												Mature Feather

* All, GE, and GBH appear in the boxes if the correlation was statistically significant ($P < 0.05$).

^ NS = Correlation not statistically significant ($P > 0.05$). Sample size for both species combined.

No data available.

from live chicks during the nesting season; the remaining samples of nestlings and fledglings were collected from dead birds found in colonies. The juvenile and adult egrets and herons were collected either as roadkills or from rehabilitation centers. Birds were necropsied, and several tissues (liver, brain, muscle, kidney, ovary, testes, feces, nails, pancreas, plumes, powder down, mature primaries, and growing scapular feathers) saved for mercury analysis. In a few cases, birds were bled before they died and blood was added to the list of tissues analyzed for mercury.

Pearson correlation analyses (PROC CORR, SAS Institute, 1988) were used to assess the relationship between mercury concentrations in different tissues. This analysis was performed for egrets and herons combined, and also separately by species. Analysis of Covariance (PROC GLM, SAS Institute 1988) were used to assess the effects of location, age, and sex on the dependent variables (mercury concentration in tissues). Where analyses were species-specific, bill length was used as a covariate, in order to account for variation induced by body size or age.

Results and Discussion

a. Mercury concentrations in tissues of great egrets and great blue herons

Table 5.1 summarizes the number of birds sampled, by species and age class. Table 5.2 shows a summary of the correlation analyses of tissues from egrets and great blue herons, and correlation plots in Figures 5.1, 5.2, and 5.3. Mercury blood concentrations were correlated with liver, brain, plumes, and growing feathers, only when the two species were combined (Table 5.2). In great egrets, mercury concentrations in blood were correlated only with plumes and growing feathers. These results indicate that mercury concentrations in powder down and growing feathers could be probably used to assess mercury concentrations in liver, brain, muscle, and kidney of both great egrets and great blue herons. The use of

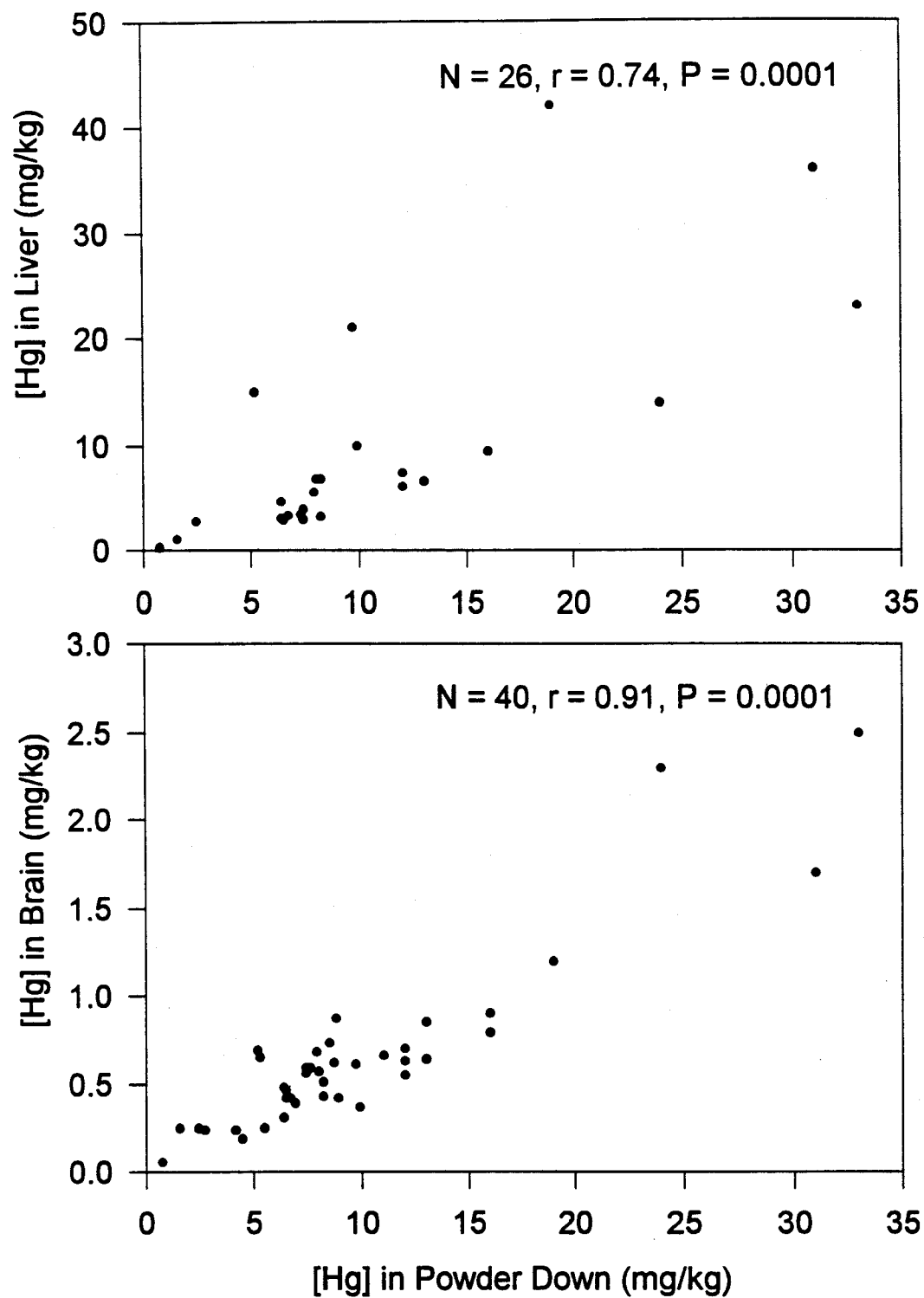


Figure 5.2 Relationship between concentration of mercury in powder down, and in liver and brain of great egrets and great blue herons.

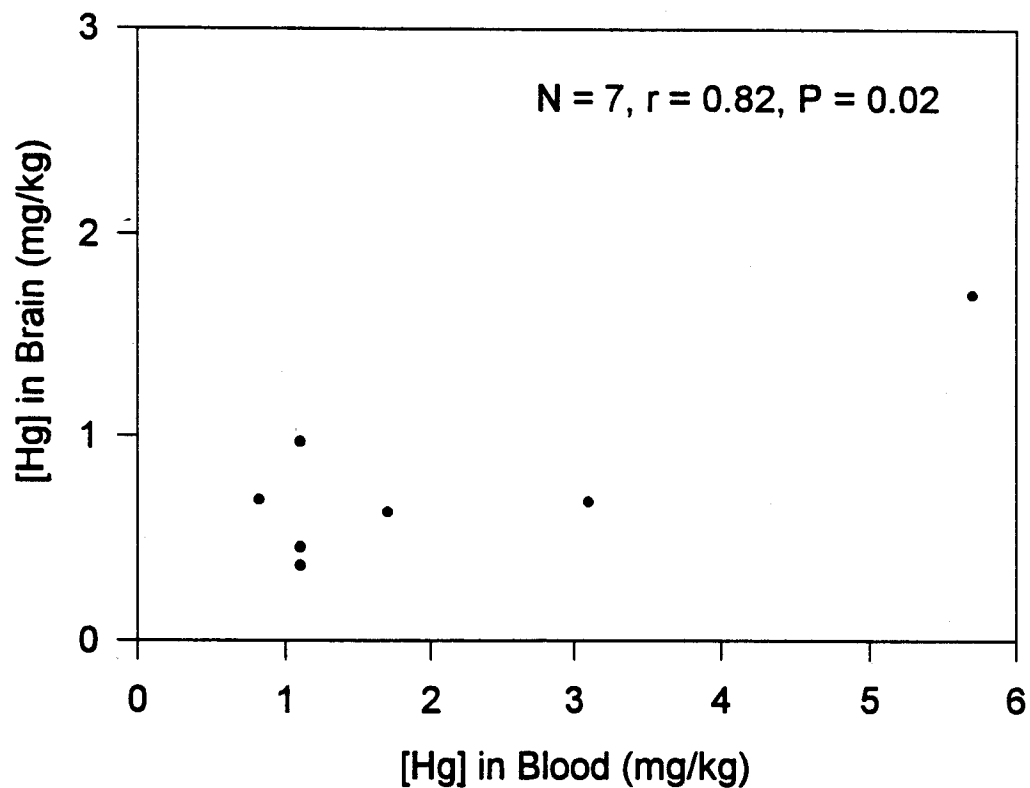
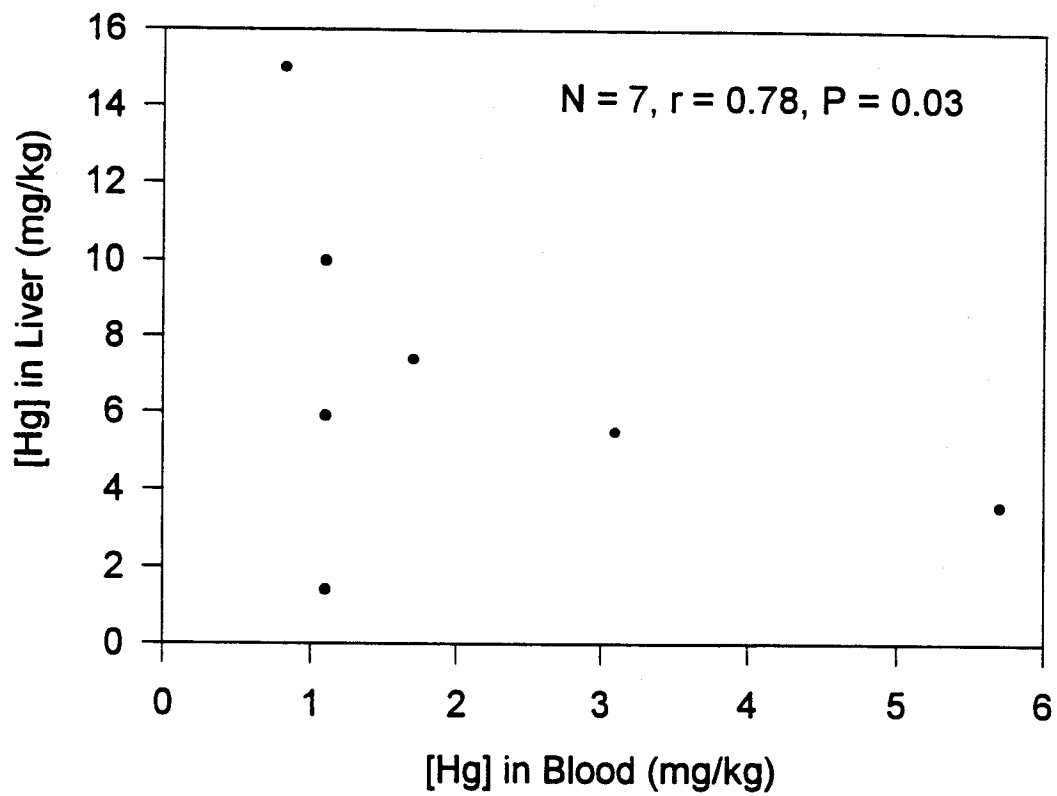


Figure 5.1 Relationship between concentration of mercury in blood, and in liver and brain of great egrets and great blue herons.

powder down as an indicator of tissue mercury concentrations in egrets and herons is of special importance, since this type of continually regenerating feather is always present in these two species of wading birds. Growing feathers, on the other hand, may or may not be present. Because of the small number of blood samples collected during 1994, the relationship between mercury concentration in blood and in other tissues of wading birds deserves further analysis.

Preliminary results indicate that mercury in great egrets and great blue herons concentrates unevenly among different tissues. Highest concentrations were present among skin keratin structures such as plumes ($n = 15$, mean = 16.90, SD = 14.61) mature feathers, ($n = 32$, mean = 16.53, SD = 41.34) growing feathers ($n = 124$, mean = 14.34, SD = 11.37), powder down ($n = 53$, mean = 11.97, SD = 10.75), and nails ($n = 3$, mean = 11.20, SD = 7.34) (Table 5.3, Figure 5.4). This phenomenon is consistent with a report by Braune and Gaskin (1987) that feathers may account for up to 93% of the total amount of mercury in a bird.

Tissues such as liver ($n = 76$, mean = 6.90, SD = 8.34), kidney ($n = 64$, mean = 2.64, SD = 3.59), ovary ($n = 6$, mean = 2.43, SD = 2.2), and pancreas ($n = 10$, mean = 2.38, SD = 2.02) had intermediate concentrations of mercury (Table 5.2, Figure 5.4) whereas muscle ($n = 55$, mean = 1.52, SD = 1.56), testes ($n = 18$, mean = 1.41, SD = 1.26), and brain ($n = 86$, mean = 0.68, SD = 0.54) were found to contain low concentrations of this metal. It is interesting to note that mercury concentrations in brains of egrets and herons were the lowest of all tissues examined. Very little information has been published regarding mercury concentrations in brain tissue of wild birds, although some information is available on mercury concentration in brains of experimentally dosed birds. It appears that relatively more mercury concentrates in brains of non-piscivorous bird species. Of a total of 13 domestic chickens (*Gallus gallus*, white leghorns) that were fed at a rate of 0.5 mgHg/kg bird for 100 days (average weekly intake of mercury of 6.5 mg), two died of possible mercury toxicosis,

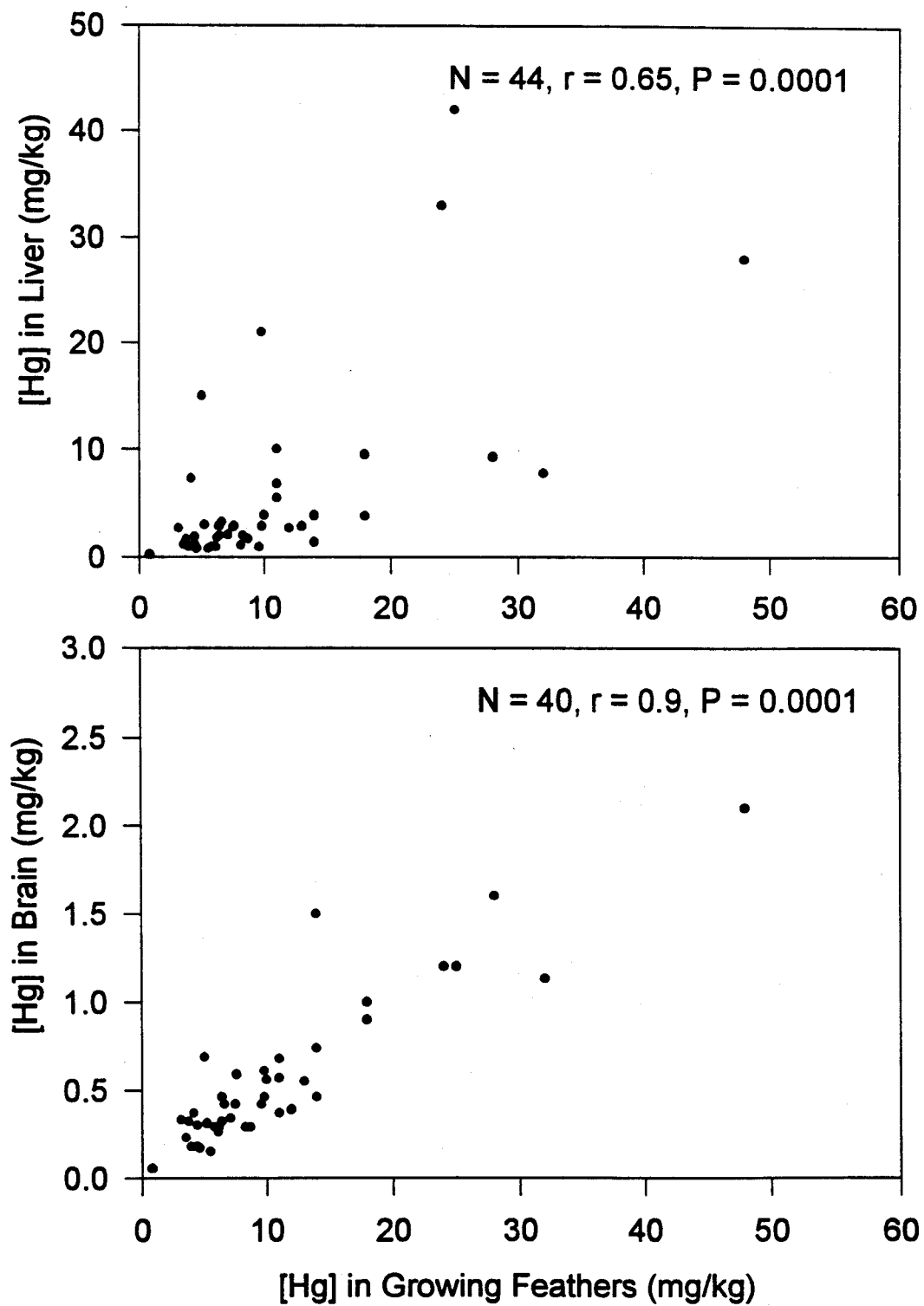


Figure 5.3 Relationship between concentration of mercury in growing feathers, and liver and brain of great egrets and great blue herons.

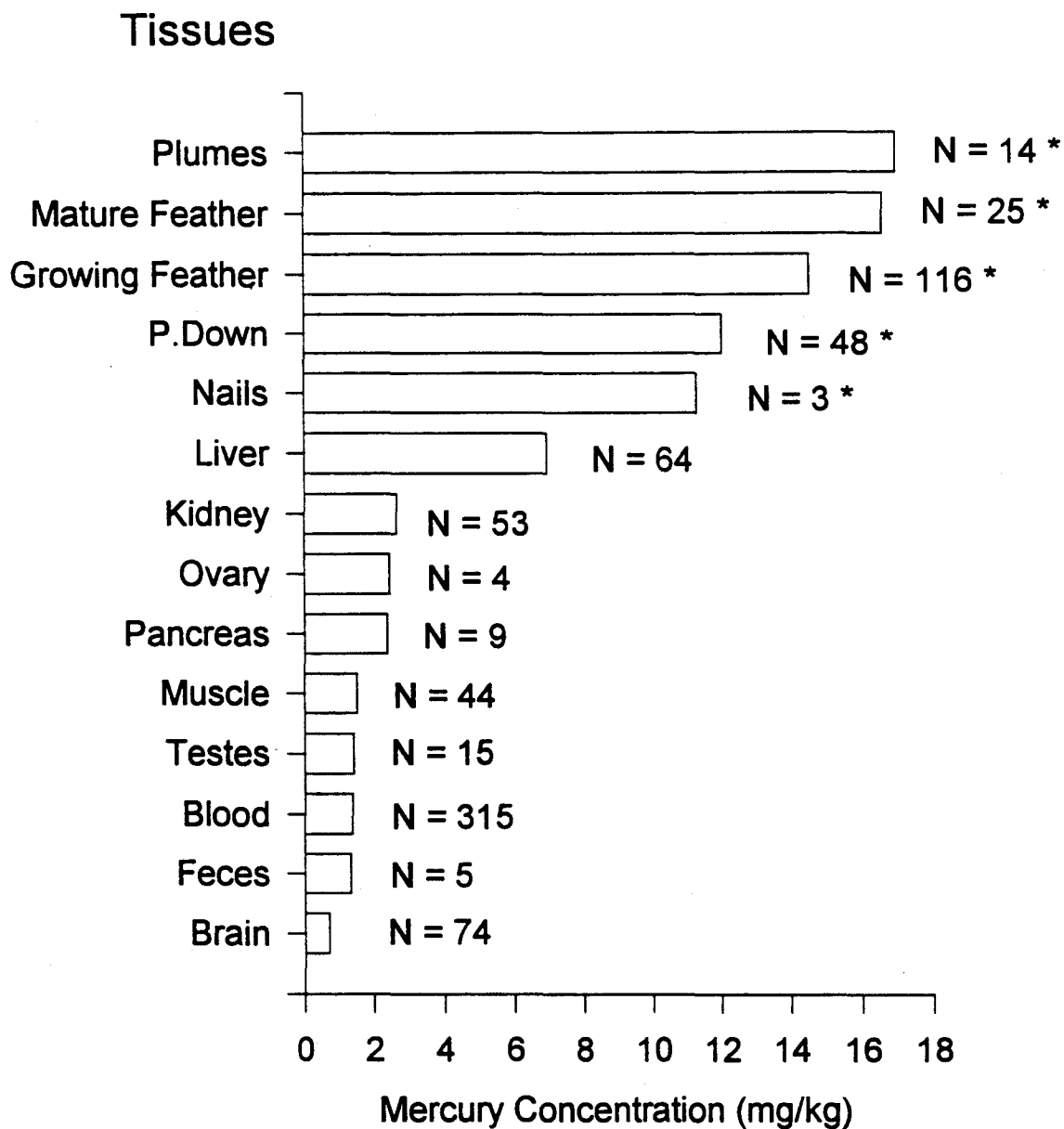


Figure 5.4 Average mercury concentration (mg/kg, dry weight for *; remaining tissues in wet weight) in tissues of great egrets and great blue herons (all ages combined).

Table 5.3 Average mercury concentrations (wet weight; mg/kg) in different tissues of great egrets and great blue herons, by age classes.

Tissue	Nestlings	Fledglings	Juveniles	Adults	All Ages	Differences between ages
Blood	1.22 (0.79)*	4.30	1.70 (1.21)	3.47 (4.08)	1.40 (1.4)	P = 0.002
Liver	2.02 (1.4)	9.50	5.85 (6.23)	11.21 (10.44)	6.75 (8.34)	NS***
Brain	0.53 (0.46)	0.90	0.58 (0.39)	0.83 (0.64)	0.68 (0.54)	P = 0.04
Muscle	0.77 (0.56)	1.60	1.36 (1.28)	2.04 (1.93)	1.48 (1.56)	P = 0.02
Kidney	1.08 (0.84)	3.0	1.91 (1.97)	4.34 (4.94)	2.57 (3.59)	P = 0.01
Ovary	-**	-	1.40 (0.56)	2.95 (2.63)	2.43 (2.2)	NS
Testes	-	-	1.63 (0.86)	1.37 (1.35)	1.41 (1.26)	NS
Feces	-	-	1.30 (1.69)	1.35 (1.52)	1.33 (1.4)	NS
Nails	-	-	2.80	15.5 (0.7)	11.2 (7.34)	NS
Pancreas	0.60 (0)	-	0.96 (0.32)	3.94 (1.75)	2.38 (2.02)	NS
Plumes	-	-	-	16.9 (14.61)	16.90 (14.61)	NS
Powder Down	-	16.0	11.43 (9.53)	12.2 (11.8)	11.97 (10.75)	NS
Growing Feathers	14.82 (11.37)	18.0	10.91 (14.16)	14.34 (10.69)	14.34 (11.37)	NS
Mature Feathers	-	15.26	9.75 (7.0)	25.55 (67.66)	16.53 (41.34)	NS

* Numbers in parentheses indicate standard deviation.

** No data available.

***Differences are not statistically significant ($P > 0.05$) (Main Effects Model Multiway ANOVA).

concentration from the different tissues studied increased with age (Table 5.3). Accumulation of mercury with age has been reported in several species of birds (Sundlof et al., 1994; Burger, 1993; Burger et al. 1992).

The collection location had an effect upon mercury concentrations in blood, liver, brain, powder down, and growing feathers. Pairwise comparisons between colonies indicated that birds from Broward County had higher mercury concentrations in brain and powder down when compared to birds from Monroe and Collier counties ($F = 4.99$, $P = 0.0002$, $DF = 3$ and $F = 5.07$, $P = 0.001$, $DF = 3$, respectively) (Table 5.4). The higher mercury in Broward County (central Everglades) is consistent with previous findings (Sundlof et al., 1994; Roelke et al., 1991).

and had 19 and 25 mg/kg (wet weight) of mercury in their brains (Wright et al., 1974). Two others that were sacrificed at the end of the experiment had 9.7 and 10.6 mg/kg of mercury in brain. In another study, three goshawks (*Accipiter g. gentilis*) were fed methylmercury at a rate of 13 mg Hg/kg food; all birds died before day 47 of dosing, and mercury concentration in brains of these birds were 36, 39 and 51 mg/kg, wet weight (Borg et al., 1970). Heinz and Locke (1976) fed methylmercury dicyandiamide to adult female mallard ducks (*Anas platyrhynchos*) through two consecutive breeding seasons at a rate of 3 mg Hg/kg food. Brains of ducklings from these ducks contained an average of 6.17 and 5.19 mg/kg of mercury (wet weight) in two successive years. In a similar study, black ducks (*Anas rubripes*) were fed methylmercury dicyandiamide for 28 weeks during two consecutive breeding seasons (Finley and Stendell, 1978). Brains from 16 dead ducklings that hatched from these eggs contained between 3.25 and 6.98 mg/kg of mercury, wet weight.

b. Differences in tissue mercury concentrations of great egrets and great blue herons in relation to age, sex, and location.

In this analysis, organ mercury data were combined for great egrets and great blue herons. The results obtained using an analysis of variance showed that mercury concentrations in blood, liver, brain, muscle, kidney, powder down and growing feathers were influenced by age and/or location. Sex did not influence any of the variables studied.

Age had an effect on blood, brain, muscle, and kidney mercury concentrations (Table 5.3). Significant differences between age classes were detected using a Hochberg's pairwise comparison for samples of unequal size (SAS Institute, 1988). Adult birds had higher mercury concentrations in blood, muscle, and kidney when compared to nestlings (3.47 mg/kg vs 1.22 mg/kg, $F = 8.64$, $P = 0.002$, $DF = 2$; 2.04 mg/kg vs 0.77 mg/kg, $F = 3.6$, $P = 0.02$, $DF = 3$; and 4.34 mg/kg vs 1.08 mg/kg, $F = 4.11$, $P = 0.01$, $DF = 3$, respectively).

The results obtained from the present study indicate that in general, mercury

Table 5.4 Average mercury concentrations (wet weight; mg/kg) in different tissues of great egrets and great blue herons, by location.

Tissue	Broward	Collier	Dade	Monroe	All Counties	Differences Between Countie
Blood	1.40 (1.05)*	-**	1.33 (1.81)	1.84 (1.37)	1.40 (1.4)	P = 0.0008
Liver	8.35 (11.45)	6.21 (2.04)	4.18 (7.23)	8.64 (9.5)	6.75 (8.34)	P = 0.03
Brain	1.16 (0.75)	0.67 (0.24)	0.49 (0.37)	0.68 (0.51)	0.68 (0.54)	P = 0.0002
Muscle	2.35 (2.24)	1.73 (0.68)	1.07 (1.16)	1.56 (1.75)	1.48 (1.56)	NS***
Kidney	2.357 (1.55)	3.75 (3.91)	1.30 (1.46)	3.84 (5.25)	2.57 (3.59)	NS
Ovary	-	-	-	3.3 (2.24)	2.43 (2.2)	NS
Testes	0.6	-	1.6	1.50 (1.4)	1.41 (1.26)	NS
Feces	-	-	2 (2.26)	0.5 (0.45)	1.33 (1.4)	NS
Nails	-	-	-	11.26 (7.34)	11.2 (7.34)	NS
Pancreas	-	-	0.6 (0)	2.37 (1.71)	2.38 (2.02)	NS
Plumes	31 (18.68)	-	6.43 (5.76)	17.45 (12.35)	16.90 (14.61)	NS
Powder Down	31	10.53 (2.69)	34.55 (22.55)	10.18 (6.48)	11.97 (10.75)	P = 0.001
Growing Feather	17.69 (13.38)	21 (15.55)	11.1 (6.21)	11.9 (8.68)	14.34 (11.37)	P = 0.005
Mature Feathers	20.92 (7.71)	5.96 (3.02)	13.53 (5.07)	26.4 (67.36)	16.53 (41.34)	NS

* Numbers in parentheses indicate standard deviation.

** Not data available.

***Differences are not statistically significant ($P > 0.05$) (Main Effects Model Multiway ANOVA).

VI. DOSE-RESPONSE RELATIONSHIPS AND FATE OF METHYLMERCURY CHLORIDE IN CAPTIVE GREAT EGRETS

Introduction

Dosing of captive adult great blue herons and great egrets was initiated as part of this study in an effort to witness any acute or sublethal effects of mercury at concentrations that could be encountered by foraging in the Everglades. Two dosing experiments were carried out, in spring of 1994 (reported on in the 1994 annual report and touched upon here) and in January of 1995. In both cases, dosing was at concentrations of mercury intake that would be high but not inconceivable in the wild condition in the Everglades. The main difference between the two dosing efforts was in the dose pathway - mercury capsules in food in 1994, and mercury solution in a subcutaneous osmotic pump in 1995.

As one of the ways of monitoring the effects of mercury on captive birds in 1995, we assessed potential effects on eye morphology and function, using electroretinography. This part of the study was done with the extensive help and collaboration of Drs. Susan M. Lorazel and Don A. Samuelson, Small Animal Clinical Sciences, College of Veterinary Medicine, Gainesville, FL.

Materials and Methods

The sublethal effects of methylmercury chloride (MeHgCl) were studied in four captive adult great egrets donated by the Florida Keys Wild Bird Rehabilitation Center. Two of the egrets (A and B) had been dosed previously in January of 1994. A and B were re-dosed in this series, and the other two (C and D) were used as controls. All birds had amputated wings, but were otherwise healthy.

The birds were housed at facilities provided by the College of Veterinary Medicine, University of Florida, Gainesville. Birds were housed in semi-enclosed concrete cages, and acclimatized for a period of two weeks prior to the start of the experiment. Before dosing began, all birds were weighed, and a sample of blood and feathers collected for mercury analysis. Mercury was administered to the birds using an osmotic pump (ALZET[®], Model 2ML4). This is a miniature pump (5.1 cm x 1.4 cm) that releases 60 ul/day of solution for a total of 28 days. We attempted to dose birds at a rate that would closely mimic a diet that contained 5 mg/kg. An 8.37 mg/ml solution of MeHgCl (MeHgCl was dissolved with a few drops of acetone and the solution was then mixed with 10 ml of propylene glycol) was used to fill the 2 ml pumps implanted in egrets A and B; egrets C and D were implanted with pumps filled with a solution that contained the same solvents but no mercury. Pumps were implanted subcutaneously in the inguinal area.

Birds were offered approximately 35 to 40 Atlantic silversides (*Menidia menidia*) every day (approximately 100 g), and the daily amount of fish eaten was recorded. Egrets were weighed and bled (for mercury, packed cell volume, plasma proteins and white cell counts) every four days for 28 days.

Electrophysiology

With the objective of determining possible effects on vision due to mercury toxicosis, we performed electroretinograms (ERG) and visual evoked potentials.

Electroretinograms test the function of the retina by recording the electrical potential difference generated as a result of light stimulation. The main components of the ERG that are evaluated clinically are the a-wave and the b-wave (Figure 6.1). The a-wave is generated by the photoreceptors (rod and cone cells), and the b-wave arises primarily from Müller cells in the retina (Berson, 1981). Various disease states involving retinal degeneration can cause alterations in the amplitudes and latencies of the a- and b-waves. Additionally, the rod and

cone function can be separated with various ERG techniques, enabling detection of disease states that preferentially affect one of these cell types (Acland, 1988).

The visual evoked potential (VEP) is an electrophysiological test which evaluates the postretinal visual pathways. VEPs have been used in a variety of animals and humans for the study of disease, toxicology and physiology (Strain et al., 1990). ERGs and VEPs can potentially be powerful, noninvasive tools in evaluation of subclinical effects of environmental toxins.

Both VEP and ERG were conducted in all four great egrets prior to the start of the experiment. Since three of the four birds died during the course of the experiment, post-dosing exams were conducted only for egret A. Each bird was anesthetized with isoflurane, and pupils were dilated to a maximal diameter of 7 mm with 4 mg/ml topical vecuronium bromide (Norcuron®). ERGs were conducted with

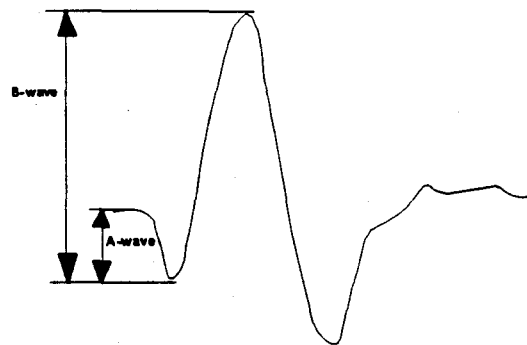


Figure 6.1 Basic form of ERG wave form.

RetinoGraphics® programming, using an LED monochromatic red light source (RetinoGraphics BPM-100 system). Electrodes were placed consistently in each bird and consisted of the ground electrode at the midline caudal aspect of the skull, the reference electrode at the lateral canthus, and a corneal contact electrode. Recordings were taken under light adaptation and after 15 min of dark adaptation. Signal averaged ERGs (8 flash average, 2 seconds apart) were recorded for three light intensities, with the highest intensity equivalent to approximately 1.4 cd m⁻² and decreasing in 1 log unit increments. Tests were repeated 3-6 times with 1 - 2 minutes inter-test time. Flicker ERGs were performed at high light intensity under various frequencies ranging from 30 Hz to 60 Hz. Visual evoked potentials were also conducted only under the highest light intensity, with signal averaging of 8 flashes, 2 seconds apart. Needle

Table 6.1 Results of mercury dosing experiment. Great egret A.

Cumulative Days of Experiment	Cumulative Mercury Administered (mg)	Average Food Ingested (g/day)	Mercury Dosage (mg Hg/kg fish)	Cumulative [Hg] in bird (mg Hg/kg bird)	[Hg] in blood (mg/kg)	Body Weight (g)	Packed Cell Volume (%)	Plasma Proteins (g/dl)	White Blood Cells (cells/ul)
5	2.5	75.50	6.62	3.07	4.8	815	41.5	8.0	25825
9	4.5	102.50	4.87	5.63	7.1	800	35	8.6	27368
14	7.0	78.62	6.35	8.75	12	800	37	9.2	21034
19	9.5	87.30	5.72	13.19	15	720	39	7.6	11864
23	11.5	102.60	4.15	15.86	18	725	40	7.4	11657
27*	13.5	120.33	4.87	19.57	15	690	49	7.6	11139
33	14.0	131.22	0.64	20.00	21	700	33	6.2	18434

* Osmotic pump has a life time of 28 days.

electrodes were placed at the medial canthus, caudal to the contralateral ear opening, and the ground at caudal midline. At the end of the fourth week of the experiment great egret A was euthanized and tissues saved for mercury and histopathology analysis.

Results and Discussion

a. Total amount of mercury administered in relation to blood mercury concentration and food intake.

In October 1994, two of the egrets that were dosed with mercury in January of 1994 were sampled for blood mercury concentrations. An updated graph showing mercury concentrations in blood in relationship to days in experiment in these birds is presented in Figure 6.2. Approximately 7 months after the last mercury dosing, blood concentrations had returned to background concentrations (< 1 mg/kg).

Unfortunately, in the second dosing experiment, three of the four birds (both controls, and one dosed bird) died during the second week of the experiment. The most likely cause of death for the controls was probably related to the effects of a single severe cold weather event, despite heat lamps and wind barriers. The appetite of both control birds decreased rapidly during and following the coldest nights, and they died soon thereafter. The dosed bird (egret B), however, never stopped eating and died of what appeared to be an acute process. We suspect mercury toxicosis as a possible cause of death for this bird. Mercury concentrations from tissues of this bird, however, are still pending.

The results of the second dosing experiment will only be presented for great egret A, and they are summarized in Table 6.1. The target mercury dosing rate was 5.0 mg/kg as measured in food; in practice, the averaged dose rate varied because of variable food intake rates, and ranged from 4.15 to 6.62 mg/kg (Table 6.2). Mercury concentration in blood increased steadily during the course of the experiment (from 0.51 mg/kg to 21 mg/kg) (Figure

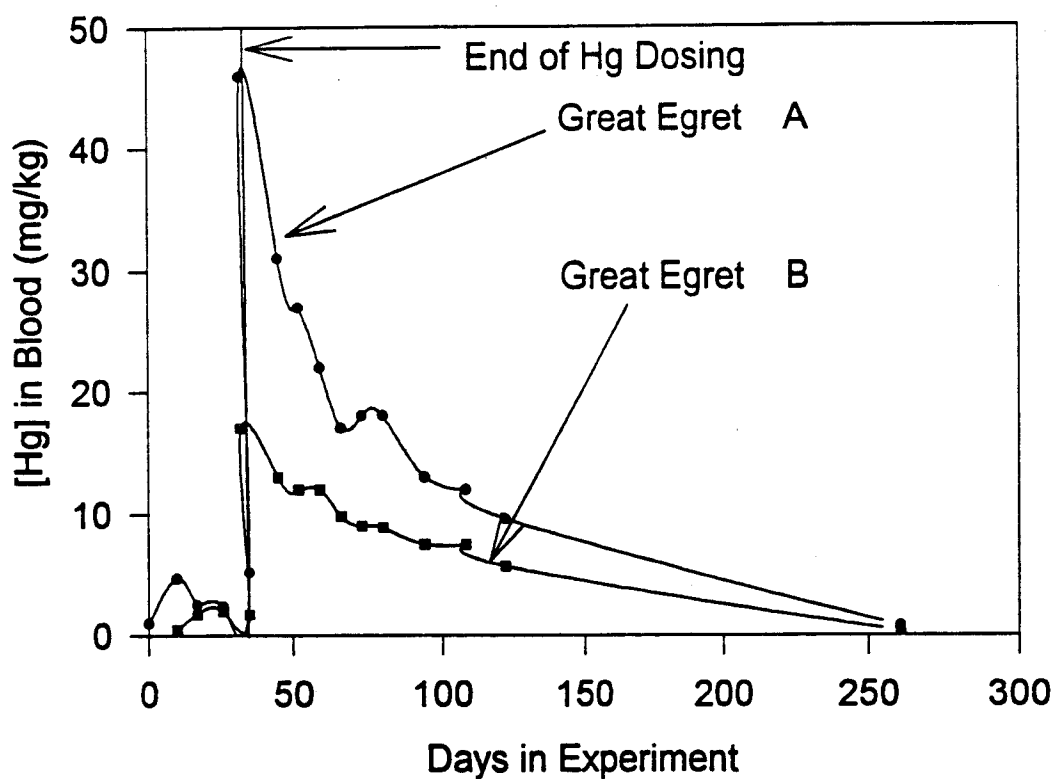


Figure 6.2 Relationship between mercury concentration in blood (mg/kg) from great egrets dosed with methylmercury and days in experiment (1994).

Table 6.2 Peak b-wave amplitudes pre-mercury dosing, all birds combined.

Intensity (log units)	Photopic (uV)	Scotopic (uV)
-2	46.35 (27.62)*	133.11 (47.32)
-1	81.03 (19.44)	181.46 (69.29)
0	145.89 (28.99)	196.38 (67.59)

Table 6.3 Peak b-wave amplitudes post-mercury dosing, great egret A.

Intensity (log units)	Photopic (uV)	Scotopic (uV)
-2	35.20 (20.14)	131.94 (79.83)
-1	91.95 (41.22)	158.00 (84.44)
0	157.15 (53.24)	163.49 (64.81)

Table 6.4 Peak b-wave latencies pre-mercury dosing, all birds combined.

Intensity (log units)	Photopic (uV)	Scotopic (uV)
-2	50.68 (12.10)	59.72 (11.73)
-1	40.70 (6.15)	50.89 (8.35)
0	38.71 (5.05)	48.81 (8.17)

* Values in parentheses indicate standard deviations.

6.3). Results for mercury concentrations from other tissues of this bird are pending.

b. Electrophysiological results.

The emphasis here will be on peak b-wave amplitude and latency, and to a lesser extent a-wave amplitude and latency, since these are the parameters most often evaluated clinically. Peak average b-wave amplitudes for the four egrets before mercury dosing, are recorded in Table 6.2. These results represent averages for both eyes on all birds. Variability between birds appeared to be slightly greater than variability within birds. Amplitudes increased with increasing intensities and the dark adapted (scotopic) state elicited higher responses than light adapted (photopic) states.

Table 6.3 describes peak b-wave amplitudes in the one surviving egret after four weeks of dosing with methylmercury. The trend of increasing amplitudes with increasing intensities remained consistent in the post treatment bird (great egret A). Although the values obtained for this bird following treatment did not differ greatly from the averages for all birds combined prior to treatment, they did differ from the pretreatment values for great egret A. Figure 6.4 shows the peak b-wave amplitudes for great egret A, pre and post treatment, for photopic and scotopic responses. It is apparent from these data, that the main change is a notable decrease in average amplitude of the scotopic response, but no change in the photopic response. Graphs for each eye were similar to both combined. An initial difference in amplitudes between left and right eye (right higher than left) remained consistent following treatment.

Peak b-wave latencies for all birds are presented in Table 6.4. The post treatment b-wave latencies for egret A revealed a (comparatively) increased scotopic latency, and little or no photopic latency change (Figure 6.5). A-wave amplitudes also exhibited a decrease in average amplitude between pre and post treatment in this bird, again mainly in scotopic responses. A-wave latencies did not appear to change as the other parameters did.

Flicker ERGs showed that the flicker fusion frequency is generally between 55 Hz and

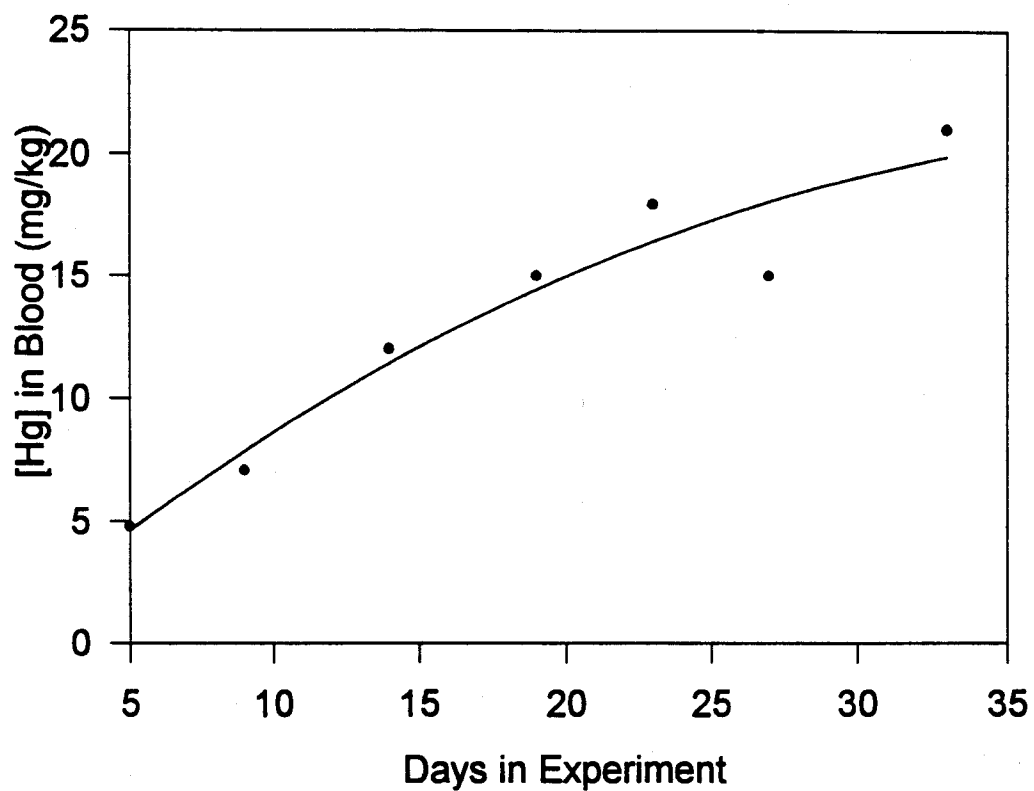


Figure 6.3 Relationship between mercury concentration in blood (mg/kg) and days in experiment for great egret A, during the captive mercury dosing experiment carried out in January 1995.

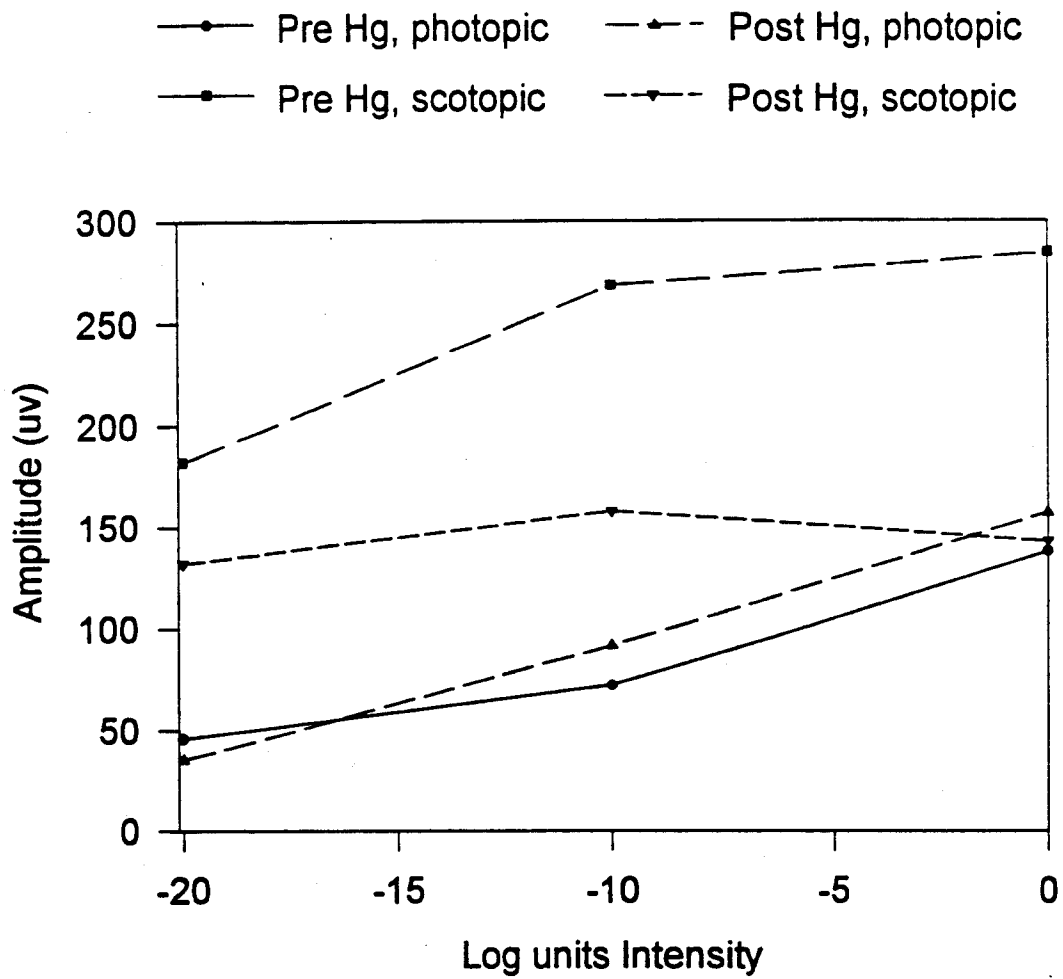


Figure 6.4 B-wave amplitudes (great egret A), right and left eyes combined.

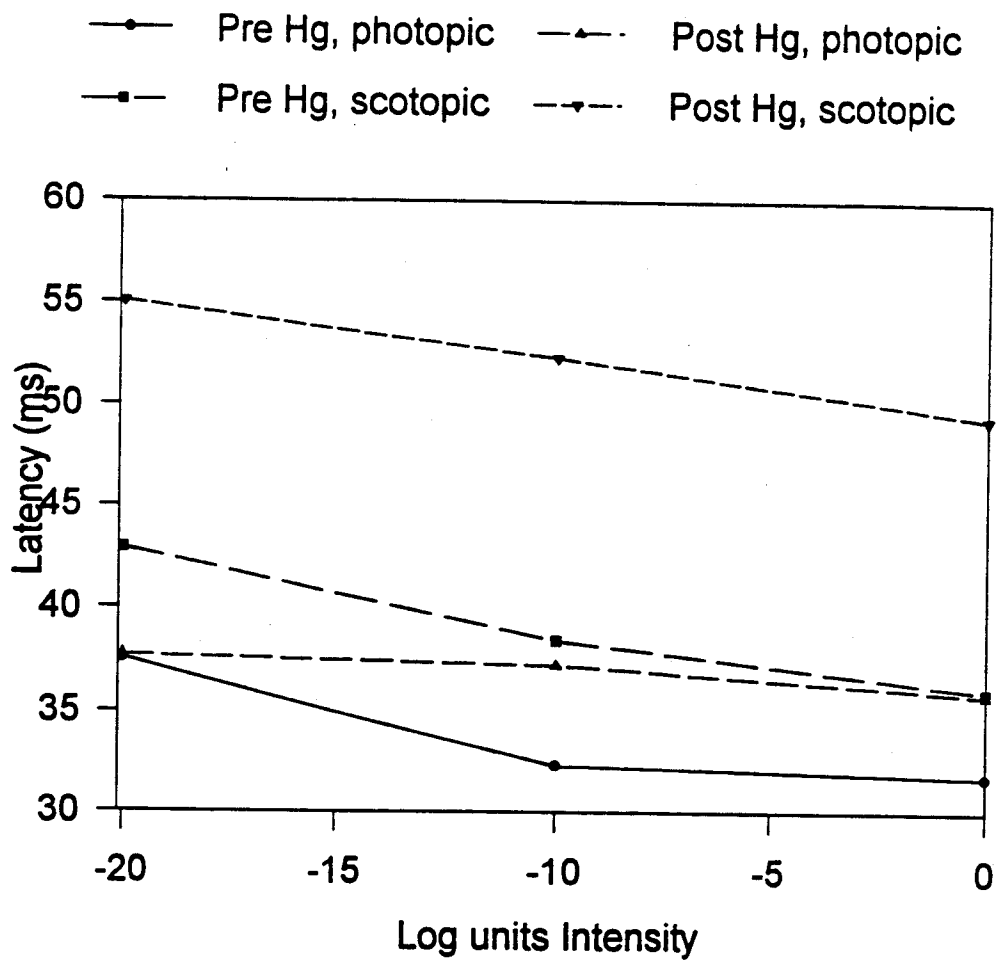


Figure 6.5 B-wave latencies (great egret A), right and left eyes combined.

65 Hz. Flickers were performed only at the high light intensity, due to time and anesthetic constraints. A more thorough analysis would require additional tests at the lower light intensities as well as at slower flicker rates of <20 Hz.

Visual evoked potential recordings elicited responses with pronounced P1 and N1 waves, and in most birds, also P2 and N2 peaks; however, responses were quite variable. At present there is no standardized protocol for VEPs in avian species and further studies will be required to obtain a consistent protocol for electrode placement in these animals.

The ERG changes seen in the surviving bird (decrease in the scotopic amplitude and the scotopic latency, but not in the photopic responses) implies a selective effect on the rod photoreceptors. This is a response that is consistent with previous studies in mammals (Fox and Sillman, 1979), but there have been no studies to date which have thoroughly explored this phenomenon in avian species. This preliminary data is at the least tantalizing, and suggests a mechanism for visual debilitation in birds with high tissue mercury concentrations. Admittedly, this particular study is weak because it is based on a single bird with no controls.

Further information may be gained by developing a more standardized protocol for the flicker ERGs and for the VEPs, as well as expanding the study to additional wavelengths and a broader range of intensities. The flicker responses have the potential for further differentiating rod and cone function, and changes in VEPs may provide useful information regarding the central visual effects of methylmercury at a subclinical level. At this stage the information gathered is very preliminary, but it provides a good basis for improving this technique and rationale for further development.

c. Histopathological results.

In addition to detecting changes in ERGs, there were ocular histological differences noted between the mercury-dosed birds and the control birds. Great egret A exhibited corneal edema compared to the control birds. Both dosed birds (egrets A and B) exhibited vacuolization

of the middle cortex of the lens. These changes were not seen in the two control animals. In addition, pronounced vacuolization in the nonpigmented epithelium of the ciliary body was present in both dosed birds. Again, the control birds did not exhibit these changes.

In summary, the ocular tissues of egrets that were dosed with mercury appeared to have some histopathological changes. Interestingly these changes were epithelial in nature and suggested injury to the cell membrane by way of damaged active transport systems, channel proteins, membrane receptors, or structural proteins. The effect of these structural changes on visual perception abilities are unknown, nor is it clear whether the structural changes are linked to the electrophysiological results.

Several other tissues from these birds are presently being analyzed for changes due to mercury toxicosis.

Table 7.1 Data on 24 regurgitations of great egret nestlings collected during 1994.

Colony	Date of Collection	Prey Items	Prey Types	Prey Species
Alley North	04/08/94	23	a,b,c	1,3,5,6,8,16
Alley North	05/02/94	1	a	10
Deer Island	04/10/94	11	a	1,5,6,10
Deer Island	04/16/94	5	a,b	7,10,19
Deer Island	04/16/94	1	a	19
Frog City South	04/23/94	Unknown *	a	19
Frog City South	05/01/94	4	a	10,11
Hidden/L-28	04/10/94	Unknown	a,c	17,20
Hidden/L-28	04/16/94	7	a	13,15
Hidden/L-28	04/16/94	Unknown	a,c	17,20
Hidden/L-28	04/18/94	Unknown	a,b,c	17,20
Hidden/L-28	04/27/94	Unknown	a	20
Hidden/L-28	04/30/94	Unknown	a,b	20
Hidden/L-28	05/03/94	Unknown	a,b	20
JW-1	04/14/94	2	a	10
JW-1	04/17/94	2	a	1
JW-1	04/29/94	1	a	10
JW-1	04/29/94	Unknown	a,b,c	17,20
JW-1	05/14/94	1	a	10
JW-1	05/14/94	1	a	10
L-67	05/21/94	1	a	10
L-67	05/21/94	22	a	1,5,9,10,18
Mud Canal	04/05/94	Unknown	a,b,c	17,20
Mud Canal	05/05/94	2	a	10

* Bolus of digested food material; identification to number of prey items was impossible.

Prey Types: a = fish; b = insect; c = crustacean (crayfish and grass shrimp);
d = frog.

Prey Species: 1 = *Fundulus chrysotus*; 2 = *F. lineolatus*; 3 = *Lucania goodei*;
4 = *Jordanella floridae*; 5 = *Gambusia affinis*; 6 = *Heterandria formosa*;
7 = *Poecilia latipinna*; 8 = *Lepomis punctatus*; 9 = *L. macrochirus*;
10 = *L. gulosus*; 11 = *Lepomis* sp.; 12 = *Micropterus salmoides*;
13 = *Belonesox belizanus*; 14 = *Tilapia* sp.; 15 = *Erimyzon succetta*;
16 = *Palaemonetes paludosa*; 17 = *Procambarus allenii*; 18 = *Rana* sp.;
19 = Odonates; 20 = Unidentified prey species.

VII. FOODS CONSUMED BY WILD GREAT EGRET NESTLINGS

Introduction

Any model of mercury intake in wading birds must be based on the amount of food consumed, and the size, species composition, and mercury content of prey animals. In order to better describe great egret food habits, we have collected and described spontaneously-regurgitated food from great egret nestlings in 1994 and 1995.

Materials and Methods

Between mid April and late May 1994, a total of 24 regurgitations were collected from great egret nestlings in 7 different colonies (Table 7.1). Regurgitations were collected from chicks in an opportunistic fashion as they regurgitated spontaneously either by being handled, or as a response to the close approach of one of us. The nest number and the chick size were recorded for each sample collected.

During 1995, a total of 51 regurgitations were collected from chicks in 6 different colonies between mid April and early June (Table 7.2). Hidden and Tamiami West colonies were visited approximately once a week during April and May, and regurgitations collected after handling of the chicks. The remaining colonies were visited with no particular schedule. All samples were stored in plastic bags and kept frozen until analysis.

In the laboratory, the number of prey items and prey types (fish, insect, crustacean, and amphibian) were determined for each regurgitation examined. Prey types were identified to species whenever possible. In addition, each prey item was weighed (weights include those of partially digested items) and measured.

Results and Discussion

The data obtained from the regurgitations of great egret nestlings are summarized in Table 7.1 and 7.2, respectively. During 1994 all of the regurgitations examined (100%) had some species of fish; 7 (29.16%) of the samples contained insect parts (Odonates, or other unidentified species of insect); 7 (29.16%) had some species of crustacean (grass shrimp or crayfish); and one sample (4.16%) had one amphibian. During 1995, all the samples contained at least one species of fish (100%); 11 (21.6%) contained insect parts; and 8 (15.6%) of the samples had crustaceans.

In 1994, a total of 10 different species of fresh water fishes were identified. The most common species was the warmouth (*Lepomis gulosus*) which was found in 41.6% of the samples. It was followed by the golden topminnow (*Fundulus chrysotus*) which was found in 16.6% of the cases. The mosquitofish (*Gambusia holbrooki*) and the least killifish (*Heterandria formosa*) were found in 12.5% and 8.3% of the samples, respectively. The remaining species of fishes (bluefin killifish, *Lucania goodei*, spotted sunfish, *Lepomis punctatus*, bluegill, *L. macrochirus*, an unidentified centrarchiid, *Lepomis* sp., sailfin molly, *Poecilia latipinna*, chubsucker, *Erimyzon succetta*, and the pike killifish, *Belonesox belizanus*) were found only in one sample each (4.2%). Of all these species, only one (*Belonesox belizanus*), is an exotic species introduced from central America. The crustaceans were identified as either grass shrimps (*Palaemonetes paludosus*, 4.2%) or crayfishes (*Procambarus alleni*, 21%). In addition, one amphibian identified as *Rana* sp. was collected from one of the samples (Figure 7.1).

During 1995, a total of at least 11 species of fishes were identified. Centrarchids (*Lepomis* spp.) were the most common group of fishes found (34.4%). The sailfin molly (*Poecilia latipinna*) was the second most common species, and was recovered from 23.5% of the samples. Killifishes were represented by flagfishes (*Jordanella floridae*, 17.6%), lined topminnows (*Fundulus lineolatus*, 15.6%), golden topminnows (13.7%), and by the bluefin

Table 7.2

Data on 51 regurgitations of great egret nestlings collected during 1995.

Colony	Date of Collection	Prey Items	Prey Types	Prey Species
Alley North	04/21/95	4	a	11,20
Alley North	04/21/95	5	a,b	1,19,20
Alley North	05/26/95	1	a	11
Hidden/L-28	04/20/95	14	a,c	1,2,5,13,17
Hidden/L-28	04/20/95	7	a,b	1,7,11,12,19
Hidden/L-28	04/20/95	18	a,b	1,2,3,4,5,7,14,19,20
Hidden/L-28	04/20/95	2	a,c	13,17
Hidden/L-28	04/20/95	2	a	7
Hidden/L-28	04/25/95	2	a	11
Hidden/L-28	04/25/95	1	a	11
Hidden/L-28	04/25/95	3	a,c	11,17,20
Hidden/L-28	04/25/95	2	a	7,14
Hidden/L-28	04/25/95	5	a,c	17,20
Hidden/L-28	04/28/95	1	a	11
Hidden/L-28	05/02/95	10	a	1,5,7,11,13,20
Hidden/L-28	05/02/95	6	a	1,7,11
Hidden/L-28	05/02/95	9	a,b,c	13,17,19,20
Hidden/L-28	05/02/95	1	a	11
Hidden/L-28	05/02/95	4	a,b,c	17,19,20
Hidden/L-28	05/02/95	1	a	4,20
Hidden/L-28	05/13/95	3	a	2,11,20
Hidden/L-28	05/13/95	2	a	2,7
Hidden/L-28	05/13/95	2	a	11,12
Hidden/L-28	05/13/95	10	a,c	4,7,11,16,20
Hidden/L-28	05/21/95	2	a	20
Hidden/L-28	05/29/95	1	a	11
Hidden/L-28	05/29/95	24	a,b	1,2,4,7,11,13,14,19,20
Hidden/L-28	05/29/95	8	a	4,5,20
Hidden/L-28	05/29/95	1	a	4
Hidden/L-28	06/05/95	3	a	11,12,20
Hidden/L-28	06/05/95	5	a	2,11,12
Hidden/L-28	06/05/95	3	a	11
JW1	04/28/95	7	a,c	11,16,20
JW1	04/28/95	28	a,b	1,4,5,7,11,14,19,20
JW1	05/06/95	7	a,b	11,19,20
L-67	05/17/95	1	a	11
Mud Canal	-	6	a	1,2
Mud Canal	04/26/95	1	a	11
Tamiami West	04/16/95	4	a	13,20
Tamiami West	04/16/95	1	a	11
Tamiami West	04/16/95	4	a	11,13,20
Tamiami West	04/16/95	2	a	4,7
Tamiami West	04/30/95	2	a,b	11,19
Tamiami West	04/30/95	Unknown**	a	20
Tamiami West	05/06/95	8	a,b	7,13,19,20
Tamiami West	05/06/95	2	a	11
Tamiami West	05/14/95	3	a	11,20
Tamiami West	05/14/95	1	a	11
Tamiami West	05/14/95	2	a	2,11
Tamiami West	05/15/95	5	a,b	11,19,20
Tamiami West	05/19/95	8	a	4,7,11

* Not recorded

** Bolus of digested food material; identification to number of prey items was impossible.

Prey Types: a = fish; b = insect; c = crustacean (crayfish and grass shrimp);
d = frog.Prey Species: 1 = *Fundulus chrysotus*; 2 = *F. lineolatus*; 3 = *Lucania goodei*;
4 = *Jordanella floridae*; 5 = *Gambusia affinis*; 6 = *Heterandria formosa*;
7 = *Poecilia latipinna*; 8 = *Lepomis punctatus*; 9 = *L. macrochirus*;
10 = *L. gulosus*; 11 = *Lepomis* sp.; 12 = *Micropterus salmoides*;
13 = *Belonesox belizanus*; 14 = *Tilapia* sp.; 15 = *Erimyzon succetta*;
16 = *Palaeomonetes paludosa*; 17 = *Procambarus allenii*; 18 = *Rana* sp.;
19 = Odonates; 20 = Unidentified prey species.

killifish (2%). The mosquitofish and the largemouth bass (*Micropterus salmoides*) were present in 9.8% and 5.8% of the samples, respectively. Exotics were represented by the pike killifish (15.6%) and by the cichlid *Tilapia* sp. (7.8%). Grass shrimp and crayfish were found in 4% and 12% of the samples, respectively (Figure 7.2).

The information on mercury concentration in some of the prey species listed above is limited to the study of Ware et al. (1990), although we expect considerably more information in the future from studies by J. Trexler and W. Loftus at Florida International University. Ware et al. found that in Water Conservation Areas 2 and 3, warmouths had an average of 0.94 mg/kg of mercury in muscle tissue, and that the mercury concentration in bluegills from a small lake in the upper Kissimmee valley was 1.01 mg/kg. Crayfish and pig frogs (*Rana grylio*) from the Everglades area showed lower mercury concentrations (< 0.25 mg/kg), but enough to biomagnify up the food chain (Ware et al. 1990). It is clear that more work on mercury concentrations are needed for a better understanding of the amounts of mercury that great egrets are receiving from the ingestion of wild freshwater fishes and invertebrates.

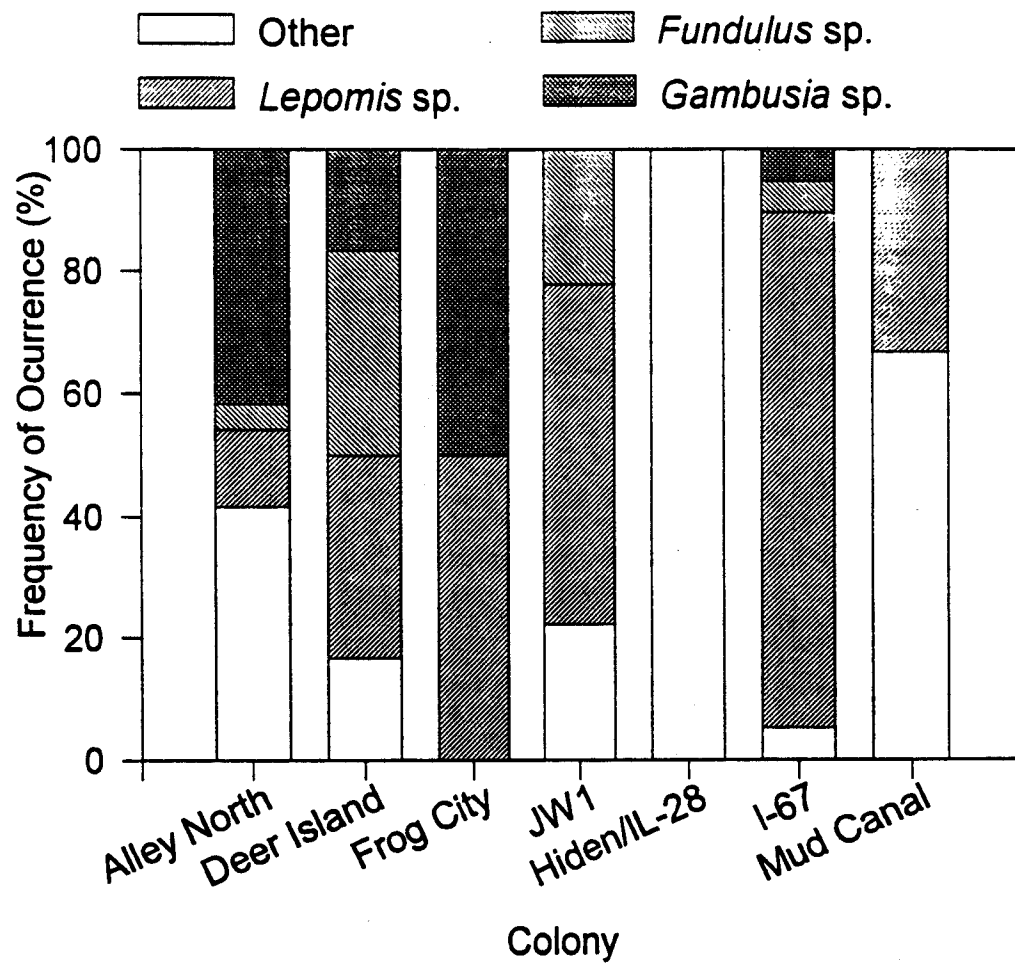


Figure 7.1 Prey composition during 1994, by colony. Prey species shown represent the three most frequent for that year.

VIII. FIELD DOSING EXPERIMENT

Introduction.

During the 1995 field season, we conducted a mercury dosing experiment on great egret nestlings with the objective of determining the effects of elevated mercury on chick food intake, growth, and first year survival. By conducting the experiment in a natural situation, we hoped that chicks would experience the natural stresses associated with weather, sibling competition, and a variable food supply, which are factors that would be impossible to duplicate in a laboratory experiment. Also, it would be impossible to measure the longer-term effects of elevated mercury on survival if the experiment had not been conducted in a field situation. Finally, by dosing, we hoped to achieve a more obvious difference in mercury concentrations of "high mercury" and "low mercury" chicks than we had recorded occurring naturally in 1994.

Materials and Methods.

A total of 72 nests, including 32 from the Tamiami East nesting colony and 40 from the Hidden/L28 nesting colonies were included in the experiment (see Figure 8.1). The experiment was divided into two transects within each colony, with each transect containing roughly equal numbers of experimental and control nests. Only first-hatched chicks approximately 12 days old (determined from nest chronologies) were used in the dosing experiment. Forty-two chicks were monitored for both food intake and survival (Survival/Food), including 21 mercury-dosed birds and 21 which recieved placebos. An additional 22 chicks (Survival) were monitored for survival and served as controls for the food monitoring procedures, including 12 mercury-dosed birds and 10 placebos. All non-experimental chicks in

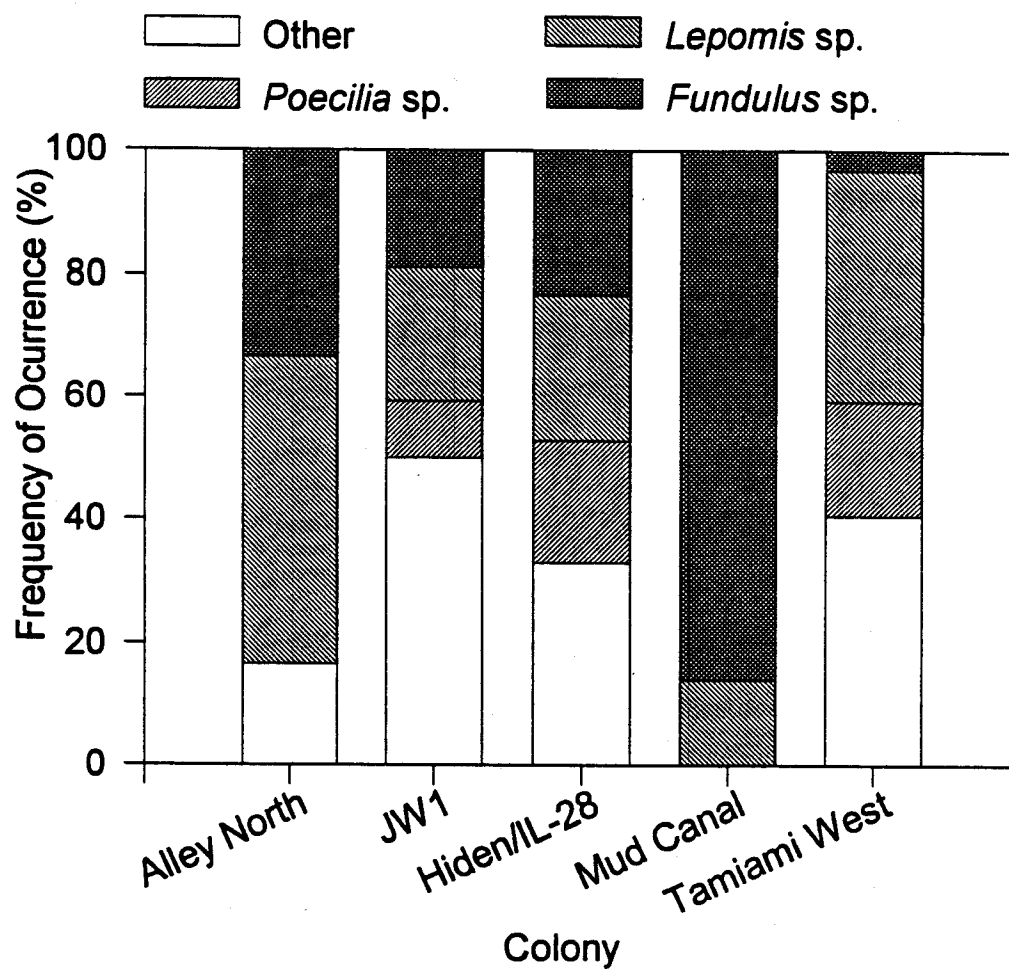


Figure 7.2 Prey composition during 1995, by colony. Prey species shown represent the three most frequent for that year.

each nest were weighed and measured to serve as within-nest controls for the effects of the experimental procedure. Finally, the oldest chicks in 8 nests (Handling Control) were weighed and handled to serve as controls for the mercury/placebo dosing procedure, and were also monitored for survival. Out of a total of 72 chicks, 14 first-hatched chicks either died or were not captured close to the end of the 15 day dosing period. Data from these chicks will be used in growth and food intake analysis. Dead chicks were necropsied.

a. Field Procedures.

First-hatched Survival/food and Survival chicks, depending on treatment, were given either empty gelatin capsules (placebo), or gelatin capsules containing 0.5 mg of methylmercury (dosed) every 2.5 days (average) for 15 days (total dose from us = 3.5 mg methylmercury). We estimate that dosed chicks received mercury (natural dietary mercury plus our dose) at a daily rate of 1.76 mg/kg of food intake, (0.63 mg methylmercury/kg food from natural food items and 1.13 mg methylmercury/kg food from dosing). We arrived at these calculations using food intake data from wild nestlings in last year's field work (see Chapter III), and mercury concentrations in great egret prey fishes in the Water Conservation Areas of the Everglades (W. Loftus and T. Lange, pers. comm.). Using this information, we calculated that dosed birds would receive a total dose that would approximately triple the rate of daily mercury intake that a great egret nestling would have been expected to ingest in 1994 in the Water Conservation Areas.

Blood samples were taken from Survival and Survival/Food chicks during the initial visit in order to determine background mercury concentrations, and were then taken every 5 days until the end of the experiment. In addition, we took growing scapular feathers from chicks in the experiment at the end of the 15 day period. All experimental chicks were fitted with radios attached to blank aluminum leg bands, and U. S. Fish and Wildlife Service aluminum leg bands at day 15, and are being monitored for dispersal patterns and survival for a one-year period.

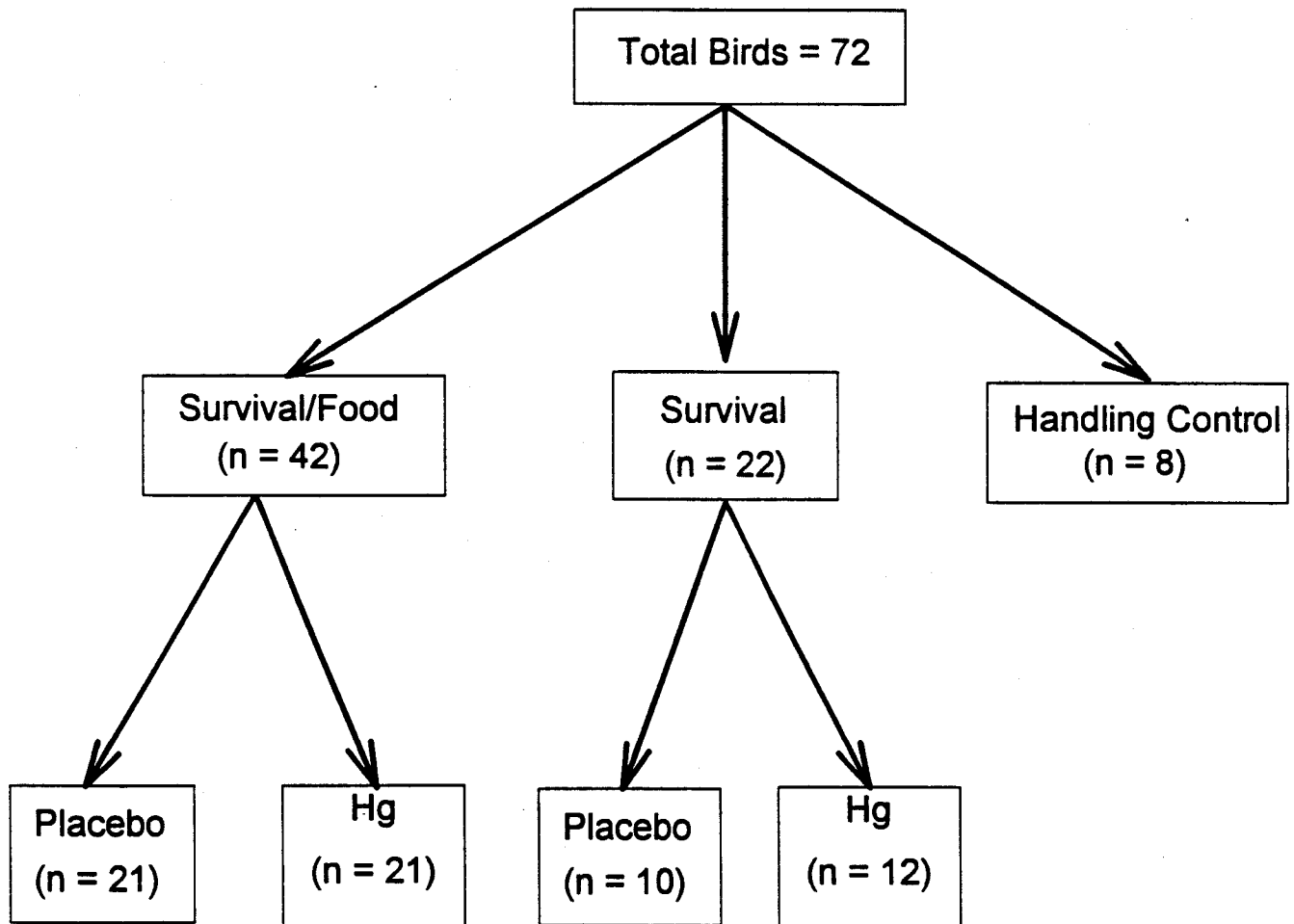


Figure 8.1 Flow diagram of treatments of first-hatched great egret chicks in mercury dosing experiment. Note that the birds were split between two colonies, Tamiami East (n = 32) and Hidden (n = 40), with appropriate distributions of controls and experimentals in each colony.

Table 8.1 Field procedures for chicks in the mercury dosing experiment.

Nest Type	Weight	Culmen	Physical		Capsule	Blood	Growing Feathers
			Exam	Tritium			
Handling Control	Yes	Yes	Yes	No	No	No	Yes*
Survival Placebo	Yes	Yes	Yes	No	Empty*	1 ml*	Yes*
Survival Hg	Yes	Yes	Yes	No	0.5 mg Methyl Hg*	1 ml*	Yes*
Survival/Food Placebo	Yes	Yes	Yes	1 mCi/kg*	Empty*	0.7 + 0.2 ml*	Yes*
Survival/Food Hg	Yes	Yes	Yes	1 mCi/kg*	0.5 mg Methyl Hg*	0.7 + 0.2 ml*	Yes*

* indicates first-hatched chick only.

The radios, which weigh 12 g, are rated to last 2 years.

In 1994, we used deuterium as a marker to measure food intake. As pointed out in Chapter III, we switched from deuterium to tritium in 1995 for three reasons: 1. Tritium is easier to measure than deuterium. 2. Tritium is cheaper than deuterium to measure. 3. The time between resampling intervals is longer for tritium than deuterium (5 days to 3 days), reducing the number of times each chick must be injected and bled.

Chicks which were monitored for food intake (Survival/food) were injected intramuscularly with tritium at the rate of 1 mCi tritiated water/kg body weight every 5 days. We took a 0.7 ml initial blood sample prior to each injection (this blood sample was also used for mercury assays) in order to measure the final concentration of tritium from the previous injection. At one hour post-injection we took another 0.2 ml of blood to measure the initial equilibrated tritium concentration for the next food intake interval. One ml of blood was taken from Survival chicks every five days for mercury assays and immunology work. All blood samples were placed in lithium heparinized vacutainers in the field, and refrigerated upon returning to the lab.

Table 8.1 summarizes the field procedures used in each treatment group. Every five days, all chicks in each experimental nest were weighed with a 300 g, 500 g, or 1000 g Pesola scale, depending on size. Culmen length was measured to the nearest 0.1 cm using a ruler. Chicks were given a brief physical exam for *Eustrongylides ignotus* and *Clinostomum* parasites, as well as any obvious injuries.

b. Laboratory Procedures.

Blood samples to be assayed for mercury content were placed in vials with teflon lids and weighed to the nearest 0.00001 g. One ml of concentrated nitric acid was then added to the blood, and the sample was thoroughly mixed. We shipped blood samples and growing scapular feathers to the DEP chemistry lab for analysis.

We measured blood samples from Survival chicks for packed-cell volume, plasma proteins, and number of white blood cells. Plasma from Survival chicks was also saved for antibody quantification, and we plan to test for differences in antibody response in dosed and placebo birds. This represents yet another potential way to assess sublethal effects of mercury.

Blood from Survival/food chicks was placed in capillary tubes which were flame-sealed and labeled according to date, nest, and sample number. Water from tritium samples will be vacuum-distilled as in Chapter III, and analyzed in triplicate for tritium content. We will use measured tritium concentrations to calculate food intake using the equations listed in Chapter III. Food intake data will be analyzed for differences between treatments and controls, and for differences between colonies.

Results and Discussion

At present, results from mercury assays and tritium work are pending. We performed an analysis of covariance (ANCOVA) to detect possible effects of colony, mercury dosing, and tritium procedures on weight and bill length (culmen) of first-hatched chicks used in the experiment, while controlling for age. Chicks from the Hidden/L28 colony had higher average weights ($F = 19.33$, $P = 0.0001$, Figure 8.2) and greater culmen lengths ($F = 12.84$, $P = 0.0004$) than those in the Tamiami East colony. No differences in weight were found between chicks dosed with mercury and controls ($F = 0.99$, $P = 0.3198$, Figure 8.3). A nearly significant difference in culmen lengths was found between mercury dosed and control chicks ($F = 3.15$, $P = 0.0774$). In comparisons between tritium and non-tritium chicks, tritium chicks had lower weights ($F = 4.31$, $P = 0.0389$), and there was a nearly significant difference in culmen length ($F = 3.39$, $P = 0.0668$). These differences in weight may have been due to the fact that tritium chicks were handled and bled twice as often as the non-tritium chicks. This

• Hidden/L-28 Colony

□ Tamiami East Colony

N = 139, $r = 0.8$, $P < 0.0001$

N = 109, $r = 0.74$, $P < 0.0001$

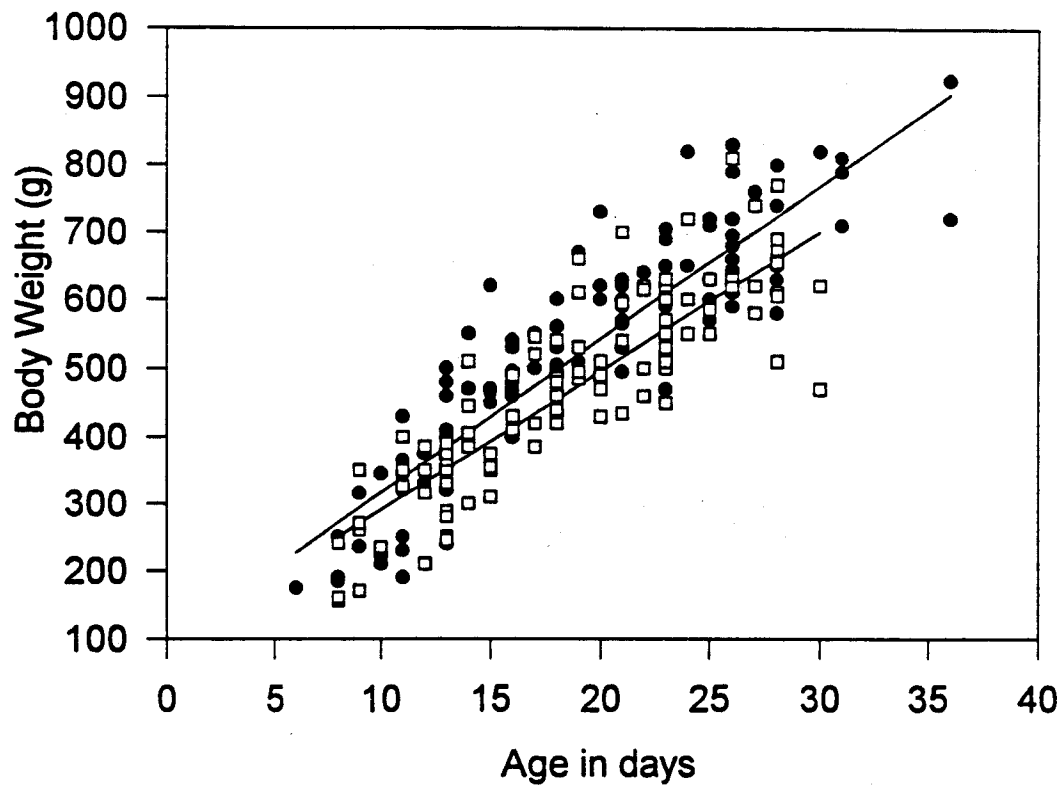


Figure 8.2 Weight of great egrets chicks, by colony in relation to age.

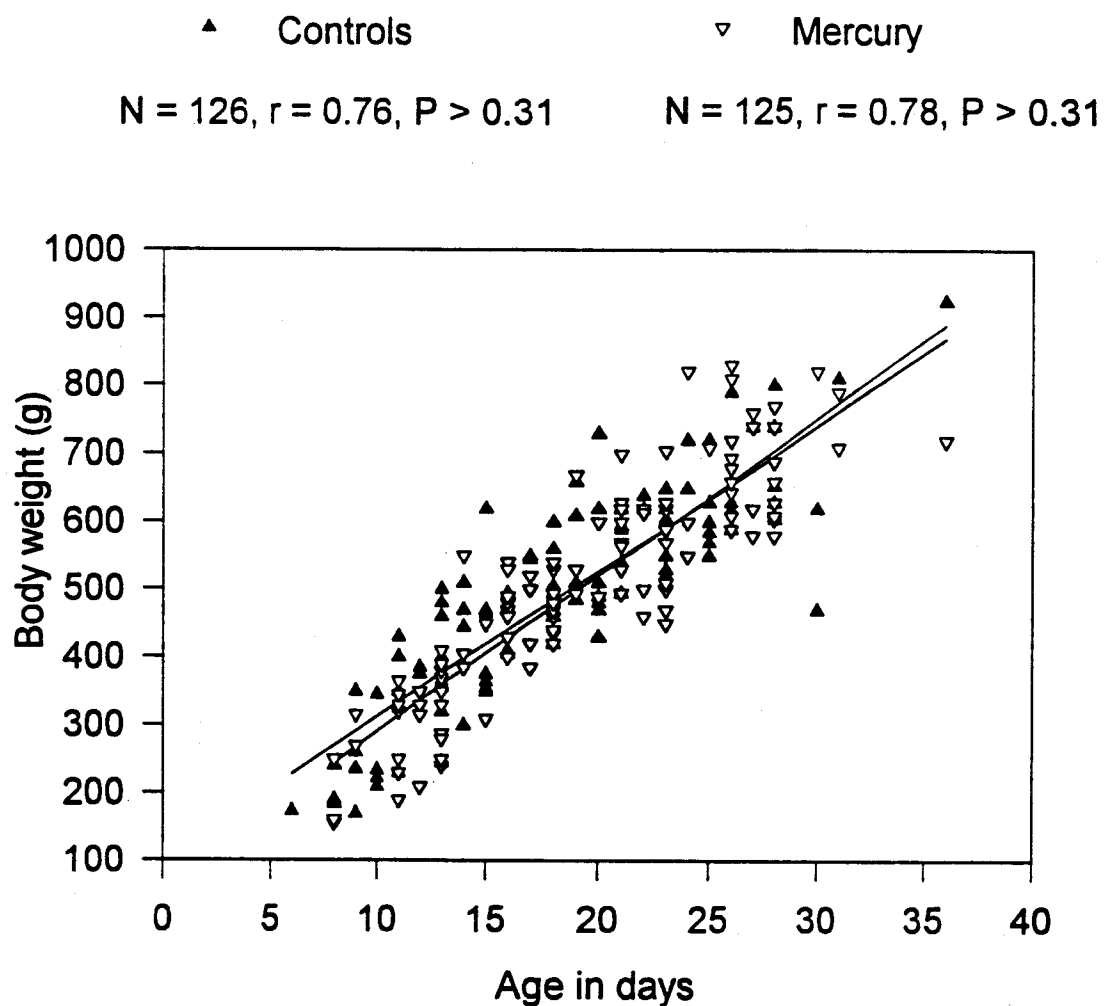


Figure 8.3 Body weight of great egret chicks, by treatment in relation to age.

hypothesis remains untestable at present.

Upon completion of tritium work, and receipt of mercury data, further analysis of the effects of elevated mercury concentrations and colony location will be possible. Telemetry data will also be analyzed as it accumulates.

IX. CONCLUSIONS

The results of our field studies in 1994/95 have provided evidence for the following conclusions:

1. Blood mercury concentrations in great egret chicks increased significantly during the first month of age.

2. Differences in concentrations of mercury in blood and growing feathers were detected between colonies within the Everglades. For both tissues, chicks from JW1 colony had the highest concentration of mercury, followed by Frog City South and L-67. There is no obvious ecological gradient that explains these patterns.

3. High mercury concentrations in blood and growing feathers were associated with health status in wild great egret chicks. Blood parameters and parasitic load were used to define a health criteria that categorized chicks as "sick" or "healthy". High mercury concentrations were associated with poor health status in the entire group of chicks only when sampled from growing feathers (> 25 mg/kg). When great egret chicks were divided into age classes based on bill length, this association was significant only in older nestlings.

4. Great egret juveniles do not remain in the central Everglades during the wet season postfledging period, but tend to emigrate to shallowly flooded agricultural lands to the north and west of the Everglades. Survival rate was estimated as 73% for the first 10.5 months of age, a much higher estimate than shown in another study.

5. Differences were found in the food intake of chicks from different colonies. There was a marginally significant inverse relationship between food intake and mercury in blood, suggesting an effect of mercury on appetite.

6. Preliminary results indicate that mercury concentrations in powder down and growing feathers can be used to assess mercury concentrations in liver, brain, muscle, and kidney of both great egrets and great blue herons. Mercury was found to concentrate unevenly

among different tissues, with highest values in feathers, and lowest in brain. Mercury concentrations in blood, liver, brain, muscle, kidney, powder down, and growing feathers were influenced by age and/or location. In addition, sex did not influence any of the variables studied, mercury concentrations generally increased with age, and were highest in birds from Broward County.

7. Mercury may cause eye pathology and affect vision. We observed changes in the Electroretinogram (ERG), with a decrease in the scotopic amplitude and latency in a single mercury-dosed adult great egret. This response is consistent with previous reports on effects of mercury on vision. This preliminary result is encouraging and warrants a more in depth evaluation of the use of ERGs as a toxicological assessment tool in wildlife.

X. LITERATURE CITED

- Aciand G. M. 1988. Diagnosis and differentiation of retinal disease in small animals by electroretinography. *Seminars in Veterinary Medicine and Surgery (Small Animal)* 3: 15-27.
- Bancroft, G. T., W. Hoffman and R. W. Sawicki. 1992. The importance of the Water Conservation Areas of the Everglades to the endangered Wood Stork (*Mycteria americana*). *Conservation Biology* 6: 392-398.
- Bancroft, G. T. , A. M. Strong, R. J. Sawicki, W. Hoffman, and S. D. Jewell. 1994. Relationships among wading bird foraging patterns, colony locations, and hydrology in the Everglades. In: *Everglades: The ecosystem and its restoration*. S. M. Davis and J. C. Ogden (Eds.). St. Lucie Press, Delray Beach, Florida. pp. 615-658.
- Berson E. L. 1981. Electrical Phenomena in the Retina. In: *Adler's Physiology of the Eye, Clinical Applications*, 7th ed. Robert A Moses (Ed.). C.V.Mosby Company.
- Borg, K. S. 1969. Alkylmercury poisoning in terrestrial Swedish wildlife. *Viltrevy* 6: 301-398.
- Borg, K., K. Erne, E. Hanko, and H. Wanntorp. 1970. Experimental secondary methyl mercury poisoning in the goshawk (*Accipiter g. gentilis* L.). *Environmental Pollution* 1: 91-104.
- Braune, B. M., and D. E. Gaskin. 1987. Mercury levels in Bonaparte's gull (*Larus philadelphia*) during autumn molt in the Quoddy region, New Brunswick, Canada. *Archives of Environmental Contamination and Toxicology* 16: 539-549.
- Burger, J. 1993. Metals in feathers of brown noddy (*Anous stolidus*): Evidence for bioaccumulation or exposure levels?. *Environmental Monitoring Assessment* 24:181-187.
- Burger, J. and M. Gochfeld. 1992. Trace element distribution in growing feathers: Additional excretion in feathers sheaths. *Archives of Environmental Toxicology* 23: 105-108.
- Burger, J., K. Parsons, T. Benson, T. Shukla, D. Rothstein, and M. Gochfeld. 1992. Heavy metal and selenium levels in young cattle egrets from nestling colonies in the

- northeastern United States, Puerto Rico, and Egypt. *Archives of Environmental Contamination and Toxicology* 23: 435-439.
- Dunn, E. H. 1975. Caloric intake of nestling double-crested cormorants. *Auk* 92: 553-565.
- Dustman, E. H., L. F. Stickel, and J. B. Elder. 1972. Mercury in wild animals, Lake St. Clare, 1970. In: *Environmental mercury contamination* (R. Hartung and B. B. Dinman, eds.). Ann Arbor Science Publishers, Ann Arbor. pp. 46-52.
- Fimreite, N. 1974. Mercury contamination of aquatic birds in Northwestern Ontario. *Journal of Wildlife Management* 35: 293-300.
- Fimreite, M. T. and R. N. Karstad. 1971. Effects of dietary methylmercury on red-tailed hawks. *Journal of Wildlife Management* 35: 293-330.
- Finley, M. T. and R. C. Stendell. 1978. Survival and reproductive success of black ducks fed methylmercury. *Environmental Pollution* 16: 51-64.
- Frederick, P. C. and M. W. Collopy. 1989. Nesting success of live ciconiform species in relation to water conditions in the Florida Everglades. *Auk* 106: 625-634.
- Frederick, P. C. and M. G. Spalding. 1994. Factors affecting reproductive success of wading birds (Ciconiiformes) in the Everglades Ecosystem. In: *Everglades: The ecosystem and its restoration*. S. M. Davis and J. C. Ogden (Eds.). St. Lucie Press, Delray Beach, Florida. pp. 659-692.
- Frederick, P. C. 1994. Wading bird nesting success studies in the Water Conservation Areas of the Everglades : 1994. Final annual report to the South Florida Water Management District, West Palm Beach, Florida.
- Heinz, G. H. 1975. Effects of methylmercury on approach and avoidance behavior of mallard ducklings. *Bulletin of Environmental Contamination and Toxicology* 13: 554-564.
- Heinz, G. H. 1979. Methylmercury: reproductive and behavioral effects on three generation of Mallard Ducks. *Journal of Wildlife Management* 43: 394-401.
- Heinz, G. H. and L. M. Locke. 1976. Brain lesions in mallard ducklings from parents fed

- methylmercury. *Avian Diseases* 20: 9-17.
- Hoffman, W., G. T. Brancoft, and R. W. Sawicki. 1991. Wading bird populations and distributions in the Water Conservation Areas of the Everglades, 1984-1988. Report to South Florida Water Management District.
- Kahl, M. P. 1963. Mortality of common egrets and other herons. *The Auk* 80: 295-300.
- Kushlan, J. A., S. A. Vorhees, W. F. Loftus, and P. C. Frohring. 1986. Length, mass and caloric relationships of Everglades animals. *Florida Scientist* 49: 65-79.
- Loftus, W. F., and A. Eklund. 1994. Long-term dynamics of an Everglades small-fish assemblage. In: *Everglades: The ecosystem and its restoration*. S. M. Davis and J. C. Ogden (Eds.). St. Lucie Press, Delray Beach, Florida. pp. 461-484.
- Nagy, K. A. 1989. Doubly-labeled water studies of vertebrate physiological ecology pp. 270-287. In Rundel, P. W., J. Ehleringer, K. Nagy (editors), *Stable Isotopes in Ecological Research*.
- Ogden, J. C., W. B. Robertson, G. E. Davis, and T. W. Schmidt. 1974. Pesticides, polychlorinated biphenyls and heavy metals in upper food chain levels, Everglades National Park and vicinity. National Park Service Report, Department of the Interior.
- Ogden, J. C. 1994. A comparison of wading bird nesting colony dynamics (1931-1946 and 1974-1989) as an indication of ecosystem conditions in the southern Everglades. In: *Everglades: The ecosystem and its restoration*. S. M. Davis and J. C. Ogden (Eds.). St. Lucie Press, Delray Beach, Florida. pp. 533-570.
- Pass, D. A. 1975. The pathology of sub acute and chronic methylmercury poisoning of the mallard duck. *Journal of Comparative Pathology* 85: 7-21.
- Pollock, K. H., S. R. Winterstein, C. M. Bunck, and P. D. Curtis. 1989. Survival analysis in telemetry studies: The staggered entry design. *Journal of Wildlife Management* 53: 7-15.
- Roelke, M. E., D. P. Schultz, C. F. Facemire, S. F. Sundlof, and H. E. Royals. 1991. Mercury

- contamination in Florida Panthers. Report of the Florida Panther Technical Subcommittee to the Florida Panther Interagency Committee, Gainesville, FL.
- SAS Institute Inc. 1988. SAS/STAT Users Guide, Release 6.03 ed. SAS Institute Inc., Cary, North Carolina. 1,028 pp.
- Spann, J. W., R. G. Heath, J. F. Kreithzer, and L. N. Locke. 1972. Ethyl mercury p-toluene sulfonanilide: lethal and reproductive effects on pheasants. *Science* 175: 328-331.
- Spalding, M. G. 1990. Antemortem diagnosis of eustrongylidosis in wading birds (Ciconiiformes). *Colonial Waterbirds* 13: 75-77.
- Spalding, M. G. and D. J. Forrester. 1993. Pathogenesis of *Eustrongylides ignotus* (Nematoda: Dioctophymatoidea) in ciconiiformes. *Journal of Wildlife Diseases* 29: 250-260.
- Spalding, M. G. and D. J. Forrester. 1991. Effects of parasitism and disease on the nesting success of colonial wading birds (Ciconiiformes) in southern Florida. Final Report # NG88-088 to the Nongame Program, Florida Game and Freshwater Fish Commission. 147 pp.
- Spalding, M. G., G. T. Bancroft, and D. J. Forrester. 1993. The epizootiology of eustrongylidosis in wading birds (Ciconiiformes) in Florida. *Journal of Wildlife Diseases* 29: 237-249.
- Spalding, M. G., R. D. Bjork, G. V. N. Powell, and S. F. Sundlof. 1994. Mercury and cause of death in great white herons. *Journal of Wildlife Management* 58: 735-739.
- Spann, J. W., R. G. Heath, J. F. Kreithzer, and L. N. Locke. 1972. Ethyl mercury p-toluene sulfonanilide: lethal and reproductive effects on pheasants. *Science* 175: 328-331.
- Strain G. M., R. M. Jackson, B. L. Tedford. 1990. Visual evoked potentials in the clinically normal dog. *Journal of Veterinary Internal Medicine* 4:222-225.
- Stoneburner, D. L., P. C. Patty, and W. B. Robertson, Jr. 1980. Evidence of heavy metal contamination in Sooty Terns. *The Science of the Total Environment* 14:147-152.
- Sundlof, S. F., M. G. Spalding, J. D. Wentworth, and C. K. Steible. 1994. Mercury in livers of

- wading birds (Ciconiiformes) in southern Florida. *Archives of Environmental Contamination and Toxicology* 27:299-305.
- Van der Molen, E. J., A. A. Blok, and G. J. DeGraaf. 1982. Winter starvation and mercury intoxication in Gray Herons (*Ardea cinerea*) in the Netherlands. *Ardea* 70: 173-184.
- Ware, J. F., H. Royals, and T. Lange. 1990. Mercury contamination in Florida largemouth bass. *Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies* 44: 5-12.
- Wilson, R. P., and M. T. J. Wilson. 1989. A minimal stress bird capture technique. *Journal of Wildlife Management* 53: 77 - 80.
- Wright R. L, R. L. Younger, and J. C. Riner. 1974. Residues of mercury in tissues and eggs of chickens given oral doses of Panogen 15. *Bulletin of Environmental Contamination and Toxicology* 12: 366-372.

