Effects of Environmental Mercury Exposure on Reproduction, Health and

Survival of Wading Birds in the Florida Everglades

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EXECUTIVE SUMMARY

This report documents the results of investigations of the effects of environmental methylmercury on the health, development, survival, and reproduction of long-legged wading birds in the Everglades ecosystem. The project was supported by the Florida Department of Environmental Protection (DEP) and the U.S. Fish and Wildlife Service, and took place between fall 1993 and spring 1997. The research involved both field and lab studies aimed at a variety of questions.

The impetus for this work came from recent findings of high levels of methylmercury in upper food chain organisms in the Everglades, and from the realization that so little was known about this contaminant in wild birds that most predictions as to its effects were virtually insupportable. Studies of other birds and other organisms indicated clearly that a number of strong effects on health, reproduction and survival were possible, but also showed that there were enormous differences in response among species at the same exposure levels. The concern for wading birds arose because this group of animals are, by virtue of their high metabolism and position in the food chain, at perhaps the highest risk of mercury exposure of any organism in the Everglades. Concern for wading birds also derived from the extreme reduction in breeding numbers in the Everglades observed during the past four decades, and the view that increased breeding numbers is a key goal for the restoration of the ecosystem.

The research has occurred in a number of different phases, each with a different purpose. These include sampling necessary to indicate sources of variation in mercury concentrations (geographic, between species, tissue type, annual, etc.), experimental field studies to investigate effects of mercury on appetite, survival and health of chicks, comparisons of mercury levels in feathers of breeding and nonbreeding birds, studies of deposition rates of mercury in various tissues, lab studies of the effects of mercury on vision, and controlled laboratory dosing experiments to elucidate the effects of mercury on post-fledging health, development, and behavior.

The field aspects of the studies were conducted almost entirely within Water Conservation Area (WCA) 3 of the Everglades, where the vast majority of wading bird nesting has occurred in recent decades. Although many species of wading birds commonly breed in the Everglades, we focused studies on the great egret (*Ardea albus*) because of its high potential exposure due to diet, because it is not threatened or endangered, and because its breeding ecology in the Everglades is particularly well understood.

Through analysis of 187 great egret and 51 great blue heron (*Ardea herodias*) carcasses of various ages, and from captive great egrets dosed with known quantities of mercury, we found that mercury concentrations are generally highest in growing feathers, powderdown, breeding plumes, and nails, with intermediate values in liver, muscle, heart and kidney. Lowest concentrations were found in brain tissue. Strong correlations between liver, feather, and blood tissue concentrations suggest that blood and especially feathers may be used to non-destructively assay mercury burdens in wading birds. We noted that mercury concentrations among tissues are to some degree dynamically interdependent, with the availability of tissues of high mercury affinity (such as growing feather) affecting concentrations in tissues of lower mercury affinity (such as blood) .

We found significant differences in the blood and feather mercury concentrations in great egret chicks among colonies within WCA 3, with samples from the JW1 colony in north-central WCA 3 consistently showing the highest mercury values. The distribution among colonies seemed to match the geographic distribution of mercury concentrations in mosquitofish (*Gambusia holbrooki*) reported by the EPA EMAP project.

Mercury concentrations increased significantly with age, both when age groups (nestling, juvenile, adult) are compared, and when nestlings of different ages are compared. Wading birds have asynchronous hatching within clutches, but there was no significant effect of hatch order on mercury concentrations of blood or feather concentrations in chicks. This work indicated that, depending on the comparison desired, the effects of age, location, and tissue sampled must be taken into account when assaying for mercury concentrations in wading birds.

We compared mercury concentrations in great egrets between the two years of field study by statistically standardizing the data for nestling age, hatch order, and colony effects. When standardized in this fashion, we found feather samples from 1994 were significantly higher in mercury than were those from 1995. This pattern followed estimated differences in mercury through food between the two years.

During 1994, we measured food consumption, health, and survival of great egret chicks, and examined the influence of mercury concentrations in blood and feathers at approximately 28 days of age, on these variables. We found food consumption decreased significantly with increased feather mercury, though only weak effects were found on health parameters, and no effects on survival through the first 7.5 months of life.

During 1995, we carried out a field dosing trial in which 72 first-hatching wild nestling great egrets were repeatedly dosed in their nests with either placebos or with gelatin capsules containing methylmercury chloride (MeHgCl) between 10 and 25 days of age. We estimated that placebo chicks received 0.6 mg/kg mercury in their diets naturally, and that dosed birds received an additional 1.2 mg/kg, for a total dose of 1.8 mg/kg for the dosed chicks. The birds were fed naturally by their parents in the colonies, and underwent all of the normal stresses associated with life in the wild. We monitored their growth, food consumption, health, and post-fledgling survival of all birds. We included control groups in this study that assayed for the effects of the dosing procedure itself, and for any effects of the labeled water technique used to measure food consumption.

We found no effects of either procedure on the growth of chicks. We found a statistically significant decrease in food consumption of dosed birds by comparison with placebos, which, when averaged over the period of study, amounted to a reduction of approximately 4% of body mass. There was no effect of mercury dose on mass or bone growth of the chicks, perhaps because the study was done in a year when food was exceptionally abundant. There were no differences in health or survival of dosed and placebo birds that were followed using radio tags for the first 8 months of life.

The apparent lack of an effect of mercury in these experiments may well have been an artifact of the timing of the dosing (and in 1994, the timing of the assay of mercury body burden), which was during a small portion of the period leading up to independence of the chicks. During this time, feather growth is extremely rapid, and we hypothesized that the effect of apparent body burdens during this time was misleading because much of the mercury ingested is rapidly sequestered into feather tissue, where it was not toxic. Our field dosing was limited to the period

prior to about the fourth week of life simply because the chicks became too mobile to catch after that point. Thus, we probably dosed birds during a time when they were best prepared to depurate the mercury.

We attempted to dose adult great egrets (two dosed, two controls) in captivity during winter 1995, using implanted osmotic pumps that delivered the daily equivalent of up to 5 mg/kg methylmercury in the diet. The vision of all four birds was tested using electroretinography, prior to dosing. Three of the four birds died during a severe cold event, and the single remaining bird (dosed) also underwent electroretinography following the end of the dosing period. The dosed bird showed a reduction in amplitude of scotopic a-waves, and increased scotopic latencies, in comparison with measurements prior to dosing. Histologically, the eyes of both dosed birds had corneal edema, and increased vacuolization of the ciliary body epithelium. These effects are consistent with studies of mercury's effect on vision in mammals.

We investigated the potential of mercury contamination to affect successful breeding in great blue herons by comparing mercury values from shed breeding plumes at nest sites, with feathers taken from birds in the same area and season that had no evidence of breeding plumage or gonadal enlargement. Generally, successfully breeding birds had higher feather mercury concentrations than nonbreeding birds. The interpretation of this result is unclear. We also found that many feather samples taken from birds in south Florida during the January - June period had much higher mercury concentrations than did those collected at other times of the year. These patterns offer no evidence to suggest that mercury affects reproduction, but because of the potential for confounding results, our findings also do little to disprove the hypothesis.

During 1996, we reared three groups of great egret chicks from hatching to 14 weeks of age on diets containing 0, 0.5, and 5 mg/kg methylmercury, wet weight. We monitored growth, food consumption, survival, mercury concentrations in blood and feathers, an array of health measures, and basic maintenance, behavior and motor abilities.

All birds on the high dose eventually became sick enough to warrant euthanasia between 10 and 12 weeks of age, and displayed many of the classic symptoms of methylmercury poisoning prior to euthanasia. The timing of symptoms, as well as dramatic increases in blood mercury, coincided with the cessation of feather growth, which supported the hypothesis that rapid feather growth enables nestling birds to rid themselves of significant amounts of mercury. In the field, these effects would be manifested at a time when young birds are first exposed to the rigors of natal dispersal. The cessation of growth (fledging period), and not the preceding period of rapid growth, therefore appears to be the time when the effects of mercury contamination should be most pronounced in great egret young.

We found significant effects of methylmercury on amount of food eaten, weight gain, and packed-cell volume (decreases in all three) when all three dose groups were compared. When the control and low-dose groups were compared, we found the same effects.

Differences were also found in biochemistry and enzyme activity in blood, liver, kidney, and brain samples of high dose and placebo groups, and in blood of low dose and placebo groups. The patterns of biochemical and enzymatic changes are similar to a profile typical of mercury toxicosis found in other avian species.

When low dose and placebo groups were compared, we found significant differences in perching location, stance, and activity, with behaviors requiring less energy expenditure more often pursued by the dosed groups than placebos. Dosed birds also spent less time in the sun

than did controls. In feeding trials, low dose birds were significantly less likely to eat fish than were controls, but, when motivated to hunt, displayed similar strike efficiencies and time required to capture fish.

We found whole-body mercury concentrations in fish regurgitated by young birds ranged from 0.035 - 1.4 mg/kg ww, and found significant relationships between body mass and mercury concentration for most fish species examined. The birds' food habits were entirely piscivorous, though marked shifts in species composition of the diet were evident among years. Using fish species-specific mercury concentrations and year specific diet data, we estimate mean annual mercury in the diet of great egret chicks at 0.33-0.43 mg/kg fish (mean across years = 0.41 mg/kg).

The cumulative effects of mercury on juvenile and post-fledging birds at levels of mercury intake common in the Everglades (0.33 - 0.75 mg/kg), are likely to result in reduced survival of offspring through reduced body mass, decreased red cell numbers, increased lethargy, increased susceptibility to disease, and increased time to capture fish. These effects are likely to occur during the fledging and post-fledging period, when feather growth has slowed, and feathers no longer absorb mercury taken in through the diet. We hypothesize that the effects of mercury measured under controlled lab conditions may actually be more acute in wild fledgings, since this is an especially stressful time for the young birds. At this time they are dispersing from natal colonies, are faced with learning to forage on their own, and are encountering risks of predation and exposure to disease. We are unable to estimate the magnitude of the effect of mercury on survival from our data.

The effects of mercury on great egrets and great blue herons are difficult to extend to other wading bird species except in a qualitative fashion. The literature indicates large differences among avian species in susceptibility to mercury, and we caution that the effects on species that have evolved at or near the top of the food chain may not be applicable to those at lower trophic levels.

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CHAPTER I. REVIEW OF THE POTENTIAL EFFECTS OF MERCURY, AND AN OVERVIEW OF THIS PROJECT

INTRODUCTION

The rapid expansion of Florida's human population during the past century has created increased demands for land, water, and other resources, placing visible stress on many of the state's ecosystems (Ewel 1990). As the most renowned ecosystem in Florida, and possibly the most famous wetland in the world, the Everglades is an important example of this process. Now crisscrossed by one of the world's largest water control systems, it is currently reduced to half its original area (Light and Dineen 1994). Habitat loss, inappropriate water management and loss of estuarine productivity, have contributed to the precipitous decline of breeding populations of ciconiiform wading birds throughout the wetland, leaving populations of some species on the verge of complete collapse (Ogden 1994, Davis 1994).

Contamination with mercury may also play a role in the continued decline of breeding by wading birds Everglades. In recent years, high concentrations of mercury have been found in several taxa of Everglades animals, including fish (Ware et al. 1990), Florida panthers (*Felis concolor coryi*) (Roelke et al. 1991, Facemire et al. 1995) and wading birds (Sundlof et al. 1994, Beyer et al. 1997). These findings have led to studies of the location of mercury hotspots within the Everglades, factors affecting mercury deposition, and mechanisms of mercury concentration in Everglades food webs. Despite this recent emphasis on studying the levels, distribution and ecology of mercury, an understanding of the actual effect of contamination on wild populations of vertebrate species exposed to mercury is almost completely lacking.

Wading birds are largely piscivorous and their position at the top of the food chain makes them particularly vulnerable to mercury accumulation. Much of their nesting effort is concentrated in the Water Conservation Areas (WCAs) of the central Everglades (Ogden 1994, Frederick 1995) where mercury concentrations in fish (Ware et al. 1990) fall within the range that appear to produce negative effects in piscivorous bird (Barr 1986, Eisler 1987). Since subadult animals are often disproportionately sensitive to mercury (Eisler 1987, Fitzgerald and Clarkson 1991), these contamination levels have the potential to affect chicks, fledglings and juveniles even more than adults. An understanding of the detrimental effects of mercury on wading bird reproduction in the Everglades therefore seems critical to the effective conservation of these birds. The purpose of this study is to investigate the potential effects of environmental mercury on wading bird reproduction and survival, especially as it pertains to the Everglades ecosystem. The following sections of the Introduction review basic information on mercury and its behavior in ecosystems, studies of the effects and mode of action of mercury in birds, and the potential role of mercury in the ecology of the Everglades and in the control of wading bird demographics. An overview of the organization and justification of each of the parts of this study are given at the end of this chapter, followed by brief descriptions of the study site, hydrological and weather conditions, and nesting responses during the study period.

Forms, cycling and toxicity of mercury

Mercury is a naturally occurring element that naturally enters the earth's atmosphere

primarily through evaporation from the surface of the earth and ocean. Mining of mercury dates back more than 2,000 years (Eisler 1987, Fitzgerald and Clarkson 1991) and anthropogenic emissions result from burning fossil fuels, incineration of waste, fungicidal seed dressings, chloralkali manufacturing, processing wood pulp, gold mining, manufacturing of plastics, pharmaceuticals, mercury arc-lamps, neon and fluorescent lamps, batteries, switches, thermometers, and paints (Eisler 1987). Such extensive human use of this element has led to elevated environmental levels so that an estimated 60 % of the global atmospheric burden of mercury comes directly and indirectly from anthropogenic sources (Fitzgerald and Clarkson 1991), and approximately 6,000 tons of mercury are released into the environment each year (Expert Panel, 1994).

Mercury serves no known biological function in animals (NAS 1978, Nims 1987) and typically produces negative effects (Fitzgerald and Clarkson 1991). Its chemical properties allow for biomagnification so that upper trophic level consumers are especially likely to accumulate enough mercury to produce negative effects on health (Eisler 1987, Zillioux et al. 1993). Although mercury can be detected in almost any ecosystem, it is often found at above-background levels in wetlands (Zillioux et al. 1993, Expert Panel 1994). Wetlands tend to accumulate more of the element than terrestrial ecosystems due in part to elevation and soil characteristics (Eisler 1987). Piscivorous birds, such as wading birds, are especially vulnerable to mercury not only because they feed at high trophic levels, but also because they often forage in relatively mercury-rich wetlands (Jurczyk 1993).

Forms of mercury

Mercury (Hg) occurs naturally in the air, water, and soil in a variety of different chemical forms from elemental mercury to complex organic molecules (Eisler 1987). It can be classified into three oxidation states: elemental (Hg°), mercurous [Hg (I)], and mercuric [Hg (II)]. Elemental mercury is the primary form of atmospheric mercury and because of its chemical and physicochemical properties, it is transported worldwide. Mercury also forms such organic compounds as phenyl, methoxy and alkyl mercury, however, methylmercury (CH_3Hg^+) is by far the most toxic of these compounds.

Methylation of mercury greatly increases its bioavailability, and as a result, methylmercury often accounts for a large percentage of the mercury in animal tissue, particularly at higher trophic levels (Scheuhammer 1987a). Methylmercury is highly stable, lipid soluble, and exhibits ionic properties that permit penetration of biological membranes (Eisler 1987). In addition, methylmercury is much more soluble in water than many inorganic forms, making it one of the most mobile forms of mercury in an aquatic environment.

Methylation/Demethylation of mercury

Methylation of mercury can occur through both biotic and abiotic processes (Eisler 1987). Almost any inorganic form of mercury entering an aquatic system can be converted into methylmercury (Jernelov 1969). The specific forms of mercury present in a wetland system are influenced by factors such as pH, redox potential, and Dissolved Organic Carbon (Scheuhammer 1991, Gambrell 1994).

In aquatic systems, the principal source of methylmercury is methylation of inorganic

mercury by bacteria and molds that are sulfate-reducing and/or methanogenic. Methylation activity of these organisms increases under anaerobic, eutrophic and acidic conditions (Compeau and Bartha 1985, Scheuhammer 1991). Under anaerobic conditions in freshwater wetlands, methylmercury may also be demethylated back to inorganic forms by the same bacteria involved in methylation. Abiotic methylation may also produce significant amounts of methylmercury in some wetlands (Lee et al. 1985).

Biomagnification and bioaccumulation

Methylmercury tends to biomagnify as it moves to higher trophic levels (Gardner et al. 1978, Hoffman and Curnow 1979), and it also bioaccumulates over time (Eisler 1987). For example, in a contaminated coastal salt marsh in Georgia, Gardner et al. (1978) found that inorganic mercury accumulated in sediments, plants and organisms at increasing concentrations. Mercury in sediments averaged 0.63 mg/kg, principally in the inorganic form. Fish averaged 1.5 mg/kg mercury, almost all of which was methylated. Therefore, mercury in the system not only biomagnified in the food web, but was also readily transformed into methylmercury, the most toxic and bioavailable form. In this system, upper trophic level consumers, such as piscivorous birds, were most likely to acquire a large mercury burden (total amount of mercury within their bodies). Further, the most toxic form of the element, methylmercury, would comprise the majority of mercury accumulated.

A regional study of mercury accumulation and biomagnification assayed tissue mercury concentrations of Belgian birds which had been found dead, and related the concentrations to trophic position and primary habitat (Delbeke et al. 1984). The highest mercury concentrations were associated with piscivorous birds. Mercury tissue concentrations in birds increased along a trophic dietary gradient: invertebrates < zooplankton < garbage < fish. For terrestrial birds, mercury concentrations increased in consumers of plants, invertebrates, mammals, and birds, respectively. On average, aquatic birds had higher mercury concentrations than terrestrial birds. Although Delbeke et al. (1984) provided only correlative information, their evidence suggests both biomagnification of mercury, and an affinity of mercury for aquatic habitats.

Mercury also bioaccumulates in birds over time. Stickel et al. (1977), fed captive mallard (*Anas platyrhynchos*) drakes methylmercury at a dietary level of 8 mg/kg (ww/dw not stated) for two weeks and then assayed them for mercury concentrations over a period of approximately four months. At the end of the dosing period, a portion of the birds were sacrificed. Mercury concentrations in their livers and kidneys both exceeded 16 mg/kg, more than twice the dietary concentration. After the dosing period ended, mercury was slow to be eliminated from the birds, with no measurable loss occurring between day 7 and day 56. In fact, approximately half of the mercury still remained at day 84. Generally, the biological half-life (the amount of time it takes for half of the mercury within an organism to be excreted) of methylmercury in birds is two to three months (Scheuhammer 1987a).

Pharmacodynamics and mechanistic effects of methylmercury

Although methylmercury has a high rate of absorption through all routes, up to 95% of ingested methylmercury is absorbed through the gastrointestinal. Once absorbed, methylmercury

is transported throughout the body, primarily in the red blood cells, and concentrates in liver, kidney, muscle, and brain (revised by Berlin 1986). In rats and guinea pigs, there is evidence that methylmercury is slowly biotransformed in the organism to inorganic mercury by cleavage of the carbon-mercury bond (Komsta-Szumska et al. 1983). Demethylation is suspected in some long-lived seabirds (Thompson and Furness 1989).

Methylmercury is slow to be excreted. Although some organic mercury is excreted via feces, urine, and hair, this process is very inefficient compared to inorganic mercury (Berlin 1986, Scheuhammer 1987a). An alternative mode of elimination of methylmercury in birds is through the feathers. Up to 90% of the total body burden of mercury is often contained in the feathers (Braune and Gaskin 1987) and subsequently can be lost during molts.

The effects of methylmercury may be due to several alterations at the cellular, systemic, and organismal levels. Methylmercury has a strong affinity for sulfhydryl groups of many proteins which inhibits cell division and causes mutations (Berlin 1986). Although methylmercury can cause damage to organs such as the liver and kidneys (Bhatnager et al. 1982, Nicholson and Osborn 1983), the most serious consequences of methylmercury poisoning are its effects on the central nervous system (Fitzgerald and Clarkson 1991). Here, methylmercury inhibits normal maturation of fetal neurons (Choi et al. 1978) and causes death of neurons (Chang and Hartmann 1972). Pathological effects in the nervous system that are associated with methylmercury intoxication in birds include demyelination and necrosis of the central and peripheral nervous system (Borg et al. 1970, Pass et al. 1975, Heinz 1976, Heinz and Locke 1976). In the kidney, mercury causes necrosis of the proximal tubular cells (Ware et al. 1975). Histologic lesions in the nervous, renal, and hepatic systems have been observed after mercury dosing in different species of captive birds (Tejning 1967, Borg et al. 1969, Borg et al. 1970, Fimreite and Karstad 1971, Pass 1973, Pass 1975, Pass et al. 1975, Heinz 1976, Heinz and Locke, 1976, Finley and Stendell 1978, Bhatnager et al. 1982, Nicholson and Osborn 1984).

Effects of mercury in birds

The effects of mercury in birds can be classified as either acute/chronic, or sublethal. Acute/chronic intoxication with mercury may be exhibited through any number of a wide range of symptoms. These include reduced food intake leading to weight loss, progressive weakness in wings and legs with loss of coordination, fluffed feathers, lethargy, eyelid drooping, difficulty in flying, walking, and standing, paralysis, convulsions, teratogenesis and death (Tejning 1967, Borg et al. 1970, Fimreite and Karstad 1971, Gardiner 1972, Pass et al. 1975, Hoffman and Moore 1979, Bhatnager et al. 1982, Eisler 1987, Scheuhammer 1988, see Table 1.1).

While acute/chronic effects tend to prevent the bird from functioning normally and often lead to death, the sublethal effects of mercury can be difficult to detect. This is especially true in free-ranging birds in which the less obvious sublethal effects of intoxication may go unnoticed unless specifically targeted for investigation. In addition, behavioral changes due to mercury poisoning have been described in mallard ducklings (*Anas platyrhynchs*) from parents fed methylmercury at a rate of 0.5 mg/kg dry weight (dw) (Heinz 1979). These birds were less responsive than controls to tape-recorded maternal calls, and were hyper-responsive to stimuli in avoidance tests (Heinz 1979). Detour learning behavior was impaired in domestic chicks (*Gallus gallus*) hatched from eggs injected with 0.5 mg methylmercury/kg egg (Rosenthal and Sparber 1972). Adult pigeons (*Columba livia*) began to exhibit behavioral changes (ataxia and reduced

operant discrimination) when blood concentrations of mercury reached 13 mg/kg (Laties and Evans 1980). Documented sublethal effects of mercury include decreased growth rates, slowed development, inhibition of reproduction, alteration of blood and tissue chemistry, and abnormal behavior (Heinz 1975, 1979, Barr 1986, Eisler 1987, Scheuhammer 1987a). There is also evidence that mercury may increase susceptibility to disease (Ensor et al. 1992, Spalding et al. 1994).

Factors Affecting Concentration and Toxicity of Mercury in Birds

Dietary methylmercury is lethal to some bird species at concentrations as low as 5.0 mg/kg (Scheuhammer 1988), while other species show sublethal effects of methylmercury up to 10 mg/kg (Fimreite 1971), or even 20 mg/kg (wet or dry weight not specified) when also treated with sodium selenite in the presence of high doses of selenium (Stoewsand et al. 1974, Table 1.1). The level of sensitivity to mercury may depend on a combination of factors including: the form of mercury ingested, species of bird, and the presence of other metals present (Van der Molen et al. 1982, Barr 1986, Eisler 1987). In addition, mercury concentration in bird tissues may vary between individuals, within and between species, between sexes, ages, localities, and as a function of physical condition. The type of tissue sampled, as well as the season (Osborn 1979, Stewart et al. 1994) during which those samples were collected could also influence total concentrations of mercury.

Factors Affecting Concentration of Mercury

Species: Differences in mercury concentrations between species can generally be attributed to dietary differences, with carnivorous or piscivorous birds exhibiting higher mercury concentrations than omnivorous or herbivorous species (Fimreite 1974, Stendell et al. 1976, Hoffman and Curnow 1979, Delbeke et al. 1984, Braune 1987, Lee et al. 1989, Ensor et al. 1992, Becker et al. 1994, Sundlof et al. 1994). Intestinal absorption rates of methylmercury are also known to vary among species of birds. Serafin (1994) compared the intestinal absorption of methylmercury in five avian species (bobwhites, *Clonus virginianus*; Eastern screech owls, *Otus asio*; American kestrels, *Falco americanus*; black-crowned night herons, *Nycticorax nycticorax*; and mallards, *Anas platyrhynchos*), and concluded that intestinal uptake of methylmercury was lowest in both wetland species (black-crowned night herons and mallards).

Sex: The concentration of mercury in tissues of female birds may be lower than in males similarly exposed, because of the ability of females to eliminate mercury through the production of eggs (Bäckstrom 1969, Hoffman and Curnow 1979, Barr 1986, Braune and Gaskin 1987, Burger 1994, Stewart et al. 1994). Some authors, however, have failed to find differences in mercury concentrations between female and male birds (Van der Molen et al. 1982, Lindberg and Odsjö 1983, Norheim 1987, Scheuhammer 1988, Furness et al. 1990, Honda et al. 1990, Thompson et al. 1991, Burger and Gochfeld 1992, Ensor et al. 1992).

Age: Differences in mercury contamination due to age may be related to differences in intestinal absorption or in toxicokinetics, as well as to differences in length and intensity of exposure (Burger 1993b). Several studies have shown that embryos and young birds have lower mercury concentrations than adults (Fimreite 1974, Hoffman and Curnow 1979, Frank et al. 1983, Lindberg and Odsjö 1983, Honda et al. 1985, 1986, Wiemeyer et al. 1989, Burger 1991, Burger

and Gochfeld 1993, Burger et al. 1992b, 1994a, b, Anthony et al. 1993, Bowerman 1993, Evans 1993, Sundlof et al. 1994). This is not universally accepted, however, since several studies have failed to establish differences in mercury concentrations among birds of different age groups (juvenile to adults) (Van der Molen et al. 1982, Norheim 1987, Custer and Mitchell 1989, Furness et al. 1990, Thompson et al. 1991, Burger and Gochfeld 1993, Burger et al. 1994a).

Physiological condition: In common loons (*Gavia immer*), mercury in tissues was higher in emaciated birds than in healthy birds (Frank et al. 1983, Ensor et al. 1992). Also, livers from emaciated wading birds in southern Florida contained an average of two to three times the concentration of mercury than found in livers from birds in good nutritional condition (Sundlof et al. 1994). These results contrast with those from Van der Molen et al. (1982), Norheim (1987) and Spalding et al. (1994), which found no significant differences in mercury concentration between birds in different nutritional condition.

Locality: Differences in mercury accumulation in birds from different locations could be related to differences in intensity of mercury exposure. This could result from differences in foraging location and type and/or size of preferred prey (Burger and Gochfeld 1991). For instance, in southern Florida, nestling wading birds collected from the central Everglades and eastern Florida Bay areas had significantly greater concentrations of mercury in livers than did nestlings from the northern Everglades and western Florida Bay (Sundlof et al. 1994).

Tissue sampled: Several studies have shown that mercury concentrates unevenly within the bodies of birds. Feathers constitute a major excretory pathway for mercury in birds, and thus the highest concentrations have generally been reported from this tissue (Tejning 1967, Osborn 1979, Honda et al. 1986, Braune and Gaskin 1987, Lee et al. 1989, Lewis and Furness 1991, Burger et al. 1992a). High mercury concentrations are also found in liver, kidney, and muscle with lower concentrations in brain tissue (Hesse et al. 1975, Finley and Stendell 1978, Osborn 1979, Nicholson 1980, Delbeke et al. 1984, Barr 1986, Honda et al. 1986, Evans 1993).

Season: Season can affect the physiological state (e.g. amount of fat, breeding, molting stage) of birds, as well as their diet. In starlings (*Sturnus vulgaris*), seasonal variations in the concentration of hepatic mercury were attributed to variations in the amount of liver (Osborn 1979). This author concluded that, because both metal and protein metabolism undergo seasonal fluctuations, the season in which the sample is taken may influence the amount of metal present in tissues and the site at which the metal is bound. In common guillemots (*Uria aalge*), a decrease in mercury tissue concentrations during the breeding season was associated with post-nuptial molt and egg laying (Stewart et al. 1994). Seasonal changes in tissue mercury concentrations have also been related to intra-annual changes in diet (Osborn 1979, Leonzio et al. 1986, Stewart et al. 1994).

Total body burden of mercury is also influenced by the time of exposure in relation to seasonal molt. Several authors have shown that mercury concentrations in soft tissues decreases as molting progresses due to an increase in mercury deposition in growing feathers (Stickel et al. 1977, Osborn 1979, Lindberg and Odsjö 1983, Goede 1985, Furness et al. 1986, Honda et al. 1986, Braune and Gaskin 1987). In addition, feather mercury concentrations are known to correlate with their relative position in a given molt, with highest mercury concentrations found

in first-molted feathers (Furness et al. 1986, Honda et al. 1986).

Factors Affecting Toxicity

Chemical form of mercury: The chemical form of mercury is one of the most important factors affecting its toxicity in animals. As stated earlier, inorganic mercury is relatively less toxic than other forms. Compared to inorganic mercury, methylmercury is more readily and has a slower excretion rate (Norseth and Clarkson 1971). Thompson and Furness (1989) found that a significantly lower proportion of total liver mercury was methylmercury in birds with higher mercury concentrations when compared to those with lower concentrations. This suggests that demethylation occurs in these birds.

Species: Experimental and field studies have shown that, even though the clinical symptomatology of methylmercury poisoning is similar for many birds, species differences exist with regard to sensitivity (Gardiner 1972, Scheuhammer 1987a). It has been postulated that these differences may be related to the ability of some species of birds to transform methylmercury into the less toxic inorganic form (Norheim et al. 1982, Thompson and Furness 1989). This phenomena is probably more prevalent in fish-eating birds, because these species are likely to be adapted to the higher concentrations of mercury present in freshwater and marine ecosystems (Smith and Armstrong 1978). This suggests that the dose levels associated with methylmercury poisoning in terrestrial birds may not be applicable to piscivorous birds.

Other metals: The presence of other metals may also influence the toxic effects of methylmercury. Several studies have demonstrated that selenium protects against the toxic effects of methylmercury in aquatic invertebrates, fish, mammals, and birds (Potter and Matrone 1974, Stoewsand et al. 1974, Chang et al. 1977, El-Begearmi et al. 1977, Heisinger et al. 1979, Cuvin-Aralar and Furness 1991). In one study, japanese quail (*Coturnix japonica*) which were fed a diet containing 20 mg/kg methylmercury suffered 90% mortality. However, the mortality rate of another group fed 20 mg/kg methylmercury, along with 5 mg/kg selenium, did not differ from controls fed a metal-free diet (Stoewsand et al. 1974, Table 1.1).

Possible mechanisms for the protective effect of selenium against mercury toxicity include: 1) formation of stable, non-toxic mercury-selenium complexes which prevent the association of mercury with the sulfhydryl groups of protein; 2) redistribution of mercury from sensitive organs (like kidney) to less sensitive ones (like muscle); 3) competition for binding sites; 4) increased conversion of methylmercury to inorganic mercury; and 5) prevention of oxidative damage by mercury (Cuvin-Aralar and Furness 1991).

In marine mammals and humans, concentrations of selenium and mercury are closely related (1:1 molar ratio) (Koeman et al. 1975, Eisler 1985). In birds, however, the molar ratio of Hg:Se varies from less than one in some marine birds (Hutton 1981), to one or greater in herons (Van der Molen et al. 1982). These variable molar ratios may be partially explained by the fact that detoxification processes are not brought into play unless a critical concentration of mercury in the liver of these birds is reached (Leonzio et al. 1986).

Effects of mercury on reproductive parameters in birds

Methylmercury is likely to affect populations of free-ranging birds through an increase in

mortality and/or a decrease in reproductive success, as measured by parameters such as fecundity, hatching and fledgling rate, or juvenile survival. Dietary methylmercury can affect reproductive success of birds at concentrations that are lower than those required to produce overt toxicity in adult birds of the same species (Heinz 1976, Heinz and Locke 1976, Finley and Stendell 1978, Finley et al. 1979, Barr 1986, Scheuhammer 1987a, 1988, 1991).

Mercury can affect reproduction both through decreases in parental reproductive effort and success, and decreases in survival of subadult birds. The relationship between mercury concentration and effects on reproductive success of various bird species has generally been studied through the sampling of eggs, feathers, and livers. For the most part, mercury concentrations of over 1.0 mg/kg wet weight (ww) in eggs and of over 20 mg/kg ww in feathers may pose a significant threat to the reproductive success of piscivorous birds (Fimreite 1974, Heinz 1979, Barr 1986, Scheuhammer 1991). The greatest variability, however, comes from comparing the effects of mercury on reproductive success of birds with different hepatic mercury concentrations. Mercury concentrations of about 2 - 12 mg/kg ww in livers of adult breeding pheasants and mallard ducks have been associated with decreased hatchability and increased embryo mortality (much lower concentrations than those associated with mortality: 30 - 130 mg/kg mercury ww) (Fimreite 1971, Heinz 1976). Similarly, liver mercury concentrations between 1 and 2 mg/kg ww in nestlings mallard ducks, have been associated with behavioral changes, while higher liver mercury concentrations (11.3 mg/kg ww) have been associated with an increase in embryo/duckling mortality (Heinz 1974, 1975, 1976, 1979). Mercury concentrations of 22.2 mg/kg ww in livers of adult American black ducks (*Anas rubripes*) have also been related to reduced hatchability, high embryo and duckling mortality, and, in addition, brain lesions (Finley and Stendell 1978). In wild bird populations, Fimreite (1974) reported reduced hatching and fledging of common terns (*Sterna hirundo*) with an average hepatic mercury concentration of 27.5 mg/kg ww, and reduced hatching in common loons (*Gavia immer*) that had an average liver mercury concentration of 51.9 mg/kg ww.

Effects of mercury on fecundity and hatching rate

In the laboratory, Spann et al. (1972) reported 50 - 80% reduction in egg production of ring-necked pheasants (*Phasianus colchicus*) fed ethyl mercury p-toluene sulfonanilide (the active ingredient in the fungicide Ceresan M) at a rate of 4.2 mg/kg for two reproductive seasons. Eggs from these birds contained a mean mercury concentration of 1.5 mg/kg ww. Fimreite (1971) fed breeding pheasants 2 - 3 mg/kg methylmercury for 12 weeks, and observed a decrease in hatchability due to early embryonic mortality (unhatched eggs contained 0.5 - 1.5 mg/kg mercury ww). Similarly, in a captive colony of American black ducks fed methylmercury at a rate of 3 mg/kg for 28 weeks, Finley and Stendell (1978) observed a decrease in egg production and in hatchability of eggs due to an increase in embryo mortality when eggs contained between 4 and 6 mg/kg mercury ww. Heinz (1974) reported a decrease in the number of eggs laid and in hatching success of mallard ducks dosed with methylmercury (0.5 to 3 mg/kg in dry mash for 21 weeks; eggs contained 6 - 9 mg/kg mercury ww).

Effects of mercury intoxication on fecundity and hatching rate have also been studied in populations of free-ranging birds. Fimreite (1974) and Conners et al. (1975), observed a decrease in hatchability in free-ranging populations of common terns nesting in a freshwater system contaminated with mercury (eggs of these birds contained from 1 to 6.5 mg/kg Hg ww).

In free-ranging populations of common loons, reproductive success was significantly affected (fewer or no eggs laid, increased nest abandonment, and impairment of territorial fidelity) at 2 - 3 mg/kg ww mercury concentrations in adult brain and eggs, and at dietary mercury of0.3 to 0.4 mg/kg mercury ww in fish (Barr 1986).

In contrast, some authors have failed to find any relationship between mercury concentrations in tissues of wild birds and hatching success. In herring gulls (*Larus argentatus*), egg mercury concentrations of up to 16 mg/kg ww have not been associated with reproductive failure (Vermeer et al. 1973). Similarly, Helander et al. (1982) found no association between reproductive success and mercury levels in eggs of white-tailed sea eagles (*Haliaeetus albicilla*). In free-ranging great skuas (*Catharacta skua*), clutch volume and hatching success was not related to their feather mercury concentrations (Thompson et al. 1991). These findings may reflect differences in sensitivity to mercury poisoning between species or contamination below the threshhold level for effect.

Effects of mercury on nestling survival and fledgling rate

Mallard ducklings of parents given a diet containing 0.5 mg/kg dw methylmercury [equivalent to approximately 0.1 mg/kg in a natural succulent diet (wet weight)], were less responsive to parental calls than controls, and were hyper-responsive to fright stimuli (Heinz 1975). These behaviors might lead to lower survival rates in a natural situation. In a comparison of survival of captive nestling black ducks from parents fed either 3.0 mg/kg methylmercury or a mercury-free diet, 70 % of ducklings from the control group survived past 7 days, whereas only 36 % of ducklings from mercury-dosed parents survived to that age (Finley and Stendell 1978). Eggs of mallard pairs fed 3.0 mg/kg (equivalent to 0.6 mg/kg in a succulent diet) methylmercury for two breeding seasons contained 7.18 and 5.46 mg/kg mercury after year one and year two, respectively (Heinz and Locke 1976). Mercury in the eggs was believed to be the cause of brain lesions in the hatched ducklings. The survival of American black ducklings was also decreased after their parents were dosed with mercury (brains from dead ducklings contained between 3.25 to 6.98 mg/kg mercury ww) (Finley and Stendell 1978).

Fimreite (1974) observed a 10 - 12 % reduction in fledging of free-ranging common terns inhabiting a mercury-contaminated freshwater system (mean liver mercury concentration of 27.5 mg/kg ww). Similarly, Connors et al. (1975) reported a decrease in fledging rate of common terns when eggs contained between 1 and 3.6 mg/kg mercury ww. Contradictory findings exist regarding the effects of mercury contamination on survival of free-ranging birds probably due to species differences, contamination below the threshold level, and other unmeasured factors. Hoffman and Curnow (1979) found no differences in liver mercury concentrations between live and dead great blue heron nestlings (*Ardea herodias*) when contamination was relatively low (mean mercury concentration of 0.96 mg/kg mercury ww). Similarly, Elliott et al. (1989) found no relationship between the number of nestling great blue herons that fledged per nest, and mercury concentrations in eggs (mercury in eggs ranged from 0.03 to 0.95 mg/kg ww). Bowerman et al. (1994a) found no relationship between nesting success (defined as the proportion of occupied breeding areas successfully fledging at least one young) of bald eagles (*Haliaeetus leucocephalus*) and mercury in feathers from adults or nestlings. In great skuas, chick survival was not related to adult feather mercury concentration (Thompson et al. 1991).

Effects on juvenile survival

In wild grey herons (*Ardea cinerea*) (mainly first year birds), liver mercury concentrations from 1.6 to 773 mg/kg dw (about 0.45 to 216 mg/kg ww) coupled with cold stress and poor nutritional condition may have contributed to a massive die-off in the Netherlands (Van der Molen et al. 1982). In common loons, juvenile healthy birds had lower mercury concentrations in brain, liver, muscle, and fat compared to emaciated juvenile birds (0.44, 1.92, 0.79, and 0.15 vs. 1.82, 26.4, 5.41, and 0.21 mg/kg mercury ww) (Frank et al. 1983). Ensor et al. (1992) also observed that juvenile common loons that died from disease had significantly higher mercury concentrations in feathers (mean of 19.8 mg/kg ww) than juveniles that died from injury (2.4 mg/kg ww).

Susceptibility to disease

In south Florida, juvenile great white herons (*Ardea herodius occidentalis*) with high hepatic mercury concentrations (geometric mean = 9.76 mg/kg ww) were more likely to die of disease than birds with low (1.77 mg/kg) hepatic mercury concentrations, suggesting that high mercury concentrations can compromise immune response (Spalding et al. 1994). Similarly, in a Minnesota study, juvenile common loons (*Gavia immer*) that died from disease had significantly higher feather mercury concentrations than juveniles that died from injury, or than live-caught juveniles (Ensor et al. 1992).

Food consumption and growth parameters

Many of the investigations noting mercury-induced changes in growth rate, body mass, or food consumption have been captive studies involving high or very high doses of mercury that, at best, represent the upper limits or above what is biologically feasible. Grissom and Thaxton (1985) noted a decrease in growth rate and food consumption in domestic chickens in response to 500 mg/l (approximately 500 mg/kg) inorganic mercury in drinking water. Captive juvenile northern goshawks (*Accipiter gentilis*) given chicken flesh containing 10 and 13 mg/kg methylmercury reduced their food consumption and lost mass, while controls gained mass (Borg et al. 1970). Captive juvenile red-tailed hawks (*Buteo jamaicensis*) that were fed a diet containing 7.2 and 10 mg/kg methylmercury generally slowed food intake and lost mass, while birds fed 3.9 mg/kg methylmercury and controls generally gained mass (Fimreite and Karstad 1971). Curiously, Fimreite and Karstad (1971) did not report any statistical analyses of their data, so there is no way to know if the differences among groups were significant.

MERCURY IN SOUTH FLORIDA

Sources of Mercury in the Everglades

Anthropogenic sources account for a substantial portion of the mercury that enters natural systems (Fitzgerald and Clarkson 1991, Jurczyk 1993), including those in Florida (KBN 1992). The level of mercury found in Everglades sediments has increased through this century. Rood et al. (1995) found that post-1985 mercury accumulation rates in sediments of the Water

Conservation Areas were an average of 5 times higher than accumulation rates at the turn of the century. Similar increases in mercury levels have been noted in other parts of the United States and in Europe (Rood et al. 1995).

The cause of elevated mercury concentrations in the Everglades is unclear (Rood et al. 1995). Agricultural activities and atmospheric deposition are two commonly hypothesized primary sources for the additional mercury inputs. The agricultural activities hypothesis postulates that, throughout geologic time, peat soils in the historic Everglades system have accumulated mercury (Davis 1994, Science Subgroup 1994, Stober et al. 1995). Extensive drainage during the past century has exposed those peat soils to air, allowing the organic matter to oxidize and subside (Stober et al. 1992). As organic matter disappeared, mercury concentrations increased in the remaining soil. Increased bulk densities in cultivated soils decreased oxygen diffusion into them (Stober et al. 1992). Periodic flooding of these oxygen-poor agricultural soils, combined with eutrophication due to agricultural phosphorus, created an environment conducive to methylation and solubilization of inorganic mercury. The resulting methylmercury is hypothesized to now flow south into the Everglades. However, this hypothesis is not so far supported by the data - less than 5% of total mercury transported into the Everglades Nutrient Removal areas (ENR) is transported via upstream flow (Stober et al. 1992).

The atmospheric deposition hypothesis attributes the high levels of mercury in the Everglades to elevated levels of mercury in the atmosphere. Mercury deposition in Everglades sediments accelerated during the 1940's, a time when global industrial use of mercury was rapidly increasing (Rood et al. 1995). This time line also coincides with increases in mercury levels in lakes in other parts of the world (Jurczyk 1993, Swain et al. 1992, Lindqvist et al. 1991, Science Subgroup 1994). Global and local atmospheric inputs of mercury due to fossil-fuel fired electrical generating plants, solid waste incinerators, medical waste incinerators, and paint, pulp and paper production, along with other forms of industrialization, are proposed as the cause of the increased burden of mercury in the Everglades (Jurczyk 1993). In support of this hypothesis, >95% of mercury deposition in the ENR is from atmospheric sources.

Surveys of mercury levels in Everglades animals have been sporadically conducted since the 1970s. High hepatic mercury concentrations of a dead Florida panther (*Felis concolor coryi*) found in the Everglades (Roelke et al. 1991), as well as high mercury levels in Everglades fish have recently stimulated interest in mercury within the Everglades ecosystem. As a result, a number of researchers are now investigating the ecology of mercury in the Everglades from a variety of perspectives.

Mercury in Everglades Animals

In 1989, a Florida panther was found dead in the Everglades. Tissue analyses revealed hepatic mercury concentrations of 98 mg/kg, a level consistent with mercury toxicosis (Technical Subcommittee of the Florida Panther Interagency Committee 1989) leading to the suggestion that contaminants might be a primary source of reproductive problems in this species (Facemier et al. 1995). During the 1980's, the Florida Game and Freshwater Fish Commission surveyed mercury levels in a variety of Everglades animals, including alligators (*Alligator mississippiensis*), mottled ducks (*Anas fulvigula*), soft-shell turtles (*Apalone ferox*), raccoons (*Procyon lotor*), white-tailed deer (*Odocoileus virginianus*), pig frogs (*Rana grylio*), and crayfish (*Procambarus*

alleni). All species averaged less than 0.5 mg/kg mercury except alligators (Figure 1.1), which had mean muscle concentrations of 2.92 mg/kg, and soft-shell turtles, with mean concentrations of approximately 0.6 mg/kg (extrapolated from figure in Ware et al. 1990). It was noted, however, that the mercury concentrations in crayfish and pig frogs were high enough to biomagnify in upper trophic level organisms (Ware et al. 1990).

Mercury in Everglades Fish

As of 1992, over 400,000 hectares of the Everglades watershed were subject to Florida Department of Health advisories (state action level = 0.5 mg/kg mercury) which urged the public to avoid consumption of sport fish. This area is now the largest continuous area in Florida where fish consumption is banned (Stober et al. 1992). The advisory resulted from a 1980s statewide study of mercury in largemouth bass (*Micropterus salmoides*) (Ware et al. 1990), which reported that the three areas where bass contained the highest mercury concentrations (in muscle) were all located within the Everglades (Figure 1.2): WCA III bass averaged 2.73 mg/kg mercury, Everglades National Park bass averaged 1.85 mg/kg mercury, and WCA II bass averaged 1.73 mg/kg mercury. In the Ware et al. (1990) study, other predatory fishes, including Florida gar (*Lepisosteus platyrhincus*) and bowfin (*Amia calva*), were also found to have high (> 1.5 mg/kg) mercury concentrations. In fact, one bowfin from WCA III contained 7 mg/kg mercury, the highest value ever recorded in a freshwater fish from Florida. Muscle concentrations of fish feeding at lower trophic levels averaged less than 0.5 mg/kg mercury with the exception of yellow bullheads (*Ictalurus natalis)* in Everglades National Park, and oscars (*Astronotus ocellatus*) and warmouth (*Lepomis gulosus*) in Water Conservation Areas II and III (Ware et al. 1990). More recent measurements of mercury in mosquitofish (*Gambusia holbrooki*) ranged from 0.1 to 0.4 mg/kg mercury (whole body) (W. Loftus pers. comm., Figure 1.1).

Mercury in Everglades Birds

Between 1971 and 1973, Everglades National Park conducted a survey of contaminant levels (mercury and other trace elements, chlorinated pesticides) in animals feeding at various trophic levels (including birds) and concluded that the mercury concentrations (1.0 to 2.62 mg/kg in white ibis muscle and brain tissue; up to 0.91 mg/kg in great egret eggs) were too low to have acute effects, but long term, chronic effects were possible (Ogden et al. 1974). A 1986 U. S. Fish and Wildlife Service study in Loxahatchee National Wildlife Refuge found mercury levels in anhinga (*Anhinga anhinga*) livers ranged from 0.42 to 2.72 mg/kg (mean = 1.5 mg/kg) (U.S. FWS unpublished data). Elevated liver mercury values (range 0.6 to 59.4 mg/kg) were found in a late 1980s study of great white herons (*Ardea herodius occidentalis*) in southern Florida, including the Everglades (Spalding et al. 1994). Sundlof et al. (1994) surveyed liver mercury concentrations of seven common wading bird species in south Florida. In their study, they found liver mercury levels as high as 74 mg/kg, with means for individual species ranging from 0.42 to 2.31 mg/kg. Hepatic mercury concentrations varied geographically, with the highest concentrations found in birds from the central Everglades and the eastern Florida Bay region. Higher liver mercury values were associated with increasing age and trophic status, and with decreasing amounts of body fat (Sundlof et al. 1994).

POSSIBLE ROLE OF MERCURY IN WADING BIRD REPRODUCTION

Breeding populations of wading birds in the Everglades have undergone a drastic decline since the turn of the century. There is general agreement that, since the 1930's, there has been at least a 90% reduction in the number of nesting pairs of four ciconiiform species: wood storks (*Mycteria americana*), great egrets, white ibises (*Eudocimus albus*), and snowy egrets (*Egretta thula*) (Bancroft et al. 1994, Ogden 1994). Further, a mid-1980s study of nesting ardeids in the Everglades found that the combined breeding numbers of six wading bird species, (those previously mentioned plus little blue herons (*Egretta caerulea)* and great blue herons), declined by at least 90% compared to the period 1940-1960 (Frederick and Collopy 1988). In some cases the 1980s numbers were low even in comparison with surveys conducted during the 1970s. An understanding of the possible role of mercury in this decline requires a review of the suite of potential ecological causes of the collapse in wading bird reproduction in the Everglades. Several factors have been suggested as prime contributors to the this decline, all of them related to human alterations of the system:

1. Habitat Loss: Since the beginning of the twentieth century, approximately half of the area of original Everglades marsh has been lost due to conversion to agriculture and residential uses (Davis et al. 1994). The area encompassing the historic Everglades is now partitioned among Water Conservation Areas (33%), agricultural production (27%), Everglades National Park (21%), urban areas (12%), and various undeveloped areas (7%) (Gunderson and Loftus 1993). Losses in total habitat translate into losses of foraging area. Browder (1978) estimated a 35% loss of five important wading bird feeding habitats since 1900. It is likely that losses of such magnitude have significantly contributed to the decline in wading bird breeding numbers.

2. Loss of Short-Hydroperiod Marsh: Originally, the Everglades was a mosaic of wetlands with varying hydroperiods, including substantial areas of short-hydroperiod (higher elevation) marsh which flanked deeper areas of marsh. During this century, short-hydroperiod marsh has disproportionately been lost to urban and agricultural uses or has become long-hydroperiod marsh due to water management (Fleming et al. 1993). Wading birds in the Everglades depend upon short-hydroperiod marsh for two primary reasons. First, at the beginning of the winter-spring dry season, when Everglades wading birds initiate breeding (Bancroft et al. 1994), short-hydroperiod marshes dry down earlier than other foraging areas, providing a source of food during courtship and nest building. This allows for early nesting so that young can fledge well before the dispersal of prey at the beginning of the wet season (Fleming et al. 1993). Second, during wetter years, short-hydroperiod marshes probably provide feeding alternatives when other areas of marsh are too deep, and prey items too dispersed, for successful foraging. Losses of short-hydroperiod marsh therefore compress the time window available for breeding, and limit feeding alternatives during wetter years (Fleming et al. 1993).

3. Compartmentalization of Large Areas of Marsh: Over 2,330 kilometers of canals and levees

have been constructed within the Everglades this century (Whitfield 1988). As a result, huge areas of marsh are impounded. The outcome of this impoundment has been to change much of the Everglades into largely homogeneous habitat so that water depths are not as variable across space as they were in the past (Fleming et al. 1993). Therefore feeding conditions of large areas of the system are uniformly acceptable or unacceptable, depending on rainfall and manipulation of water levels in the Water Conservation Areas. Birds simply cannot move between wetter and drier areas as easily as they once could.

4. Unpredictable Hydroperiods Due to Water Management Practices: Historically, Everglades wading birds could generally depend on adequate food supplies for the 3-4 months needed to complete nesting (Bancroft et al. 1994). Food supply for wading birds is limited both by the availability of suitable foraging habitat (concentrated prey in areas of suitable foraging depth) and the total abundance of prey items. Water management practices have influenced both of these factors (Ogden 1994). As previously noted, wading birds time their nesting attempts to coincide with the dry season in the Everglades. During the dry season, marshes are shallow and prey items are concentrated in smaller areas. Due to the impounded nature of much of the Everglades, single weather events can have a much greater influence on water depth than they did in the unaltered system, and suitable foraging habitat can disappear much more quickly than it did in the past. Currently, dry season rains cause rapid and marked reversals in the drying trend (Bancroft et al. 1994, Ogden 1994), resulting in the dispersal of prey items and causing reductions in nesting success, or complete colony failure (Frederick and Collopy 1989).

There is also evidence that total prey abundance, especially in historical wading bird breeding habitat in Everglades National Park, has decreased as a result of shortened hydroperiods in the deeper marsh areas of Taylor and Shark River sloughs (Ogden 1994). Deep marshes provide refugia for ardeid prey items during periods of low water. Loftus and Eklund (1994) suggested that marshes with annual hydroperiods of less than 9-10 months produce fewer fish than marshes with longer hydroperiods. Water management practices have shortened hydroperiods in Taylor Slough and Shark River Slough, producing frequent and extensive dry outs, with a resulting decrease in wading bird prey in these areas (Ogden 1994). This decrease in prey biomass is likely to contribute to reduced wading bird breeding in Everglades National Park (Ogden 1994).

5. Salinization of Estuarine Feeding Areas: In the earlier part of this century (1931- 1946), before the bulk of human alterations to the Everglades, the largest wading bird colonies were located in the mangrove estuarine zone of what is now Everglades National Park (Bancroft et al. 1994, Ogden 1994), with approximately 90% of the nesting concentrated along the freshwater-mangrove ecotone at the lower end of Shark River Slough, north of Florida Bay (Ogden 1994). During the period 1974-1988, the Shark River Slough estuary accounted for 15% of Everglades nesting pairs, and in 1989, only 10% (Ogden 1994).

Presently, all the large breeding colonies, which are much smaller than in the past, are concentrated inland in the Water Conservation Areas of the Everglades (Bancroft et al. 1994, Ogden 1994). It is thought that this shift in colony location has resulted from a decrease in prey availability in the estuarine zone for several reasons. First, present freshwater flow to Everglades estuaries is greatly restricted when compared to historical rates, resulting in increased salinity

(McIvor et al. 1994). Second, there is strong evidence linking secondary productivity of many fish and invertebrates to adequate freshwater inflow in estuaries (McIvor et al. 1994, Lorenz 1997). Finally, lowered recruitment of several commercial fish species is also associated with decreased freshwater inflow to Florida Bay (McIvor et al. 1994, Lorenz 1997). In light of this evidence, it seems more than reasonable to conclude that the estuarine prey base for nesting wading birds has declined as well. Wading bird breeding is now largely restricted to inland marsh areas with high annual variation in food availability (Frederick 1995).

6. Increasing Mercury Contamination: There is circumstantial evidence both for and against mercury as a factor in the decline of breeding wading birds in the Everglades. The argument for mercury playing a role in wading bird declines stems from research which found that mercury deposition in Everglades sediments began accelerating sometime around 1940, prior to the steep declines in wading bird breeding in the area (Rood et al. 1995). This line of evidence is confounded by the fact that much of the drainage and impoundment of the Everglades was also initiated during the 1940s. Presently there is no information available to show that mercury contamination has changed with time in Everglades wading birds. Analyses of feather samples in museums hold perhaps the greatest promise for answering this question definitively. Mercury concentrations in tissues of wading birds collected during the 1970's are within the range of current studies (Ogden et al. 1974, Sundlof et al. 1994). While it is unclear whether mercury contributed to the decline in Everglades wading bird reproduction, it is possible that, given the present concentrations of mercury in ardeid prey items, mercury may pose a significant barrier to reproductive success and increases in reproductive effort.

PURPOSE OF THIS STUDY

This research was initiated in August of 1993 to study the effects of mercury contamination on wading birds in the Everglades ecosystem. The goals of this work have been to assay for any effects of mercury that might affect the development, health, survival, reproductive effort and success of wading birds. We have designed our studies to assay for effects at mercury levels that are biologically relevant to those occurring in the Everglades ecosystem, or that could conceivably occur in the near future. As shown below, our work has taken many different routes. This makes our report somewhat complex, and we feel it is important that the reader's attention be directed to the following section, which describes the rough outline of separate research projects and the logic behind the pursuit of each.

OUTLINE OF THE WORK PERFORMED

In order to understand whether, where, and how any effects of mercury contamination might be occurring in wading birds in the Everglades, it was obvious that we first needed to gather some basic information about contamination. Specifically, we wished to understand whether mercury was uniformly distributed among wading bird species within the environment, what the range of contamination levels might be, and whether contamination changed with time. In the course of pursuing this goal, we also found that we needed to find a standard, non-destructively sampled, and representative tissue that could be inexpensively obtained from live birds. This pursuit became complex, and has required studies of the dynamics of mercury in

tissues over time, as well as studies of relative concentration between tissues. Further, the interpretation of any effects of mercury seemed likely to involve an understanding of differences due to age of birds, which we pursued both on a short time scale (during the first two months of life), as well as by comparing various age classes (nestling, juvenile, adult). The results of these investigations are described in Chapter II. Two separate analyses were performed. One on the subset of fledged and adult great blue herons and the other on the entire set of great blue herons and great egrets of all ages. Lastly, we needed to be able to link measurements of mercury contamination with exposure. We did this by studying the diet of great egrets over a four-year period, and by determining the mercury content in food fishes. By putting these two pieces of information together, we have been able to estimate the exposure of great egret chicks to mercury, and to match mercury contamination and exposure at the level of the colony (Chapter VII).

Having outlined the basic distribution of mercury in the Everglades, and having some understanding of the effects of age, location, hatch order and tissue type on mercury concentrations in great egrets, we turned to experimental studies to explore the effects of methylmercury in a more controlled fashion. The first attempt was to dose several great egret adults with methylmercury, using implanted osmotic pumps. Although this experiment failed largely for logistical reasons (poor survival due to extreme cold weather), we did find differences in the evoked vision potentials and eye morphology of one dosed bird, suggesting that mercury may have effects on vision (Chapter III).

One of our initial goals had also been to examine the effect of mercury on both reproductive success of adult birds and the ability of adults to come into reproductive condition. We had proposed to address this by comparing mercury levels in successfully breeding and nonbreeding birds. This work was hampered to a large degree by the extreme difficulty of live-trapping adult birds on the nest for collection of feather and blood samples. However, we eventually found that we could associate shed feathers with individual pairs of successfully breeding adult great blue herons, and we compared the feather values with a sample of non-breeding birds (without breeding plumes and inactive gonads) collected as roadkills or from rehabilitation centers. This was possible only because adult great blue herons often nest solitarily in the Everglades, and there was therefore no ambiguity in associating shed feathers with pairs that were known to have bred successfully. These results are described in Chapter IV.

Preliminary work and the existing literature had also suggested that mercury might affect on appetite and health of young birds. We hypothesized that these effects would lead to a reduction in survival of young birds during their first year of life. Since we wished to preserve as much of the natural stresses as possible, we attempted to study these effects by dosing great egret nestlings in the wild, and comparing the food consumption, growth, health, and survival of placebo and mercury-dosed birds (reported in Chapter V). This work involved controls to account for the effect of the dosing procedure, and the food measurement procedure (using labeled water), as well as the long-term tracking of over 70 chicks using radio telemetry, during the first 9 months of life. The lack of differences between dosed and placebo groups, and the fact of rapid feather growth during the period of study, led us to the conclusion that chicks were able to dispose of mercury very effectively during this period by depositing it in feathers. Since dosing in the field is limited to the early, pre-mobile part of the chick period when routine capture is possible, we turned to a captive dosing experiment to test the feather deposition

hypothesis, and to systematically answer questions about effects of health, tissue deposition, and effects on behavior under controlled conditions.

This captive dosing was perhaps the most definitive part of our studies (Chapter VI). Chicks were raised by hand from the hatchling stage to well past normal fledging and independence from the nest, and we examined the effects of three dose levels (placebo, 0.5 mg/kg in diet, and 5 mg/kg in diet) in an effort to begin to develop a crude dose-response relationship. We examined the effects of these doses on growth and development, appetite, blood parameters, liver enzymes, neurological competence, feather development, maintenance and movement behaviors, foraging behaviors, and repeatedly assayed mercury concentrations in blood, growing feathers and powderdown through time. This study allowed us to compare the effects of these doses on a number of potential responses, which led to better characterization of the nature of mercury effects in egrets at different dose levels. The dynamics of mercury among tissues in conjunction with these responses has given further clues about the physiological systems upon which mercury is acting, as well as providing a direct test of the feather deposition hypothesis.

Finally, Chapter VIII synthesizes the results of each of the chapters in an effort to present the results comprehensively, and to integrate our work into the current state of knowledge on the subject of effects of mercury exposure on populations of birds.

STUDY AREA- THE EVERGLADES ECOSYSTEM

We chose to study effects of mercury in the Everglades ecosystem of southern Florida, because, as previously mentioned, many animals have high tissue concentrations (Figure 1.1). The Everglades has also been characterized by its large populations of wading birds, which seem to be at high risk of contamination by virtue of their position in the food chain. The restoration of their breeding populations is a defining issue for the restoration of the ecosystem, and the effects of mercury contamination might result in significant impairment of reproductive abilities. Lastly, the Everglades seemed a natural choice because there are a number of other mercury studies occurring concurrently with ours, and because a great deal is known about the ecology of wading birds there.

We studied wading birds in the freshwater Everglades (Figure 1.2), a region dominated by freshwater marshes, including extensive stands of sawgrass (*Cladium jamaicense*), open sloughs and wet prairies, and in places, large invasive monomorphic stands of cattail (*Typha* spp.). This marsh area is quite dynamic in terms of hydrology, nutrient cycling, aquatic animal community composition, and vegetative composition (Gunderson and Loftus 1993). The ecology of the region is dominated by a pronounced wet-dry cycle, with the vast majority of rainfall occurring between June and October and an obvious dry period from November to May. Wading bird nesting occurs almost entirely during the dry season, and appears to be dependent in part upon the recession of surface water to concentrate and make available large quantities of aquatic prey (Frederick 1995).

The freshwater Everglades has undergone marked changes in the past century, including extensive canalization and impoundment. The overland flow of water containing elevated phosphorus levels from upstream of the Everglades has become widespread and, in some regions, has resulted in profound changes in vegetation and water chemistry (Gunderson and Loftus

1993). In addition, the recent spread of exotic fishes in the freshwater Everglades has altered community composition of freshwater fishes, and has in some years affected the diet of wading birds considerably (Frederick 1995).

We studied wading birds in the freshwater region because that is where nesting is currently concentrated. Although the large, historically significant colonies of wading birds were almost uniformly located in the mangrove forests and bays of the coastal region of the Everglades, those colonies have largely been abandoned due to the ecological degradation of the coastal region (Ogden 1994, McIvor et al. 1994). Wading bird colonies are now concentrated in the impounded, freshwater areas of the Everglades (60-90% of nesting pairs, 1986 - 1995, see Frederick 1995).

Although we made use of bird carcasses from throughout the south Florida region in some surveys, our studies of the geographic distribution of mercury, and of effects on birds were conducted almost completely from the colonies in Water Conservation Area 3A (Figure 1.2). Histories of these colonies, and descriptions of fluctuations of population sizes and nesting success in relation to environmental conditions may be found in Frederick (1995).

WEATHER AND HYDROLOGICAL CONDITIONS DURING THE STUDY

The decade of the 1990s began with a very severe and persistent drought (1989-1991), which ended with normal to heavy rainfall and a fully wetted marsh in 1991. Strong and persistent drying trends in the winter and spring of 1992 helped fuel an abnormally large nesting of wading birds, probably cued by some aspect of the preceding severe drought (Frederick 1995). Since that time, water levels have, with little exception, remained high in the WCAs and, during fall of 1994 and winter of 1995, resulted in an extreme flood condition.

All years of the 1993-1995 period have been wet by any definition. All three spring breeding seasons have been preceded by above to well above normal rainfall during the October - December period (see Figure 1.3). This was particularly true of the fall of 1994, when a series of tropical storms and depressions dropped over 8 inches of rainfall at most stations in south Florida. The winter and spring of 1993 also were peppered with an abnormal number of rainfall events, leading to frequent reversals in water trend and slow recession of surface waters.

The net result has been a generally wet marsh (long hydroperiod) since the summer of 1991. WCA 3 has had the highest stage among the three Water Conservation Area impoundments, relative to its long-term mean stages. For example, the stage at the 3-4 station in WCA 3 has remained above the mean monthly maximum for every month since June of 1992, and has exceeded one standard deviation in excess of the monthly maximum for 23 of the 45 months since September of 1991. The flood of 1994/5 is most clearly evident from the 3-4 hydrograph, beginning in September or October of 1994, and persisting perhaps until June of 1995. This flood qualifies as an extreme water event in the Everglades (Figure 1.4).

The rate at which surface water recedes in the Everglades (the "drying rate" of Kushlan et al. 1975) has been found to be a correlate of wood stork (*Mycteria americana*) and white ibis (*Eudocimus albus*) annual nesting effort (numbers of nests) in the Everglades (Kushlan et al. 1975, Frederick and Collopy 1988), and may have some effect on nesting by other species. Early drying rates (November - January) were well in excess of the threshold 2.0 mm/day thought necessary to stimulate nesting (Frederick and Collopy 1989) in all three WCAs in 1992 and 1994,

and well below it in 1993 and 1995 (Table 1.2). Late drying rates (January - March) were more variable among WCAs, and were consistently greater than 2 mm/d in 1992 at all stations, consistently less than this amount in 1994, and no clear trend was obvious across stations in the other two years. The 1995 season was one of hyperbole, with the slowest early drying rate and the fastest late drying rate on record for the station. Conversely, the 1994 season had the opposite extremes - among the fastest early drying rate and among the very slowest late drying rate.

Cold temperatures, often accompanied by wind, appear to have a strong negative effect on nesting, particularly by great egrets. This may result in both delayed and interrupted nesting (Frederick and Loftus 1993). The general pattern during 1993-1995 was warmer than average conditions during the winter and spring period (Frederick 1995). The fall of 1992 showed considerably warmer conditions than average, the months of January through June of 1994 were considerably more than one standard deviation warmer than the long term monthly means, and the same was true for nearly every month since.

Wind speeds at Tamiami Trail Ranger Station (40-mile Bend) were well below average during most months of the 1992 winter, spring, and summer, and well above average during the winter of 1992/3, and the fall of 1994.

These characterizations may be summarized as follows. The 1992 nesting season was preceded by the first full water year following a severe drought, had stronger than usual drying patterns, below average rainfall during the spring, and much less wind than usual. The 1993 nesting season was preceded by above average rainfall during the fall, and by Hurricane Andrew during the previous summer, very slow drying rates during winter and spring of 1993, extremely windy conditions, and higher than average temperatures. The 1994 season was preceded by at least 20 months of relatively wet conditions and abnormally high rainfall, had fast early winter drying, very slow spring drying rates, and exceptionally warm conditions. The 1995 spring season was preceded by at least two years of wet conditions followed by extremely heavy rainfall in the preceding fall and an exceptionally warm summer, and had extremely high stages in all compartments, slow early drying rates, extremely fast late drying rates, and exceptionally warm winter and spring conditions.

NESTING DURING THE STUDY PERIOD

During the period 1993-1996, a number of trends in wading bird nesting have been observed, which may be relevant to the present study. These observations are supported by data and analyses presented in Frederick (1995). It should be noted that the majority of our field work occurred between 1993 and 1995, with only maintenance surveys conducted during 1996. Overall, there was little change in the total numbers of nests of great egrets during this period, as compared with the previous decade. Numbers of great egret nests, however, were well above the previous 6-year mean in all of the years of this study (60% greater overall), and have increased during each year of study from 1993-1995 (Figure 1.5). Great egrets have nested predominantly (67-84% of Everglades populations in WCA 3) during this time. The reasons for the increasing population are not known, but it seems likely that this has something to do with the very high water levels that were experienced during this period. We have hypothesized that long hydroperiod in the Everglades leads to an increase in the size of fishes available, which would favor great egrets.

In 1993, great egrets showed a clutch size in the middle of the range for the Everglades, and in the upper end of the range of values from studies in other locations. Similarly, nest success was in the middle of the range of reported figures, and brood size at 21 days of age was in the upper end of the range for the Everglades, and in the middle of the range for the species.

In 1994, great egret clutch sizes were again in the middle of the range for the species, nest success was in the middle of the reported range, and brood size was virtually the same as in 1993.

In 1995, great egret clutch size and nest success were the highest so far discovered in the Everglades, and in the high end of the range for the species. Brood size at 21 days of age was in the middle to lower end of the range for the species. This distinct increase in reproductive parameters for 1995 is likely due to the combination of warm temperatures (Frederick and Loftus 1993) and strong, uninterrupted drying pattern seen in the spring of 1995.

STUDY SPECIES

We chose to study effects of mercury in great egrets and great blue herons for a number of reasons. Both species nest regularly in the Everglades, and both are at or near the top of the aquatic food web by virtue of consuming large, partially or wholly predatory fish species. This puts them at highest risk of mercury contamination among wading birds. Both species are common, and neither are on state or federal lists of endangered, threatened or special concern species. In addition, both species are regular nesters, with consistent nesting colonies from year to year, unlike white ibises, snowy egrets, and in recent years, wood storks. For both species, nesting phenology, ecology and distribution in the Everglades are well studied.

The only downside of working with these species was that neither has shown any sign of reproductive problems in the Everglades. In fact, these are the only two well-monitored wading bird species in the Everglades that have *not* shown declines in breeding populations during the past 40 years. Although this may not seem a desirable attribute in study species, we were faced with a clear tradeoff. We felt it more important to study species that were at highest risk of mercury exposure and with which we could work, than to focus on species with recognized reproductive declines, but for which sample sizes might be small or sporadically available.

Table 1.1. A summary of captive experimental studies investigating lethal concentrations of methylmercury in avian diets. Unless otherwise noted, dietary concentrations of methylmercury are expressed in terms of wet mass.

Species	*Dietary Concentration*	*Mortality Rate*	*Duration of Dosing (d)*	*Source*
Mallard	3.0 mg/kg[1]	16.7 % (year 1) 10.5 % (year 2)	ca 60 ca 425	Heinz and Locke 1975
Black Duck	3.0 mg/kg	0 %	196	Finley and Stendell 1978
Zebra Finch	5.0 mg/kg	25 %	77	Scheuhammer 1988
Japanese Quail	8.0 mg/kg	ca 10 %	21	Hill and Soares 1984
Red-tailed Hawk	7.2 and 10 mg/kg	25 %	84	Fimreite and Karstad 1971
Goshawk	10 and 13 mg/kg	100 %	47	Borg et al. 1970
Japanese Quail	20 mg/kg 20 mg/kg[2]	90 % equal to controls	14 14	Stoewsand et al. 1974
Chicken	33 mg/kg	7.5 %	35	Gardiner 1972

[1]Dry mass concentration equivalent to approximately 0.6 mg/kg in a succulent diet. Mortality rate reported is for ducklings of parents fed mercury. [2]Given selenium at 5 mg/kg along with 20 mg/kg MeHg.

Year	Station	Early Dry	Late Dry	% Exceedance Early Drying Rate*	% Exceedance Late Drying Rate*	% Exceedance Both Early and Late Drying Rate*
1996	3-4	6.99	5.68	100	100	100
1996	1-9	0.14	0.383	25.0	3.5	0.0
1996	2A 1-7	11.50	0.646	96.9	34.4	34.4
1995	3-4	-0.90	5.95	0.0	100.0	0.0
1995	1-9	0.97	0.21	32.1	10.7	3.6
1995	2A 1-7	0.55	3.50	28.1	87.5	29.0
1994	3-4	2.56	-1.08	58.6	6.9	3.6
1994	1-9	1.49	0.42	21.8	9.3	3.1
1994	2A 1-7	3.32	-4.67	90.0	3.3	3.3
1993	3-4	0.22	-0.40	10.0	10.0	3.3
1993	1-9	-0.33	3.91	14.8	7.8	0.0
1993	2A 1-7	-1.45	0.22	12.9	29.0	3.2
1992	3-4	2.29	2.63	24	38	14
1992	1-9	2.01	1.47	46	54	21
1992	2A 1-7	3.16	2.09	82.1	53.5	44.4

Table 1.2. Water level recession rates in mm/day in the Water Conservation Areas of the Everglades, with comparisons of drying rate in the year in question with period-of-record statistics at each station. Negative values indicate rising water, positive values indicate falling water. Percent exceedance is the percent of years in the record in which the drying rate was less than that of the focal year.

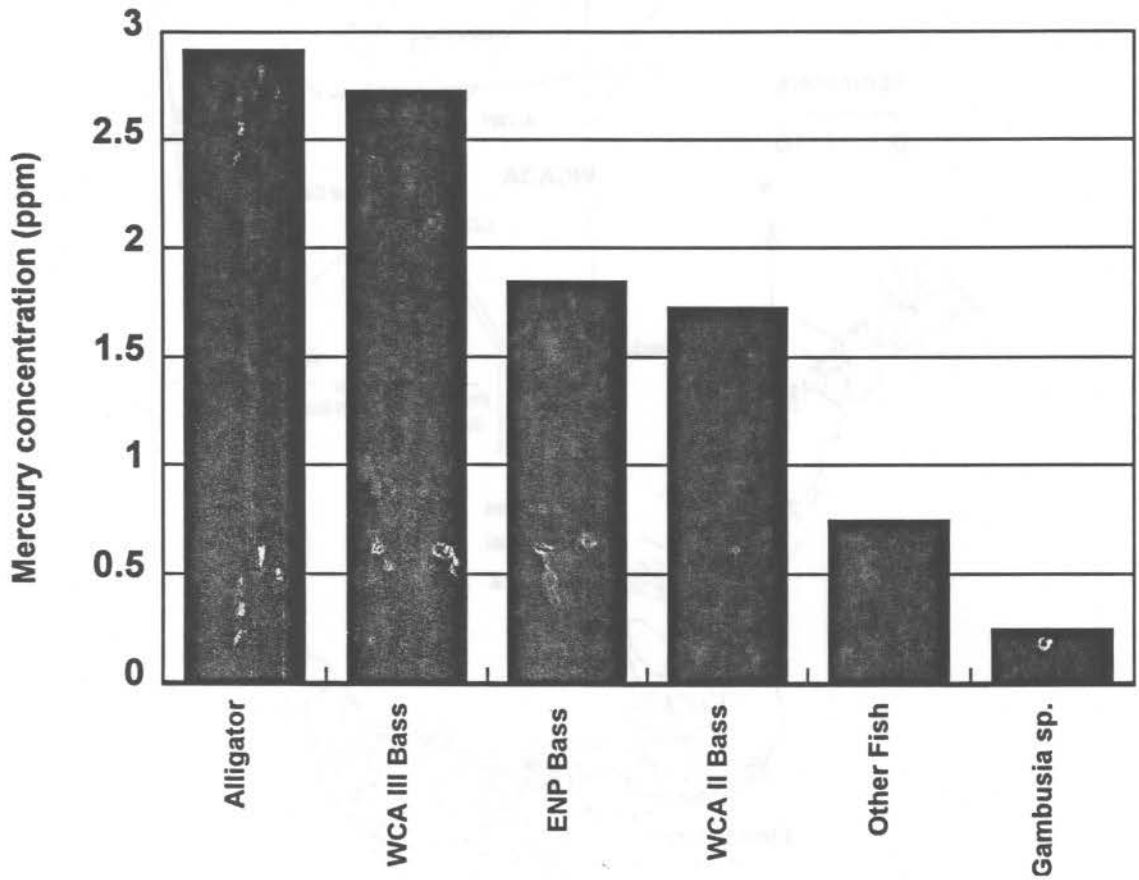


Figure 1.1. Mercury concentrations (ppm) of selected Everglades animals. Alligator and bass data are from Ware et al. 1990. Other fish and *Gambusia* data are from W. Loftus (pers. comm.).

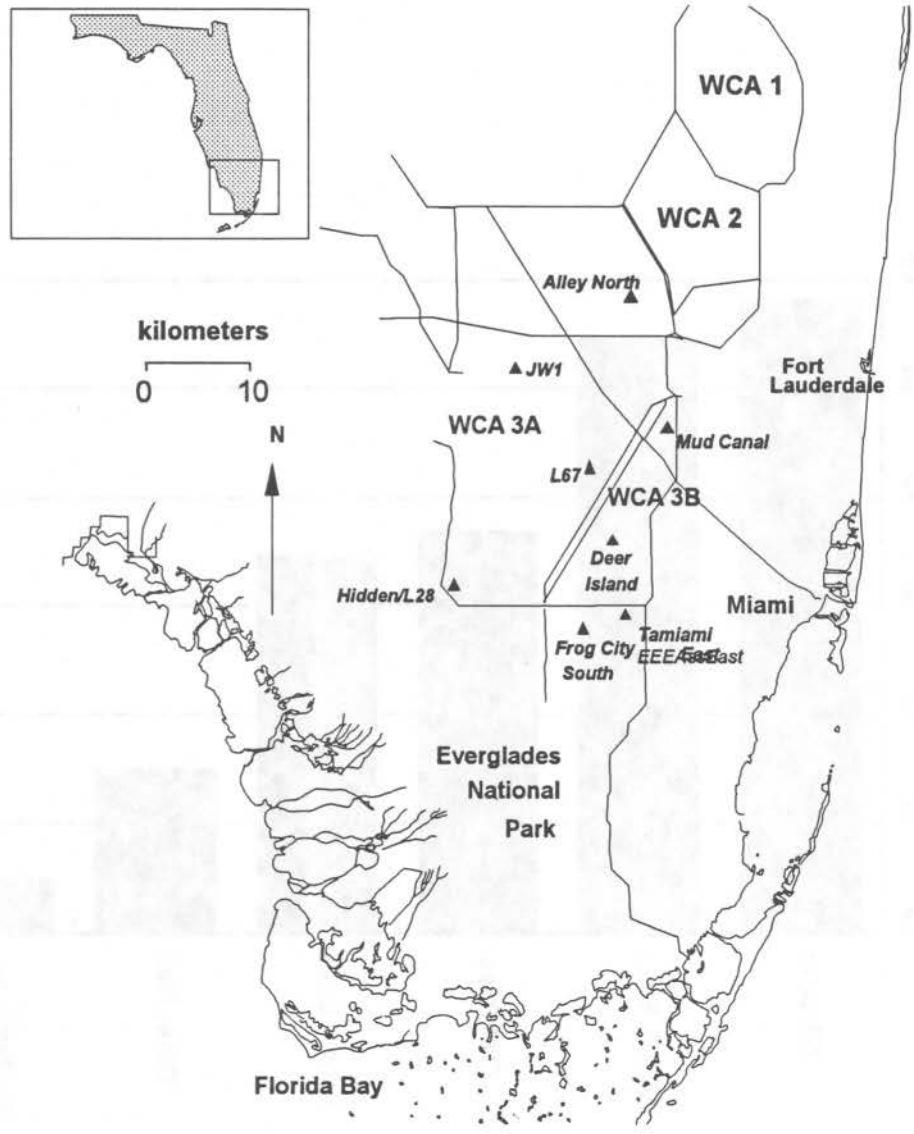


Figure 1.2. Map of study area in southern Florida, showing locations of colonies sampled (triangles) in Water Conservation Area 3, in relation to access, water management boundaries and areas of major habitation.

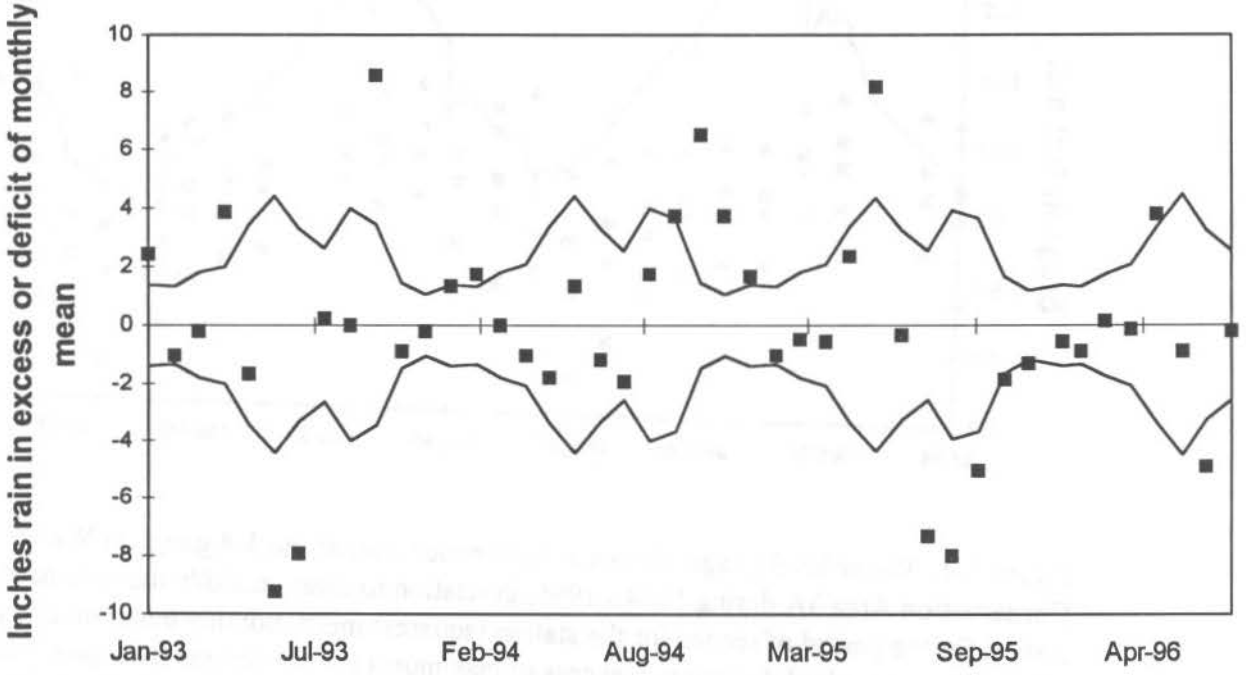


Figure 1.3. Monthly rainfall measured at the Tamiami Trail Ranger Station (40 mile bend) during the study period, displayed in inches in excess or deficit of monthly means for the period of record of that station. The two continuous lines depict one standard deviation in excess or deficit of the monthly mean, and the dark squares indicate monthly rainfall totals during the study period.

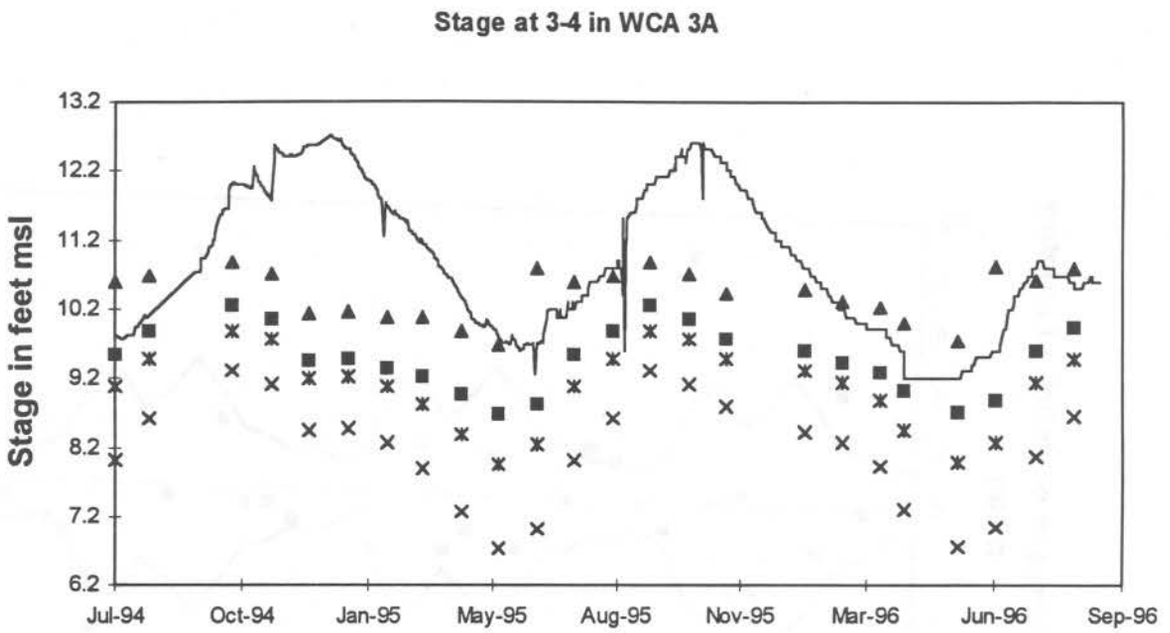


Figure 1.4. Water level (stage, shown as continuous line) at the 3-4 gauge in Water Conservation Area 3A during 1994 - 1996, in relation to mean monthly maximums at that station for the period of record for the station (squares) mean monthly minimums (dark x's), and one standard deviation in excess of maximums and minimums (triangles and light x's, respectively). Note that stages were well in excess of one standard deviation of maximums for much of the study period.

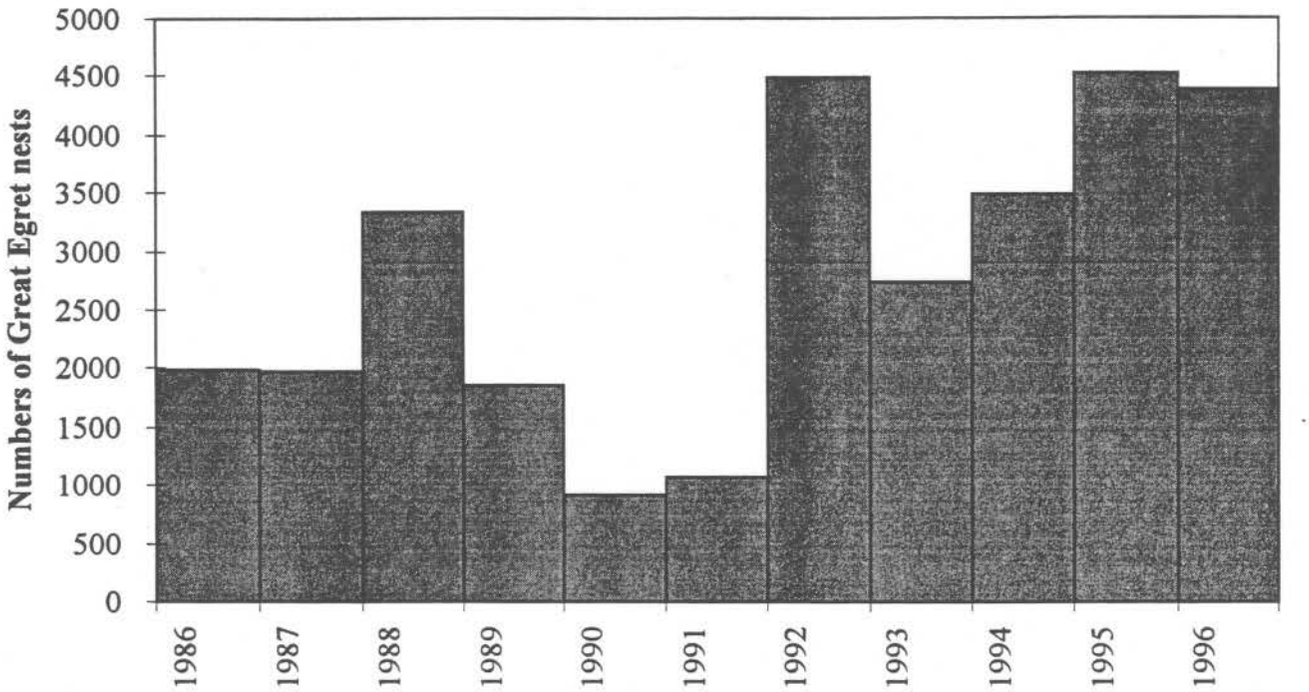


Figure 1.5. Numbers of Great Egret nests counted in the Everglades (Everglades National Park and all Water Conservation Areas) during the period 1986 - 1996. Note that during the period of study, Great Egrets have been increasing steadily in breeding population size in the study area.

CHAPTER II. EFFECTS OF SPECIES, GEOGRAPHIC LOCATION, AGE AND TISSUE TYPE ON MERCURY CONCENTRATIONS IN WADING BIRDS

INTRODUCTION

This section focuses on the distribution of mercury in various tissues of wild great egrets and great blue herons. This topic comes up again for captive dosed great egrets in Chapter VI.

Although mercury concentrations in livers of wading birds from southern Florida have been documented to some extent (Spalding et al. 1994, Sundlof et al. 1994), the focus of most studies has been to use liver concentrations to compare contamination between species, ages and locations. The relationship between hepatic mercury concentration and the concentration of mercury in other tissues, especially those that can be readily and non destructively sampled from live birds (e. g. blood and feathers), remains poorly understood (Beyer et al. 1997). 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.

As a result of bioaccumulation, the concentrations of mercury in the tissues of tertiary predators are likely to reflect the degree of contamination in aquatic food webs (Stendell et al. 1976, Delbeke et al. 1984). As a result, mercury concentrations in piscivorous birds, such as wading birds, constitute an excellent indicator of ecosystem contamination. However, wide-ranging or migratory birds, can be problematic as local monitors because it is difficult to determine where their exposure occurred. This problem can be overcome by monitoring mercury concentrations in the tissues of chicks and fledglings since these birds feed exclusively on prey items captured in the vicinity of the breeding colony (Bancroft et al. 1994, Frederick 1995). An understanding of sampling biases that are attributable to methods and tissue typ are essential to using bird samples for bioindication. Consequently we wished to identify a tissue or tissues, and a sampling method, that could be used to accurately monitor mercury burdens in wild populations of herons and egrets, and to provide predictive relationships among concentrations in different tissues so that would could compare our results with other studies. The reader should note that relationships between tissue concentrations are also treated in detail in Chapter VI.

Similarly, factors such as age, sex, geographic location and year may also account for differences in mercury concentrations between individual birds. A basic understanding of the variability in contamination and accumulation of mercury is necessary to interpret all of our other investigations, as well as for making predictions about the population effects of mercury. As a result, we analyzed tissue samples from great egret nestlings in order to examine the effects of age, hatch order, colony location and year.

METHODS

Distribution of mercury among tissues of fledged and adult great blue herons

Various tissues were collected from 104 blue (n = 66) and white (n = 38) color morphs of the great blue heron (Ardea herodias) found sick or dead. These birds were either juvenile birds

that had fledged from their colony (n = 16) or adults (n = 88). Most were from the Everglades or from Florida Bay (Dade, Broward and Monroe counties). A few were included from other locations in Florida. Not all tissues were collected from all birds. Blood, for instance, was only collected from live or very recently dead birds. Some of these same birds are included in the analysis that follows which includes birds of two species and all ages.

Distribution of mercury among tissues of great egrets and great blue herons

A total of 299 great egrets and 51 great blue herons (30 blue and 21 white color morphs of the latter) were collected from four different counties in Florida (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. but it is known that they were collected in south Florida. The sample of great egrets included a total of 252 nestlings, 6 fledglings, 14 juveniles, 14 adults, 8 adult-breeding birds, and 5 birds of unknown age. Tissues from the great blue herons included a total of 20 juveniles, 16 adults, 13 adult-breeding, and 2 birds of unknown age. Birds were assigned to age classes based on bill length and plumage. Adult birds were considered to be in breeding condition if they had both breeding plumes and enlarged gonads.

A large proportion of the blood and feather samples from nestling great egrets were collected from live chicks during the nesting season; the remaining samples of nestlings and fledglings were collected from birds found dead in nesting colonies. The juvenile and adult egrets and herons were collected either as roadkills or were obtained as carcasses from rehabilitation centers. Following necropsy, several tissues (liver, brain, muscle, kidney, ovary, testes, feces, nails, pancreas, plumes, powderdown, mature primaries, and growing scapular feathers) were 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.

Collection of feathers and blood from great egret nestlings

Great egret 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, it is difficult to collect blood even from chicks with large veins. Total amounts of food ingested during this period were extremely small, and it is likely that chicks of up to 5 days of age receive almost all of their mercury burden from the egg rather than from food. The average frequency of bleeding varied among colonies (Tables 2.1 and 2.2), and ranged from every 3 to every 14 days. These variable collection times were designed to record both short term variation in blood mercury concentrations, and geographic variation among colonies. Growing scapular feathers were collected once from each chick, usually during the last visit to the nest (approximately 28 days of age). Several aspects of data collection differed between 1994 and 1995, as below.

1994: From late March to mid-May 1994, a total of 125 great egret chicks (58 nests) from seven colonies located in Water Conservation Areas (WCA's) 3A and 3B were sampled for mercury concentrations in blood and feathers (Frog City South, 25°43.27'N, 80°35.90'W, n = 2 chicks; Hidden/L28, 25°47.93'N, 80°50.58'W, n = 27; Deer Island, 25°51.29'N, 80°33.30'W, n = 4; L67, 25°57.33'N, 80°33.92'W, n = 46; Mud Canal, 26°00.60'N, 80°27.65'W, n = 13; JW1, 26°09.01'N, 80°43.15'W, n = 19; and Alley North, 26°11.35'N, 80°31.55'W, n = 14, Figure 1.2

and Table 2.1). Except for three nests in which the smallest chick was not handled, all chicks within each nest were sampled for mercury.

Ages were determined by known hatch or laying dates for 98 of the 122 chicks sampled (46 of the 58 nests), and ranged from one to 44 days (mean = 17.8 days; SD = 9.2). Bill length was measured from the skin/bill demarcation on the dorsal surface of the upper mandible to the tip of the maxilla every time a bird was handled. Bill length was measured in all birds, and ranged from 1.1 to 8.1 cm (mean = 4.2 cm; SD = 1.7).

1995: From mid-April to early June 1995, a total of 246 blood samples and 121 growing scapular feathers were collected from 127 first hatched great egret chicks (127 nests) (Table 2.2). Samples were from birds in six different colonies located within WCA 3A and 3B in the Everglades (Figure 1.2): Tamiami East 25°45.52'N, 80°30.51'W, n = 31; Hidden/L28, n = 47; L67, n = 14; Mud Canal, n = 7; JW1, n = 14; and Alley North, n = 14. Only the largest chick in each nest ("A" chick) was sampled for blood and feathers during 1995. Blood samples were taken every five days from chicks at Tamiami East and Hidden/L28 colonies for a total of 15 days per chick, and growing scapular feathers were collected on the last visit to the nest. (These chicks were the "control" chicks of the field dosing study outlined in Chapter V).

During 1995, ages were determined by known hatch or laying dates for 92 of the 127 chicks sampled, and ranged from two to 37 days (Mean = 17.1 days; SD = 6.8) at the time of sampling. Bill length was measured in all but two of the chicks, and ranged from 1.3 to 10.2 cm (Mean = 5.1 cm; SD = 1.5).

During both years, blood was drawn from the jugular vein using 1 ml insulin syringes and 27 gauge needles, and stored in 3 ml lithium heparinized glass tubes. The amount of blood collected ranged from 0.3 to 0.6 ml. During 1994, blood was kept refrigerated until it was sent to the Department of Environmental Protection (DEP) laboratory for mercury determination (see below for a detailed description of this analysis). The time 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 scale, respectively. Volumes between 0.3 and 0.4 ml (approximately 0.3 to 0.4 g) of blood were saved for mercury analysis. Blood was then pippeted into Teflon-sealed glass vials and digested with 1 ml of trace metal grade nitric acid. Blood was allowed to react with the nitric acid for approximately 15 minutes before vials were fitted with their caps. Samples were then stored at room temperature until submitted for analysis.

Determination of total mercury concentration in blood and tissues

Total mercury concentrations in blood and tissues of great egret nestlings were determined by the Department of Environmental Protection chemistry laboratory in Tallahassee, Florida. At least 0.1 ml of field-prepared blood sample was pippeted into a Nalgene tube, and both the volume and weight of the blood recorded. The feather samples were treated with 30 ml sulfuric acid, 12 ml of nitric acid and allowed to sit for at least 24 hours. After that time, a 7 ml aliquot of the feather-acid solution was placed in a Nalgene bottle and heated in a water bath at 58 degrees for 30 minutes. The tissue samples were removed from the bath and 40 mls deionized water, 10 ml 8% potassium permanganate, and 4 ml potassium persulfate were added to each

sample. The tissue samples were then allowed to react overnight.

Prior to analysis, all samples (blood and tissue) were treated with 4 ml of 12% hydroxylamine hydrochloride solution and were placed in a sonicator bath for 10-15 minutes to remove excess permanganate and chlorine interferences respectively. Finally the samples were analyzed using cold vapor atomic absorption spectroscopy on a Varian 30/40 atomic absorption spectrometer with deuterium background correction. The spectrometer was fitted with a cold vapor/hydride generator using stannous chloride reducant and was automated with an SPS5 autosampler.

At the beginning of each day, a new 4-5 point calibration curve was created at the following levels: 1.25, 3.75, 6.25, 12.5, 25 μg/L. The following quality control samples were analyzed with every 20 samples or less:

1. A digestion blank which consisted of 40 ml of deionized water.
2. A duplicate sample for each different type of matrix.
3. A high (4 μg/ml of methylmercury chloride) and a low (1 μg/ml methylmercury chloride) organic mercury sample matrix spike.
4. A fish tissue standard (DORM-1, 0.15 - 0.2 g).
5. A practical quantitation level (PQL) standard inorganic mercury solution (0.25 μg/L).

Unless specified otherwise, reported values are total mercury concentrations on a wet weight basis (ww) for blood and tissue samples and on a dry weight basis (dw) for feathers. Methylmercury was analyzed in frozen tissues by Frontier Geosciences in Seattle, Washington.

Determination of selenium in livers of great blue herons and great egrets

Selenium concentrations were measured by Dr. Lee McDowell at the University of Florida using frozen livers of 24 great egrets, 20 great blue herons, and 21 great white herons using a fluorometric method (Whetter and Ullrey 1978). The sample consisted of birds of all ages.

Data Analysis

For the combined set of great egrets and great blue/white herons, Pearson correlation analyses were used to assess the strength of relationships between mercury concentrations in different tissues. This analysis was performed for egrets and herons combined, and also separately by species. Linear models (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 possible differences in body size.

For chicks sampled repeatedly in 1994, we calculated Pearson correlation coefficients between age and blood mercury concentrations for each bird sampled. The proportion of chicks that had positive correlation coefficients was then calculated and tested for significance using a two-sided sign Z test to determine if mercury concentration in blood increased with age (Sokal and Rohlf 1995). Chicks that were sampled only once were not included in this analysis. Since

growing feathers were sampled only once, intra-individual correlation coefficients were calculated for blood mercury concentrations only. This analysis was performed by year, colony, and hatch order.

For blood samples collected during the 1995 field mercury dosing experiment, we calculated correlation coefficients between mercury concentration in blood and age separately for the control and the mercury-dosed great egret chicks. We used Pearson correlation coefficients to determine if mercury in blood and feathers were significantly correlated with each other.

Analyses of covariance (ANCOVA, PROC GLM, SAS Institute 1988) were used to assess the effects of year, colony, and hatch order on the dependent variables (mercury concentration in blood and feathers). To control for differences in age, bill length was used as a covariate in all of these analyses. Assessment of differences between groups (colonies, dose groups, etc.) was accomplished using a Tukey's Studentized Range test (SAS Institute, 1988).

Comparisons between years were done only withfirst-hatched or "A" chicks since samples were only collected from "A" chicks in 1995. In addition, no blood or feather mercury concentrations from dosed chicks were included in the annual comparison. Since blood was sampled in both years only at Hidden/L28 colony, samples from this colony were the only ones used to assess for year differences in blood mercury concentrations. Inter-year differences in feather mercury concentrations were determined using samples from Hidden/L28, L67, JW1, Mud Canal, and Alley North colonies.

When using data from 1994, differences among colonies were analyzed both by controlling for hatch order, and by combining all hatch orders. These analyses included samples from Hidden/L28, L67, JW1, Mud Canal, Frog City South, Alley North, and Deer Island colonies. For 1995, differences in blood mercury concentrations among colonies were determined only for Hidden/L8 and Tamiami East colonies, and JW1, L67, Mud Canal, and Alley North colonies were added when comparing differences in feather mercury concentrations.

Since all birds from each nest were sampled for mercury only during 1994, the effect of hatch order on mercury concentrations was analyzed using only data from that year. This analysis was done only in those nests that had either two or three chicks, that were sampled for mercury in blood and/or feathers at least twice, and whose brood size remained unchanged during the sampling period. We also tested the hypothesis that body weight and mercury concentration in blood increases at a higher rate in the surviving chick or chicks when one or more sibling dies, than in nests where no mortality occurs. Blood mercury concentrations were compared in "A" chicks from two-chick broods where no mortalities occurred, with "A" chicks from similar nests but in which the "B" chick died. Both types of nests were sampled for mercury at comparable frequencies and periods of time. Final body weight and the last measurement of mercury in blood minus initial body weight and blood mercury concentration were calculated for each chick, and we used a paired t-test to compare these parameters among the two groups.

RESULTS

Tissue Distribution

Mercury distribution in tissues of fledged and adult great blue/white herons

Mercury concentrations were generally greater in feathers than in other tissues (Table 2.3). However, unlike the captive reared great egrets (see Chapter VI), liver mercury concentrations were greater than that of some feather types (when only those birds with both tissues available were evaluated) (Table 2.5). Significant correlations were evident between mercury concentratiions of most tissue types (Table 2.4). Low sample sizes were frequently associated with insignificant correlations. Despite good sample sizes, mercury concentrations in blood could only be significantly correlated with powderdown, kidney, muscle, brain, and bile. Similarly, despite good sample sizes for liver and blood, no correlation was found concentrations in those tissues. Relative to powderdown, liver and growing scapular feathers had higher mercury concentrations, and breeding plumes, kidney, pancreas, muscle, blood, brain, and bile, had lower concentrations (Table 2.5).

The proportion of total mercury occurring as methylmercury was relatively low in the few samples tested. Two liver samples from an adult and a juvenile great blue heron analyzed for methylmercury contained 17% (methylmercury = 0.58 mg/kg, total =3.3 mg/kg) and 23% (methylmercury =1.4 mg/kg, total Hg = 6.0 mg/kg) methylmercury respectively.

Selenium concentrations in the livers of great egrets and great blue herons

Selenium concentrations were directly and significantly correlated with mercury concentrations in livers of herons and egrets (Fig. 2.1). The liver Hg:Se ratio ranged from 0.01 to 7.45 (ave. = 1.2). For birds with mercury liver concentrations greater than 10 mg/kg this ratio increased to 2.2.

Concentrations of mercury in blood and feathers of great egret nestlings

Of the 558 blood samples collected during 1994 and 1995, a total of 56 samples (26 from 1994 and 30 from 1995) could not be analyzed for mercury. In the case of feathers, only seven samples (all from 1995) were not analyzed for mercury. Reasons for the loss of samples included: samples that were too small in volume or mass for analysis; clotting of blood and inability to collect it from vacutainer; and during 1995, explosion of vials with blood and nitric acid during shipment or when opened upon arrival to the laboratory.

Mercury concentrations in blood and growing feathers of all great egret nestlings sampled in 1994, when controlled for age (Least Squares Means, LSM, n = 286, SD = 0.65), averaged 1.19 mg/kg and 14.75 mg/kg (LSM) (n = 81, SD = 6.59), respectively. A summary of mercury concentrations in blood and feathers of great egret chicks sampled during 1994 are presented by colony and hatch order in Table 2.6.

Excluding those chicks that were dosed with mercury, first-hatched chicks in 1995 had average blood and feather mercury concentrations of 0.75 mg/kg (LSM adjusted for age of chick) (n = 107, SD = 0.33) and 9.68 mg/kg (LSM) (n = 83, SD = 5.02), respectively. Table 2.7

summarizes mercury concentrations in blood and feathers by colony collected from un-dosed chicks during 1995.

Correlation between mercury in blood and feathers

Mercury concentration in blood and feathers of great egret nestlings were significantly correlated with each other. This correlation was significant for 1994 ($n = 77$, $r^2 = 0.67$, $P = 0.0001$), for 1995 when excluding mercury-dosed chicks from that year ($n = 25$, $r^2 = 0.71$, $P = 0.0001$), and for both years combined ($n = 102$, $r^2 = 0.72$, $P = 0.0001$) (Figure 2.2). When mercury-dosed chicks from 1995 were included in this analysis, the correlation coefficient increased ($n = 45$, $r^2 = 0.90$, $P = 0.0001$). Note that the data used in these correlations sometimes involved multiple measurements from the same individual.

Mercury distribution in great egrets and great blue herons of all ages

Table 2.8 summarizes the numbers of all birds sampled for mercury, by species and age class. A summary of the correlation analyses of tissues from great egrets and great blue herons is presented Table 2.9, and correlation plots are shown in Figures 2.3, 2.4, and 2.5, respectively. In Figure 2.3 the sample size for blood was relatively small, because unclotted blood was difficult to obtain from carcasses. When the two species were combined, mercury blood concentrations were significantly negatively correlated with concentrations in liver, and positively correlated with concentrations in brain and growing feathers (Figure 2.5). When the sample was restricted to great egrets, mercury concentrations in blood were correlated only with plumes and growing feathers.

Age Specific Differences In Mercury Concentrations

Changes in blood mercury concentration with age

Great blue herons and great egrets

For combined samples from great egrets and great blue herons, age had a significant effect on blood, brain, muscle, kidney, and growing feather mercury concentrations (Table 2.10). Significant differences in mercury between age classes were detected using a Hochberg's pairwise comparison for samples of unequal size (SAS Institute, 1988).

Blood and feathers sampled from great egret nestlings

1994: When great egrets from all hatch orders and colonies where included from the 1994 sample, the proportion of nestlings that had positive correlations between blood mercury and age was significantly higher than chance ($n = 82$, $Z = 3.63$, z-test, $P < 0.0001$). Within colonies, this proportion was significant only for birds from Alley North ($n = 12$, $Z = 2.31$, $P = 0.005$), Hidden/L28 ($n = 24$, $Z = 2.03$, $P = 0.01$), and L67 ($n = 26$, $Z = 3.13$, $P < 0.0005$). The absence of the same effect in other colonies is probably attributable to the very small numbers of birds sampled more than once (e.g. Frog City South $n = 2$; JW1 $n = 4$; Deer Island $n = 4$; and Mud Canal $n = 10$).

The proportion of chicks whose blood mercury increased with was significant for all chick hatch order categories we tested ($n = 43$, $Z = 2.89$, $P = 0.0009$, for first-hatched chicks, and $n = 31$, $Z = 3.05$, $P = 0.0005$, for second-hatched chicks). There were not enough samples to test this hypothesis for third-hatched chicks.

1995: The proportion of first-hatched chicks with positive blood mercury/age correlations was significantly higher than chance both when mercury-dosed birds were included in the analysis ($n = 62$, $Z = 5.32$, $P < 0.0001$) and when they were excluded ($n = 36$, $Z = 2.41$, $P = 0.004$). Mercury in blood from dosed birds from both Tamiami East and Hidden/L28 colonies increased with age ($n = 13$, $Z = 3.6$, $P < 0.0001$, and $n = 18$, $Z = 4.24$, $P < 0.0001$, respectively) (Figure 2.6). However, correlation coefficients between mercury in blood and age were significant only for control birds from Tamiami East colony ($n = 14$, $Z = 2.67$, $P = 0.002$) (Figure 2.7), and not from controls from Hidden/L28.

Effect Of Hatch Order

For nests with two nestlings, mercury in blood and feathers did not differ between first-hatched "A" and second-hatched "B" chicks (ANCOVA, DF = 1, $F = 0.59$, $P = 0.4462$, and DF = 1, $F = 0.57$, $P = 0.4551$, respectively). Similarly, for nests with three chicks no differences were found between blood and feather mercury concentrations of "A", "B", and "C" chicks (ANCOVAS, DF = 2, $F = 0.14$, $P = 0.8674$, and DF = 2, $F = 1.52$, $P = 0.3221$, respectively).

Effect of brood reduction on blood mercury concentration and body weights of the surviving chick

Changes in blood mercury concentrations and body weights from eight first-hatched chicks from nests with two birds where chick "B" died during the study period were compared to those from eight first-hatched chicks from nests with two birds, where no birds died during the course of the study. Even though mean blood mercury concentrations and body weights were higher for the birds that had their only sibling die (0.18 vs. 0.14 mg/kg and 361 vs. 273 g, respectively), these differences were not significant (DF = 7, $t = 0.19$, $P = 0.4236$ for the blood mercury comparison, and DF = 7, $t = 1.39$, $P = 0.1030$ for the body weight comparison).

Geographic Differences in the Everglades

Effects of geographic location on mercury concentrations

Collection location had a significant effect upon mercury concentrations in blood, liver, brain, powder down, and growing feathers of all great egrets and great blue herons when data for these two species were combined. Pairwise comparisons between colonies indicated that birds from Broward County had significantly 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).

Comparison of blood mercury in 1994

When only first-hatched chicks were included in the analysis, JW1 chicks had higher blood mercury concentrations compared to all other colonies, with the exception of Frog City

South (ANCOVA, DF = 6, F = 5.53, P = 0.0001; Tukey's Studentized Range, DF = 139, MSE = 0.32) (see Figure 2.8 and Tables 2.6 - 2.7 for mean values). "B" chicks from JW1 and L67 colonies had higher mercury concentrations in blood when compared to "B" chicks from Hidden/L28, Alley North, and Mud Canal colonies (ANCOVA, DF = 6, F = 6.09, *P* = 0.0001; Tukey's Studentized Range, DF = 113, MSE = 0.35). No differences in blood mercury concentration were detected among colonies when using only values from "C" chicks (ANCOVA, DF = 4, F = 1.54, *P* = 0.2588). When all hatch orders were used in the comparison between colonies in 1994, birds from JW1 had more mercury in blood than did chicks from all other colonies except Frog City South; chicks from L67 colony had a higher blood mercury concentration when compared to chicks from Hidden/L28, Mud Canal, and Alley North; and birds from Frog City South had higher blood mercury than birds from Alley North (ANCOVA, DF = 6, F = 11.55, *P* = 0.0001; Tukey's Studentized Range, DF = 278, MSE = 0.34) (see Table 2.11 for mean values).

Comparisons of feather mercury in 1994 and 1995

During 1994, first-hatched great egret chicks from JW1 had higher feather mercury concentrations than did first hatched chicks from all other colonies (ANCOVA, DF = 5, F = 10.18, *P* = 0.0001; Tukey's Studentized Range, DF = 37, MSE = 24.77) (see Figure 2.8b and Table 2.6 and 2.7 for mean values). No feather samples were collected from Frog City South "A" chicks during 1994. For "B" chicks, differences in feather mercury concentrations were detected only between JW1 and both Hidden/L28 and Mud Canal colonies (ANCOVA, DF = 5, F = 3.35, *P* = 0.0188; Tukey's Studentized Range, DF = 25, MSE = 24.60). No differences in feather mercury concentration were detected among colonies when using data collected from "C" chicks (ANCOVA, DF = 2, F = 0.44, *P* = 0.7297). When all hatch orders were used to compare colonies, birds from JW1 had higher mercury concentrations in feathers than all other colonies except Frog City South, and L67 chicks had a higher feather mercury content when compared to Hidden/L28 and Mud Canal (ANCOVA, DF = 6, F = 12.26, *P* = 0.0001; Tukey's Studentized Range, DF = 74, MSE = 23.81) (see Tables 2.6 and 2.7 for mean values).

During 1995, blood mercury concentrations did not differ between first-hatched un-dosed chicks from Hidden/L28 and Tamiami East colonies (ANCOVA, DF = 1, F = 0.92, *P* = 0.3396) (see Table 2.7 for mean values). First-hatched chicks from JW1 and L67 colonies had a significantly higher mercury concentration in their feathers when compared to birds from Tamiami East, Hidden/L28, Alley North, and Mud Canal colonies (ANCOVA, DF = 5, F = 12.97, *P* = 0.0001; Tukey's Studentized Range, DF = 66, MSE = 13.13) (see Figure 2.9 and Table 2.7 for mean values).

Annual Differences in Mercury Concentrations

Differences in mercury concentrations between years for great egret chicks

Blood mercury concentrations of first-hatched chicks at Hidden/L28 colonies differed between years (ANCOVA, DF = 1, F = 10.23, *P* = 0.0019), averaging 1.04 mg/kg (LSM) during 1994 and 0.78 mg/kg (LSM) during 1995 (Tukey's Studentized Range, DF = 99, MSE = 0.21).

Feather mercury concentrations from un-dosed "A" chicks in Hidden/L28, L67, JW1,

Mud Canal, and Alley North colonies during 1994 and 1995 also showed differences between years (ANCOVA, DF = 1, F = 11.75, $P = 0.00029$). Combining data from these colonies, mean mercury concentrations were 15.97 mg/kg (LSM) in 1994, and 9.68 mg/kg (LSM) in 1995 (Tukey's Studentized Range, DF = 97, MSE = 36.59). Mercury concentrations in feathers were higher in all five colonies during 1994 compared to 1995 (Hidden/L28 = 13.26 vs. 7.94 mg/kg; L67 = 17.23 vs. 15.51 mg/kg; JW1 = 30.07 vs. 14.51 mg/kg; Mud Canal = 9.64 vs. 6.39; Alley North = 13.30 vs. 7.32 mg/kg) (LSM) (Tables 2.6 and 2.7).

Only first-hatched chicks with similar visitation schedules (Food/Placebo + Food Measurement Control/Placebo from 1995 and all first-hatched chicks from 1994) that were not dosed with methylmercury were used in the following analyses. Concentrations of mercury in blood (ANCOVA, F = 18.36, $P < 0.0001$, controlled for age and individual nest effects) and growing feathers (ANCOVA, F = 22.16, $P < 0.0001$, controlled for age and individual nest effects) were significantly higher in 1994 than in 1995. Within chicks from Hidden colony, there was also yearly variability in blood (ANCOVA, F = 21.66, $P < 0.0001$, controlled for age and individual nest effects) and feather (ANCOVA, F = 31.35, $P < 0.0001$, controlled for age and individual nest effects) mercury concentrations in first-hatched chicks, with concentrations for both being higher in 1994 than in 1995.

DISCUSSION

Tissue Distribution

Mercury distribution in fledged and adult great blue herons

The results of this study indicate that mercury concentrations in powderdown and growing feathers could be used to assess mercury concentrations in most other tissues. Powderdown is an especially important resource because it is present at all times, even when other types of growing body or flight feathers are absent. Powderdown feathers occur in several groups of birds, however in herons they grow and produce powder continuously, not just during molt as in Columbiformes (Lucas and Stettenheim 1972). There is the added advantage that powderdown or growing feathers, when compared with mature feathers, can tell a history of exposure, especially if it can be determined when the mature feather was grown; for example breeding plumes, which are grown seasonally, can be used in this fashion (see Chapter IV).

Mercury measured in feathers appears to be almost entirely methylmercury (Thompson and Furness 1989). The mercury deposited in feathers appeasr to be closely linked with mercury body burden and exposure at the time of feather growth (Braune and Gaskin 1987, Honda et al. 1986, Furness et al. 1986, Chapter IV this report). Thus for nestlings, feather mercury is a measure of egg contamination and food contamination up until time of collection. Feathers collected from adults represent body burden and exposure at the time of growth and this might be affected by the molt sequence, i.e. the first feathers to begin growing might have higher mercury concentrations.

Blood mercury concentrations had a less predictable relationship with concentrations in other tissues. These results differ from the captive great egret study (see Chapter VI) in that relative tissue concentrations were less tightly correlated and frequently reversed. This could be

easily explained by temporal factors that were controlled for in the captive experiment and that are unknown in most of the wild birds. Wild birds, especially those that migrate, are exposed to different concentrations of mercury in their diet over time. Feathers grown during periods of high exposure should be higher in mercury concentration than feathers grown during periods of low exposure. Also, it appears from the experimental work (Chapter VI) that blood acts as a storage reservoir only when exposure is high and other tissues (such as feathers or liver) are saturated, or not growing. Thus a bird with a history of high exposure might have higher liver concentrations than blood concentrations, if recent exposure has also been low. Variation in exposure history would explain the failure to establish a significant correlation between liver and blood mercury in wild birds. The captive dosing experiment section of this report (Chapter VI) deals with this issue in more detail.

It is interesting to note that brains of egrets and herons had the lowest concentrations of all tissues examined. Very little information has been published regarding mercury concentrations in brain of wild birds. The proportion of total mercury that was methylmercury was lower in great blue heron liver (17 and 23%) in this study than in others (40%, smowy egret) (Gardner et al. 1978). However, the sample size of two birds prevents specualtion regarding the significance of this finding.

Selenium concentrations in liver relative to mercury concentrations

Selenium accumulated in liver of great egrets and great blue herons at about half the rate of mercury. This is slightly higher than was observed for captive great egrets, which was about 1/3 the rate (see Chapter VIII). Explanations for this difference include 1) age differences 2) species differences and/or 3) differences in selenium concentrations in the food. The Hg:Se ratio (1.8) is relatively high when compared with other species (Cuvin-Aralar and Furness 1991).

Mercury concentrations in the blood and feathers of nestling great egrets

Blood samples from free-ranging great egret nestlings from southern Florida for 1994 and 1995 combined had mercury concentrations averaging 1.7 mg/kg (n = 393, SD = 0.56) (Table 2.12). In comparison to other tertiary predators, this contamination level is within the range found in other studies. For example, our concentrations are lower than those reported for common terns (*Sterna hirundo*) on Long Island, New York (Gochfeld 1980) and higher than those reported for bald eagles (*Haliaeetus leucocephalus*) from Florida, Oregon, Washington, and Florida (Wiemeyer et al. 1989, Anthony et al. 1993, Wood et al. 1996) (see Table 2.12 for values). Beyond this rather superficial calibration, there are no logical comparisons for our reported values since very few studies of nestlings of other species exist, and no studies of mercury concentrations in blood of piscivorous freshwater birds have ever been published.

The average growing feather mercury concentration of great egret chicks in this study was 12.16 mg/kg (n = 165, SD = 5.8). This is as much as 11 times greater than the concentrations reported for nestling wading birds sampled elsewhere (average = 1.03 dw and 1.26 mg/kg ww) (Table 2.12) but similar to those reported for great egret nestlings by Beyer et al. from southern Florida (mean = 7.1 mg/kg dw) (1997). In comparison to other species, mercury concentrations in the feathers of egrets in this study were higher than those found in any other study of young

birds. The only concentrations that approximated our findings were those found in young raptors with an average of 6.28 mg/kg dw and 9.3 mg/kg ww (Table 2.12) which is still considerably lower than the great egrets (12.15 mg/kg). Thus it appears that great egret nestlings in the WCAs of the Everglades have very high mercury exposure relative to birds in other ecosystems. We have no information about species differences in the ability to excrete mercury into feathers.

Several authors have reported differences in mercury concentrations between feathers of different types and growth stages (Applequist et al. 1984, Honda et al. 1985, Furness et al. 1986, Braune 1987, Braune and Gaskin 1987). In general, higher mercury concentrations have been documented in abdominal and down feathers than for contour and wing feathers (Frank et al. 1983, Honda et al. 1985, Braune and Gaskin 1987, Becker et al. 1994). Table 2.12 summarizes many of the studies which, combined, have sampled almost every type of feather. In contrast to this study, most authors report mercury concentrations of mature feathers. In one study it was shown that mercury concentrations were higher in fully formed or mature feathers than in growing feathers (Burger et al. 1992a). Our results show this is an inconsistent finding.

Differences in mercury levels between different species of nestlings or different areas may arise from differences in prey size and type (larger, piscivorous fish usually accumulate more mercury than smaller fish or invertebrates) (Cutshall et al. 1978, Hoffman and Curnow 1979, Lange et al. 1994 see Chapter VII), and/or to differences in levels of mercury contamination of prey items between different geographic areas. Only one of the studies presented in Table 2.12 reports mercury levels from wild-caught food items (Hoffman and Curnow 1979). These authors report mean mercury concentrations from 10 species of fish from Lake Erie, with a range from 0.02 to 0.29 mg/kg ww. Mercury in feathers from egrets and herons in Lake Erie averaged 2.0 mg/kg ww which is less than 20 percent of the concentrations we have reported. As a result, it is logical to presume that birds from southern Florida are eating fish that contain considerably higher concentrations of mercury than those reported by Hoffman and Curnow in 1979 (see Chapter VII).

Chick feathers as indicators of local mercury contamination

Growing feathers collected from nestling wading birds are useful indicators of local contamination. During the time when young rely on parents for food, the adults forage in the vicinity of the colony. Frederick (1995) found that birds nesting in WCA 3 flew an average distance of 6.21 km from the colony during 1994 and of 8.50 km in 1995. Similarly, Bancroft et al. (1994) reported that during 1983, and from 1986 to 1989, adult breeding great egrets flew an average distance of 6.3 km away from their colonies in the Everglades. This indicates that chicks are being fed only prey items collected close to the breeding colonies, making them excellent indicators of mercury contamination levels in relatively local areas.

According to the results of this study, mercury concentrations in growing feathers are strongly predictive of concentrations in various tissues and of cumulative mercury examined. This conclusion is also supported by the results of the captive dosing study (Chapter VI). Our results suggest that growing feathers may serve as a medium with which to accurately monitor large numbers of free-ranging great egret nestlings for mercury contamination. Collection of

feathers is a much less intrusive, safer, easier, and faster technique than drawing blood (analyses of feather mercury through time in Chapter VI).

Age Specific Differences in Mercury Concentrations

The results obtained from this study indicate that in general, mercury concentrations in the different tissues studied increased with age (Table 2.10). Accumulation of mercury with age has been reported in several species of birds (Sundlof et al. 1994, Burger 1993a, Burger et al. 1992b).

Mercury concentration in the blood of great egret chicks increases steadily during the first month after hatching. This increase of mercury in blood with age occurs despite the fact that large amounts of mercury are being deposited into growing feathers. This is illustrated by the fact that average blood mercury concentration in great egret chicks, for all ages combined, was approximately 12 times lower than that of growing feathers during 1994 (1.19 mg/kg vs. 14.75 mg/kg), and 13 times lower during 1995 (0.75 mg/kg vs. 9.61 mg/kg). This phenomenon was also reported by Honda et al. (1986) for eastern great white egret (*Egretta alba modesta*) nestlings. In that study, whole-body concentrations of mercury (feathers removed) increased until the 45th day of age, which corresponds to fledging, and decreased thereafter. The authors concluded that this decrease was due to the fact that mercury was being excreted at a relatively high rate through molting. Our studies with captive great egrets suggest that the dynamics of feather and blood mercury are inter-related. A more detailed treatment of the age related dynamics of feather and blood mercury are given in Chapter VI.

Effects of Hatch Order

No differences in mercury tissue concentration between siblings were detected, despite the fact that a large sample was analyzed. These results indicate that great egret siblings have similar concentrations of mercury, at least during the first month of age. Whether eggs or sibling great egrets have similar amounts of mercury at hatching is not known.

In studies of herring gulls (*Larus argentatus*) and common terns, egg mercury levels have been shown to decline with laying sequence (Becker 1992). In addition, first-hatched herring gulls and common terns had significantly higher mercury in their down than did their younger siblings (20% and 31% higher concentrations respectively) (Becker et al. 1994). Fully grown back feathers from these birds, however, showed no significant differences in mercury contamination between siblings. These results suggest that differences in mercury contamination between siblings, if any, are more likely to be detected at very early stages of development (embryo and downy stage). Stendell et al. (1976) found no differences in egg mercury concentrations within clutches of great egrets nesting in Lake St. Clair and the Detroit River, although the levels of contamination were lower than in Becker's (1992) study. The fact that great egret chicks were sampled for mercury at a later stage could explain the absence of a similar pattern in the present study.

Geographic Differences in the Everglades

Adult and juvenile great egrets and great blue herons from Broward County had significantly higher mercury concentrations in brain and powderdown than did birds from

Monroe and Collier counties. During 1994, chicks from JW1, L67, and Frog City South had the highest concentrations of mercury in blood and growing feathers. A similar pattern was observed during 1995, (no samples from birds from Frog City South were collected during 1995). For both years, chicks from Hidden/L28, Mud Canal, and Alley North, together with Deer Island colony in 1994, and Tamiami East colony in 1995, had the lowest concentrations of mercury.

There is some information on foraging dispersion of adult great egrets during the breeding seasons of 1994 and 1995 from three of the colonies that had relatively low mercury concentrations (Hidden/L28, Alley North, and Tamiami East) and from one colony with nestlings that had higher mercury concentrations (L67). For both years, the majority of flights from Hidden/L28 colony were to the south and southwest, ending outside WCA 3A, either in Big Cypress National Preserve, or in Everglades National Park (Frederick 1995). Flights from Alley North were in all directions during 1994 and 1995, and ended primarily in WCA 3A. Great egrets from Tamiami East foraged mainly in southeastern WCA 3B and northeastern Shark Slough. From the L67 colony, adult great egrets flew in all directions, and all the flights ended in WCA 3A and 3B (Frederick 1995).

These flight patterns were compared to mercury concentrations reported for largemouth bass collected from different areas in southern Florida (Ware et al. 1990). Fish collected south of WCA3 (Everglades National Park) had lower concentrations of mercury in muscle (mean = 1.85 mg/kg ww) than did fish collected within the WCA3 (mean = 2.73 mg/kg ww). A similar pattern was observed in birds in this study: colonies in which breeding egrets were feeding outside WCA3 (Hidden/L28 and Tamiami) had chicks with lower mercury concentrations than colonies in which adult egrets were foraging inside WCA3 (L67 and JW1). Alley North is an exception to this rule.

At this time it is unclear whether the observed differences in mercury concentrations between colonies are due to differences in preferred prey consumed by the breeding egrets, to differences in mercury contamination of the selected food items, or a combination of both. The differences in feather mercury concentrations do seem to match local differences in fish contamination, however. Using a comprehensive grid of sampling stations within the Everglades, the EPE EMAP program has been able to map mercury concentrations in mosquitofish (Stober et al. 1996). The differences in mercury in great egret feathers overlay the fish data quite well. Thus it is likely that mercury in feathers of great egret nestlings is indicative of differences in mercury concentrations in food items rather than differences in diet.

Annual Differences in Mercury Concentration

The primary mode of mercury contamination in great egret nestlings is by ingestion of contaminated prey. As a result, the differences in tissue mercury concentrations between years are probably explained by differences in the amount of mercury that these birds are exposed to through their diets. Higher mercury concentrations found in great egrets during 1994 might be explained by an increased availability of larger prey due to lower water levels that year (Frederick 1995). Since larger fish tend to be older, and tend to forage at higher trophic levels, these fish probably contain greater concentrations of contaminants than smaller fish. As shown in Chpater VII, differences in diet between 1994 and 1995 are enough to explain the differences in feather contamination between the two years. Other possible explanations for interannual

differences in mercury concentration in food items include changes in the availability of mercury between years due to changes in atmospheric mercury deposition or changes in the biogeochemistry of the study area (e.g. pH, phosphorus content, sulfate levels, temperature, dissolved organic carbon, aeration, etc.) (Science Subgroup 1994).

Table 2.1. Summary of mercury sampling of great egret nestlings during 1994, by colony.

Colony [a]	Chicks Sampled	Nests Sampled	Average Frequency of Sampling	Number of Collection Events [b]	Blood Samples Collected	Feather Samples Collected [c]
Frog City South	2	1	Every 7 days	4	8	1
Hidden/L28	27	12	Every 3 days	20	101	25
Deer Island	4	2	Every 6 days	5	16	3
L67	46	22	Every 3 days	12	90	27
Mud Canal	13	6	Every 6 days	5	40	8
JW1	19	8	Every 7 days	5	23	9
Alley North	14	7	Every 14 days	4	34	8
Total	125	58		55	312	81

[a] Location of colonies can be found in Figure 1.2.

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

[c] Growing scapular feathers were collected during the last visit to the colony.

Table 2.2. Summary of mercury sampling of great egret nestlings during 1995, by colony.

Colony [a]	Chicks Sampled [b]	Nests Sampled	Average Frequency of Sampling	Collection Events [c]	Blood Samples Collected	Feather Samples Collected [d]
Tamiami East	31	31	Every 5 days	18	106	29
Hidden/L28	47	47	Every 5 days	20	140	43
L67	14	14	-	1	0	14
Mud Canal	7	7	-	2	0	7
JW1	14	14	-	2	0	14
Alley North	14	14	-	2	0	14
Total	127	127		45	246	121

[a] Location of colonies can be found in Figure 1.2.

[b] Only first-hatched chicks in each nest were sampled for mercury.

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

[d] Growing scapular feathers were collected during the last visit to the colony.

Table 2.3. Number examined, mean, and range of mercury concentrations (mg/kg ww) for tissues collected from 104 juvenile and adult great blue and great white herons from south Florida between 1991 and 1994.

Tissue	# Examined	Mean	Range
Plume	32	26.82	1.7-49
Mature primary	13	22.66	0.84-240
Growing scapular	36	11.60	0.48-48
Powderdown	56	11.28	1.3-75
Liver	65	11.07	0.24-77
Blood	28	2.42	0.48-18
Ovary	3	2.34	0.31-5.6
Kidney	38	2.28	0.092-12
Pancreas	19	1.41	0.37-4.2
Muscle	19	1.33	0.13-4.8
Testes	9	0.74	0.077-1.6
Brain	62	0.55	0.056-2.1
Bile	19	0.45	0.18-1.4

Table 2.4. Correlation analysis of mercury in tissues collected from 104 wild great blue herons. Each cell contains the slope of the predictive equation over the number of samples/Pearson correlation coeficient. NS = a P value > 0.05. The tissue in the left column is on the y-axis of the graph.

	Liver	Gr. scapular	Powderdown	Plume	Blood	Kidney	Ovary	Muscle	Pancreas	Testes	Brain	Bile
Mature primary	14.05 12/.061	NS 6/	NS 7/	NS 1/	NS 3/	NS 8/	NS 1/	NS 8/	NS 4/	NS 4/	NS 10/	NS 2/
Liver		0.703 34/0.76	1.268 51/0.57	2.228 7/0.89	NS 20/	5.919 38/0.90	NS 3/	6.764 19/0.82	7.71 19/0.71	NS 9/	15.81 62/0.59	21.98 19/0.57
Growing scapular			1.23 27/0.63	NS 1/	NS 13/	5.558 21/0.73	NS 3/	7.678 13/0.92	10.674 11/0.96	16.958 5/0.97	24.35 34/0.85	38.95 9/0.83
Powderdown				1.447 9/0.95	9.14 22/0.97	2.353 32/0.71	NS 1/	8.877 13/0.92	4.066 19/0.60	NS 6/	15.95 51/0.87	28.67 17/0.89
Plume					NS 5/	2.252 6/0.84	NS 0/	NS 2/	NS 3/	NS 2/	15.64 6/0.97	26.95 4/1.00
Blood						0.563 16/0.85	NS 1/	2.604 4/0.99	NS 12/	NS 2/	2.497 20/0.76	0.163 11/0.90
Kidney							0.703 3/1.00	1.325 15/0.89	0.794 19/0.68	NS 8/	3.471 37/0.92	4.957 18/0.81
Ovary								NS 3/	NS 0/	NS 0/	NS 3/	NS 0/
Muscle									NS 4/	2.07 9/0.87	2.215 19/0.87	NS 5/
Pancreas										NS 2/	2.058 19/0.74	0.147 14/0.69
Testes											NS 9/	NS 2/
Brain												0.053 19/0.92
Bile												

Table 2.5. Comparison of relative proprtion of mercury in powderdown to various tissues of experimentally dosed great egrets and wild great blue herons.

Tissue	Wild Adult GBH	Captive fledgling GE
Growing scapular	1.23	1.17
Liver	1.27	0.23
Plume	0.69	-
Kidney	0.42	0.19
Pancreas	0.25	0.18
Mature scapular	-	0.22
Blood	0.11	0.22
Muscle	0.11	0.1
Brain	0.06	0.05
Bile	0.03	0.05
Fat	-	0.01
Eye	-	0.01

Table 2.6. Summary of total mercury concentrations in blood and growing scapular feathers of great egret nestlings during 1994, by colony and hatch order. Note mean values are corrected for age.

Colony	Mercury in Blood (mg/kg ww) A Chicks	Mercury in Feathers (mg/kg dw) A Chicks	Mercury in Blood B Chicks	Mercury in Feathers B Chicks	Mercury in Blood C Chicks	Mercury in Feathers C Chicks
Frog City South	1.47(4;0.17)[a]	-[b]	1.65 (4;0.49)	16.24 (1;-)	-	
Hidden/L28	1.05(43;0.58)	13.26(11;0.60)	0.95(41;0.53)	12.13(11;6.22)	1.03(5;0.73)	8.65(3;3.43)
Deer Island	0.96 (9;0.26)	9.39 (3;1.36)	1.40 (7;0.98)	-	-	-
L67	1.31(44;0.63)	17.23(15;4.67)	1.53(37;0.68)	15.37(10;4.30)	1.23(2;0.60)	13.18 (1;-)
Mud Canal	1.10(21;0.81)	9.64 (5;2.49)	0.85(11;0.42)	8.44 (2;0.63)	1.13(4;0.91)	7.88 (1;-)
JW1	2.06 (9;0.33)	30.07 (4;6.55)	1.82 (9;0.72)	23.07 (4;3.69)	1.84(3;0.20)	-
Alley North	0.88(17;0.24)	13.30 (6;2.00)	0.80(12;0.22)	14.97 (4;2.94)	0.59(3;0.06)	-
Overall	1.18 (148;0.62)	15.47 (44;7.12)	1.22(121;0.66)	14.74(32;5.95)	1.14(17;0.73)	9.40(5;3.54)

[a] Least Square Mean (LSM), corrected by age (n; SD).
[b] No samples collected.

Table 2.7. Summary of total mercury concentrations in blood and growing scapular feathers of first-hatched, un-dosed great egret nestlings during 1995, by colony.

Colony	Mercury in Blood (mg/kg)	Mercury in Feathers (mg/kg)
Tamiami East	0.72 (46; 0.28)[a]	7.75 (14; 3.36)
Hidden/L28	0.78 (61; 0.36)	7.94 (26; 2.86)
L67	-[b]	15.51 (14; 6.16)
Mud Canal	-	6.39 (7; 1.73)
JW1	-	14.51 (8; 3.31)
Alley North	-	7.32 (14; 3.39)
Overall	0.75 (107; 0.33)	9.68 (83; 5.02)

[a] Least Square Mean (LSM; corrected by age) (n, SD).
[b] No samples collected.

Table 2.8 Number of individual birds sampled for tissue mercury concentrations, by species and age during both years of study.

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

	Blood	Liver	Brain	Muscle	Kidney	Ovary	Testes	Feces	Pancreas	Plumes	P. Down	Growing Feather	Mature Feather
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(19)GE(36)	All(63)GBH(19)GE(44)	All(6)	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(19)GE(34)	All(61)GBH(20)GE(41)	NS(5)	All(18)GE(9)	NS(5)	All(9)GE(9)	NS(9)	All(40)GBH(29)GE(11)	All(42)GBH(13)GE(29)	NS(19)			
Muscle	All(49)GBH(15)GE(34)	NS(8)	All(16)GBH(9)GE(7)	NS(5)	All(8)GE(8)	NS(7)	All(21)GBH(12)GE(9)	All(35)GBH(12)GE(23)	NS(18)				
Kidney	All(6)GBH(3)	All(17)GE(9)	NS(5)	All(10)GE(10)	NS(9)	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(9)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(16)GBH(6)GE(10)	NS(16)											
Growing Feather	NS(9)												
Mature Feather													

* All, GE, and GBHI 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.

Table 2.10. 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).

Table 2.11. Summary of total mercury concentrations in blood and growing scapular feathers of great egret nestlings (all chicks in the nest) during 1994, by colony.

Colony	Mercury in Blood (mg/kg) All Chicks	Mercury in Feathers (mg/kg) All Chicks
Frog City South	1.56 (8; 0.35)[a]	16.24 (1; -)
Hidden/L28	1.00 (90; 0.56)	12.03 (25; 6.15)
Deer Island	1.12 (16; 0.68)	10.16 (3; 1.36)
L67	1.39 (83; 0.66)	16.29 (27; 4.53)
Mud Canal	1.04 (36; 0.72)	10.06 (8; 2.03)
JW1	1.94 (21; 0.52)	21.12 (9; 6.10)
Alley North	0.83 (32; 0.25)	12.47 (8; 2.34)
Overall	1.19 (286; 0.65)	14.75 (81; 6.59)

[a] Least Square Mean (LSM; means corrected by age) (n, SD).

Table 2.12. Mercury concentrations in feathers and/or blood of piscivorous freshwater, piscivorous marine, insectivorous, and raptor nestlings. Concentrations of Hg in feathers and blood (mg/kg) are expressed as dry or wet weight, respectively, unless otherwise specified.

Species	Age (days)	Country	Exact Location	Year(s)	Type of Feather	Mercury Concentration in Feathers	Mercury Concentration in Blood	Source
Piscivorous Freshwater Birds								
Great Egret *Ardea albus*	– [a]	United States	Ohio	1972-1973	Wing	2.64 [b, e] (1.28-3.67) (n = 11)	–	Hoffman and Curnow, 1979
Great Egret	**1-44**	**United States**	**Southern Florida**	**1994-1995**	**Growing Scapulars**	**12.16 ± 5.80 [c] (n = 165)**	**1.7 ± 0.56 (n = 393)**	**This study**
Great Egret	1-60	United States	Southern Florida	1987-1990	Primary, Tail	7.1 ± 5.1 (n = 9)		Beyer et al., 1997
Great Blue Heron *Ardea herodias*	–	United States	Ohio	1972-1973	Wing	1.90 [e] (0.54-4.32) (n = 7)	–	Hoffman and Curnow, 1979
Great Blue Heron	1-60	United States	Southern Florida	1987-1990	Primary, Tail	3.5 ± 2.3 (n = 7)		Beyer et al., 1997
Great White Heron	1-60	United States	Southern Florida	1987-1990	Primary, Tail	4.7 ± 2.6 (n = 10)		Beyer et al., 1997
Roseate Spoonbill	1-60	United States	Southern Florida	1987-1990	Primary, Tail	2.0 ± 1.5 (n = 32)		Beyer et al., 1997
Black-crowned Night Heron *Nycticorax nycticorax*	–	United States	Ohio	1972-1973	Wing	2.74 [e] (2.25-4.25) (n = 7)	–	Hoffman and Curnow, 1979
Black-crowned Night Heron	21-28	China	Szechuan, Hong Kong	1992	Breast	0.87 ± 0.30 (n = 16)	–	Burger and Gochfeld, 1993

Table 2.12 continued.

Eastern Great White Egret *Egretta alba modesta*	1-70	Korea	Cheonan City	1981	Coverts, Abdominals, Remiges	0.54 ± 0.71 [e] (n = 25)	–	Honda et al., 1986
Eastern Great White Egret	12	Korea	Cheonan City	1981	Coverts	0.23 [e] (n = 1)	–	Honda et al., 1985
Eastern Great White Egret	21-28	China	Hong Kong	1992	Breast	0.27 ± 0.03 (n = 8)	–	Burger and Gochfeld, 1993
Wood Stork *Mycteria americana*	–	United States	Florida	1991	Breast	1.87 ± 0.27 (n = 15)	–	Burger et al., 1993
Wood Stork	1-90	United States	Central Florida	1993	-	3.8 (n = 1)		Beyer et al., 1997
Wood Stork	–	Costa Rica	Tempisque River	1990-1992	Breast	0.51 ± 0.05 (n=36)	–	Burger et al., 1993
Little Egret *Egretta garzetta*	21-28	China	Hong Kong	1992	Breast	2.20 ± 0.88 (n = 7)	–	Burger and Gochfeld, 1993
Pond Heron *Ardeola bacchus*	21-28	China	Szechuan	1992	Breast	2.40 ± 0.70 (n = 5)	–	Burger and Gochfeld, 1993
Piscivorous Marine Birds								
Common Tern *Sterna hirundo*	< 28	United States	New York	1980	Breast	1.40 ± 0.60 (n = 16)	4.41 ± 2.64 (n = 16)	Gochfeld, 1980
Common Tern	–	United States	New York	–	Wing Breast	2.1 ± 0.24 (n = 14) 2.60 ± 0.25 (n = 21)	–	Burger and Gochfeld, 1992
Common Tern	20-23	United States	Massa-chusetts	–	Breast	3.10 ± 0.0002 (n = 21)	–	Burger et al., 1994b
Common Tern	15-22	Germany	Wadden Sea	1991	Back	3.0 ± 0.50 [e] (n = 13)	–	Becker et al., 1994
Common Loon *Gavia immer*	–	United States	Minnesota	1984-1990	Breast	0.86 ± 1.95 [e] (n = 8)	–	Ensor et al., 1992

Table 2.12 continued.

Great Skua *Catharacta skua*	–	United Kingdom	Shetland	1987	Body	1.30 ± 0.40 [e] (n = 40)	–	Thompson et al., 1991
Red-billed Gull *Larus novae-hollandiae scopulinus*	16-26	New Zealand	Kaikoura Peninsula	1988	Body	2.2 ± 1.16 [e] (n = 27)	–	Furness et al., 1990
Black-headed gull *Larus ridibundus*	15-30	Germany	Wadden Sea	1991	Back	0.88 ± 0.53 [e] (n = 36)	–	Becker et al., 1994
Herring gull *Larus argentatus*	24-37	Germany	Wadden Sea	1991	Back	1.27 ± 0.60 [e] (n = 39)	–	Becker et al., 1994
Brown Noddy *Anous stolidus*	–	United States	Hawaii	1990	Breast	0.6 ± 0.003 (n = 20)	–	Burger, 1993b
Franklin's Gull *Larus pipixcan*	–	United States	Minnesota, South and North Dakota, Montana	1994	Breast	1.04 ± 0.11 (n = ?)	–	Burger, 1996
Insectivorous Birds								
Cattle Egret *Bubulcus ibis*	21-28	Puerto Rico	Humacao	1989	Breast	0.28 ± 0.06 (n = 10)	–	Burger et al., 1992b
Cattle Egret	21-28	United States	New York	1991	Breast	1.15 ± 0.35 (n = 24)	–	Burger et al., 1992b
Cattle Egret	21-28	Egypt	Cairo, Aswan	1991	Breast	1.42 ± 0.32 (n = 23)	–	Burger et al., 1992b
Cattle Egret	21-28	China	Hong Kong	1992	Breast	1.30 ± 0.28 (n = 9)	–	Burger and Gochfeld, 1993
Birds of prey								
Bald Eagle *Haliaeetus leucocephalus*	49-77	United States	Oregon, Washington	1979-1981	–	–	1.10(0.07-4.2) [d] (n = 91)	Wiemeyer et al., 1989

Table 2.12 continued.

Bald Eagle	56-77	United States	Oregon	1980-1987	–	–	0.47(0.19-1.4) (n = 15)	Anthony et al., 1993
Bald Eagle	–	United States, Canada	Great Lakes Basin	1985-1989	Breast	9.00(1.5-27.0) (n = ?)	–	Bowerman, 1993
Bald Eagle	42-63	United States	Central Florida	1991-93	Breast	3.23(0.76-14.30)[d] (n = 61)	0.13(0.02-0.61) (n = 48)	Wood et al., 1996
Bald Eagle	–	United States, Canada	Alaska, Great Lakes Basin	1985-1989	Breast, Body, Primary	9.30 ± 6.39 [e] (n = 163)	–	Evans, 1993
Peregrine Falcon Falco peregrinus	23-25	Sweden	Northern	1971-1978	Secondary Rectrix	6.28 ± 2.35 (n = 23)	–	Lindberg and Odsjö, 1983

[a] Not reported.
[b] Mean ± standard deviation, and number of samples examined.
[c] Mean, range in parenthesis, and number of samples examined (standard deviation not reported).
[d] Geometric mean, range in parenthesis, and number of samples examined.
[e] Mercury feather concentration (mg/kg) expressed on a wet weight basis.
[f] Not reported if Hg feather concentration was expressed as dry or wet weight.

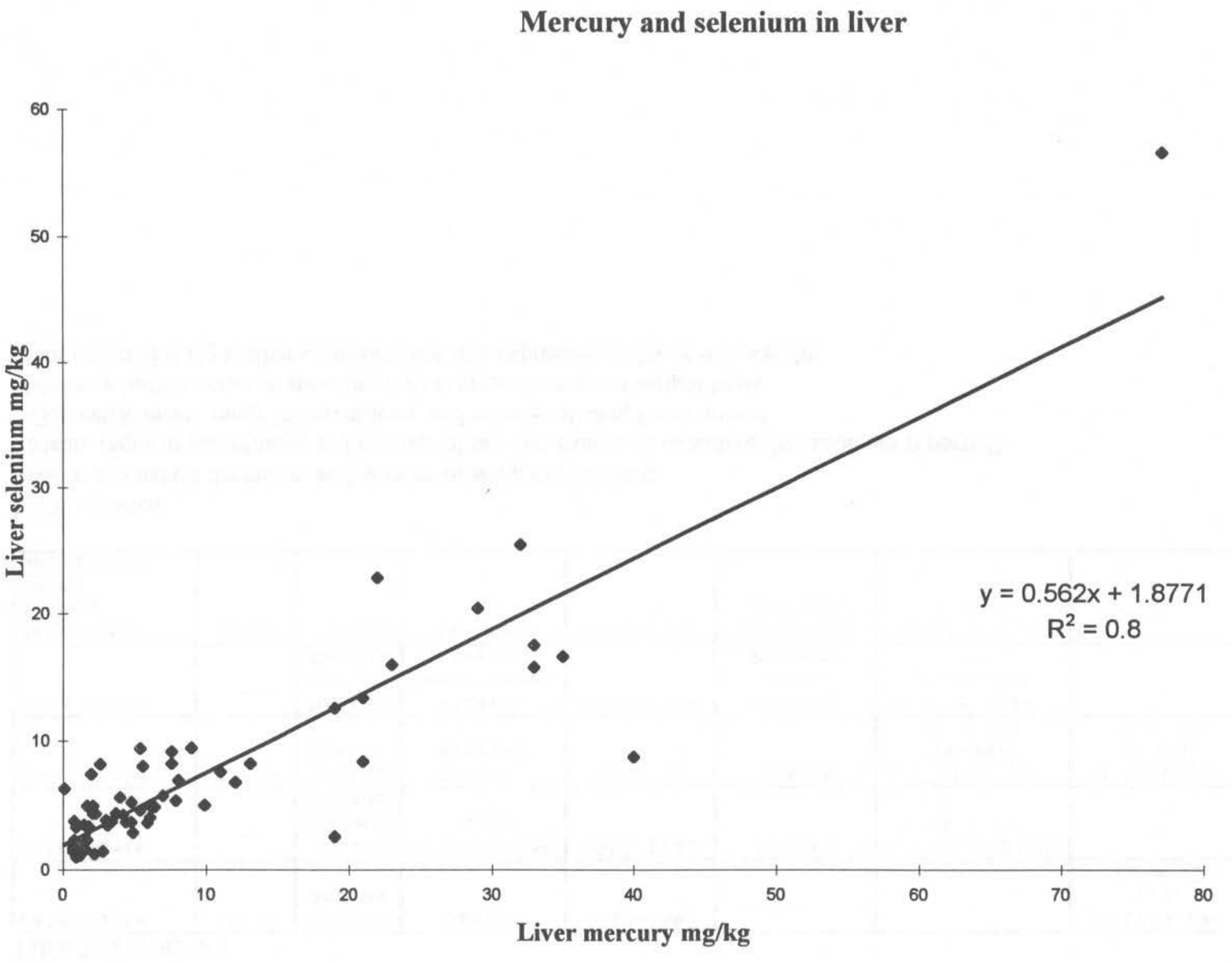


Figure 2.1. Correlation of mercury and selenium in livers of great egrets and great blue/white herons.

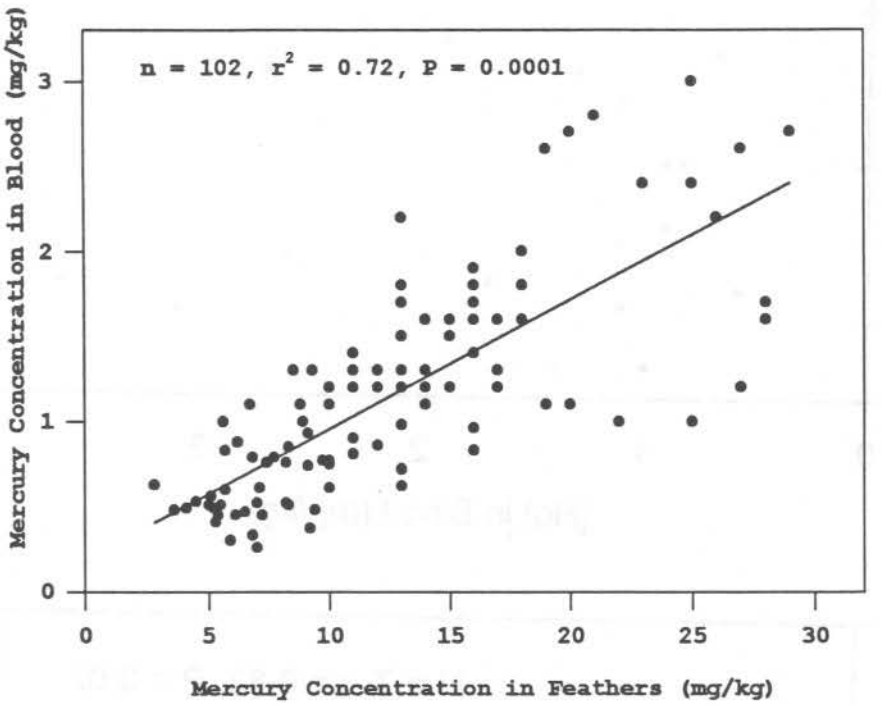
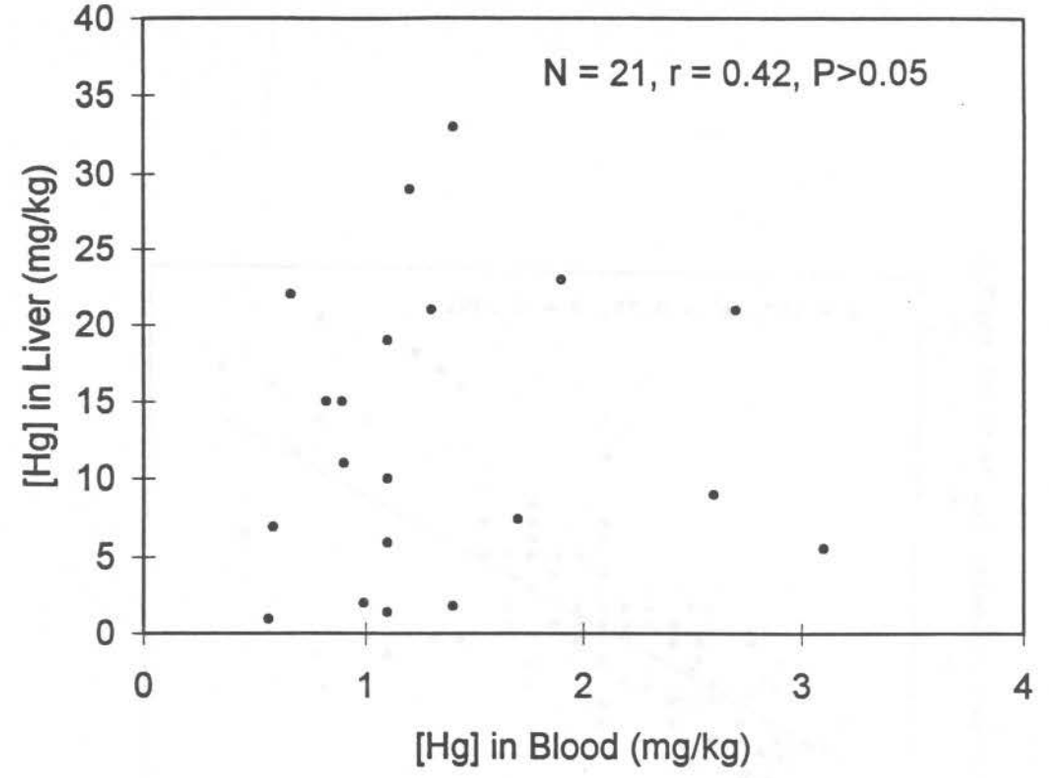


Figure 2.2. Correlation between total mercury concentration in blood and growing scapular feathers (mg/kg) from great egret nestlings. Note data from both 1994 and 1995 are combined, and mercury-dosed birds from 1995 are excluded. Correlations include multiple measurements from the same individuals.



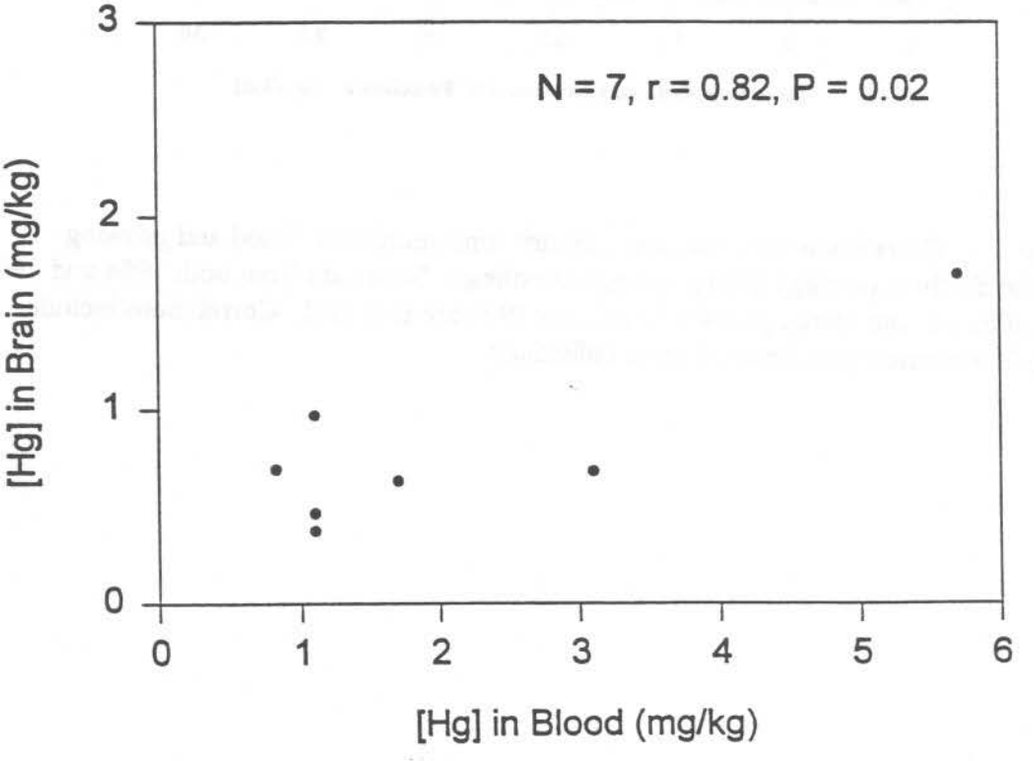


Figure 2.3. Relationship between concentration of mercury in blood, and in liver and brain of great egrets and great blue herons.

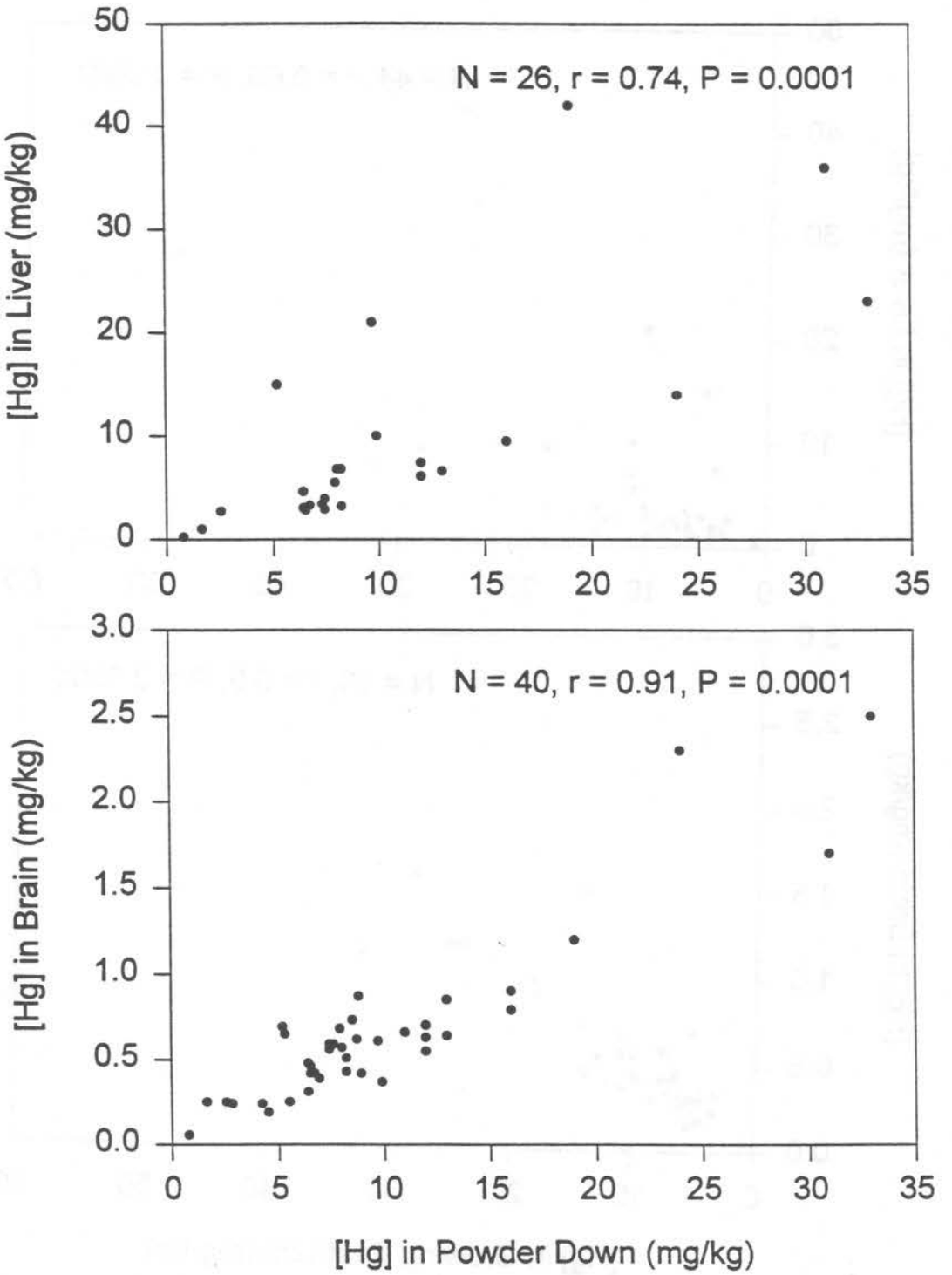


Figure 2.4. Relationship between concentration of mercury in powderdown, and in liver and brain of great egrets and great blue herons.

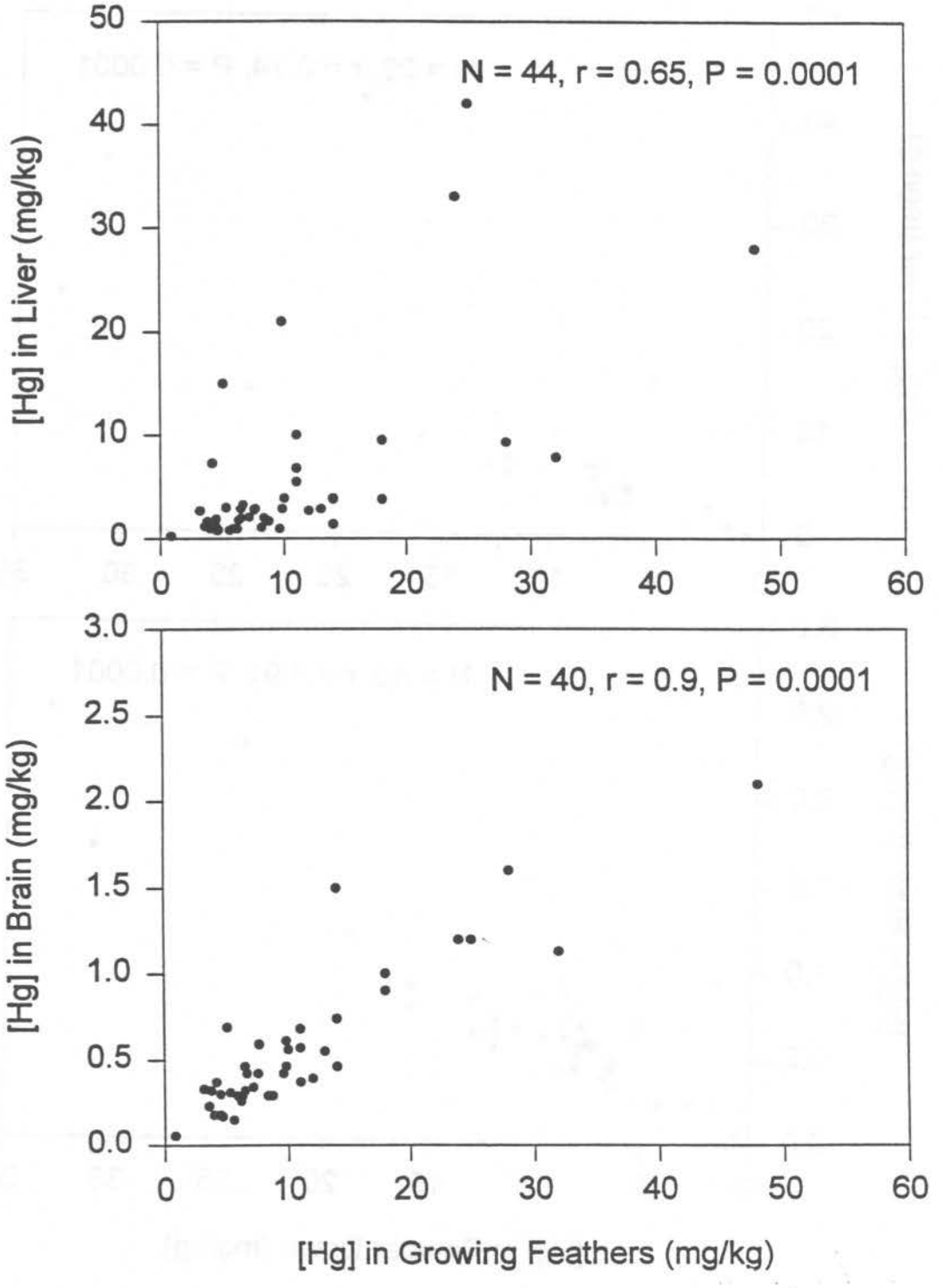


Figure 2.5. Relationship between concentration of mercury in growing feathers, and in liver and brain of great egrets and great blue herons.

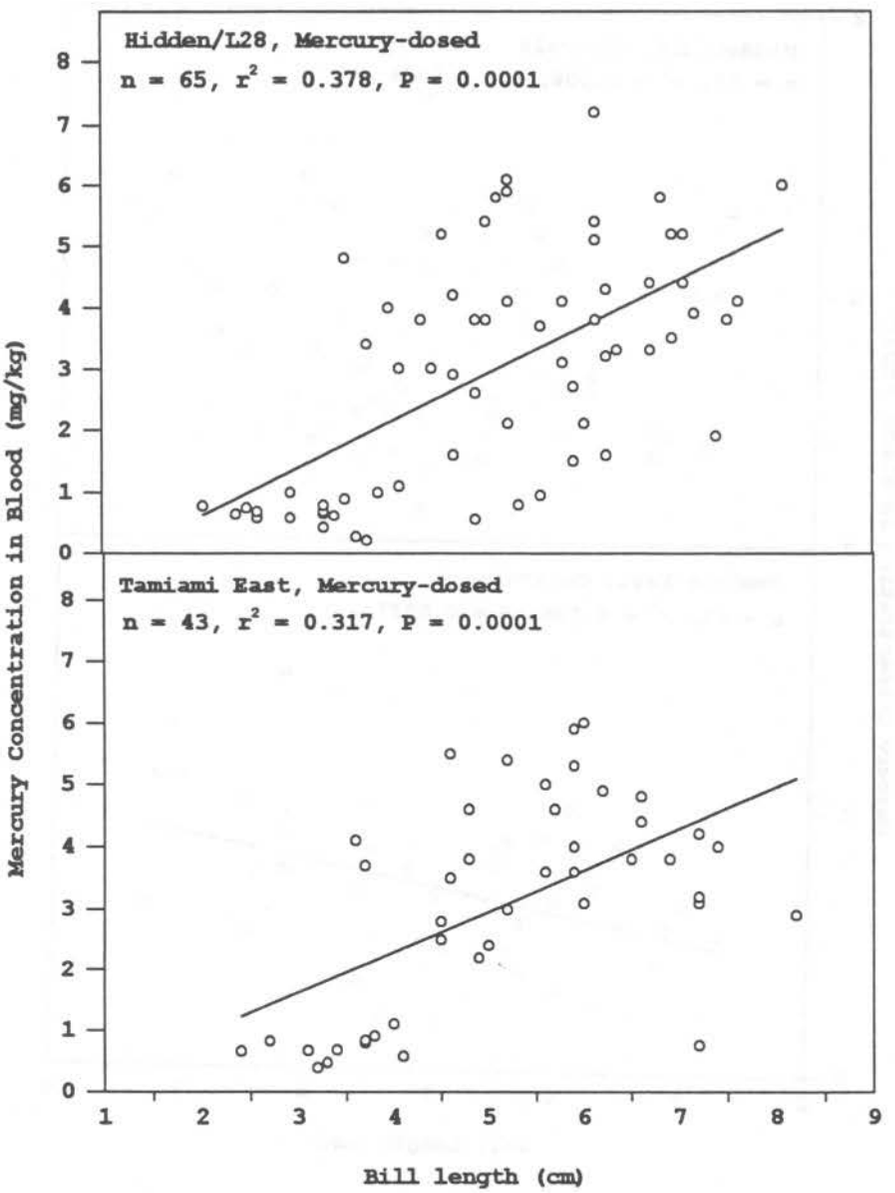


Figure 2.6. Relationship between total mercury concentration in blood (mg/kg) and age (expressed as bill length, cm) of great egret chicks dosed with mercury during the 1995 field mercury dosing experiment, by colony.

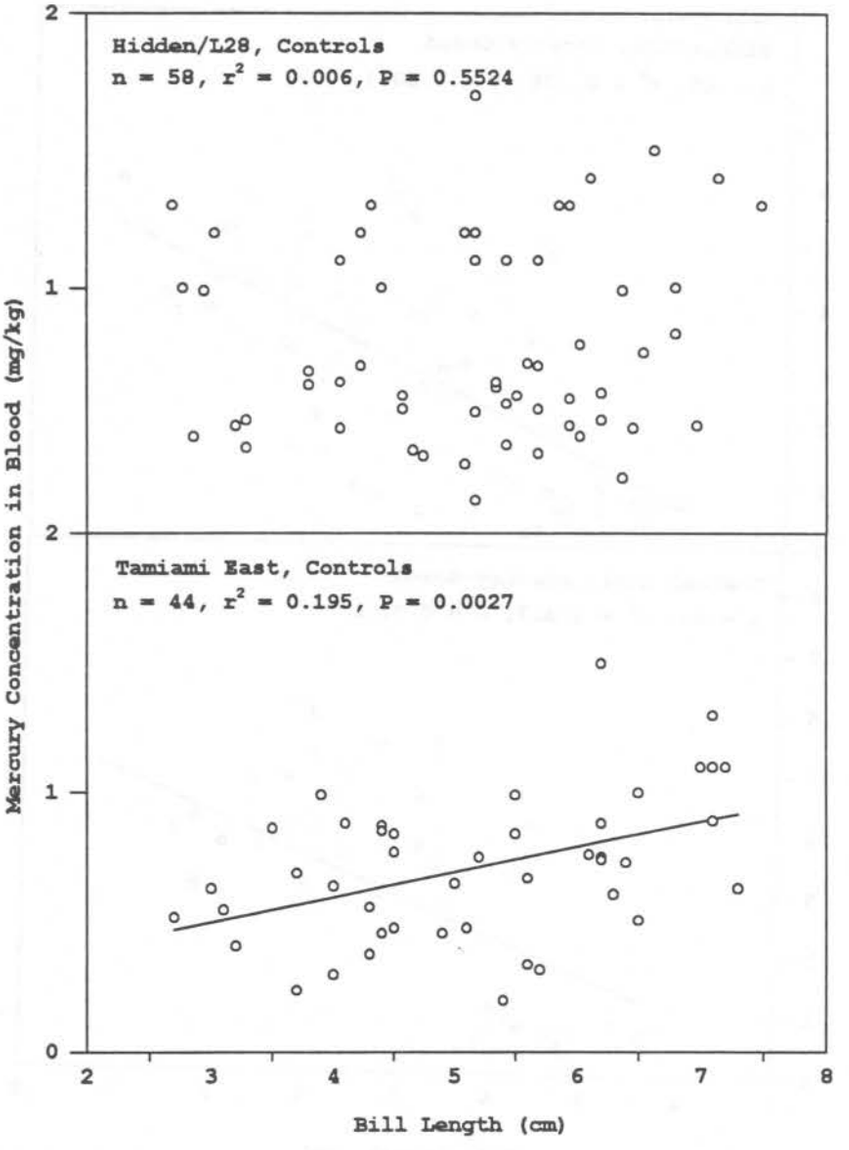


Figure 2.7. Relationship between total mercury concentration (mg/kg) in blood and age (expressed as bill length, cm) of great egret chicks used as controls during the 1995 field mercury dosing experiment, by colony.

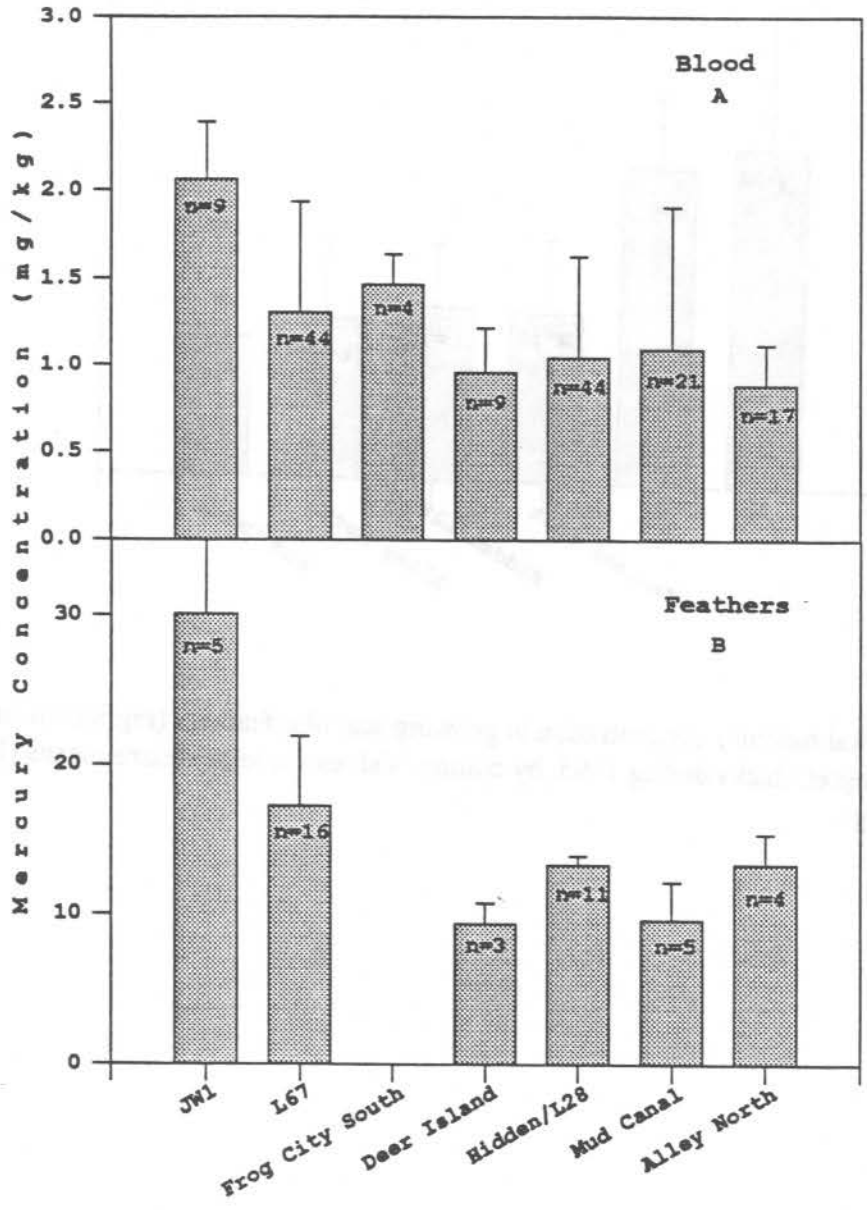


Figure 2.8. Total mercury concentrations in blood (A) and growing scapular feathers (B)(mg/kg) of first-hatched great egret chicks during 1994, by colony. Values are least square means (LSM) corrected by age.

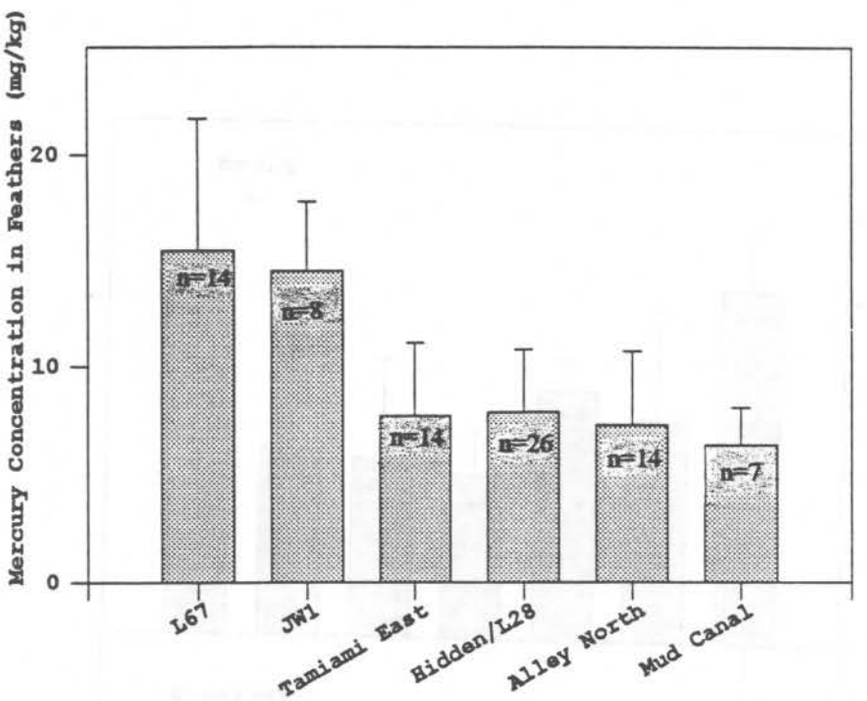


Figure 2.9. Total mercury concentration in growing scapular feathers (mg/kg) of first-hatched great egret chicks during 1995, by colony. Values are least square means (LSM) corrected by age.

CHAPTER III. EFFECTS OF MERCURY ON VISION OF GREAT EGRETS

INTRODUCTION

Organic mercurials are known to affect the visual systems of several species including cats (Davies and Nielsen 1977, Gitter et al. 1988), dogs (Mattsson et al. 1981), rats (Gramoni 1980), swine (Davies et al. 1976, Miller et al. 1976), monkeys (Merigan et al. 1983) and humans (Sabelaish and Hilmi 1979). The effects of methylmercury on vision in avian species have not been extensively studied. Birds have highly developed visual systems on which they rely extensively for foraging and survival. Electrophysiological techniques have been used in many species to evaluate the effects of organic mercury compounds on neuro-opthalmic function. This pilot study assessed effects of mercury on ocular morphology and function using electroretinography, visual evoked potentials, and histopathology in captive egrets dosed with methylmercury.

METHODS

The sublethal effects of methylmercury chloride 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 (Sepúlveda et al. 1995), and had depurated to the point that their blood mercury was well below background levels (<1 mg/kg in blood) (Fig. 3.1). For the vision studies, the A and B birds were re-dosed, and the other two birds (C and D) were used as controls. All birds had one amputated wing, but were otherwise healthy adults.

The birds were housed at facilities provided by the College of Veterinary Medicine, University of Florida, Gainesville. Birds were housed in semi-enclosed concrete-floored chain link fence cages, and were allowed to acclimate 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 implanted osmotic pump (ALZET^{R}, Model 2ML4). This is a miniature pump (5.1 cm x 1.4 cm) released 60 µl/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 of mercury in food consumed, wet weight. We dissolved methylmercury chloride in very small amounts of acetone and propylene glycol to arrive at 8.37 mg methylmercury per mL solution. We then filled the 2 ml pumps with this solution, and implanted them in egrets A and B; egrets C and D were implanted with pumps filled with a solution that contained the same volume of 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. At the end of the fourth week of the experiment great egret A was euthanized and tissues saved for mercury and histopathology analysis.

Electrophysiology

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

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. The a-wave is generated by the photoreceptors (rod and cone cells), and the b-wave arises primarily from Müller and bipolar 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, allowing 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 potentially can be powerful, noninvasive tools in the 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 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 cup-shaped contact electrode placed on the cornea. Recordings were taken under light adaptation and after 15 minutes 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 candela 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 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 electrodes were placed at the medial canthus, caudal to the contralateral ear opening, and the ground at caudal midline.

RESULTS AND DISCUSSION

Three of the four birds (both controls, and one dosed bird) died during the second week of the experiment. Despite liberal use of heating equipment and wind barriers, the most likely cause of death for the controls was probably related to the effects of a single severe cold weather event. 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.

The results of the second dosing experiment are presented for only great egret A (Table 3.1). 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. Mercury concentration in blood increased steadily during the course of the experiment (from 0.51 mg/kg to 21 mg/kg) (Figure 3.2).

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 3.2. These results represent averages for both eyes for 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 3.3 describes peak b-wave amplitudes in the one surviving egret (A) 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 pre-treatment values for great egret A. Figure 3.3 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 3.4. The post treatment b-wave latencies for egret A revealed a comparatively increased scotopic latency, and little or no photopic latency change (Figure 3.4). A-wave amplitudes also exhibited a decrease in average amplitude before and after 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 and 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 an effect on the rod function. Other studies have also concluded that photoreceptor function may be altered (Fox and Sillman 1979, Gitter et al. 1988) and that mercury may act as a phosphodiesterase inhibitor in these cells (Tessier-Lavigne et al. 1985). No studies to date have thoroughly explored this phenomenon in avian species. These preliminary data are at the least suggestive of an effect of mercury, and imply a mechanism for visual debilitation in birds with high tissue mercury concentrations. However, this particular study is weak because it is based on a single bird with few 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 a rationale for further study.

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 high doses of methylmercury 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, and it is unclear whether the structural changes are linked to the electrophysiological results.

Table 3.1 Results of mercury dosing experiment for great egret A during the investigation of the effects of methylmercury on vision.

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	87.3	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 lifetime of 28 days.

Table 3.2 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 3.3 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 3.4 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.

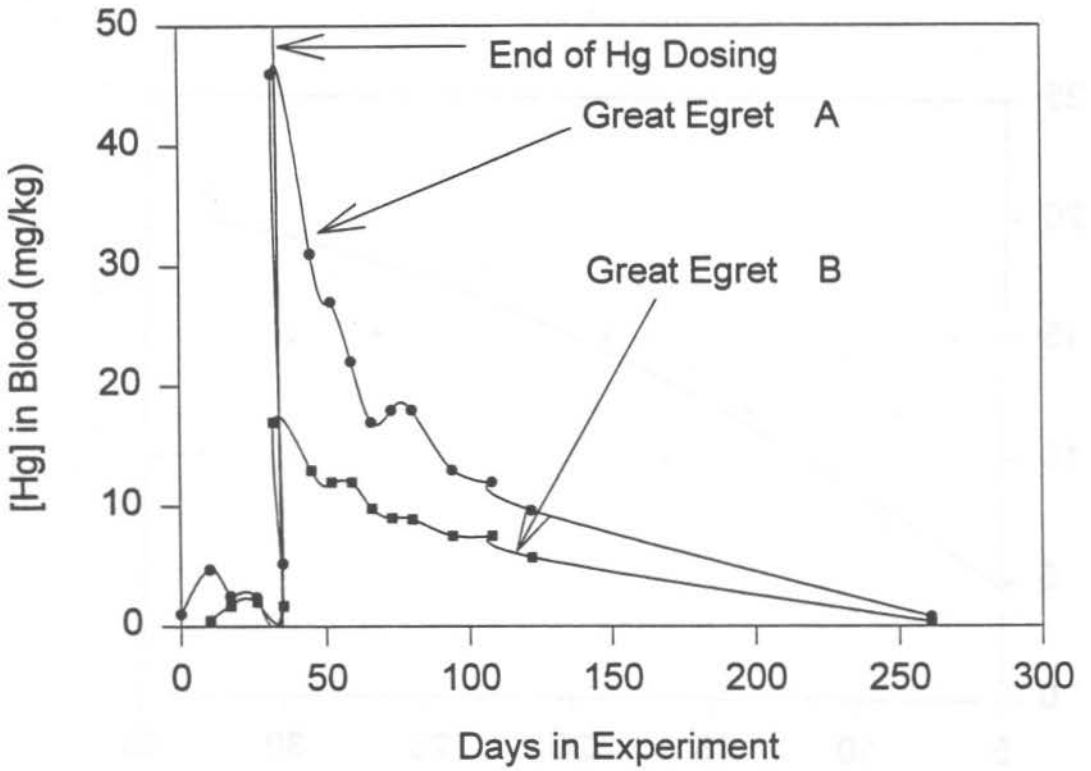


Figure 3.1. Relationship between mercury concentration in blood (mg/kg) from two great egrets (A and B) dosed with methylmercury during 1994, and days since dosing began. See Sepúlveda et al. (1995) for details. Note that egrets A and B had apparently depurated mercury in the bloodstream to pre-experimental levels by 250 days following the end of dosing.

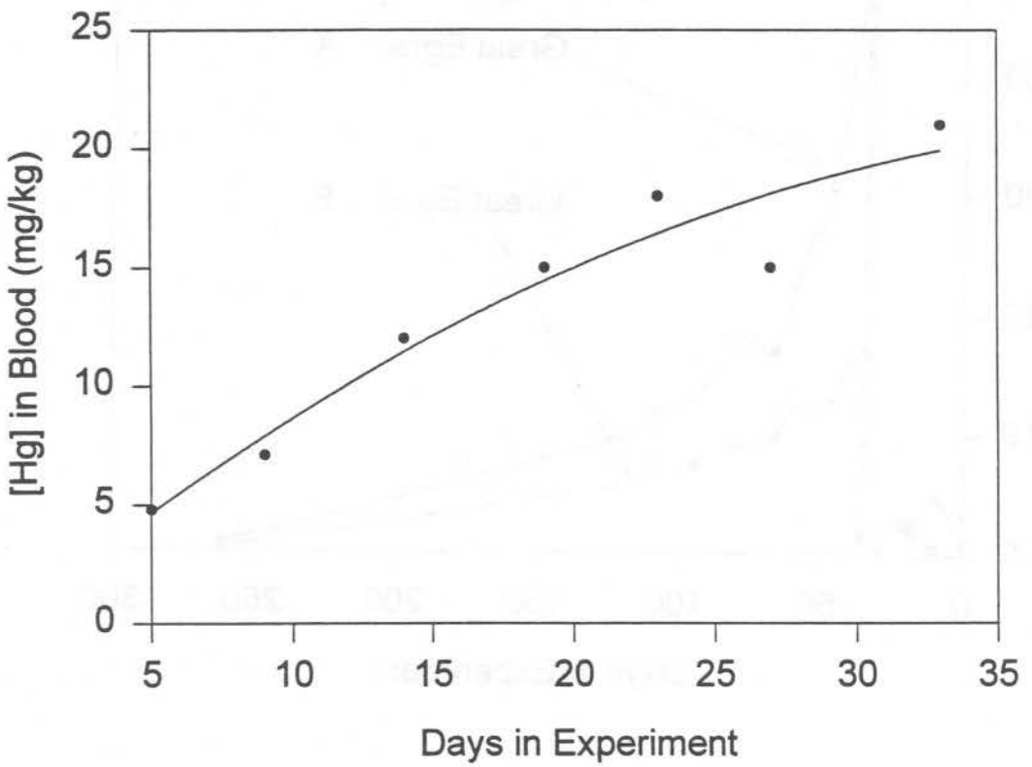


Figure 3.2. Relationship between mercury concentration in blood and days in experiment for great egret A, during the captive mercury dosing experiment carried out in January 1995.

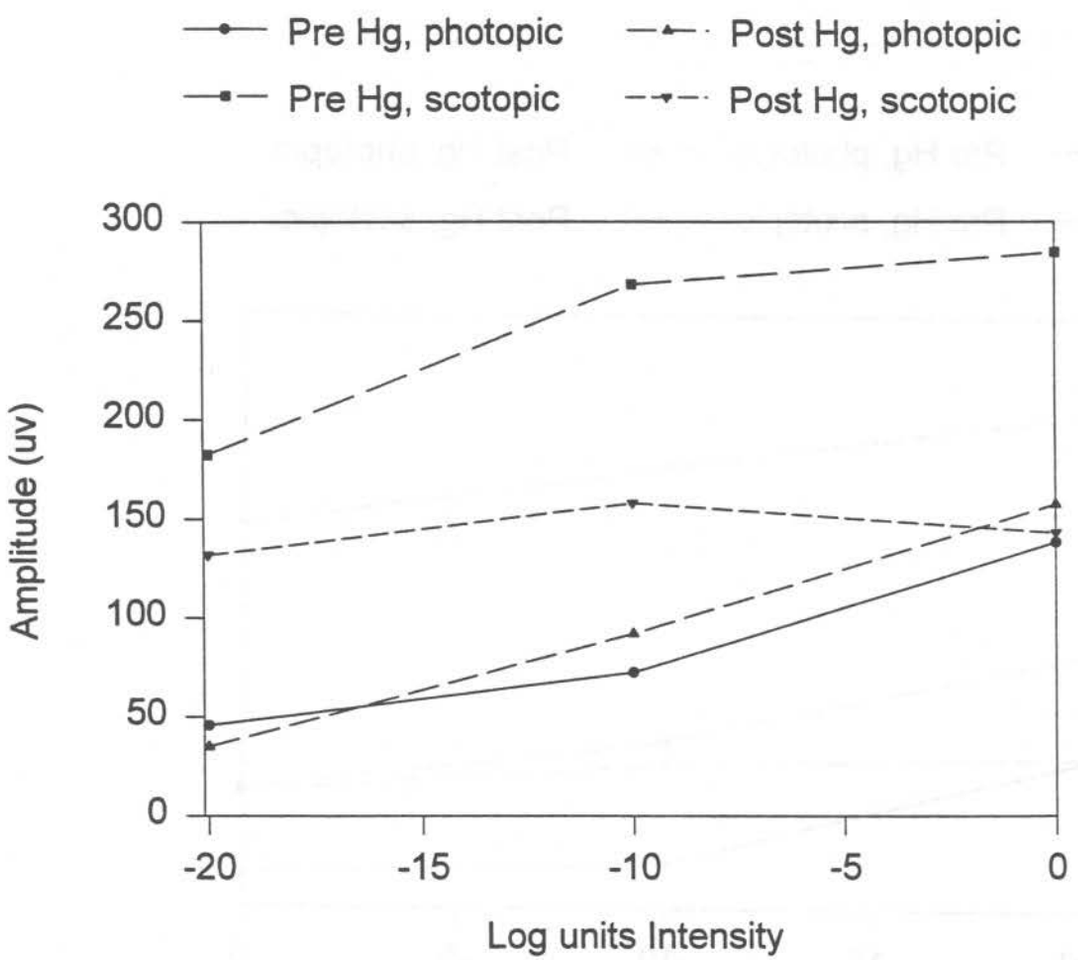


Figure 3.3. B-wave amplitudes (great egret A), right and left eyes combined.

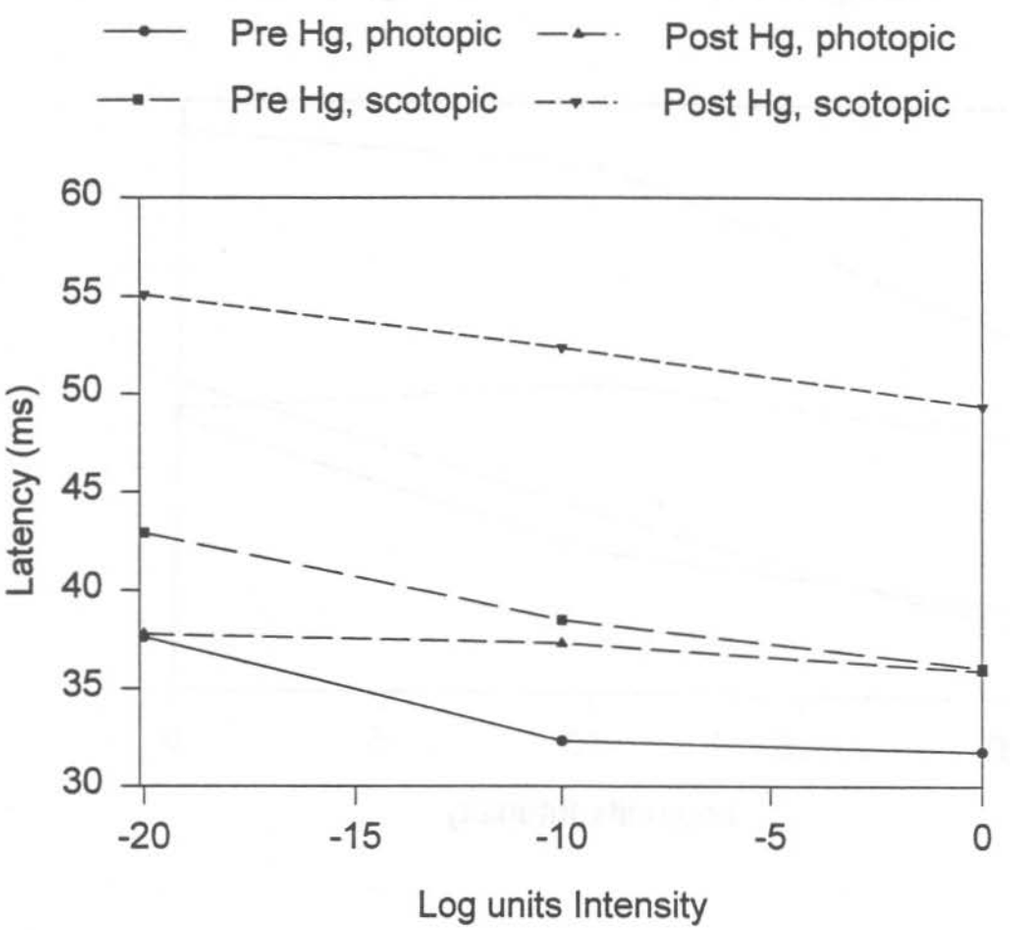


Figure 3.4. B-wave latencies (great egret A), right and left eyes combined.

CHAPTER IV. EFFECTS OF MERCURY ON REPRODUCTION BY GREAT BLUE HERONS

INTRODUCTION

This section focuses on the question of whether exposure of great blue herons to methylmercury can result in poor reproduction and, especially, the inability to come into reproductive condition. One of the most dramatic of the reporductive problems in the Everglades is the low proportion of the birds present that actually come into reporductive condition (20-50%) (Frederick unpubl. data); this implies that the ability to come into reproductive condition is somehow impaired. Mercury contamination has been associated with decreased appetite, increased susceptibility to disease, decreased locomotor skills, and reduced reproductive success (see review in Chapter I). Courtship, nest building, and egg laying are all energetically very demanding activities, and birds must be in excellent body condition to accomplish them. Reduced appetite and increased susceptibility to disease could well result in poorer body condition, and hence, late reproduction or no reproduction. Locomotor skills are undoubtedly important in hunting success, and impaired locomotor abilities may result in the inability to come into reproductive condition. Similarly, reduced locomotor skills could strongly affect the ability to pair with a member of the opposite sex, since mate selection may be based on the performance of extremely stereotyped courtship behaviors.

We therefore predicted that if mercury had effects on the ability to come into reproductive condition, that breeding birds would tend to have lower tissue mercury concentrations than nonbreeding birds. We attempted to test this by collecting tissue samples in breeding and nonbreeding great egrets, and later, great blue herons.

Feathers are accurate indicators of mercury exposure at the time of feather development (see Chapter VI). All members of the family Ardeidae develop specialized feathers early in the breeding season that are important in courtship display. In great egrets these are long plumes emerging from the scapular region. In great blue herons these consist of long thin feathers located in the crown, neck, and scapular region. Most of these feathers then fall out at the end of breeding season. The feathers are grown during the early breeding season, probably August through February, and thus they should be good indicators of mercury exposure immediately prior to pair formation and egg development, a critical time in the reproductive cycle.

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, we realized that the plumes we collected in colonies could not be unambiguously attributed to breeding birds, since nonbreeding birds may roost near active nests and shed feathers.

Great blue herons became the focus of this investigation in 1995 for several reasons. First, there were indications that this species would be more susceptible to trapping than were the great egrets. Second, great blues often nest solitarily in isolated tree islands in the Everglades, presenting the opportunity for collecting feathers that could be unambiguously associated with birds of known breeding status and known breeding success. Finally, a large sample of traumatically injured birds from rehabilitation centers and roadkill collections was available to form a comparable body of samples that were more likely to contain non-breeding birds.

METHODS

In March and April of 1995, we located solitary nests of great blue herons via airboat survey, and used a motorized, radio controlled syringe positioned in nests to immobilize adult birds while sitting on their eggs (Wilson and Wilson 1989). Despite excellent reported success with this device with seabirds and considerable effort (27 attempts involving approximately 400 man-hours), only two adults were captured. 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 end of the eggs to avoid intra-abdominal injection. Because this was difficult to control and because the high rate of nest abandonment was unacceptable, this method was discarded.

During the course of repeated visits to great blue heron nests in 1995, we discovered that adults begin to shed feathers during the postnuptual molt while the chicks are still in the nest. Since the great blue herons nest solitarily, it was therefore possible to collect breeding plumes from these breeding adults, and be sure that there would be no confusion with nestling feathers or with roosting birds (a problem with great egrets in colonies). Published molt chronologies (Palmer 1962, Butler 1992) suggest that any plumes shed at this time would have been grown during the few months or weeks preceding courtship. A review of museum specimens and birds that we collected indicated that growing plumes could be found on birds between the months of August and February. As indicated in other chapters (II and VI), feathers grown early in the nesting cycle might be a more appropriate sample of mercury contamination than a blood sample taken at the time of capture. Shed breeding plumes collected at nests with large young would also ensure that samples would only be collected from birds that had successfully accomplished courtship, nest building, and chick rearing.

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 with half to full-sized chicks were visited early in the morning and the area around the nest was examined for adult breeding plumes (which are easily distinguished from other body feathers and from feathers shed by chicks). Feathers were collected, stored in envelopes, and submitted for mercury analysis.

Feathers were collected from adult great blue herons (n=39) found dead along the road (n=5), dead in the field (n=1), submitted to rehabilitation centers (n=11), sick in the field (n=3), from birds trapped on the nest (n=2), and breeding feathers collected under nests (n=22) from the mainland Everglades. Birds with juvenal plumage were excluded (gray crest feathers). Feathers were categorized as: growing scapular feathers, powderdown, breeding plumes, and mature primaries. Preparation of feathers and analysis was similar to that described in Chapter II. For the analysis of breeding birds, feathers which were collected between January and June were placed into one of three categories 1) at nest with chicks, 2) collected away from nest, with gonad enlargement or breeding plumage or skin color (red/pink legs and feet), indicating strong likelihood of breeding, and 3) collected away from nest with no evidence of breeding plumage,

color, or gonad enlargement. No mature primaries were collected between January and June.

RESULTS

The group of birds with the highest probability of being successfully breeding birds (those at nests with chicks) had higher feather mercury concentrations than those less likely to be successfully breeding (those collected during Jan-June with no evidence of breeding plumage, skin color, or gonad enlargement), Figure 4.1, Table 4.1 and Table 4.2. Birds collected away from nests between January and June with gonad enlargement or breeding plumage had feather mercury concentrations intermediate to, and significantly different from, the other two groups.

Feathers with greater than 30 mg/kg mercury were found only during March-June (Figure 4.2). A similar pattern was found when growing and mature feathers were examined separately.

DISCUSSION

Our finding of higher concentrations of mercury in the feathers of successfully breeding great blue herons than in feathers of non-breeding great blue herons is directly counter to the hypothesis that mercury contamination limits pair formation, egg laying, and the production of live chicks. This conclusion is the first piece of evidence in the search for a link between mercury contamination of the Everglades and reduced breeding, and needs to be supported with additional samples.

Several aspects of these results should be further investigated. We compared mercury concentrations in breeding plumes from breeding birds, with feathers in many cases from the same region (but not breeding plumes) in non-breeding birds. If breeding plumes are fundamentally different in their ability to concentrate mercury than non-breeding feathers, and non-breeders do not grow plumes, then it is not possible to compare breeding and non-breeding birds directly. Powderdown had higher concentrations than plumes according to our correlation analysis in Chapter II (Table 2.4). The relative mercury values in growing breeding plumes and other feather types growing at the same time need to be compared within individual breeding birds in order to test this question. Another unknown factor that needs to be considered is that birds with no evidence of breeding or even those with plumage changes may be sexually immature birds with a shorter exposure history and, thus, lower feather mercury concentrations.

Feather mercury in adult great blue herons was highest during the breeding season (Figure 4.2). This suggests that exposure during these months is greater, and the apparent association with breeding status may therefore be simply an artifact of timing of collection. This greater exposure could be explained by several mechanisms, or by a combination of them. First, larger fish with higher mercury contamination might be particularly available during this time of year, simply because they are exposed during the drying trend typical of this time of year in south Florida. Successful breeders may be more efficient at capturing these larger fish. Second, the greater energy costs associated with breeding could result in greater food consumption and thus greater mercury exposure for breeding birds. We thus have no evidence that mercury exposure, as measured by feather mercury, correlates with the ability of ardeids to come into reproductive condition, but it is equally clear that our results may be confounded by any one of a number of artifacts. In an attempt to use feathers to demonstrate an association between mercury and reproductive success, Bowerman et al. (1994a) found no relationship between mercury

concentrations and nesting success in bald eagles in the Great Lakes region, even in areas where the geometric mean feather mercury concentration was lower (21 mg/kg) than in our study (33 mg/kg at successful nests).

Table 4.1 Summary of mercury concentrations in feathers of adult great blue herons in the Everglades between January and June, by breeding status.

Breeding status*	Mean feather mercury, ppm	Standard error
1	32.92	2.678
2	21.12	3.216
3	5.98	1.346

* Category 1 birds were known to have bred successfully, category 2 birds were carcasses found with evidence of recent breeding (breeding plumes and/or enlarged gonads), and category 3 birds were adult birds found with no evidence of breeding.

Table 4.2. Summary of results of one-way ANOVAs testing for differences in mean feather mercury among adult great blue herons of different breeding status in the Everglades.

Comparison*	df	p
Groups 1 and 3	1	0.0001
Groups1 and 2	1	0.0454
Groups 2 and 3	1	0.0030

* Category 1 birds were known to have bred successfully, category 2 birds were carcasses found with evidence of recent breeding (breeding plumes and/or enlarged gonads), and category 3 birds were adult birds found with no evidence of breeding.

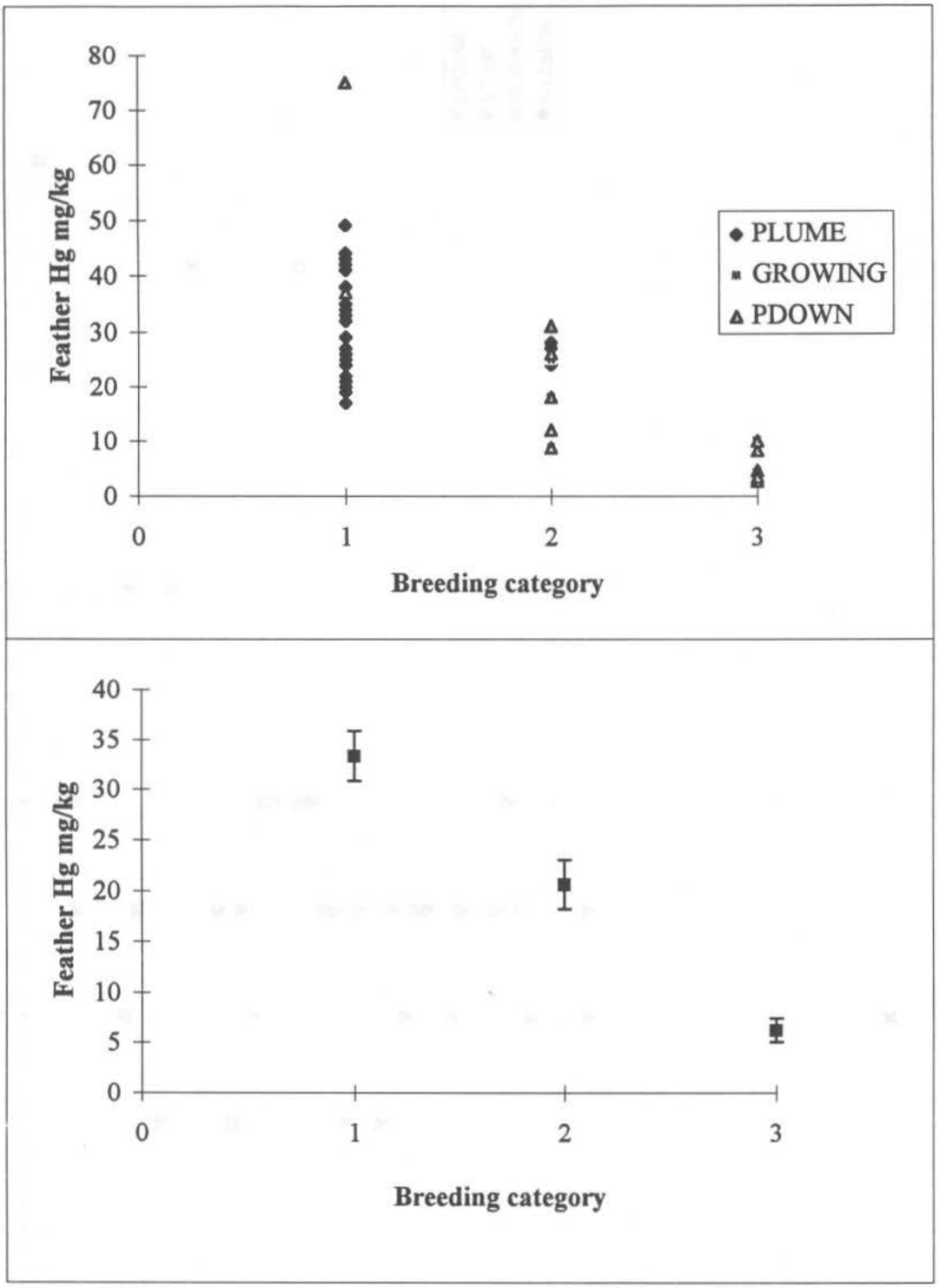


Figure 4.1. Graph of feather mercury concentrations from great blue herons from the Everglades collected between January and June, shown by breeding category (1 = known successful breeder, 2 = breeding plumes or enlarged gonads, 3 = no evidence of breeding). Lower graph shows means and standard error for the same data.

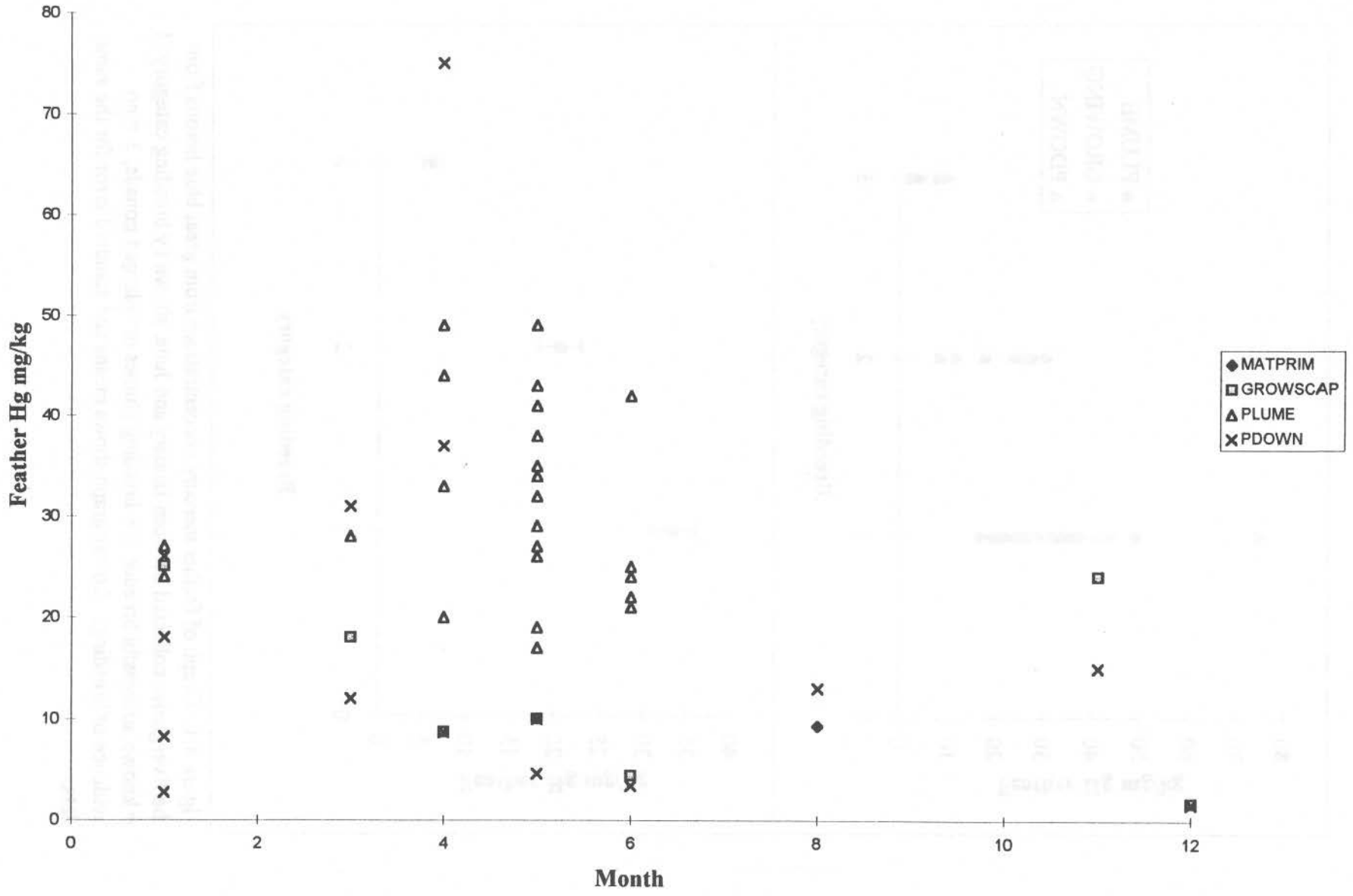


Figure 4.2 Graph of feather mercury concentrations in great blue herons by time of year. All feathers were collected from birds in the mainland Everglades ecosystem.

CHAPTER V. EXPERIMENTAL FIELD STUDIES

INTRODUCTION

This section explores the effects of mercury contamination on nestling, fledgling and juvenile survival, and on pre-fledging growth rates, food consumption and health, in wild, young great egrets. During 1994, health, survival and food intake were measured in a group of young great egrets with naturally variable mercury contamination levels. In 1995, a field dosing experiment was designed to continue this line of inquiry by artificially elevating methylmercury concentrations and comparing the effects to those at ambient levels.

It is important to understand the biological relevance of the comparison of dosed and un-dosed birds in the 1995 field study. In 1994 and 1995, there were high water levels in the Everglades (Frederick 1995). There is evidence that great egrets take smaller prey during wet years (Smith 1994) as a result of deeper surface waters which make larger fish less accessible. Methylmercury concentrations are generally lower in smaller fish (Ware et al. 1990, also see our results on fish mercury concentrations, Chapter VII) so that great egret nestlings fledged during a "high water year," such as 1994 and 1995, are likely to have a lower methylmercury intake than would nestlings in a dry year. Our experiment in 1995 relied on this natural fluctuation, by using undosed birds in a high water year as a "low dose" group, and comparing them to birds dosed to a level that might occur in a dry, higher exposure year.

Artificial elevation of mercury levels through dosing was a logical next step in our work for three reasons. First, the results of field observations are inherently difficult to interpret because of the great variety of uncontrolled influences on the organism. Through experimental dosing with controls, it is possible to isolate more accurately the effects of mercury from other uncontrolled factors. Second, in order to accurately understand the effects of mercury exposure, it is necessary to know the effects of contamination over the range of concentrations to which a wild animal is likely to be exposed. As above, our dosing experiment was designed to mimic the higher methylmercury intake that might occur in a low water year, in order to determine the effects of the upper range of possible exposure levels.

There is evidence in fish that contaminants (including heavy metals) promote increased parasitism, either by impairing the host's immune system or by favoring the survival and reproduction of the intermediate hosts (Khan and Thulin 1991). There is only one report on the effects of mercury intoxication on parasitism in birds. In common loons, high mercury concentrations have been associated with abnormally high infestations with internal trematodes (Graham 1984). Following this line of evidence, the number of oral trematodes (*Clinostomum* spp.) in great egret nestlings was used as an indicator of general health.

Clinostomum spp. is a fairly common trematode parasite of fish-eating birds in southern Florida (Bush and Forrester 1976, Sepúlveda et al. 1994), and birds acquire the oral infection after ingesting fish that harbor the intermediate stages of the parasite. This conspicuous parasite (red-brown in color and about 1 cm long) is confined to the oral cavity and is very easy to count in live birds.

Finally, while the effects of mercury on growth and development, motor coordination, behavior, blood and plasma chemistry, and other health parameters can be

studied under captive or laboratory conditions, its effects on survival, and its effects in combination with natural stresses, cannot. Survival rates have a marked effect on population dynamics on a regional scale, and the effect of mercury on survival of young is therefore critical to management and conservation of wading birds in the Everglades.

METHODS

Design of field studies

Overview of experimental design: 1994 and 1995

During 1994, 46 great egret nestlings from two nesting colonies (Hidden, n = 13 nests, and L67, n = 11 nests, see Figure 5.3) were monitored for growth, food consumption, survival, and mercury concentrations in blood and feathers. All siblings within each nest were monitored. This allowed us to later compare the effect of mercury concentration in blood and feathers with food consumption, growth and survival.

In 1995, we compared of chicks exposed to naturally occurring dietary methylmercury and chicks dosed to an estimated total of 1.8 mg/kg ww diet during a controlled, fifteen day long methylmercury dosing experiment. In this experiment, we included controls to examine the effects of both the dosing procedure, as well as the procedure used to measure food consumption. We compared growth, food consumption, health and survival among dosed and undosed groups.

Food consumption and growth

The labeled water technique for measuring food consumption

Food consumption of great egret nestlings was measured using isotopically-labeled water (deuterium in 1994 and tritium in 1995). This method confers several advantages over other food measurement techniques such as nest scales (n, inferring food deliveries to young from instantaneous weight gain using changes in nest mass) or collaring (c, constriction of the esophagus to prevent passage of food to the stomach):

1. Food consumption can be measured continuously, rather than periodically (n,c).
2. Individual food consumption can be measured (n).
3. Nestlings are not visited daily, thereby reducing colony disturbance (n,c).
4. Since measuring food with labeled water method does not require observer attendance, a larger sample can be achieved with the same amount of effort (n,c).
5. The labeled water method is an accurate way to measure food consumption (Nagy 1989, Table 5.1), while the accuracy of nest scales and collaring is unknown (n,c).

How the labeled water technique works

Isotopically-labeled water, when injected into an organism, can serve as a marker of the volume of water contained within its body. By measuring the change in concentration of

such a marker over a known interval, it is possible to calculate the amount of water that has passed through the organism (influx + efflux = water turnover, Lifson and McClintock 1966, Nagy and Costa 1980). The most common way to measure these changes in marker concentration is through an assay of the water contained in the organism's blood. Food consumption may be calculated from water turnover if the following assumptions are met:

1. The organism does not drink water during the measurement interval. Great egret nestlings, at the ages studied in our project, are largely confined to the nest and have no access to drinking water.

2. Water turnover not due to food consumption can be accounted for. The only other sources of water for nestling birds are metabolic water produced during digestion, and water exchanged with the atmosphere through respiration and across skin. Using equations that take into account the mean size of great egret nestlings at the ages used in our study, the high relative humidity of breeding colonies in the Everglades, and a diet comprised primarily of fish, it was estimated that the proportion of water turnover due to metabolic water production and exchange across skin and lungs would be approximately 15 percent.

3. Water content of food items is known. Water content of aquatic Everglades animals, including many great egret prey items, has been measured (Kushlan et al. 1986). Using water content data from Kushlan et al. (1986), and knowing the composition of fish species found in regurgitant samples of great egret nestlings in 1994 (see Chapter VII), we estimated prey consumed by great egret nestlings to contain an average of 72 percent water, and used this percentage in our calculations.

4. Measured mass of the animal includes only the mass of body components i.e., stomach is empty. For great egret nestlings, this assumption is met by entering nesting colonies shortly after daylight before nestlings have been given their first meal of the day.

5. The deuterium, or tritium, labels body water only. There is evidence that a portion of the injected tritium label can bind to other molecules besides water within an organism (Nagy and Costa 1980), a phenomenon that may be more pronounced in growing organisms (Williams and Nagy 1985). However, in an experiment with growing partridge chicks (*Alectoris chukar*), it was found that <2 % of injected tritium was incorporated into organic molecules over a 24 hour period (J. B. Williams unpubl. data). Even if 10% of injected tritium were incorporated into organic molecules (an extreme example) water flux would only be overestimated by approximately 15 %, which would still compare favorably with other field methods of determining food consumption (Williams and Nagy 1985). Additionally, such an error would be systematic and would not affect statistical comparisons of food consumption between chicks.

Isotopes used

Deuterium and tritium, which are isotopes of hydrogen, can both serve to label water contained within an organism. Tritium is radioactive, while deuterium is not. In 1994, we used deuterium-labeled (deuterated) water for food consumption measurements. We switched to tritium-labeled (tritiated) water in 1995 for three reasons. First, tritiated water has a much longer biological half-life than deuterated water, allowing a sampling interval of five days, rather than three. Second, tritium is less time-consuming to analyze than deuterium. Finally, tritium analysis is substantially less expensive than deuterium analysis.

Use of tritium in the field

As a radioactive isotope, tritium is a hazardous material that must be handled with care. The tritiated water used in our field work was refrigerated at 4° C inside a locked field laboratory trailer. In accordance with University of Florida policy, the laboratory trailer displayed the required radiation warning signs, both inside and out. Weekly swipe surveys were conducted to ensure there was no contamination of field transport equipment or laboratory surfaces. Latex gloves and protective clothing were worn at all times where tritium was, or had been, used. Tritium was transported to and from field sites in properly labeled containers. Radioactive waste generated in the field was properly labeled, double-bagged, and transported directly to labeled waste containers in the field laboratory. All personnel involved with the use of tritium submitted monthly urine samples to University of Florida Radiation Control and Radiological Services to monitor for internal exposure to radiation.

Determining labeled water concentrations and calculating food consumption

The following is a description of the laboratory procedures and calculations used to estimate food consumption of nestlings. The distillation procedure and food consumption calculations are the same for both tritium and deuterium. Concentrations of the two isotopes are measured quite differently, however.

Distillation of water from blood samples

To measure deuterium or tritium concentrations of water contained in blood samples, it is necessary to first distill the water from the blood. We extracted water from nestling blood samples through vacuum distillation. Distilling water from blood under a vacuum both accelerates the distillation process, and more importantly, greatly reduces contamination of the sample from water in ambient air.

Capillary tubes containing blood samples were placed in ball-and-socket joints which were connected to a vacuum manifold (Figure 5.1). After the vacuum line reached a pressure of 2.5×10^{-2} TORR, the stopcock connecting the capillary tube to the vacuum line was closed. The ball-and-socket joint was then twisted, cracking the capillary tube, and releasing the blood sample. Heat was applied to the blood, causing the water within it to vaporize. Water vapor condensed into an ampule which was immersed in a cold trap of liquid nitrogen. The ampule containing the frozen water sample was then flame-sealed and removed from the vacuum manifold.

Deuterium analysis

Flame-sealed ampules containing distillate were broken and 4 μl of the water sample was pipetted into a new ampule containing 400 mg of zinc reagent (Biogeochemical Laboratories, Indiana University). The water sample in the new ampule was then quick-frozen by immersing the tip of the ampule in liquid nitrogen, and once again placed under a vacuum and flame-sealed. After vacuum-sealing, the water/zinc mixture was baked at 500°

C for 30 minutes, causing the water to react with the zinc reagent to produce zinc oxide and deuterium/hydrogen gas.

Sample deuterium/hydrogen ratios were measured at the University of Miami, using a VG Isotech™ mass spectrometer. The mass spectrometer was equipped with a tube-cracking apparatus which broke the ampule and released the deuterium/hydrogen gas sample into the spectrometer. Deuterium/hydrogen ratios were then converted to atom excess percentages (Hayes 1992) used in calculating water turnover.

Tritium analysis

Three 10 µl subsamples of each sample of tritiated water distillate were pipetted into separate 7 ml borosilicate glass scintillation vials. Five ml of Scintiverse™ biodegradable scintillation cocktail was placed in each vial. Sealed vials were agitated for thirty seconds to thoroughly mix the distillate and scintillation cocktail. Above-background tritium levels were then measured using a Beckman™ scintillation counter. Because the distillate subsamples consisted only of tritiated water, and equal amounts of subsample were placed in each scintillation vial, there was no need to control for differences in quench among subsamples. Means of the triplicate samples (counts per minute) were used in food consumption calculations.

Blood samples containing tritium were stored, handled, and disposed of in accordance with safety protocols established by the Radiation Control and Radiological Services Department of the University of Florida's Division of Environmental Health and Safety. In addition, weekly swipe surveys were conducted to ensure no contamination of laboratory surfaces or equipment as per Division of Environmental Health and Safety regulations.

Calculating food consumption

The following two equations were used to calculate food consumption, one to calculate water turnover, and a second to convert water turnover to a daily rate of food consumption:

1. Water turnover (Nagy and Costa 1980):

$$TO = (2000 \times (Bw_f - Bw_i) \times LN ((H_i \times Bw_i) / (H_f \times Bw_f)) / ((M_i + M_f) \times LN (BW_f/BW_i) \times t) + (((2000 \times (Bw_f - Bw_i)) / (t \times (M_i + M_f)))$$

where:

TO = water turnover (ml/kg/d)

BW_i[1] = initial body water volume of chick (ml); $0.7 \times M_i$

Bw_f[1] = final body water volume of chick (ml); $0.7 \times M_f$

[1] This estimate of % body water was not validated, and is based on body water measurements of other bird species. Adult body water volume of most species of birds is equal to approximately 65-70 % of body mass (Ellis and Jehl, Jr. 1991). However, water content of some muscles in domestic fowl decreases from nestling to adult (Ricklefs 1983) and body water content was found to be 73 % in a study of nestling savannah sparrows (Williams and Nagy 1985). We chose to use 70 % as a compromise, representing the upper range of adult body water percentages, but less than the Williams and Nagy (1985) estimate for savannah sparrow nestlings.

H_i = initial concentration of labeled water in chick (expressed as atom excess percentage for deuterium or counts per minute for tritium)

H_f = final concentration of labeled water in chick (expressed as atom excess percentage for deuterium or counts per minute for tritium)

M_i = mass of chick at time of injection (g)

M_f = mass of chick at the end of the food measurement interval (g)

t = length of interval (d)

2. Food consumption:

$FC = (TO / 0.87) \times (((0.001 \times M_i) + (0.001 \times M_f) / 2)$ where:

FC = food consumption (g/d)

TO = water turnover (ml/kg/d)

0.87 = mean water content of food items (72 %) + metabolic water (7.5 %) + respiration water (7.5 %)

M_i = mass of chick at time of injection(g)

M_f = mass of chick at the end of the food measurement interval (g)

Field Procedures

All nestlings from 1994 and 1995

Colonies were entered just after daylight every third (1994) or fifth (1995) day in order to minimize heat stress on the chicks, and to meet the empty stomach assumption for food consumption calculations (see above). We weighed each chick to the nearest gram, or 5 g, using a 300 g or 1000 g Pesola™ scale, depending on size. Culmen (bill) measurements were taken to the nearest millimeter using rulers. We palpated the abdomens of chicks to check for *Eustrongylides ignotus* parasites (Spalding 1990), and visually checked for the oral parasite, *Clinostomum* sp. During the last visit to each chick (approximately 28 days of age) we placed radio transmitters affixed to blank aluminum leg bands on one tarsometatarsis, and a U. S. Fish and Wildlife Service leg bands on the other.

Field Techniques for Food Consumption

Nestlings were injected intramuscularly in the thigh with either deuterated water (1994, 3 % or 10 % ^{2}HHO solution at the rate of 0.09 g^2 H/kg body mass, Nagy pers. comm.) or with tritiated water (1995, 1 mCi ^{3}H/kg body mass, Williams and Nagy 1985). Younger nestlings were immediately returned to their nest after the injection, while older, ambulatory chicks (ca > 21 d) were detained in cloth pillow cases hung in the shade under the nest tree. A 0.3 ml blood sample, representing the initial, equilibrated label concentration, was taken

from the nestling's jugular vein one hour post-injection. Afterward, nestlings were immediately replaced in their nests. Blood samples were flame-sealed in capillary tubes for later analysis. In 1994, capillary tubes were flame sealed in the field, but in 1995, blood was placed in heparinized 3 ml Vacutainers™ and flame-sealed in capillary tubes upon returning to the field lab. On each ensuing visit to a nest, a blood sample was taken, pre-injection, for use as a measure of the final label concentration for the previous interval (Figure 5.2). The chick was then reinjected with labeled water and re-bled according to the procedure above.

Field techniques for mercury analysis

A tuberculin syringe was used to take a 0.5 ml blood sample from the jugular vein for measurement of mercury concentration. Blood samples were placed in a 3 ml heparinized Vacutainer™ for later mercury analysis. During the last visit to a chick, two or three growing scapular feathers were also taken for mercury analysis.

1995 field dosing experiment

Seventy-seven chicks from two colonies were used in the 1995 mercury field dosing experiment, including 45 from Hidden colony and 32 from Tamiami East colony (Figure 5.4). Of these chicks, five either died during the experiment or were not captured close to the end of the 15-day dosing period so that only 72 were used in the analysis of food consumption results.

Within each colony, the sample was divided between two transects or paths through the colony, with each transect containing approximately equal numbers of experimental and control nests. In order to avoid biases due to hatching order within a nest, only first-hatched chicks were used in the experiment. The age of the chicks was determined from egg laying and hatching chronologies and they were an average of 11.6±5.2 days old (3.7 cm bill length) at the beginning of the experiment. Each chick was involved in the experiment for fifteen consecutive days, or until they proved too mobile to recapture.

Chicks were divided among three treatment groups (Figures 5.4 and 5.5), two of which were included in the mercury dosing procedure. The groups were:

1. Food Measurement: Chicks in this group (n = 42) were monitored for food consumption using tritiated water. Every 2.5 days for 15 days, half of the chicks were dosed with gelatin capsules containing 0.5 mg of methylmercury chloride (*see Rationale for Methylmercury Dose Rate* below) and the other half received empty capsules or placebos. Every five days, blood samples (2) were taken for estimation of food consumption and mercury. The weight and culmen length of Food chicks were taken every five days. Growing scapular feathers were collected for mercury analysis during the last visit to a nest. U. S. Fish and Wildlife Service leg bands and radio transmitters were placed on nestlings during the last visit to a nest to monitor later survival.
2. Food Measurement Control: To test for an effect of the food consumption monitoring procedure (injecting with tritiated water; taking two, rather than one, blood samples every five days) in Food group chicks, Food Measurement Control chicks (n = 22-g/27-m) were treated identically to Food chicks except for the food monitoring procedure. Food Measurement Control chicks were measured, growing scapulars were collected and each bird was banded with a radio. Twelve chicks were dosed with methylmercury (Hg) and

ten received placebos (Placebos).

3. Handling Control: This group (n = 8) was used to test for effects of the mercury dosing procedure (force-feeding capsules to chicks, taking a single blood sample every five days). Note that this is not the same as testing for the effects of methylmercury, which was the role of the placebos. Handling Control chicks were treated exactly the same as Food Measurement Control chicks except no blood samples were taken and they were not force-fed capsules; otherwise, they did get the same measurement schedule.

Rationale for the methylmercury dose rate

The supplemental dose of methylmercury was designed to mimic the methylmercury burden nestlings would presumably receive in a dry year, as explained above. The methylmercury dose rate was calculated using information on preferred sizes and species of fish eaten by great egret nestlings (see Chapter VII), available data on mercury content of Everglades fishes (W. Loftus pers. comm.), and the mean rates of food intake that we calculated were ingested by great egret chicks during 1994 (Chapter VII). It was estimated that great egret chicks, at the ages to be included in the experiment, ingested methylmercury at a rate of 0.63 mg Hg/kg food (wet weight).

In total, dosed birds received 1.76 mg methylmercury/kg food which approximately tripled the rate of daily mercury intake that a great egret nestling (e.g. placebos) would have been expected to ingest during 1994 in the Water Conservation Areas (0.63 mg Hg/kg food from natural ingestion of fish + 1.13 mg Hg/kg food from supplement). The total dose rate of 1.76 mg Hg/kg food (wet weight) is within the range of current mercury concentrations for larger fish in the Everglades (Ware et al. 1990, Figure 5.6), and could be a typical dose rate even during wet years if concentrations of mercury increase from their present levels in the Everglades.

Methylmercury: dosing and handling

Food chicks and Food Measurement Control chicks were given either 100 µl gelatin capsules containing 0.5 mg methylmercury (Hg), or identical empty capsules (placebo) respectively, every 2.5 days for 15 days (7 doses, total supplemental dose = 3.5 mg methylmercury). The 0.5 mg dose of methylmercury corresponded to the desired supplemental dose rate of 1.13 mg Hg/kg food [dose rate (mg/kg food) = dose (mg/unit time)/food consumption rate (kg/unit time)].

Capsules were filled with methylmercury by first placing them under a fume hood and then pipetting a methylmercury/acetone solution of known concentration into them. The acetone evaporated in minutes, leaving only methylmercury inside the capsules. While still under the fume hood, capsules were fitted together and placed in airtight containers.

In the field, latex gloves and forceps were used when dosing chicks with methylmercury capsules. Capsules (held in forceps) were coated with a drop of vegetable oil, placed inside the nestling's mouth, and gently massaged down its throat. The birds were monitored for at least one minute to ensure that the nestling did not regurgitate the capsule.

Mercury analysis

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 digest the blood, and the sample was thoroughly mixed. Total mercury concentration

(mg/kg) of blood and feather samples was measured by Florida Department of Environmental Protection (DEP) chemists using flame atomic absorption spectrophotometry (Hatch and Ott 1968, see Chapter II of this report for a detailed description of this procedure).

Health and survival

Methods for assessing health

During 1994 and 1995 we monitored the health of a sample of the experimental and control chicks. The general health of great egret nestlings was also evaluated by counting the number of oral trematodes (*Clinostomum* sp.), monitoring packed cell volume, refractive index of plasma (a measure of the concentration of plasma proteins), and number of white blood cells. In general, the health of great egret chicks was evaluated at least twice, at the beginning and at the end of the sampling of each nest, and from all chicks within a nest.

For the determination of packed cell volume, approximately 30 µl of blood was collected in a 40 µl microhematocrit plain capillary tube and centrifuged at 10,000 r.p.m. for 10 minutes in a hematocrit centrifuge. After each packed cell volume determination, the capillary tube was broken at the plasma level, and a couple of plasma drops were used to determine the refractive index. The number of white blood cells was determined using a commercially prepared diluent (Eosinophil Unopette Test 5877, Becton-Dickinson Vacutainer Systems, Rutherford, New Jersey 07070, USA) by first counting the number of eosinophils in a Neubauer hemacytometer (granulocytes were counted in 10 cells, five in each counting area), multiplying this number by 32, and dividing it by the proportion of granulocytes (eosinophils, heterophils, and basophils). The proportion of granulocytes was calculated by counting 100 white blood cells (differential count) after fixing and staining a thin blood smear with LeukoStat Stain Kit© (Fisher Scientific, Hampton, New Hampshire, USA).

During 1995, chicks from the field dosing experiment were monitored and packed cell volume, refractive index, number of white blood cells, and number of *Clinostomum* spp. were determined using the methods above. Eleven of the chicks were dosed chicks (Tamiami East colony, n = 6, and Hidden/L28 colony, n = 5) (Food Measurement Control/Hg group) and 10 were Food Measurement Control/Placebo group chicks (Tamiami East colony, n = 4, and Hidden/L28 colony, n = 6) . All health indicators were measured once prior to the start of the dosing experiment (day 0), and at days 5, 10, and 15 after the start of the experiment.

Analysis of data on health indicators

1994: Packed cell volume, refractive index, number of white blood cells, and oral parasitic load were used to define a criteria that divided great egret chicks into two groups: "sick" birds and "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 43% were considered dehydrated, while those with a packed cell volume of less than 28% were considered anemic. Similarly, chicks with more than 7.4 g/dl or less than 5.2 g/dl of plasma proteins were considered "sick". It was also assumed that chicks that had more than 35,500 white blood cells/µl were experiencing an immunological response, while those that had less than 10,500 white blood cells/µl were immunodepressed. Parasitic loads of over 11

individual *Clinostomum* spp. were considered 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 scapular feathers of great egret nestlings were divided into two categories: "high" (> 2.01 mg/kg and > 25.01 mg/kg, respectively) and "normal" (< 2.00 mg/kg and < 25.00 mg/kg, respectively). All these groups were defined so that "sick" or "high" corresponded to the highest 10% of values, and "healthy" or "normal" to the remaining 90%. Since some of the variables used in this analysis are known to be affected by age of the chicks (packed cell volume, plasma proteins, number of oral parasites), birds were divided into two age classes based on bill length. 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 greater than 4.1 cm. Contingency 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 by hatch order.

1995: The effect of treatment (mercury and control) on blood parameters (packed cell volume, refractive index, number of white blood cells, and number of eosinophils, heterophils, and lymphocytes) and on number of oral parasites was studied using a Repeated Measures ANOVA (PROC GLM, SAS Institute, 1988). Basophils and monocytes were rarely found in blood smears, and thus were not included in this analysis. This procedure compared the means for all these variables between treatments (11 mercury-dosed birds and 10 controls) at days 0, 5, 10, and 15 of treatment.

Methods of monitoring survival

1994: Between mid-April and mid-May 1994, a total of 46 great egret chicks (30 nests) were marked 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; range of frequencies 165.034 - 165.915) attached to them. The plastic band and the radio transmitter weighed approximately 15 g (less than 4% of the weight of the chick in all cases). 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 25 days of age. Nestlings were marked in five different colonies: Hidden/L28 (n = 15), Alley North (n = 12), Deer Island (n =1), L-67 (n = 16), and Frog City South (n = 2). Usually, more than one chick from each nest was tagged. These chicks were first bled for mercury determination at approximately five days of age, and then sampled again (blood and growing scapular feathers) at the time of radio-tagging.

Between radio-tagging and the departure of the young from the colony (fledging), survival was documented through signal checks on the ground every three days. Any bird that died during this period was collected, a complete necropsy performed to determine cause of death, and several tissues saved for mercury analysis. A bird was considered to have fledged on the midpoint date between the last day recorded in the colony and the first day it was missed. After departure from the colony area, post-fledgling 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 from September to November, and once in February 1995. Flights were designed to cover all the WCAs, but

also extended north up to the Lake Okeechobee area, and south to Florida Bay. Given the one year life of the batteries, transmitters were considered unreliable after February 1995 and birds were not followed after this point. Not all birds were located on every survey flight, and most individuals were rarely located more than once every 14 days, even during the period of most intense tracking. When a bird was located both the location (in coordinates, using a Global Position System 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. Birds were assumed to have died at the midpoint date between the last day recorded alive and the first day a mortality signal was heard. Birds that were not located, and birds that had transmitters fail or fall from the bands were "censored" for the survival analysis on the day they were last known to be alive.

1995: Between late April and early June 1995, a total of 70 first-hatched great egret chicks (70 nests) that were included in the mercury dosing experiment were marked with aluminum leg bands (fitted above the tarsometatarsal joint on the right leg) that had radio tags (American Wildlife Enterprises, 10 g, with mortality switches attached to them; range of frequencies 164.113 - 164.735). All chicks were also fitted with U.S. Fish and Wildlife Service numbered aluminum bands below the tarsometatarsal joint on the left leg at an average age of 27 days. Of the 70 birds, 30 were dosed with methylmercury chloride (20 were monitored for food intake and survival, and 10 were monitored for health and survival) and 40 were used as controls (nine for health and survival, 12 for growth and survival, and the remaining 19 were monitored for food intake and survival) (see above for more details about this experiment).

Nestlings were marked in two different colonies in 1995: Hidden/L28 (n = 40), and Tamiami East colony (n = 30). The aluminum band and the radio transmitter combined weighed approximately 10 g (less than 2.3% of the weight of the chick in all cases). These chicks were first bled for mercury determination at approximately 12 days of age, and then re-sampled every five days for a total of 15 days (n= 4 samples). In addition, growing scapular feathers were collected on the last visit at the time of radio-tagging.

Between radio-tagging and the departure of the young from the colony (fledging), survival was documented through signals heard, as in 1994. In contrast to 1994, post-fledging survival was monitored through aerial surveys (n = 31), flown approximately every four days during the first three months post-tagging (June, July, and August), once a week during September, and every 40 days thereafter until the end of the study (once in October, once in December, and once in February/March 1996). Flights were performed to cover all the WCAs, and also extended north up to the Lake Okeechobee area, and south to Florida Bay. When a bird was located both the position (in coordinates, using a Global Position System unit) as well as a general description of the area were recorded. Carcasses were collected and birds were censored from the survival analysis as in 1994.

Analysis of survival

1994: 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 or feathers at the time of radio-tagging (approximately 30 days of age) increased the probability of dying during the first 7.5 months of age. Since mercury

concentrations differed significantly between colonies (see Chapter II), colony was added as a cofactor in this analysis.

1995: A log-rank test (PROC LIFETEST, SAS Institute, 1988) was used to compare survival during the first eight months of age between egrets that were dosed with mercury those that were used as controls. The LIFETEST program calculates Kaplan-Meier probabilities of survival through time, incorporating censored birds.

RESULTS

Effects of mercury on growth and food consumption

1994 field season

During 1994, a total of 154 blood samples, representing 77 three-day food measurement intervals, were collected and analyzed, including 40 intervals from 11 first-hatched or "A" chicks, 34 intervals from 10 second-hatched or "B" chicks, and 3 intervals from 3 third-hatched or "C" chicks. Food consumption, which ranged from 26.5 g/d for an 8 day old chick to 240.1 g/d for a 22 day old chick, varied significantly with age (ANCOVA, $F = 60.75$, $P < 0.0001$, when controlled for individual chick effects and hatch order; Figures 5.7 and 5.8). We found no difference in food consumption between chicks from Hidden and L-67 colonies (ANCOVA, $F = 0.03$, $P = 0.86$, controlled for age, hatch order, and individual chick effects; Table 5.2). Similarly, we found no differences in masses of chicks between colonies when age, hatch order, and individual chick effects were taken into account (ANCOVA, $F = 0.15$, $P = 0.70$; Figure 5.9). We found no significant differences in masses of first-hatched chicks among colonies (ANCOVA, $F = 0.40$, $P = 0.53$, controlled for age and individual chick effects), and a marginally significant difference in masses of second-hatched chicks among colonies (Hidden > L-67, ANCOVA, $F = 3.84$, $P = 0.060$, controlled for age and individual chick effects). Food consumption varied significantly with hatch order, with first- and second-hatched nestlings eating more than third-hatched nestlings (ANCOVA, $F = 6.68$, $P = 0.012$, controlled for age, individual chick effects).

The relation of blood and growing scapular feather mercury concentrations to food consumption, body mass, and colony was also examined. Blood mercury concentrations did not account for a significant amount of variation in food consumption among chicks (ANCOVA, $F = 1.34$, $P = 0.21$, controlled for age, hatch order, individual chick effects). There was, however, a significant negative association between chick food consumption and concentrations of mercury in growing scapular feathers (ANCOVA, $F = 2.04$, $P = 0.033$, controlled for age, hatch order, individual chick effects). A significant inverse relationship existed between mercury concentrations in both blood and growing scapular feathers and body mass (ANCOVA, $F = 2.41, 2.22$, $P = 0.009, 0.024$, controlled for age, hatch order, and individual chick effects). There was no evidence to support the hypothesis that there was an effect of colony on mercury concentrations in blood (ANCOVA, $F = 0.04$, $P = 0.84$, controlled for age, hatch order, individual chick effects), or growing scapular feathers (ANCOVA, $F = 1.60$, $P = 0.21$, controlled for age, hatch order, individual chick effects).

1995 field season

The 1995 field dosing experiment was designed to isolate potential differences in food consumption and growth rate to one of three main effects: the effect of the dosing procedure, the effect of the food consumption measurement technique, and the effect of mercury itself.

Effects of the methylmercury dosing procedure

Potential effects of the dosing procedure (giving chicks capsules and taking blood samples) were assessed by comparing masses and culmen lengths of Food Measurement Control/Placebo chicks and Handling Control chicks (see Fig. 5.5 from methods). Note that a summary of all tests comparing dose groups for differences in mass, culmen, and food consumption are given in Table 5.3. Handling Control chicks received identical treatments as the Food Measurement Control/Placebo chicks with the exception of the dosing procedure. The masses of chicks in these groups (Table 5.4) were not significantly different (SMM, T = 1.01, adj. P = 0.97, controlled for age and individual chick effects; Figure 5.10). Also, no significant differences in culmen length were found between the two groups (SMM[2], T = 0.73, adj. *P* = 1.00). Since there were no differences between the age-adjusted masses or culmen lengths of the two groups, there was no evidence to suggest that the dosing procedure affected chick growth.

Effects of the food consumption measurement procedure

The food consumption measurement procedure involved giving chicks injections of tritiated water and taking pre- and post-injection blood samples. We tested for differences in the masses and culmen lengths of Food and Food Measurement Control chicks in order to assess possible effects of the food measurement procedure on chick growth. A comparison of Food/Hg and Food Measurement Control/Hg chicks revealed no significant differences in their masses or culmen lengths (SMM, T = 0.80, adj. *P* = 1.00 (masses), T = 0.46, adj. *P* = 0.81 (culmen lengths), controlled for age and individual chick effects). Further, in a similar analysis of Food/Placebo and Food Measurement Control/Placebo chicks, no significant differences were found between their masses (SMM, T = 0.25, adj. *P* = 1.00, controlled for age and individual chick effects) or culmen lengths (SMM, T = 0.60, adj. *P* = 0.97, controlled for age and individual chick effects). Since no growth parameters were significantly different between groups, there was no indication that the food consumption measurement procedure affected growth.

Methylmercury, Chick Growth, and Food Consumption

Since we found no evidence of effects resulting from the dosing and food measurement procedures, we assumed that any differences in growth and food consumption among dosed and undosed birds can be related directly to the dose of methylmercury.

Our food consumption data included 53 five-day measurement intervals for Food/Placebo chicks (n = 21) and 57 for Food/Hg chicks (n = 21). There was an effect of methylmercury on nestling food consumption: Food/Placebo chicks ate significantly more than Food/Hg chicks (ANCOVA, F = 4.17, *P* = 0.048, controlled for age and individual chick

[2] SMM (Studentized Maximum Modulus) is a very conservative method for making multiple ad-hoc comparisons (adjusted P values reported are often higher (approaching 1) than those of other tests).

effects; Table 5.4, Figure 5.11). The difference between the mean food consumption of the two Food groups, although significant, was only about 4 % (calculated from overall means). When considering colonies individually, however, differences in food consumption between dosing groups were not significant (SMM, T = 2.07, adj. $P = 0.24$ (Tamiami East), T = 0.74, adj. $P = 0.97$ (Hidden)). The large difference in P-values between within and across colony comparisons of Food groups is suggestive of an interaction between location and experimental group, but no evidence of interaction was found (ANCOVA, F = 1.16, $P = 0.29$, controlled for age and individual chick effects). Perhaps the difference in significance was due to the reduction in statistical power resulting from the smaller sample sizes of the individual colonies.

Differences in food consumption were not reflected in differences in growth between groups. There were no significant differences in masses or culmen lengths of Food/Placebo and Food/Hg chicks (SMM, T = 1.61, 1.04, adj. $P = 0.68$, 0.97, controlled for age and individual nest effects; Figure 5.12; Table 5.4). This pattern was mirrored for Hg and Placebo chicks in the Food Measurement Control group: masses (Figure 5.13) and culmen lengths did not vary according to methylmercury dose (SMM, T = 0.66, 0.87, adj. $P = 1.00$, 1.00, controlled for age and individual nest effects). Similarly, there were no significant growth parameter differences among groups within individual colonies. In Hidden colony, masses and culmen lengths did not differ between Hg and Placebo chicks in the Food group (SMM, T = 0.66, 0.45, adj. $P = 1.00$, 1.00, controlled for age and individual nest effects), or the Food Measurement Control group (SMM, T = 1.52, 1.61, adj. $P = 0.99$, 0.99, controlled for age and individual nest effects). Finally, in Tamiami East colony, masses and culmen lengths did not differ between Hg and Placebo chicks in the Food group (SMM, T = 1.56, 0.99, adj. $P = 0.99$, 1.00, controlled for age and individual nest effects), or the Food Measurement Control group (SMM, T = 0.44, 0.24, adj. $P = 1.00$, 1.00, controlled for age and individual nest effects).

Colony effects

Since sample composition differed slightly between colonies (i. e., more Food/Hg and less Food/Placebo chicks in one colony vs. the other), we performed analyses comparing individual treatment groups between colonies to test for effects of colony on food consumption and growth parameters. Food consumption did not differ among Hidden and Tamiami East colony Food/Placebo chicks (SMM, T = 0.30, adj. $P = 1.00$, controlled for age and individual chick effects) or Food/Hg chicks (SMM, T = 1.82, adj. $P = 0.37$, controlled for age and individual chick effects) at the two colonies. Similarly, masses (SMM, T = 1.32, 2.34, adj. $P = 1.00$, 0.59, controlled for age and individual chick effects) and culmen lengths (SMM, T = 0.93, 1.54, adj. $P = 1.00$, 0.99, controlled for age and individual chick effects) of these two colony groups did not vary between Hidden and Tamiami East colonies. Additionally, there were no significant differences in the masses (SMM, T = 2.10, 0.30, adj. $P = 0.79$, 1.00, controlled for age and individual chick effects) or culmen lengths (SMM, T = 2.02, 0.35, adj. $P = 0.84$, 1.00, controlled for age and individual chick effects) of Food Measurement Control/Hg and Food Measurement Control/Placebo groups in the two colonies. Finally, masses and culmen lengths of Handling Control chicks did not differ among colonies (SMM, T = 1.16, 0.67, adj. $P = 1.00$, 1.00, controlled for age and individual chick effects), suggesting that the control populations were similar at the two colonies.

Because great egret parents from Hidden and Tamiami East colonies forage in

different locations (Frederick 1995), differences in mercury concentrations of prey items might exist. In order to assess this possibility, mercury concentrations in blood and growing feathers of Placebo (Food + Food Measurement Control lumped together) chicks were examined for variability between colonies. No significant differences were found in blood (ANCOVA, F = 0.24, $P = 0.62$, controlled for age and individual chick effects), or feather (ANCOVA, F = 0.52, $P = 0.48$, controlled for age and individual chick effects) mercury concentrations among colonies.

Blood and feather mercury concentrations

Mercury concentrations in blood and feathers from mercury-dosed and control chicks are summarized in Table 5.5. Prior to the start of the experiment (Day 0 = 11.6 d of age), chicks from Tamiami East and Hidden/L28 colonies had an average blood mercury concentration of 0.61 and 0.82 mg/kg, respectively (Figure 5.14, Table 5.5). During the first five days of the experiment, the total mercury concentration in blood increased from 0.71 to 3.34 mg/kg in chicks from Tamiami East, and from 0.67 to 3.44 mg/kg in birds from Hidden/L28. Mercury concentration in blood continued increasing through days 10 and 15 of the experiment (16.6 - 21.6 d of age), although at a much lower rate (from 3.34 to 4.43 mg/kg (24.6%) in chicks from Tamiami East, and from 3.44 to 4.6 mg/kg (25.2%) in chicks from Hidden/L28) (see Table 5.5 and Figure 5.14). Control nestlings maintained a more or less constant concentration of mercury in blood throughout the experiment (Figure 5.14). Growing feathers from mercury-dosed chicks had approximately three times the concentrations of mercury compared to control birds from the same colonies (52.5 vs. 7.21 and 49.18 vs. 7.28 mg/kg for Tamiami East and Hidden/L28, respectively).

Growth and food consumption

Only Food/Placebo chicks (Hidden and Tamiami East colonies in 1995), and all first-hatched chicks from 1994 were used in analyses of the effect of year on growth and food consumption. Both groups had similar visitation schedules and were not dosed with methylmercury. Masses of chicks varied by year (ANCOVA, F = 22.52, $P < 0.0001$, controlled for age, individual chick effects); chicks from 1995 were heavier (Table 5.6). Culmen lengths were also longer in 1995 (ANCOVA, F = 8.35, $P < 0.0001$, controlled for age, individual chick effects). In addition, we performed separate analyses of growth of first-hatched chicks (Food/Placebo from 1995 and all first-hatched from 1994) from Hidden colony between years and in 1995 found significantly greater masses and culmen lengths (ANCOVA, F = 10.46, 6.60, $P = 0.002$, 0.013 respectively, controlled for age and individual chick effects).

Although the overall yearly means for food consumption differed by nearly 10 g, this difference was not significant (ANCOVA, F = 3.31, $P = 0.073$, controlled for age and individual chick effects). Similarly, there were no statistically significant annual differences in food consumption within Hidden colony (ANCOVA, F = 1.37, $P = 0.25$, controlled for age and individual chick effects). The comparisons of food consumption data between years should, however, be cautiously interpreted, since different hydrogen isotopes (deuterium and tritium) were used to measure food consumption in 1994 and 1995. Deuterated and tritiated water both function in the same way within an animal (they label body water and their dilution over time is used to calculate water turnover) and are identical aside from differing

atomic weights. To the best of our knowledge no studies have been conducted which directly compared the performance of the deuterated and tritiated water within the same animal. We made no such comparison because the mass spectrometer we used for deuterium analyses was not equipped to safely dispose of the radioactive tritium gas that would be generated in deuterium-tritium analyses. Thus, even though we know of no difference in the performance of deuterium and tritium labels, it is possible that the lack of a significant difference in food consumption between years might be an artifact of the different hydrogen labels used to measure it in the two years.

Effects of mercury on health

1994: During 1994, packed cell volume of wild great egret nestlings averaged 35.42 % (n = 286, SD = 7.03), the concentration of plasma proteins averaged 6.24 g/dl (n = 284, SD = 1.01), the number of white blood cells averaged 22,444.04 cell/μl (n = 177, SD = 12,110.8), and the number of oral parasites averaged 3.91 (n = 235, SD = 4.87), (Table 5.7). With the exception of white blood cell counts, all these variables increased with the age of the chicks (Figures 5.15 and 5.16).

Contingency analyses between chicks considered "healthy" and "sick", and between chicks that had "high" and "normal" mercury concentrations in blood and growing feathers (hatch orders "A", "B", and "C" combined), showed that there was a marginally significant association between health status and mercury in blood, but not feathers of "old" chicks (n = 157, $X^2 = 3.541$, $P = 0.06$, for blood, and n = 73, $X^2 = 1.333$, $P = 0.248$, for feathers) (Table 5.8). Mercury concentration in blood of "young" egrets was not associated with health condition (n = 129, $X^2 = 0.523$, $P = 0.469$) (Table 5.8). There were not enough feather samples from "young" chicks to test this hypothesis.

This same analysis was also performed by hatch order. Even though a higher mercury concentration in blood of "young" first-hatched chicks was not associated with sickness (n = 57, $X^2 = 3.229$, $P = 0.072$), a higher mercury concentration in blood of "old" first-hatched chicks was significant (n = 90, $X^2 = 4.05$, $P = 0.044$) (Table 5.9). Of the eight birds that had "high" mercury in their blood, four had higher than normal numbers of oral parasites and plasma protein values; two had higher than normal packed cell volume values; and one had lower than normal number of white blood cells. The mean blood mercury concentrations for these birds was 2.33 mg/kg.

"High" mercury in feathers of "old" "A" chicks was not associated with poor health status (n = 42, $X^2 = 1.736$, $P = 0.188$), and there were not enough feather samples from "young" "A" chicks to perform this analysis. There was no association between health status and mercury concentrations in blood of either "old" or "young" "B" chicks (n = 64, $X^2 = 0.238$, $P = 0.625$, and n = 57, $X^2 = 0.621$, $P = 0.431$, respectively). Similarly, there was no association between mercury in feathers and health status of "old" "B" great egret chicks (n = 30, $X^2 < 0.001$, $P > 1.00$). This analysis could not be performed for mercury in feathers of "young" "B" chicks, nor for mercury in blood and feathers of "old" and "young" "C" chicks because of lack of sufficient sample size.

1995: Table 5.10 summarizes measured health parameters obtained from control and mercury-dosed egret nestlings at days 0, 5, 10, and 15 of the field dosing experiment. The

relationship between age (= bill length) and all of the health parameters studied are plotted by treatment (control vs. mercury-dosed egrets) in Figures 5.14 and 5.17-5.19.

The results obtained from the repeated measures ANOVA indicated that there were no differences in any of the health parameters studied between the control and the mercury-dosed birds. Even though the number of white blood cells and number of lymphocytes was greater in the control group (see Figures 5.15a and 5.15b), these differences were not statistically significant when the effects of age, colony, and individual differences were accounted for (DF = 3, F = 0.31, $P = 0.8201$, and DF = 3, F = 0.72, $P = 0.5485$, respectively). On the other hand, mercury-dosed birds had slightly higher numbers of heterophils and eosinophils compared to controls (see Figures 5.15a and 5.15b), but again these differences were not significant (DF = 3, F = 0.41, $P = 0.7472$, and DF = 3, F = 0.97, $P = 0.4143$, respectively). There were no obvious patterns in the percentage of packed cell volume, concentration of plasma proteins (Figure 5.17), or number of oral parasites between the control and the mercury-dosed egrets (DF = 3, F = 0.34, $P = 0.7969$ for packed cell volume; DF = 3, F = 0.34, $P = 0.7936$ for plasma proteins; and DF = 3, F = 0.21, $P = 0.7127$ for number of oral parasites).

Effects of mercury on survival

1994: A summary of the radio-telemetry study of survival conducted in 1994 is presented in Table 5.11. During the last survey (February 18, 1995), 10 of the 46 radio tagged birds were still known to be alive, 12 were known to have died, and 24 were lost from the study because of different causes (one had a broken radio, four had broken plastic bands, and signals from the remaining 19 birds could not be located during the last flight). Of the 46 birds originally radioed, nine birds were never located after leaving their natal colonies; seven birds were located once; nine were located twice; 12 birds were found between three and five times; eight were located between six and eight times; and a single individual was found nine times.

A map with the movements of the radio-tagged birds is presented in Figure 5.20a In general, post fledging dispersal was to the north of natal colonies and towards shallowly flooded agricultural lands in southwest Florida. Several birds were sighted near vegetable 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.

Fledging (departure from colony) of radiotagged birds is plotted against age in Figure 5.20b. Of the 46 egrets radioed, only one died prior to leaving its natal colony. Birds began to leave their natal colonies at about two months of age, and the peak of fledging occurred when egrets were between 75 and 85 days old (n = 27, 60% of the birds). The oldest two birds to fledge were over 100 days old.

Twelve birds died during the course of the study. The first three mortalities occurred when birds were between two and three months of age (25%). The largest number of mortalities (n = 4, 33%) occurred approximately one month after the peak of fledging, decreasing thereafter until the birds were 330 days old (n = 5, 42%) (Figure 5.20b).

After a mortality signal was heard from the air, a search for the carcass was initiated as soon as possible. Autolysis and scavenging, however, occurred at extremely rapid rates, and in almost all cases the cause of death could not be determined, and the only remains

recovered were bones, mature wing feathers, radios, and aluminum bands. On one occasion a partially scavenged carcass was recovered, and a complete necropsy conducted. This egret was found to be infected with large numbers of feather lice, and with the parasite *Eustrongylides ignotus*, a nematode known to be a frequent cause of death of free-ranging nestlings egrets and herons (Spalding and Forrester 1993). In addition, the stomach contained some insect parts, and no fish parts. Liver, brain, muscle, and feathers were collected from this bird and analyzed for mercury concentrations. Mercury concentrations from tissues of this bird and from mature feathers of another radioed six birds are presented in Table 5.12.

The relationship between mercury concentration in blood and growing feathers at the time of radio-tagging and survival during the first 7.5 months of life was examined using a maximum likelihood analysis. Mercury concentration in blood at the time of last visit (approximately 28 days) was not related to the probability of surviving (DF = 16, $X^2 = 24.69$, $P = 0.0754$). Similarly, mercury concentration in growing scapular feathers at the last visit (approximately 28 days) was also not associated with survivorship (DF = 15, $X^2 = 4.99$, $P = 0.9922$).

1995: A summary of the radio-telemetry data for 1995 is presented in Table 5.13. During the last survey (February/March, 1996), five of the original cohort of 70 birds were known to be alive, seven were known to have died, and 58 were lost from the study because of different causes (in four birds the radio fell off the aluminum band, and signals from the remaining 54 birds could not be located during the last flight). Of the 70 birds radioed, 29 birds were never located after leaving their natal colonies (14 mercury-dosed and 15 controls; 13 from Hidden/L28 and 16 from Tamiami); 17 birds were located between one and three times (five mercury-dosed and 12 controls; eight from Hidden/L28 and nine from Tamiami); 10 egrets were located between four and seven times (three mercury-dosed and seven controls; nine from Hidden/L28 and one from Tamiami); and 14 birds were found between eight and 14 times (eight mercury-dosed and six controls; 13 from Hidden/L28 and only one from Tamiami).

Fledging of radiotagged birds is plotted against age in 1995 (Figure 5.21). Of the 70 birds radioed, one died prior to leaving the colony, and three lost their radios while still in the colony. Birds began to leave their natal colonies at a similar age as those monitored in 1994 (about two months of age), and the peak of fledging during 1995 also occurred when egrets were between 75 and 85 days old (n = 48, 73% of the birds). The oldest bird to fledge was 95 days old. Mercury-dosed birds fledged at 73 days of age on average, while control egrets left their natal colonies when they were on average 70 days old. Movements of birds within the south Florida area were wide-ranging, and fewer birds were found moving to southwest Florida than in 1994 (Figure 5.22a and 5.22b).

Seven birds were found dead during the course of the study. One bird died prior to fledging (19 days). This was a control female egret being monitored for food intake. At necropsy, the liver and spleen were enlarged, and the glandular stomach was found to be infected with the parasite *Eustrongylides ignotus*. This nestling had mercury concentrations of 1.7 mg/kg in the liver; 1.5 mg/kg in the pancreas; 1.2 mg/kg in the kidney; and 0.36 mg/kg in the brain. The second and third mortalities occurred when birds were between two and three months of age (29%) (Figure 5.21). The remaining four egrets died between six and

seven months of age (57%). Of the seven birds that were found dead, six were controls and only one was a mercury-dosed bird (this latter bird died at age 211 days) (Figure 5.21). Since the only remains found from the six birds that died outside their natal colonies were a few bones and mature feathers, no tissues from these birds were analyzed for mercury concentrations.

The effect of mercury on survival in 1995 was studied using a log-rank test. The results from this test showed that there was no difference in the probability of surviving during the first eight months of age between egrets that were dosed with mercury (66.6 % survival) and those that were not (55.5 % survival) (DF = 1, $X^2 = 0.5543$, $P = 0.4565$).

DISCUSSION

Effects of methylmercury on growth and food consumption

The 1995 dosing experiment demonstrated that there are measurable differences in food consumption between nestlings with ca 1.76 mg/kg methylmercury and ca 0.63 mg/kg methylmercury in their diets. Curiously, differences in food consumption were small, and did not affect chick growth during the fifteen days they were monitored. This lack of an effect of decreased food consumption on body mass could be due to a number of factors.

Why did dosed birds eat less?

Mercury-related mechanisms that could explain the difference in food consumption between mercury dosing groups include:

1. *Loss of coordination*: Great egret nestlings must aggressively compete with siblings for food (Mock 1987, Mock et al. 1987). If methylmercury affected their coordination, impaired nestlings might not be able to obtain as much food. It is worth noting that we found food consumption differences among first-hatched chicks, who are normally able to out-compete their younger siblings for food (Wiese 1975, Mock 1987, Mock et al. 1987). It is possible that the dose of methylmercury reduced the competitive advantage of first-hatched chicks in the Food/Hg group via loss of coordination (Fimreite and Karstad 1971), resulting in a greater proportion of total food deliveries eaten by the undosed second- and third-hatched chicks. In a natural situation where high mercury concentrations in prey items affected coordination of *all* nestlings in a brood, one of two things could be expected to occur: either the normally dominant first-hatched chick would command an even greater proportion of food deliveries, or all chicks would eat proportionally less food.

2. *Lethargy*: Birds with high mercury burdens often become lethargic (Scheuhammer 1988). Once again, this symptom could affect a chick's ability to compete for food.

3. *Abnormal behaviors*:: Heinz and Locke (1976) found that ducklings of "high" mercury parents had abnormal reactions to common stimuli, including slower responses to parental calls and hyper-responsiveness to predator silhouettes. If great egret nestlings were slow to react to a parent approaching the nest, it is likely their food consumption would decrease.

4. *Perception:*: High mercury burdens may simply cause a chick to "feel sick", and subsequently have less of an appetite.

Behavioral observations were not formally recorded during this study and would be necessary to determine which of the above factors are contributing to lower food consumption in the dosed chicks. Coordination and alertness, however, were not obviously different between dosing groups when we handled the birds, and both Hg and Placebo chicks became extremely difficult to capture toward the end of the experimental period. These hypotheses are addressed in more detail by the captive dosing experiment (Chapter VI).

Why didn't reduction in food intake result in slower growth?

Methylmercury-dosed nestlings ate significantly less food than did controls, yet masses and culmen lengths did not differ between dosing groups. There are several conceivable reasons for this result including length of the experiment, timing of the experiment, the magnitude of differences in food consumption between groups, lack of physiological stress, and selenium-mercury interactions.

1) *Length of the experiment.* The methylmercury field dosing experiment lasted only 15 days, limited by our inability to capture the older, mobile chicks. Dosing periods of studies reporting a methylmercury-induced slowing of growth are often much longer than 15 days. For instance, Fimreite and Karstad (1971) dosed juvenile red-tailed hawks for 84 days, and did not begin to note slower growth until after 30 days of dosing. Similarly, goshawks reported as losing weight due to mercury intake were dosed for 47 days (Borg et al. 1970). Furthermore, both of these experiments involved much higher doses of dietary methylmercury (7-13 mg/kg vs. our 1.76 mg/kg) than we used and, under similar conditions, should have elicited symptoms sooner than in our experiment. The fact that both studies showed first effects well after the fifteen day dosing period of our experiment suggests strongly that this experiment was too short to detect effects on growth.

2) *Timing of the experiment.* The age range of chicks (ca 12-27 d) used in this experiment has important implications concerning the potential effects of methylmercury. Chicks at these ages are rapidly growing new feathers. While they are growing, feathers are connected to the circulatory system, and provide a major excretory pathway for mercury (Burger and Gochfeld 1991). Out of 10 tissues sampled in great blue herons and great egrets from southern Florida, feathers contained the highest concentrations of mercury (Chapter II). In fact, as much as 93% of the mercury within a bird's body may be stored in feathers (Braune and Gaskin 1987). In young Korean great egrets, it was found that 48% of their mercury was stored in feathers (Honda et al. 1986). Therefore, the chicks in the experiment were at an age where they could presumably sequester a substantial portion of their mercury intake in a place where it could no longer affect them. The nestlings might have become more vulnerable during the post-fledging period after feathers had stopped growing, as is strongly suggested by the results of our captive dosing experiments (Chapter VI).

3) *Differences in food consumption.*

There was only a 4% difference in food consumption between Food/Placebo and Food/Hg chicks. Perhaps this small a difference, combined with error inherent in growth measurements, is not enough to produce statistically significant differences in growth parameters.

4) Lack of environmental stress during 1995.

It is possible that most first-hatched nestlings from 1995 were not subjected to food-related physiological stress. Since physiological condition and environmental stress may

modify the effects of mercury on ardeids (Van der Molen et al. 1982), lack of food stress could have important implications i.e., even though the Food/Hg group was consuming less food than the Food/Placebo group, perhaps they were still eating enough to grow at a nearly maximal rate (see Custer and Peterson 1991). Three lines of evidence suggest that prey items were unusually available during the 1995 breeding season. First, clutch size and nest success in 1995 were the highest in the >10 year record for great egrets in the Everglades (Frederick 1995). Second, the 1995 nesting season was characterized by an uninterrupted drying trend, which generally produces favorable foraging conditions (Frederick 1995). Finally, in 1995 we noted a number of nests containing four old (ca 2-3 weeks) and apparently healthy nestlings (an unusually high clutch size), while none were found in 1994. If food was consistently available, chicks would likely have been in better physical condition than if food supplies were less predictable. In other words, while no effects were noted in 1995, the dose rate of supplementary methylmercury might have had a measurable impact on growth in a year where food supplies were less predictable.

4) *Selenium-mercury interactions*. The antagonistic effect of selenium on the toxicity of inorganic mercury has long been known (Parizek and Ostadalova 1967). While the mechanism has not been conclusively demonstrated, it has nonetheless been shown that selenium reduces the effects of given concentrations of methylmercury in a number of mammals (e. g., Rana and Boora 1992) and birds (e. g., Stoewsand et al. 1974, Di Simplicio and Leonzio 1989). Selenium concentrations were not measured in nestlings in this field study, and this variable therefore remains as a potential explanation for the observed results.

Effects of geographic location

1994: We found no evidence of differences in food consumption, growth, and mercury concentrations in blood and feathers when comparing nestlings from Hidden and L-67 colonies during 1994. It should be noted, however, that the ability to detect differences between groups suffered due to small sample sizes (13 nestlings from Hidden colony and 11 from L-67 colony). While working in the two colonies, we noted that nestlings in L-67 colony appeared to be less healthy (less fat, more lethargic) than those from Hidden Colony. Further, 1994 great egret nest success was significantly lower in the L-67 colony than in Hidden colony (Frederick 1995). Differences in nesting substrate between the two colonies might account for the apparent differences in nestling condition. Nestlings from the L-67 colony were more exposed to heat stress than nestlings from Hidden colony. Great egret nestlings, with their white plumage, are afforded some measure of protection from solar radiation, but there are substantial metabolic costs associated with thermoregulation in hot environments (Ellis 1976). Willow trees in L-67 colony afforded nestlings much less shade than the cypress and coco plum trees in Hidden colony. In general, the survival of both nests and chicks of great egrets from Hidden colony has been higher than most other colonies in the Everglades (Frederick 1995), and it is possible that protection from heat stress is among the factors contributing to this trend. Alternatively, the L-67 colony may be located in less productive foraging areas than the Hidden Colony (Frederick 1995).

1995: Chick growth varied among colonies in 1995. Chicks from Hidden colony were heavier, and had longer culmens than chicks from Tamiami East colony. There was a

marginally significant difference in food consumption between colonies. We have no evidence that mercury in natural prey items played a role in these differences, as mercury concentrations in blood and feathers were similar between colonies. Hidden and Tamiami East colony differ both in parental foraging ranges (Frederick 1995) and nesting substrate, with Hidden colony providing more protection from direct sunlight (above). Without whole-nest food consumption data (we monitored only "A" chicks in 1995), it is difficult to speculate on differences in food availability between the colonies, because first-hatched great egrets tend to dominate food supplies, even in times of food shortages (Mock 1987, Mock et al. 1987). Future investigations, using doubly labeled water (see below) could help determine the relative importance of both heat stress and food shortages.

Food consumption estimates from other studies

Field studies of the food consumption of great egret nestlings are almost completely lacking. Mock et al. (1987) estimated food consumption of entire three-chick broods of great egrets, aged 0 to 25 days, by estimating through observation the volumes of boluses delivered by parents. The volumes of boluses were assessed by visually comparing them to adult head morphology. Volumes were converted to masses using a conversion factor derived from measurements of wads of prey species and weights of fresh boluses. This study estimated that entire three-chick broods received an average of 70 g food/day. Assuming first-hatched chicks eat half of all food delivered (Mock et al. 1987), this would mean that the first-hatched chicks in Mock's study eat over 75 % less than Food/Placebo chicks did in our study (mean = 163.4 g/d). Since visual estimates of bolus size provide only a coarse estimate of food consumption, the results reported by Mock et al. (1987) can provide only a rough index of relative food distribution within a nest and are not dependable estimates of mass of food consumed.

Min et al. (1984) estimated food consumption of five free-ranging great egret nestlings using the collar method. Despite potentially strong biases associated with this method (i.e., if chicks are unable to swallow a first bolus, they make be less likely to take a second), their results were very similar to those found in this study. Food consumption ranged from an average of ca 110 g/d to ca 170g/d for chicks between the ages of 10 and 28 days (estimated from a figure of food consumption vs. age in Min et al. 1984). This average is similar to the average consumption of 163.4 g/d found for the Food/Placebo chicks from 8-31 days old. The chicks from the Min et al. (1984) study also weighed less than the chicks in this study, suggesting that the lower mean food consumption was a reflection of either slower growth or smaller body size. Alternatively, it is possible that the apparent differences in food consumption are simply an artifact of differing methodologies.

There have been a number of captive studies measuring the food consumption of nestlings of ciconiiform species (Table 5.14). While the data from such studies must be interpreted with caution since chicks are not subject to natural stresses, or limits of parental food provisioning, they nonetheless provide an index for the relative amounts of food consumption between species. As would be predicted from adult masses, the data from this study lie in between the food consumption values for white ibises and little egrets (*Egretta garzetta*).

Effects of Mercury on Health

Overall, the effects of mercury contamination on health parameters were generally weak and differed between years. During 1994, egret chicks that had a naturally higher concentration of mercury in blood were more likely to be found sick, but this relationship was only significant for first-hatched chicks that had a bill length of over 4.1 cm. These "sick" birds had higher numbers of oral parasites, higher plasma proteins, higher PCV and lower white blood cell counts than "normal" chicks. Three similar comparisons with younger chicks and "B" chicks showed no significant effects. During 1995, when the concentrations of mercury in tissues of free-ranging great egret chicks were artificially elevated through the administration of methylmercury, no significant effects of mercury contamination on health parameters were detected. However, because of differences in the methodology and analysis employed to measure the effects of mercury on health between 1994 and 1995, any direct comparison of the results obtained among years is inappropriate.

The lack of any effect of mercury on health parameters during 1995 emphasizes the difficulty in interpreting results obtained under field conditions, without eliminating possible effects of confounding variables. In this respect, it is known that malnutrition and/or elevation of corticosteroids in response to chronic stress can cause suppression of the immune system (Fairbrother 1994). If there was less food available in 1994 than in 1995, as previously suggested, then it could be hypothesized that during 1994 chicks would have been under a higher physiological stress which could have negatively affected several physiological parameters, including those studied here. Conversely, these effects would not have been expressed under conditions of high food availability as in 1995.

It is interesting to note, however, that even though no effects of mercury on any of the health parameters studied were detected in 1995, some trends were observed. The most intriguing ones had to do with the leukocyte and differential cell counts. Egrets that were dosed with mercury had fewer white blood cells, probably due to a decrease in the number of lymphocytes. A decrease in lymphocyte numbers were also observed in captively dosed great egrets at much higher concentrations (see Chapter VI). In mice which have been exposed to methylmercury during embryonic development and up to nine weeks of age, both primary and secondary immune responses were suppressed (Ohi et al. 1976). Unfortunately, there are few reports on the effects of heavy metals on the immune system of free-ranging wildlife. Mallards (*Anas platyrhynchos*) exposed to field concentrations of lead had significantly lower circulating numbers of white blood cells, with lower numbers of heterophils, lymphocytes, and monocytes (Rocke and Samuel 1991).

There are some indications that methylmercury can affect hematological parameters in animals. Mice dosed with 24 mg/kg body weight of methylmercury chloride (intraperitoneal injection) for 14 days had a significant decrease in hemoglobin content, red blood cell count, and packed cell volume when compared to the control mice (Shaw et al. 1991). In fish dosed with sublethal concentrations of inorganic mercury, changes in erythrocyte morphology have also been reported (Chang et al. 1977, Panigrahi and Misra 1979). Presently, there are no studies on the effects of mercury intoxication on the hematology of birds.

The lack of any significant effect of mercury on the health condition of great egret nestlings during the course of the field dosing experiment could also be explained by the length of the experiment. Due to the fact that great egret chicks move out of the nest and

become too mobile to catch after approximately 30 days of age, birds were dosed with mercury for only a relatively short period of time (15 days). In addition, during the time birds were being dosed, they were growing a complete new set of feathers. Feathers constitute an important excretion pathway of mercury in birds, and more than 50% of the body burden of mercury in egret nestlings can be found in this tissue (Honda et al. 1986). At this point in our studies, it became obvious that the "feather excretion hypothesis" needed to be tested via experimental means, if we were to understand the results of our field dosing experiment. We needed to be able to dose birds throughout the nestling and fledging periods, and monitor responses to methylmercury during and after the time that feathers stopped growing (see Chapter VI).

Effects of Mercury on Survival

For both 1994 and 1995 the concentration of mercury in tissues of great egrets was unrelated to the probability of great egret nestlings surviving the first eight months of age. Most of the studies on the effects of mercury contamination on survival of nestling and juvenile birds have been conducted with non-piscivorous birds under laboratory conditions. In addition, the length, amount and type of mercury administered, and tissues used to evaluate concentration of mercury vary greatly between experiments. All these factors make any comparisons with the results obtained in the present study very difficult. However, it can be stated with some certainty, that mercury doses of 1.86 mg/kg between 12 and 28 days of age seem to have very little effect on later survival, even though these doses are occurring during a period of rapid growth and development.

Mercury is known to negatively affect survival of captive birds, and has also been implicated in mortalities of free-ranging birds. In the laboratory, Heinz and Locke (1976) and Heinz (1974) observed an increase in early mortality of mallard ducklings dosed with methylmercury (3 mg/kg wet weight in dry mash). Similarly, an increase in duckling mortality was observed when American black ducks (*Anas rubripes*) were dosed with 3 mg/kg of methylmercury in mash (Finley and Stendell 1978). When pheasants, ducks and chickens were dosed at a rate of 33 mg/kg of methylmercury for 35 days, it resulted in 90%, 85% and 7.5% mortality, respectively (Gardiner 1972). Fimreite (1974) observed a 10-12% reduction in fledging rate of free-ranging common terns inhabiting a mercury contaminated freshwater system (mean liver mercury concentration of 27 mg/kg). In wild grey herons (*Ardea cinera*), mainly first year birds, liver mercury concentrations of over 160 mg/kg coupled with cold stress and poor nutritional condition may have contributed to a massive die-off in the Netherlands (Van der Molen et al. 1982). Hoffman and Curnow (1979) found no differences in liver mercury concentrations between live and dead wild great blue heron nestlings (*Ardea herodias*) when contamination was much lower (mean mercury concentration was 0.96 mg/kg) in the Great Lakes.

Despite the different methods employed in each of these studies, these results suggest that interspecific differences exist in sensitivity to the toxic effects of mercury. Such variation may be the result of differences in the ability of various species to decompose methylmercury compared to the less toxic and more readily excreted inorganic form of mercury (Scheuhammer 1987a). Other factors, such as the presence of selenium, can also reduce the toxicity of methylmercury (Potter and Matrone 1974). It remains unknown whether these factors, coupled with the limitations in timing and duration of our field dosing

experiment (as discussed above), could have played a role in the results obtained.

High concentrations of mercury in tissues of wild birds have also been associated with an increased probability of dying of chronic, debilitating diseases. Ensor et al. (1992) observed that juvenile common loons that died from disease had significantly higher mercury concentrations in feathers (mean of 19.8 mg/kg) than did juveniles that died from injury (2.4 mg/kg). Similarly, Spalding et al. (1994) reported that juvenile great white herons (*Ardea herodias occidentalis*) that died from acute causes had lower liver mercury concentrations (1.77 mg/kg) compared to birds that died of chronic diseases (9.76 mg/kg). These are interesting findings, and suggest a negative effect of mercury on the immune system of these species. In the present study, great egret nestlings that were dosed with mercury had higher mercury concentrations in feathers (average of 44 mg/kg) at time of banding than those reported by Ensor et al. (1992). However, because of the inability to recover fresh carcasses, the cause of death of these birds could rarely be established. The lack of relationship between tissue mercury concentrations and survival may therefore be an artifact of the fact that we were unable to use mercury concentrations at the time of banding (approximately 28 days of age) to predict mercury concentrations at time of death. It could also be an artifact of the timing of the experiment, (as above under discussion of mercury and food consumption). The dosing ceased after a 15 day period, during which extremely rapid feather growth may have provided the birds with an efficient way of sequestering mercury outside the body. This significant depuration could have occurred between the time of banding (approximately 28 days) and fledging (e.g. 75 days). Thus, the mercury burden at time of fledging could have been negligible, even in mercury dosed birds.

Our of juvenile great egret survival also indicates relatively high survival of nestlings during the period that they spend in the colony, followed by a period of much higher risk of mortality immediately following fledging. This pattern is echoed by other studies of survival in ardeids (Frederick et al. 1993). The finding of greatly increased risk of mortality in control birds at the time of fledging is of particular interest, since this coincides with the period when young birds are suddenly unable to excrete mercury through growing feathers (Chapter VI).

Table 5.1. Percent measurement error of the isotopically labeled water technique, in validation studies of avian material and energy balance. Modified from Nagy 1989.

Species	*% Error*	*Source*
Penguin	+ 6.5	Gales 1989
Sparrow	+ 6.5	Williams and Nagy 1985
Sparrow	+ 6.1	Williams 1985
Pigeon	+ 3.6	LeFebvre 1964
Martin	+ 3.6	Hails 1979
Starling	+ 2.5	Williams 1985
Parakeet	- 0.04	Buttemer et al. 1986
Quail	- 4.9	Goldstein and Nagy 1985

	Food Consumption (g/d)	Blood Hg Concentration (mg/kg)	Feather Hg Concentration (mg/kg)	
Group	*Mean*	*Mean*	*Mean*	*SD*
All Hidden	141.8	1.36	15.70	4.01
All L-67	120.8	1.67	19.21	6.50
All First-hatched	147.1	1.41	17.59	5.90
All Second-hatched	122.1	1.63	17.05	5.24

Table 5.2. Raw mean food consumption and mercury concentrations in blood and growing scapular feathers of great egret nestlings in 1994. Data are segregated by nesting colony and hatching order; third-hatched nestlings are not included in the summaries. Note that these means are not adjusted for age. Since food consumption and blood mercury concentrations were measured multiple times in the same individuals, no standard deviations are reported for these variables.

Table 5.3. Results of tests of null hypotheses from the 1995 field methylmercury dosing experiment. P-values are reported for analyses of covariance, while adjusted p-values are reported for Studentized Maximum Modulus tests.

Groups Compared		Measure	Result
F/Hg[1]	F/P[2]	food consumed	0.048
F/Hg	F/P	mass	0.68
F/Hg	F/P	culmen	0.97
FMC/P[3]	HC[4]	mass	0.97*
FMC/P	HC	culmen	1.00*
F/Hg	FMC/Hg[5]	mass	1.00*
F/Hg	FMC/Hg	culmen	0.81*
F/P	FMC/P	mass	1.00*
F/P	FMC/P	culmen	0.97*
F/Hg-Hid[6]	F/Hg-TE[7]	food consumed	1.00*
F/P-Hid	F/P-TE	food consumed	0.37*
F/Hg-Hid	F/Hg-TE	mass	0.59*
FMC/Hg-Hid	FMC/Hg-TE	mass	0.79*
F/P-Hid	F/P-TE	mass	1.00*
FMC/P-Hid	FMC/P-TE	mass	1.00*
F/Hg-Hid	F/Hg-TE	culmen	0.99*
FMC/Hg-Hid	FMC/Hg-TE	culmen	0.84*
F/P-Hid	F/P-TE	culmen	1.00*
FMC/P-Hid	FMC/P-TE	culmen	1.00*
HC-Hid	HC-TE	mass	1.00*
HC-Hid	HC-TE	culmen	1.00*

[1]Food/Hg. [2]Food/Placebo. [3]Food Measurement Control/Placebo. [4]Handling Control. [5]Food Measurement Control/Hg. [6]Hidden Colony. [7]Tamiami East Colony. *P-value adjusted to account for increased probability of Type I error in SMM (multiple comparison) analyses.

Table 5.4. Summary of overall means of measured parameters by treatment group and colony for the 1995 mercury dosing experiment. Since multiple measurements were taken from individuals over a period of fifteen days (except feathers), standard deviations are not reported.

Group	Mass (g)	Culmen Length (cm)	Food Consumption (g/d)	Blood Hg Conc. (mg/kg)	Feather Hg Conc. (mg/kg)	
	Mean	*Mean*	*Mean*	*Mean*	*Mean*	*SD*
F/P[1]	500.2	5.43	163.4	0.78	7.94	3.74
F/Hg[2]	497.0	5.50	156.9	3.23	49.09	14.04
FMC/P[3]	518.8	5.61	na[8]	0.74	7.30	3.43
FMC/Hg[4]	497.0	5.51	na[8]	3.01	50.13	11.19
HC[5]	445.2	5.23	na[8]	na[9]	9.08	3.03
Hid[6] *(all)*	520.0	5.60	163.6	na[10]	na[10]	
TE[7] *(all)*	469.2	5.29	155.3	na[10]	na[10]	

[1]Food/Placebo. [2]Food/Hg. [3]Food Measurement Control/Placebo. [4]Food Measurement Control/Hg. [5]Handling Control. [6]Hidden Colony. [7]Tamiami East Colony. [8]Food consumption was not measured for these groups. [9]No blood mercury assays were performed for this group. [10]Since a portion of nestlings in both of these colonies were dosed with methylmercury, overall means are not reported.

Table 5.5. Summary of mercury concentrations in blood and growing scapular feathers of great egret nestlings from the 1995 field dosing experiment, by colony and treatment.

Colony	Treatment	Mercury Blood (mg/kg) Day 0[a]	Mercury Feathers (mg/kg) Day 0	Mercury Blood (mg/kg) Day 5	Mercury Feathers (mg/kg) Day 5	Mercury Blood (mg/kg) Day 10	Mercury Feathers (mg/kg) Day 10	Mercury Blood (mg/kg) Day 15	Mercury Feathers (mg/kg) Day 15
Tamiami East	**Placebos** [b]	0.61 (13;0.23)[d]	–[e]	0.62 (13;0.19)	–	0.86 (10;0.35)	–	0.86 (9;0.27)	7.21 (13;2.54)
	Dosed with Hg [c]	0.71 (12;0.12)	–	3.34 (11;1.26)	–	3.98 (12;1.07)	–	4.43 (9;0.94)	52.50 (10;13.39)
	Food Measurement Control/Placebo	0.57 (4;0.22)	–	0.58 (4;0.30)	–	0.70 (3;0.31)	–	0.69 (2;0.09)	5.50 (2;3.82)
	Food/Placebo	0.63 (9;0.25)	–	0.63 (9;0.15)	–	0.93 (7;0.37)	–	0.90 (7;0.29)	7.70 (7;2.23)
	Handling Control	–[f]	–	–	–	–	–	–	11.0 (4;4.10)
	Food Measurement Control/ Mercury	0.68 (6;0.25)	–	2.90 (5;0.55)	–	4.01 (6;0.68)	–	4.14 (5;0.98)	52.25 (4;6.80)
	Food/Mercury	0.74 (6;0.12)	–	3.71 (6;1.60)	–	3.95 (6;1.43)	–	4.80 (4;0.86)	52.66 (6;17.17)
Hidden/ L28	**Placebos**	0.82 (14;0.33)	–	0.74 (15;0.34)	–	0.84 (16;0.43)	–	0.74 (15;0.33)	7.28 (18;2.83)
	Dosed with Hg	0.67 (18;0.23)	–	3.44 (18;1.49)	–	3.68 (17;1.31)	–	4.60 (12;1.39)	49.18 (16;14.07)
	Food Measurement Control/Placebo	0.88 (5;0.31)	–	0.67 (5;0.43)	–	0.81 (5;0.52)	–	0.87 (5;0.30)	8.02 (5;3.80)

Food/Placebo	0.78 (9;0.35)	–	0.77 (10;0.32)	–	0.85 (11;0.41)	–	0.68 (10;0.35)	6.91 (10;2.38)
Handling Control	-	–	–	–	–	–	–	7.50 (3;2.21)
Food Measurement Control/ Mercury	0.72 (6;0.33)	–	2.93 (6;1.15)	–	3.95 (4;1.26)	–	3.93 (3;0.57)	48.75 (4;15.41)
Food/Mercury	0.65 (12;0.17)	–	3.70 (12;1.62)	–	3.61 (13;1.36)	–	4.82 (9;1.53)	49.33 (12;14.32)

Table 5.5. Continued.

[a] Mercury dosing experiment lasted 15 days, and mercury concentrations were measured every 5 days. First sample (Day 0) was collected prior to the administration of mercury (approximately 11.6 days of age).
[b] Placebos = Handling Control, Food Measurement COntrol/Placebos and Foo/Placebos (see Figure 5.5).
[c] Doesed withHg = Food Measurement Control/Mercury and Food/Mercury (see Figure 5.5).
[d] Mean (n, SD).
[e] Feathers were collected only at Day 15.
[f] No blood samples were collected from Handling Controls.

Table 5.6. Unadjusted means of age, growth parameters, and food consumption of great egret nestlings by colony and year. Since multiple measurements were taken from chicks over a period of weeks, standard deviations are not reported.

Group	Mean Age (d)	Mean Mass (g)	Mean Culmen Length (cm)	Mean Food Consumption (g/d)
T. East[1]	18.75	484.7	5.38	164.2
Hidden '95[1]	18.16	513.8	5.47	162.6
1995[1]	18.43	500.2	5.43	163.4
Hidden '94[2]	17.82	436.4	5.19	147.6
L-67[2]	19.90	420.6	5.21	161.9
1994[2]	18.71	429.7	5.20	153.5

[1]Placebo chicks (Food + Food Measurement Control) only. [2]First-hatched chicks only.

Table 5.7. Average packed cell volumes (%), plasma proteins (ug/dl), white blood cells (cells/ul), and total number of oral parasites (Clinostomum sp.) in great egret nestlings during 1994, by colony.

Colony	Packed Cell Volume (%)	Plasma Proteins (ug/dl)	Number of White Blood Cells (cells/ul)	Number of Oral Parasites (Clinostomum spp.)
Frog City South	6 (2)[a]	6 (2)	0	8 (2)
Hidden/L28	92 (25)	91 (25)	55 (24)	76 (20)
Deer Island	14 (4)	14 (4)	14 (4)	10 (4)
L67	80 (42)	79 (42)	35 (19)	96 (45)
Mud Canal	42 (13)	41 (13)	39 (13)	16 (10)
JW1	20 (17)	21 (17)	15 (11)	16 (16)
Alley North	32 (14)	32 (14)	19 (12)	13 (13)
Total	286(117)	284(117)	177(83)	235(110)

[a] Total number of samples collected, and in parentheses, number of chicks sampled.

"YOUNG" CHICKS

Health Status	Hg in Blood		
	"Normal"	**"High"**	Total
"Healthy"	67	5	72
"Sick"	51	6	57
Total	118	11	129

Chi-square = 0.52, P = 0.469

"OLD" CHICKS

Health Status	Hg in Blood		
	"Normal"	**"High"**	Total
"Healthy"	75	5	80
"Sick"	65	12	77
Total	140	17	157

Chi-square = 3.54, P = 0.06.

"OLD" CHICKS

Health Status	Hg in Feathers		
	"Normal"	**"High"**	Total
"Healthy"	34	2	36
"Sick"	32	5	37
Total	66	7	73

Chi-square = 1.33, P = 0.248

Table 5.8. Contingency tables comparing frequencies of healthy and sick chicks in normal and high mercury catagories, by tissue mercury type and age ("young" < 4.1 cm bill length; "old" > 4.1 cm).

"YOUNG" CHICKS

Health Status	Hg in Blood "Normal"	"High"	Total
"Healthy"	32	1	33
"Sick"	20	4	24
Total	52	5	57

Chi-square = 3.23, p = 0.07.

"OLD" CHICKS

Health Status	Hg in Blood "Normal"	"High"	Total
"Healthy"	43	2	45
"Sick"	37	8	45
Total	80	10	90

Chi-square = 4.05, p = 0.04.

"OLD" CHICKS

Health Status	Hg in Feathers "Normal"	"High"	Total
"Healthy"	19	1	20
"Sick"	18	4	22
Total	37	5	42

Chi-square = 1.73, p = 0.188.

Table 5.9. Contingency tables comparing frequencies of healthy and sick first-hatched chicks in normal and high mercury catagories, by tissue mercury type and age ("young" < 4.1 cm bill length; "old" > 4.1 cm).

Table 5.10. Health indicators from great egret nestlings included in a field mercury dosing experiment during 1995, by treatment. Indicators were calculated prior to the start of the experiment (Day 0), and at 5-day intervals thereafter. Note that chicks averaged 11.6 days of age at day 0 of the experiment.

Variable Measured, by Treatment	Average (n, SD) Day 0	Average (n, SD) Day 5	Average (n, SD) Day 10	Average (n, SD) Day 15
Clinostomum (C)[a]	1.66 (9; 2.29)	4.80 (10; 7.55)	6.70 (10; 8.57)	6.00 (6; 6.13)
Clinostomum (Hg)[b]	2.27 (11; 2.83)	3.00 (11; 3.19)	5.72 (11; 6.29)	8.30(10; 8.74)
Packed Cell Volume, % (C)	43.65 (10; 4.33)	36.94 (9; 5.10)	35.95 (10; 3.66)	36.14 (7; 3.13)
Packed Cell Volume, % (Hg)	34.50 (11; 4.75)	36.87 (11; 2.59)	35.00 (11; 3.68)	34.05 (9; 6.54)
Plasma Proteins, g/dl (C)	4.88 (10; 0.53)	4.95 (8; 0.41)	5.43 (10; 0.30)	5.52 (7; 0.49)
Plasma Proteins, g/dl (Hg)	4.86 (11; 0.53)	4.99 (11; 0.56)	5.45 (11; 0.36)	5.15 (9; 0.30)
White Blood Cells, cells/ul (C)	2,743(10; 15,639)	32,693(10; 13,184)	52,679(10; 37,206)	42,321(7; 11,484)
White Blood Cells, cells/ul (Hg)	22,798 (11; 6,303)	35,159 (11; 8,807)	40,492(11; 17,077)	31,586(9; 12,903)
Lymphocytes, cells/ul (C)	13,131 (10; 2,079)	18,602 (10; 2,347)	30,606 (10; 7,266)	27,750 (7; 1,117)
Lymphocytes, cells/ul (Hg)	11,360 (11; 843)	17,459 (11; 1,164)	22,857 (11; 1,304)	17,372 (9; 1,223)
Heterophils, cells/ul (C)	7,382 (10; 1,953)	7617 (10; 2,079)	11,010 (10; 5,395)	10,096 (7; 1,008)
Heterophils, cells/ul (Hg)	6,212 (11; 622)	8,613 (11; 939)	8,282 (11; 1,593)	9,619 (9; 1,903)
Eosinophils, cells/ul (C)	6,511 (10; 1,534)	6,146 (10; 1,439)	10,061 (10; ,844)	5,501 (7; 889)
Eosinophils, cells/ul (Hg)	4,901 (11; 714)	8,670 (11; 982)	8,908 (11; 1,585)	4,769 (9; 994)

[a] C = Controls
[b] Hg = Mercury-dosed

Table 5.11 Summary of 1994 radio-telemetry data.

Colony	Nest N°	Chick Weight(g) at banding	Hatch Order	Weight of Transmitter as a % of body mass	Date of Deployment	Last Information
Hidden/L28	25	473	Largest	2.54%	4/24/94	Last located on 07/26/94 in impounded pasture (26°29.04'N, 81°33.43'W).
	25	473	Smallest	2.54%	4/24/94	Last located on 06/26/94 at colony (approximate location 25°49.47'N, 80°45.67'W).
	26	568	Largest	2.11%	5/6/94	Found dead on 06/15/94, in cocoa plum-patch, East of colony (25°46.58'N, 80° 50.08'W).
	26	465	Smallest	2.58%	5/6/94	Heard mortality signal on 06/26/94, close to colony (25°46.50'N, 80°50.25'W). Found nothing to indicate death of bird.
	27	533	Largest	2.25%	4/24/94	Last located on 02/18/95 (26°00.61'N, 81°03.24'W).
	27	508	Smallest	2.36%	4/24/94	Last located on 08/11/94 in wet prairie (25°37.13'N, 80°54.73'W).
	114	638	Only	1.88%	5/6/94	Heard mortality signal on 08/11/94, North of Imokalle. Found nothing to indicate death of bird.
	128	670	Largest	1.79%	5/21/94	Last located on 10/29/95 in Big Cypress (26°13.23'N, 81°03.97'W).
	132	593	Largest	2.02%	5/6/94	Heard mortality signal on 08/15/94, North of colony. Found nothing to indicate death of bird.
	132	450	Smallest	2.67%	5/6/94	Found dead on 07/22/94 (25°57.85'N, 80° 55.28'W).
	215	480	Largest	2.50%	5/6/94	Last located at colony on 06/27/94.

Table 5.11. Continued.

Colony	Nest N°	Chick Weight(g)	Hatch Order	Weight of Transmitter as a % of body mass	Date of Deployment	Last Information
Hidden/L28	215	460	Smallest	2.61%	5/6/94	Found dead on 07/26/94 in Big Cypress (25°59.45'N, 81°59.84'W).
	221	595	Largest	2.02%	5/21/94	Found dead on 08/11/94 in wet forest (26°26.47'N, 81°13.53'W). Transmitter with strong indentation possibly from bill.
	221	555	Smallest	2.16%	5/21/94	Heard mortality signal on 06/29/94, 150 m West of colony, in pondapple/cypress stand. Found nothing to indicate death of bird.
	972	555	Largest	2.16%	4/11/94	Found dead on 09/14/94 in Big Cypress (26°04.19'N, 81°11.55'W).
Alley North	51	780	Largest	1.54%	5/2/94	Last located on 08/22/94 in sugarcane fields (26°33.75'N, 80°46.92'W).
	51	700	Smallest	1.71%	5/2/94	Last noted at colony on 06/14/94.
	55	540	Smallest	2.22%	5/2/94	Last located on 02/18/95 (26°27.54'N, 81°03.10'W).
	59	770	Largest	1.56%	5/2/94	Last noted at colony on 06/20/94.
	59	620	Smallest	1.94%	5/2/94	Found dead on 02/18/95 in a cypress swamp close to an orange grove (26°56.71'N, 80°27.52'W).
	64	695	Largest	1.73%	5/2/94	Last located on 02/18/95 (26°49.67'N, 80°28.87'W).
	65	765	Largest	1.57%	5/2/94	Heard mortality signal on 10/29/94 (26°27.89'N, 81°04.27'W)in pastures. Found nothing to indicate death of bird.

Table 5.11. Continued.

Colony	Nest N°	Chick Weight(g)	Hatch Order	Weight of Transmitter as a % of body mass	Date of Deployment	Last Information
Alley North	66	494	Largest	2.43%	5/8/94	Found dead at colony, under nest on 07/06/94.
	66	493	Medium	2.43%	5/8/94	Last located on 10/29/94 in canal between sugarcane fields (26°23.85'N, 80°37.76'W).
	66	492	Smallest	2.44%	5/8/94	Last located on 07/15/94 in ditch between sugarcane fields (26°42.00'N, 81°38.08'W).
	70	710	Largest	1.69%	5/2/94	Last located on 02/18/95 (26°13.60'N, 81°00.33'W).
	70	695	Smallest	1.73%	5/2/94	Last located on 02/18/95 (26°12.07'N, 80°36.39'W).
Deer Island	116	498	Largest	2.41%	4/16/94	Found dead on 06/29/94 approximately 150 m from colony.
L67	180	478	Largest	2.51%	5/10/94	Last located on 02/18/95 (26°26.13'N, 81°02.51'W).
	185	540	Largest	2.22%	5/21/94	Last located 07/06/94 in colony.
	186	740	Largest	1.62%	5/10/94	Last located on 08/22/94 in flooded area between cypress and palmettos (26°30.02'N, 81°14.84'W).
	190	619	Largest	1.94%	5/10/94	Found dead on 09/14/94 in Big Cypress (25°59.95'N, 80°59.06'W).
	190	306	Smallest	3.92%	5/10/94	Last located at colony on 07/22/94.
	191	568	Largest	2.11%	5/10/94	Last located on 02/18/95 (26°34.05'N, 81°02.81'W).
	191	316	Smallest	3.80%	5/10/94	Seen alive at colony on 05/16/94, but signal was very weak. Never heard signal again.
	234	598	Largest	2.01%	5/21/94	Found dead on 07/22/94 by I-75.

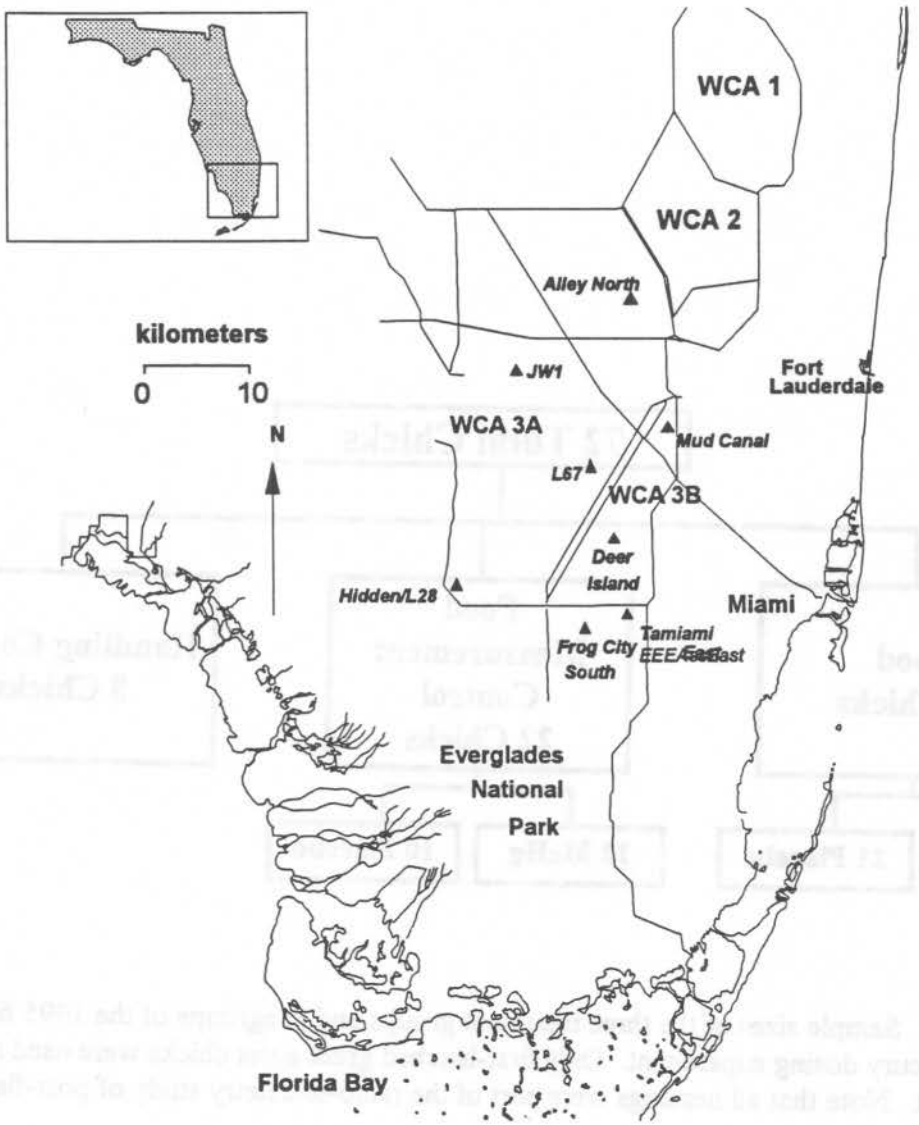


Figure 5.3. Map of study area in southern Florida, showing locations of major wading bird colonies (triangles) in Water Conservation Area 3. Hidden colony is near the southwest corner of WCA 3, Tamiami East colony is approximately 20 km east of Hidden colony, and L-67 colony lies approximately 20 km northeast of Hidden colony.

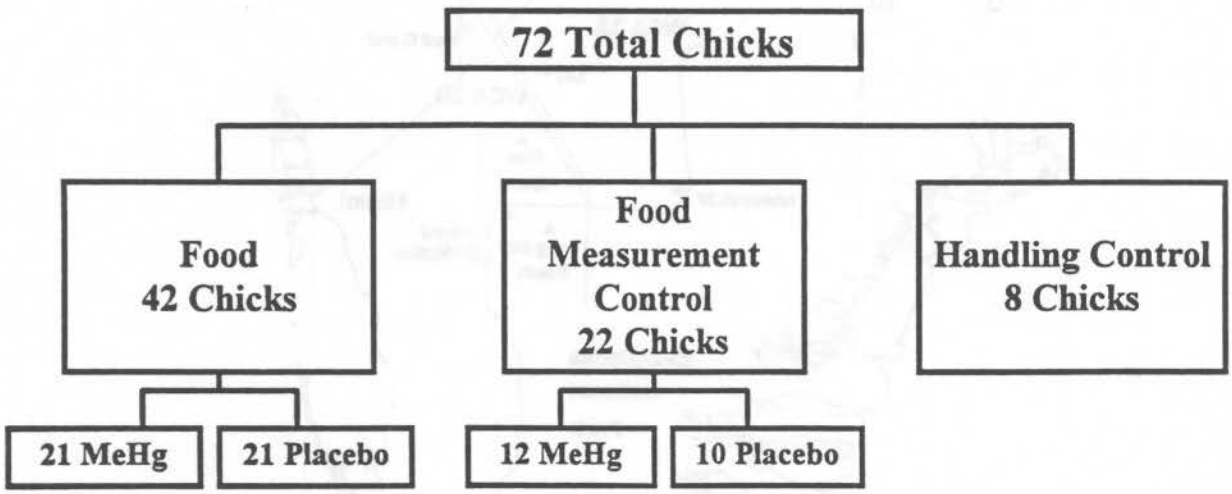


Figure 5.4. Sample sizes of the three treatment groups and subgroups of the 1995 field methylmercury dosing experiment. Only first-hatched great egret chicks were used in the experiment. Note that all nestlings were part of the radio-telemetry study of post-fledging survival.

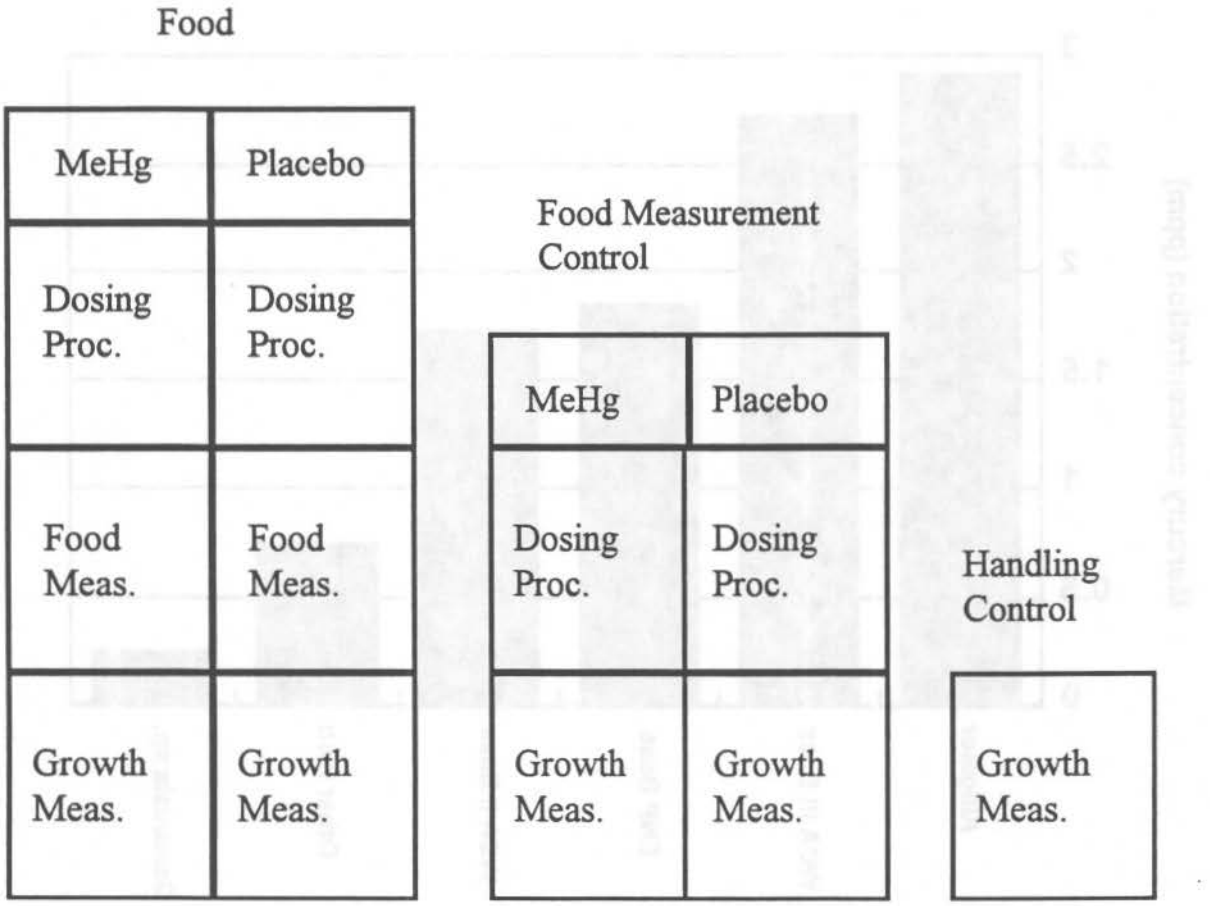


Figure 5.5. Treatments received by each of the groups and subgroups in the 1995 field mercury dosing experiment. The Food Measurement Control group serves as a control for the food consumption monitoring procedure (injecting tritium, taking a second blood sample every five days). The Handling Control group serves as a control for the dosing procedure (taking blood samples, giving capsules). Within the Food and Food Measurement Control groups, subgroups receiving placebos (Placebo) serve as controls for the dose of methylmercury (MeHg).

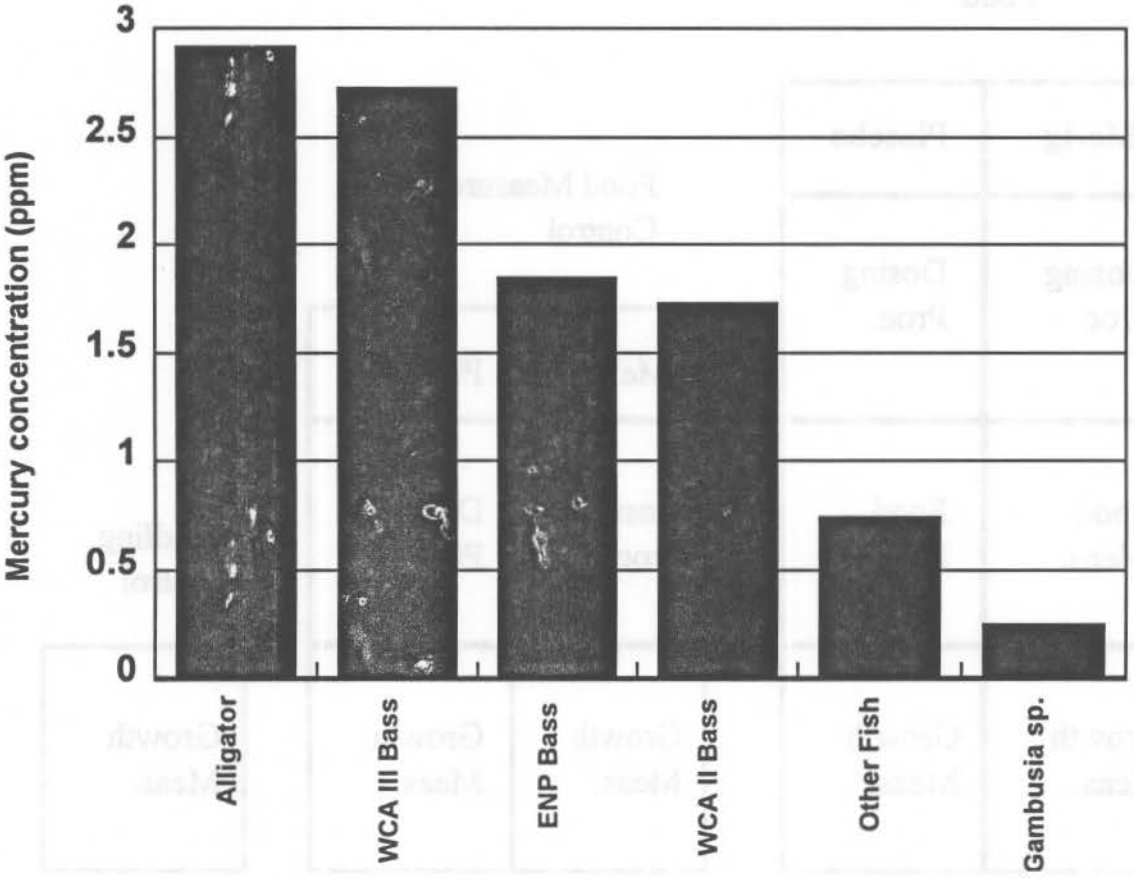


Figure 5.6. Mercury concentrations (mg/kg) of selected Everglades animals. Alligator and bass data are taken from Ware et al. 1990. Other fish and *Gambusia* data are from W. Loftus (pers. comm.).

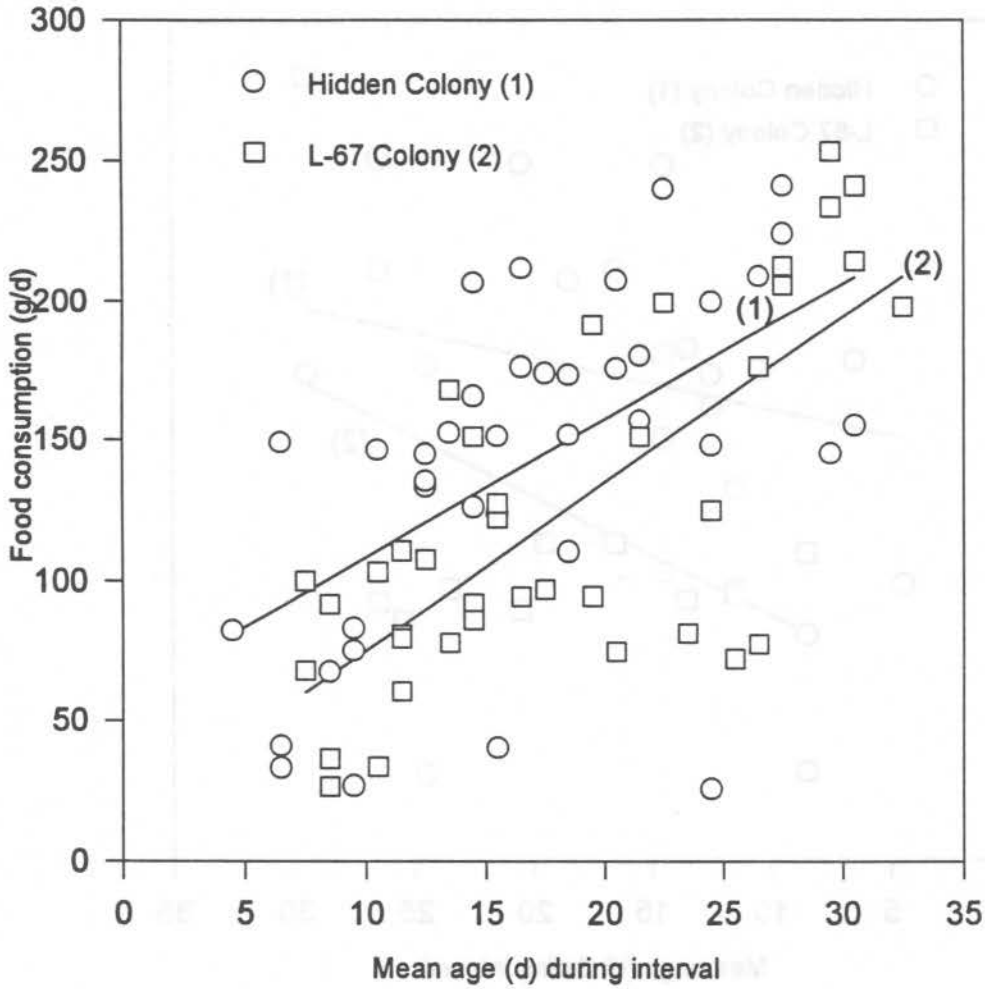


Figure 5.7. Food consumption of all great egret nestlings as a function of age at two colonies in 1994. Both food consumption and age are plotted as means of three-day measurement intervals. Individual nestlings may be represented by multiple data points in this graph. Regression lines are plotted to illustrate trends in data, and are unrelated to analyses presented in the text.

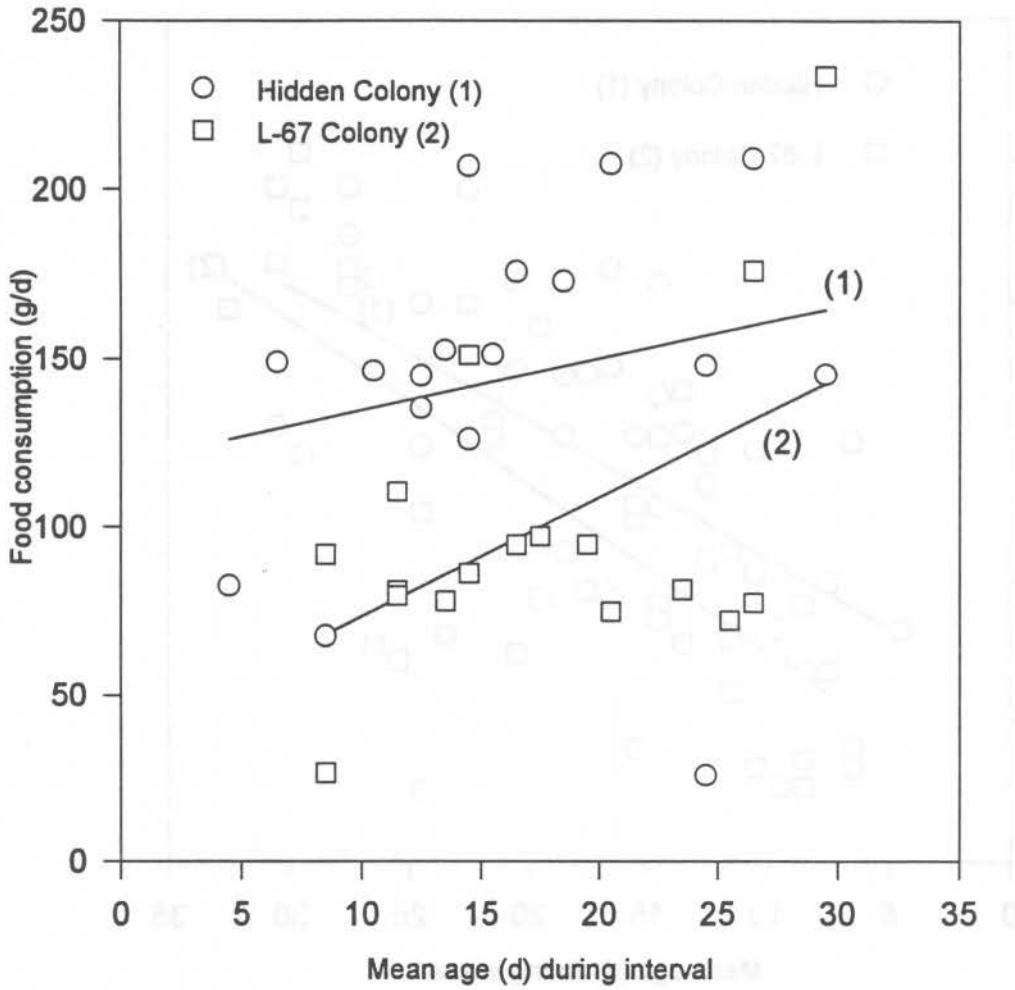


Figure 5.8. Food consumption as a function of age of all second-hatched great egret nestlings surveyed during 1994. Both food consumption and age are plotted as means of three-day measurement intervals. Individual nestlings may be represented by multiple data points in this graph. Regression lines are plotted to illustrate trends in data, and are unrelated to analyses presented in the text.

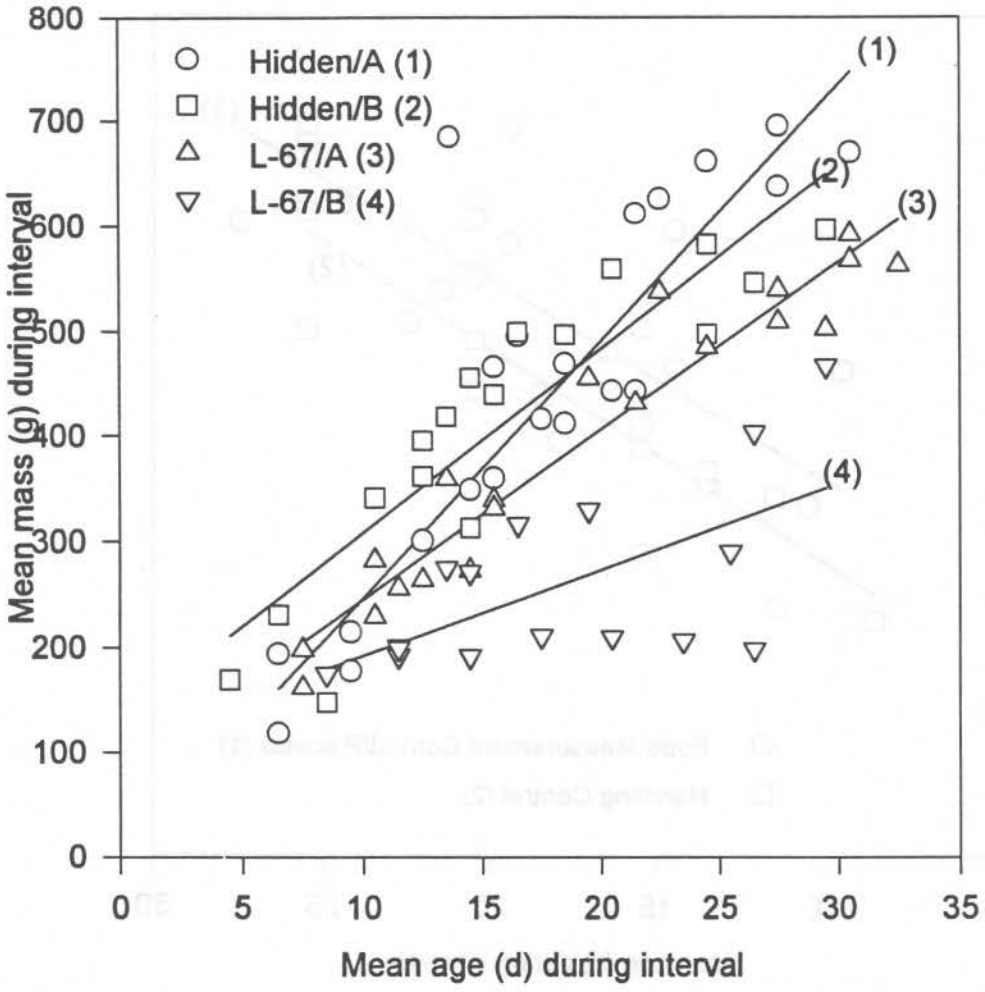


Figure 5.9. Body mass as a function age for first- and second-hatched great egret nestlings that were monitored for food consumption during 1994, grouped by hatch order and by nesting colony. Both body mass and age are plotted as means of three-day measurement intervals. Individual nestlings may be represented by multiple data points in this graph. Regression lines are plotted to illustrate trends in data, and are unrelated to analyses presented in the text.

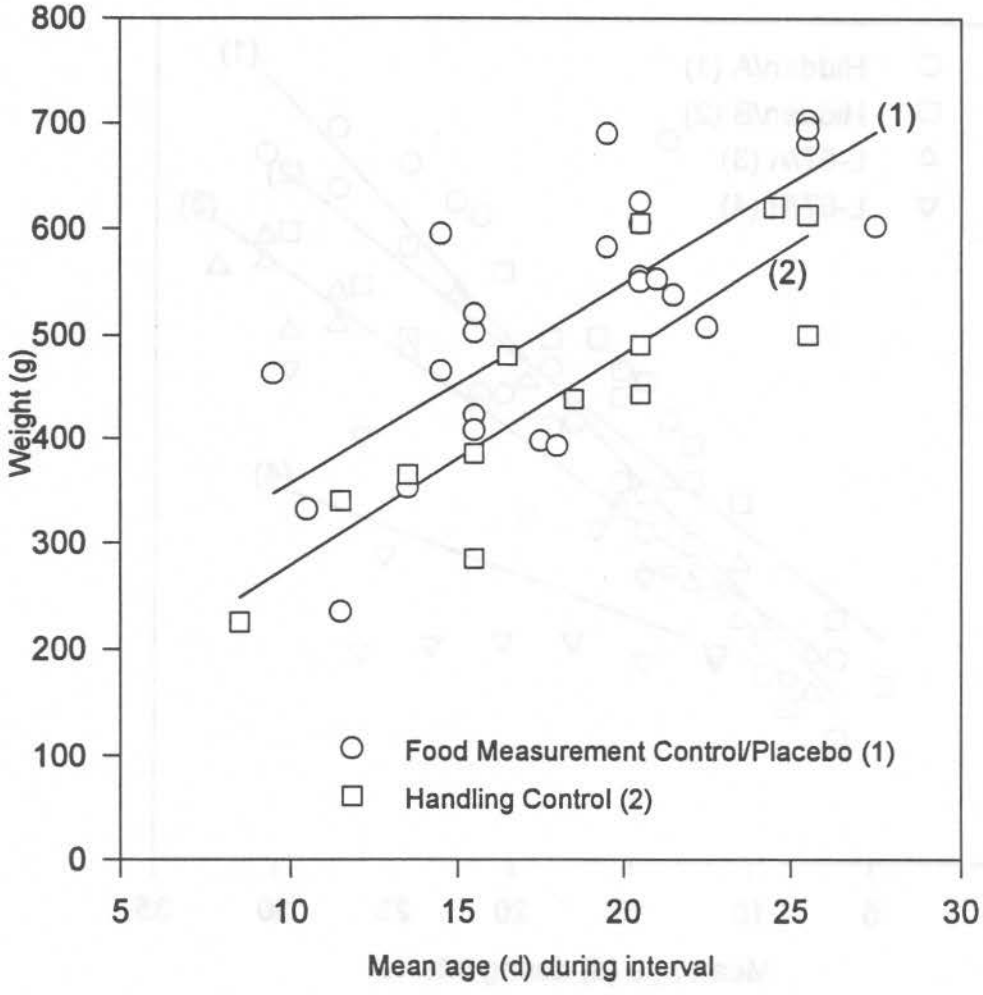


Figure 5.10. The relation of body mass to age for Food Measurement Control/Placebo and Handling Control chicks from the 1995 field experiment. Both body mass and age are plotted as means of five-day measurement intervals. Individual nestlings may be represented by multiple data points in this graph. Regressions are plotted to illustrate trends in data, and are unrelated to analyses presented in the text.

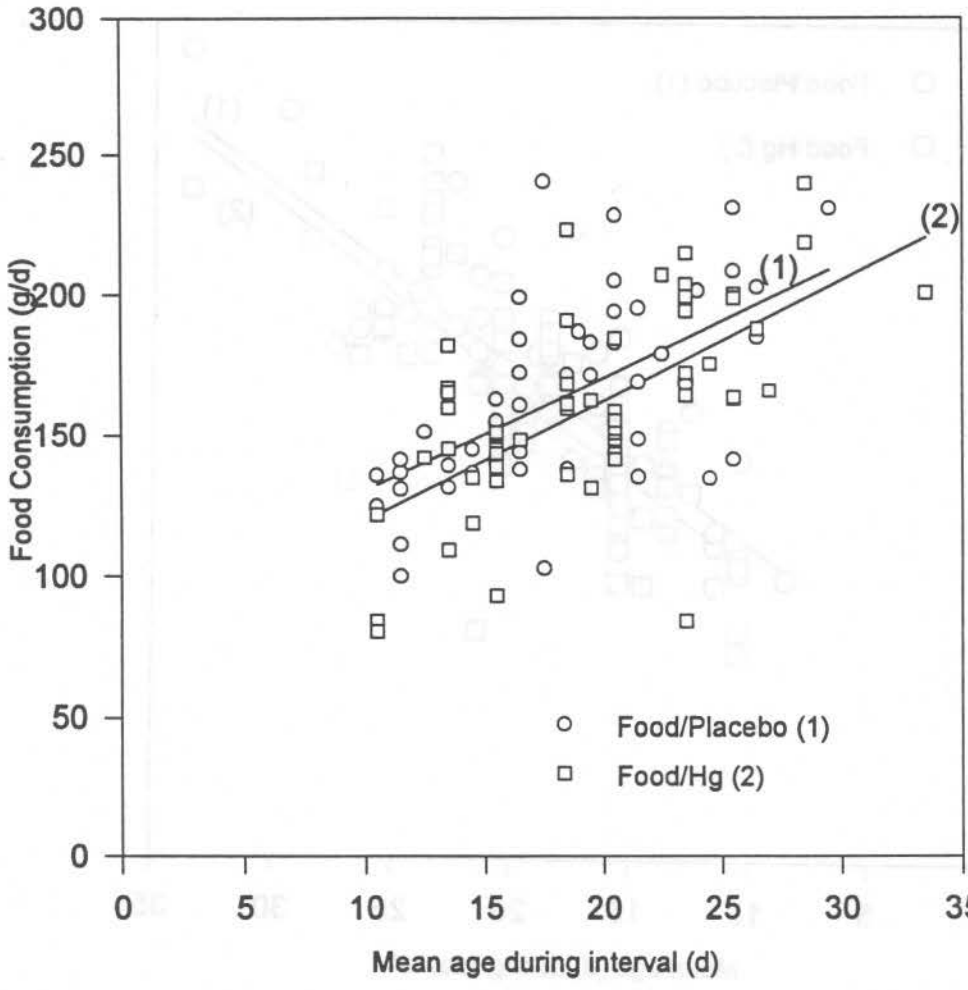


Figure 5.11. Relation of food consumption to age for Food/Placebo and Food/Hg chicks in the 1995 field experiment. Both food consumption and age are plotted as means of five-day measurement intervals. Individual nestlings may be represented by multiple data points in this graph. Regression lines are plotted to illustrate trends in data, and are unrelated to analyses presented in the text.

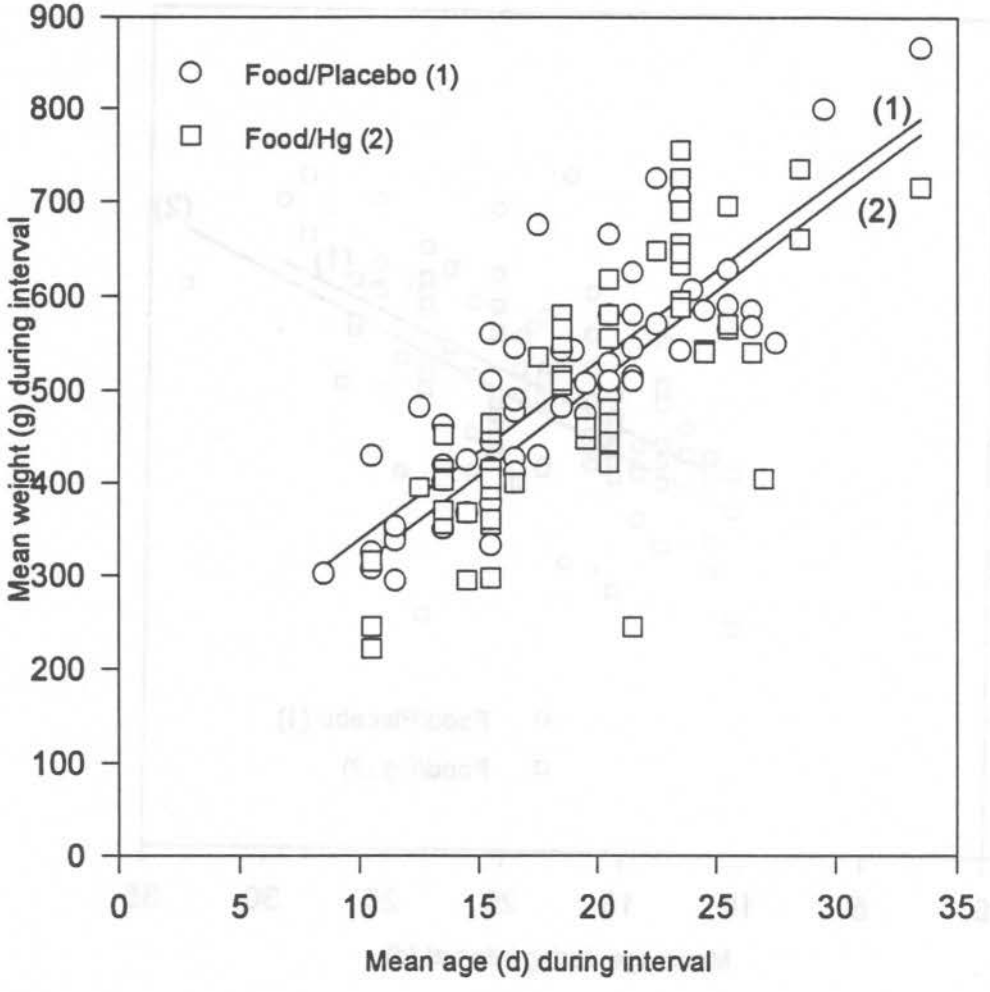


Figure 5.12. Relation of body mass to age for Food/Placebo and Food/Hg chicks used in the 1995 field experiment. Both body mass and age are plotted as means of five-day measurement intervals. Individual nestlings may be represented by multiple data points in this graph. Regression lines are plotted to illustrate trends in data, and are unrelated to analyses presented in the text.

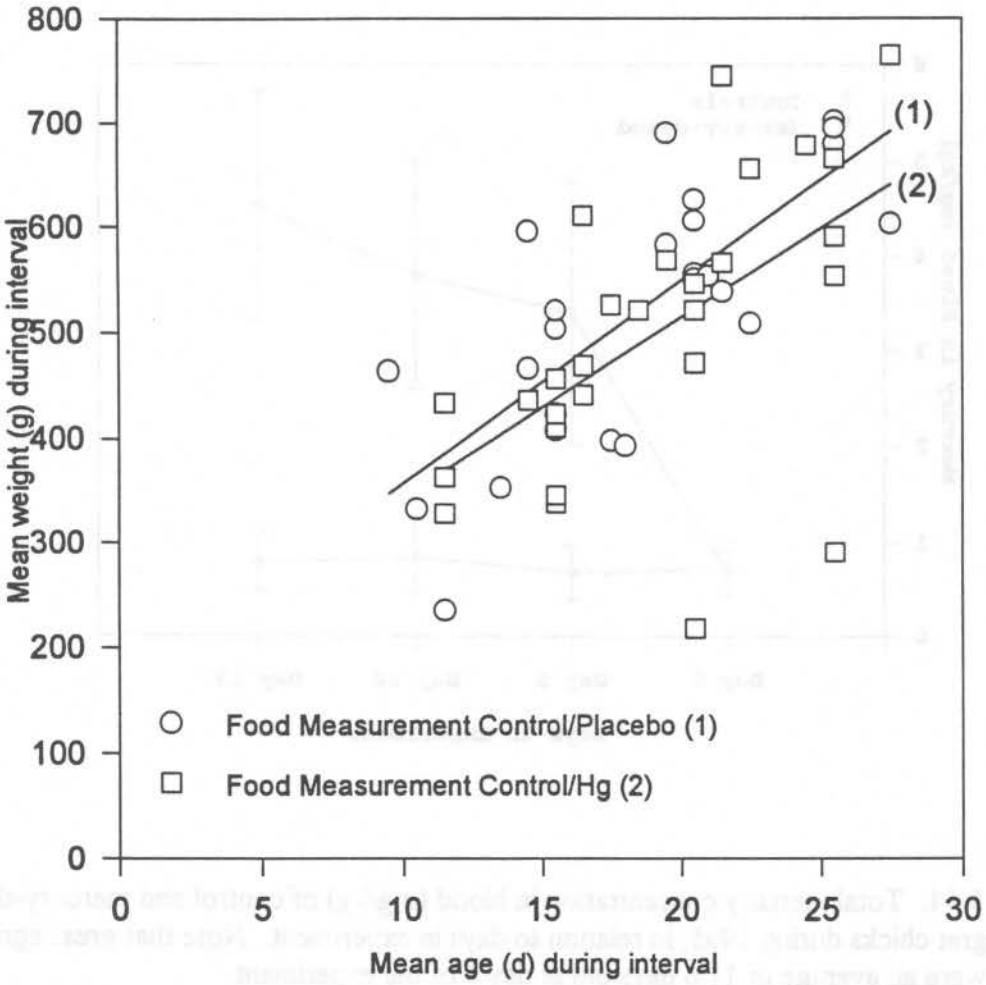


Figure 5.13. Relation of body mass to age for Food Measurement Control/Placebo and Food Measurement Control/Hg chicks in the 1995 field experiment. Both body mass and age are plotted as means of five-day measurement intervals. Individual nestlings may be represented by multiple data points in this graph. Regression lines are plotted to illustrate trends in data, and are unrelated to analyses presented in the text.

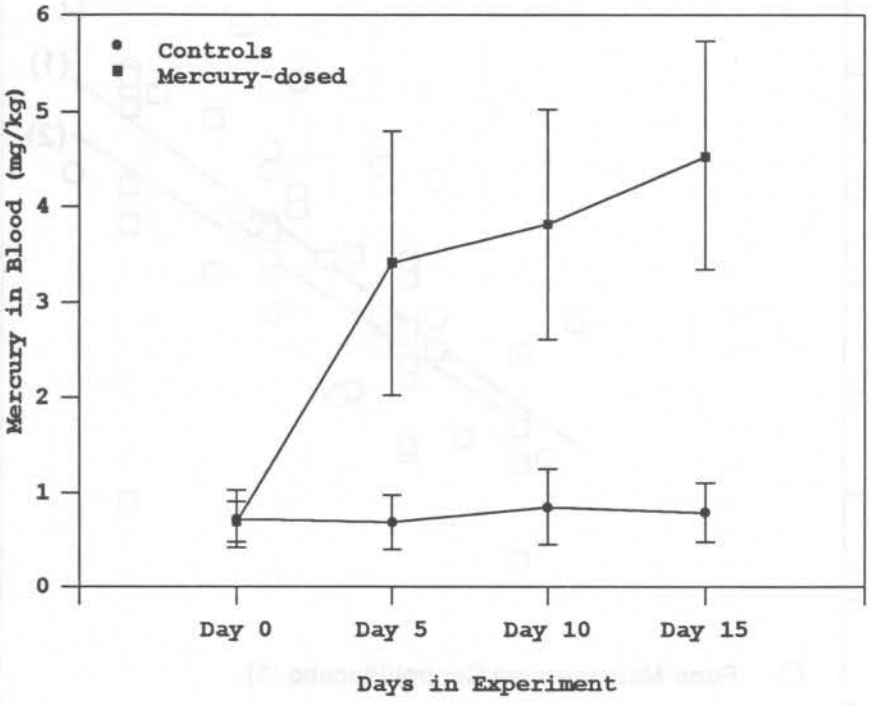


Figure 5.14. Total mercury concentration in blood (mg/kg) of control and mercury-dosed great egret chicks during 1995, in relation to days in experiment. Note that great egret chicks were an average of 11.6 days old at day 0 of the experiment.

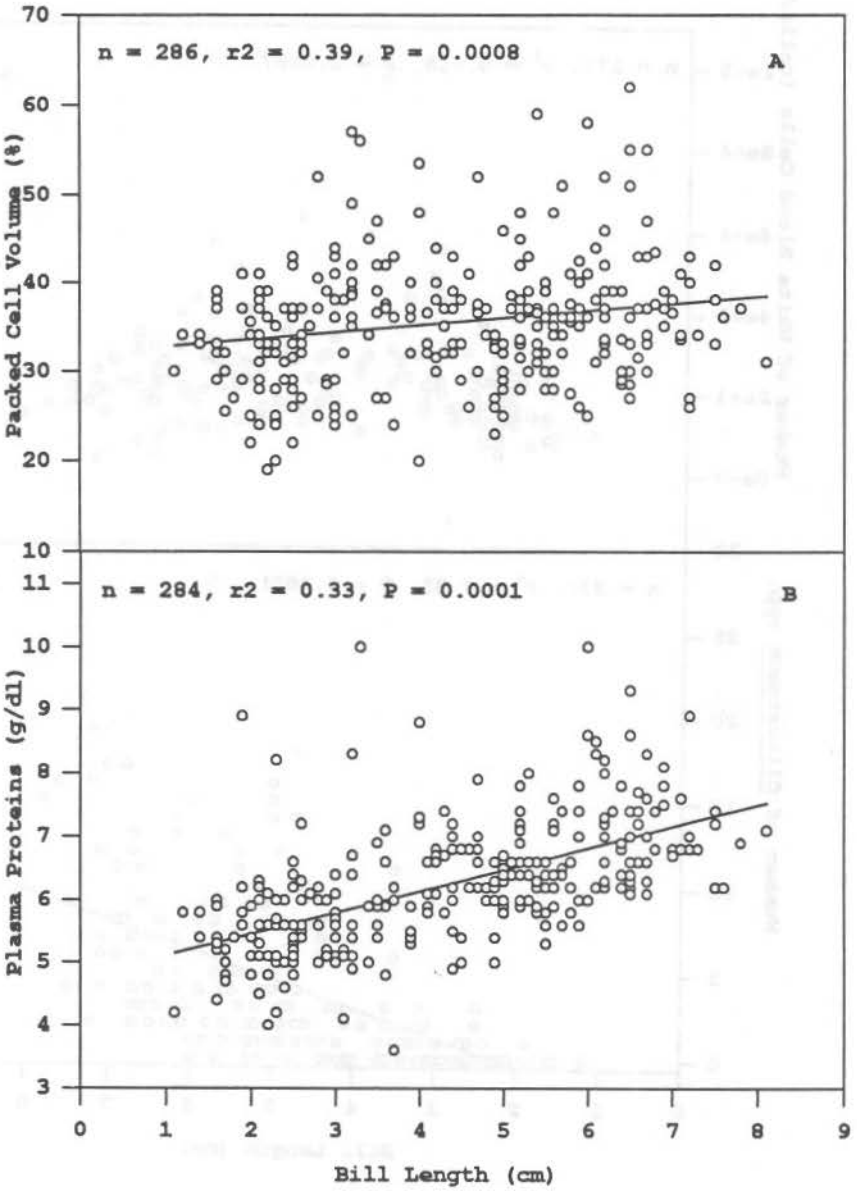


Figure 5.15. Relationship between bill length, packed cell volume (A) and plasma proteins (B) in great egret nestlings during 1994.

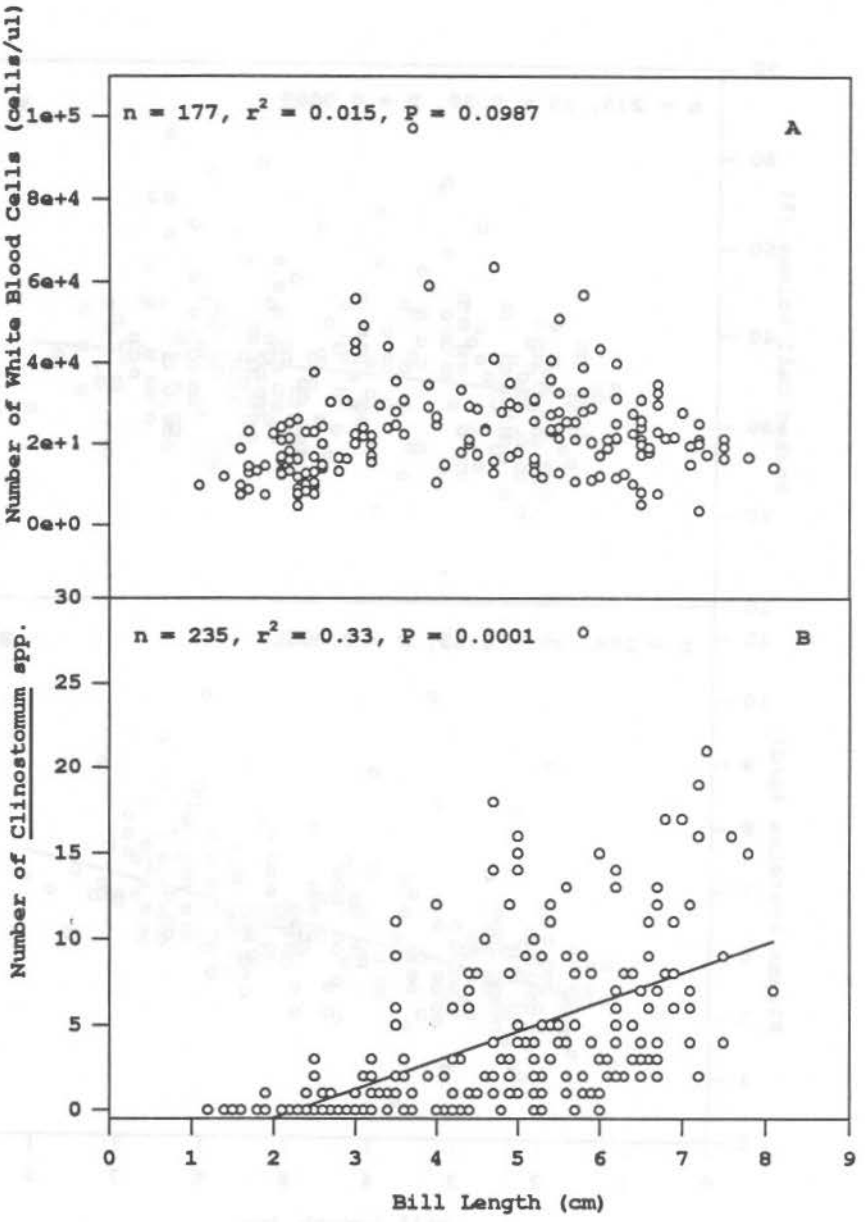


Figure 5.16. Relationship between bill length, number of white blood cells (A) and number of Clinostomum spp. (B) in great egret nestlings during 1994.

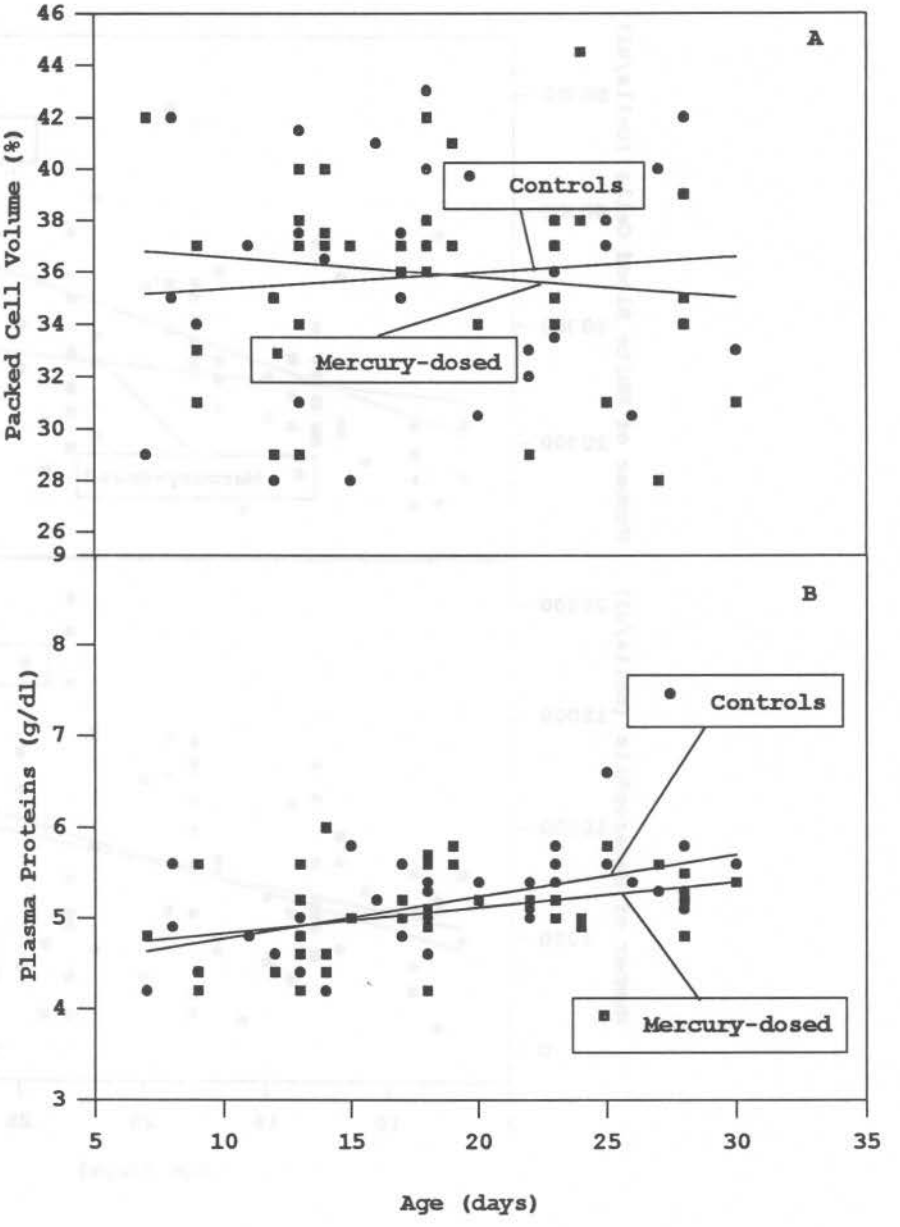


Figure 5.17. Relationship between age, packed cell volume (A) and plasma proteins (B) in great egret nestlings from the field mercury dosing experiment in 1995. Note that great egret chicks averaged 11.6 days of age on day 0 of the experiment.

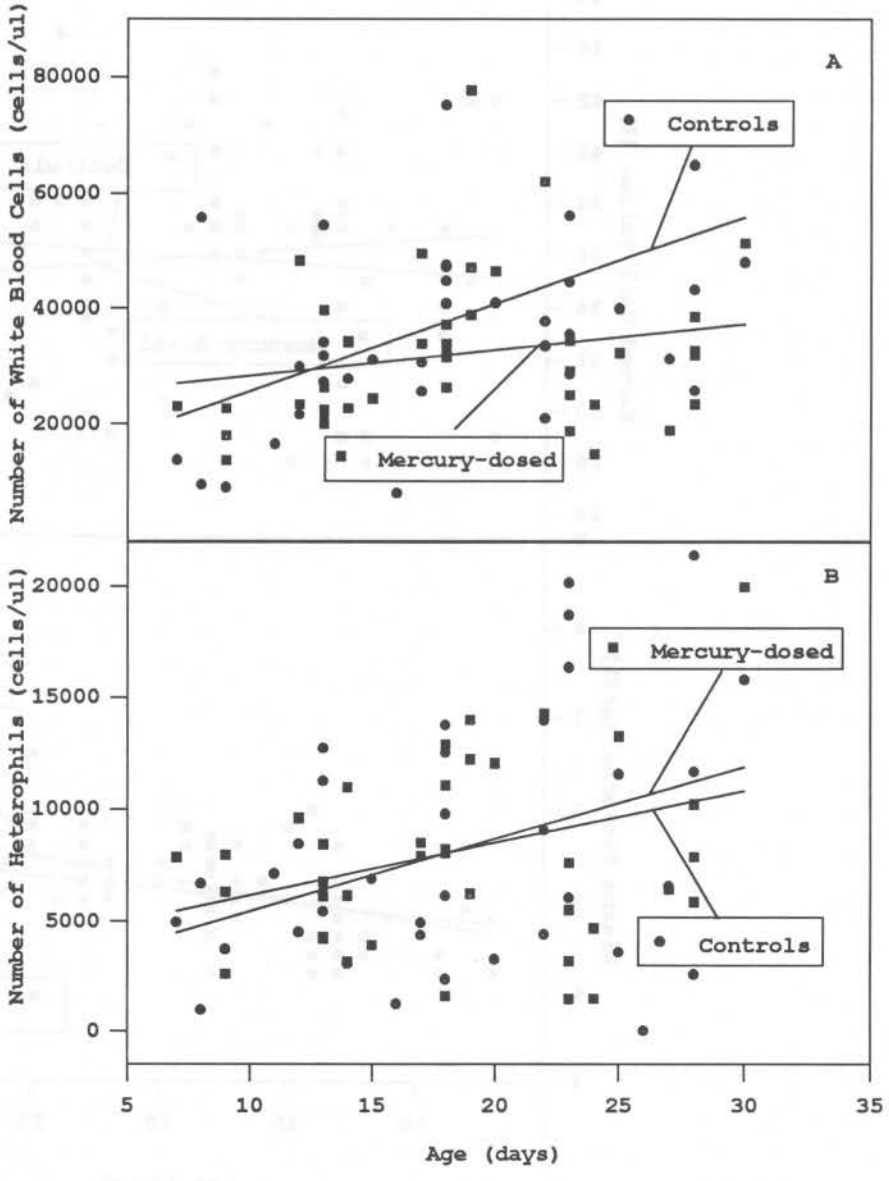


Figure 5.18. Relationship between age and number of white blood cells (A) and heterophils (B) in great egret nestlings from a field mercury dosing experiment in 1995. Note that great egret chicks averaged 11.6 days of age at day 0 of the experiment.

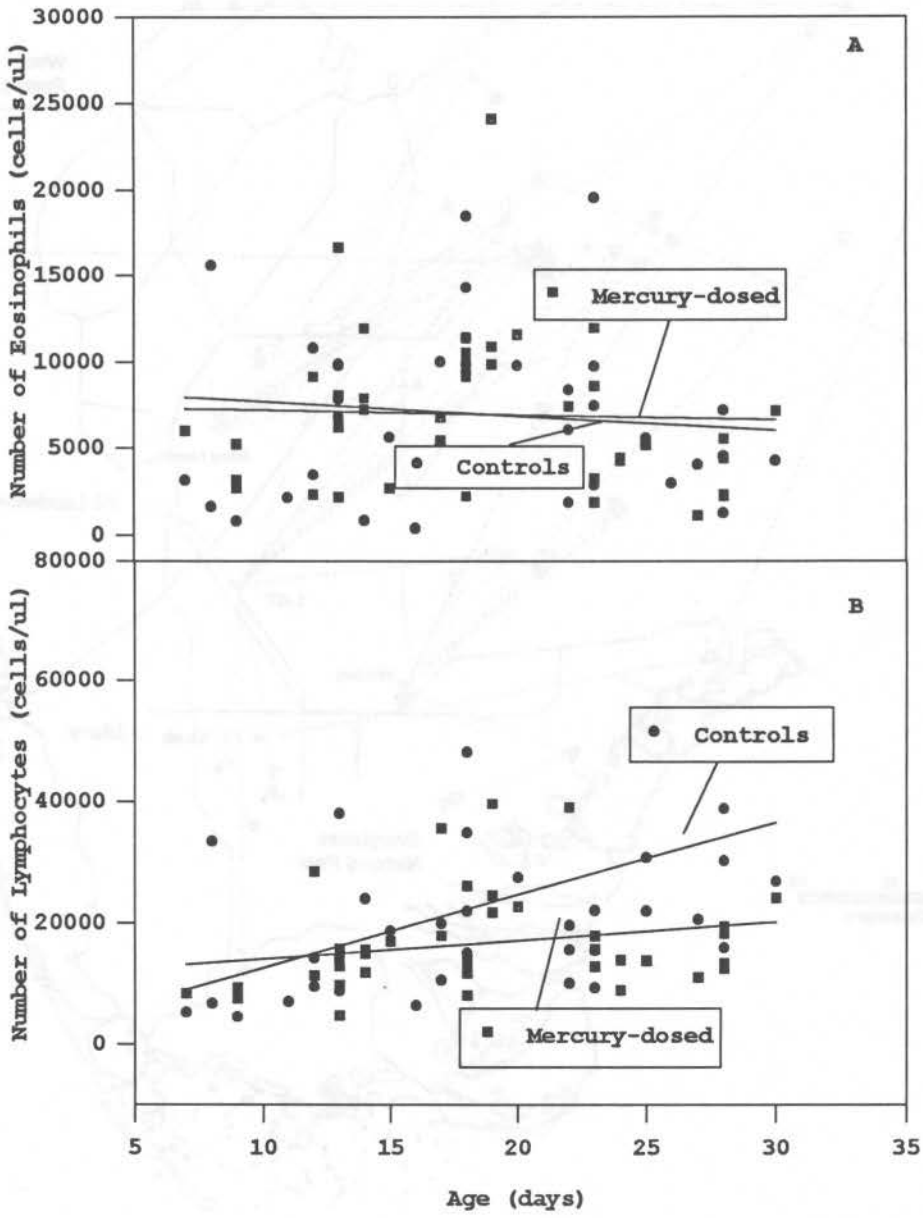


Figure 5.19. Relationship between age and eosinophils (A) and lymphocytes (B) in great egret nestlings from the field mercury dosing experiment. Note that great egret chicks averaged 11.6 days of age on day 0 of the experiment.

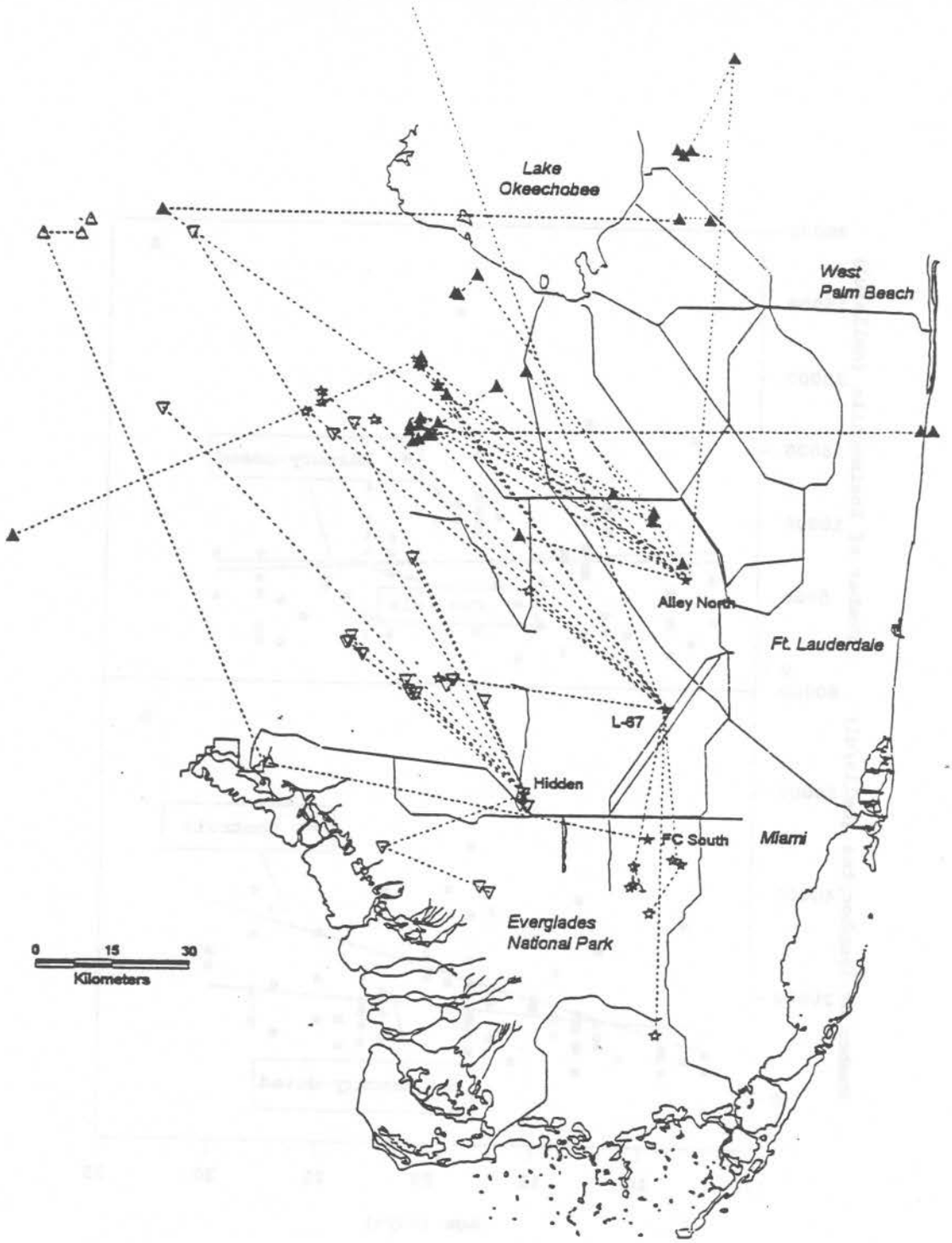


Figure 5.20a. Map of the study area showing locations of colonies where fledgling great egrets were radio-tagged and post-fledgling dispersal patterns of those juveniles as of February 18, 1995, after which the radios were deemed unreliable. Colony locations are shown with stars, and symbols of same type denote birds from the same colony.

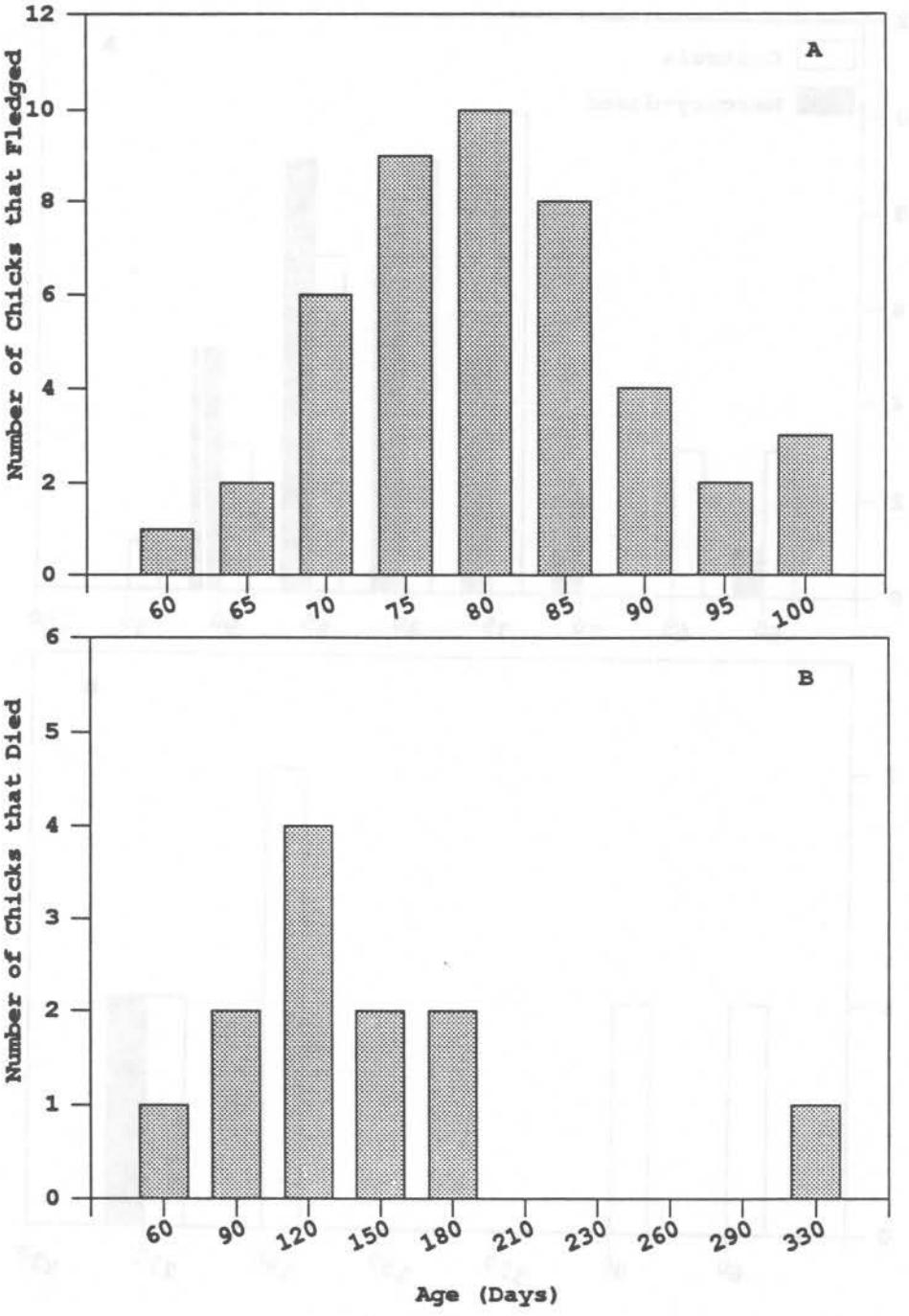


Figure 5.20b. Timing of fledging (A) and of mortality (B) of great egret chicks from colonies studied during 1994 in the Everglades.

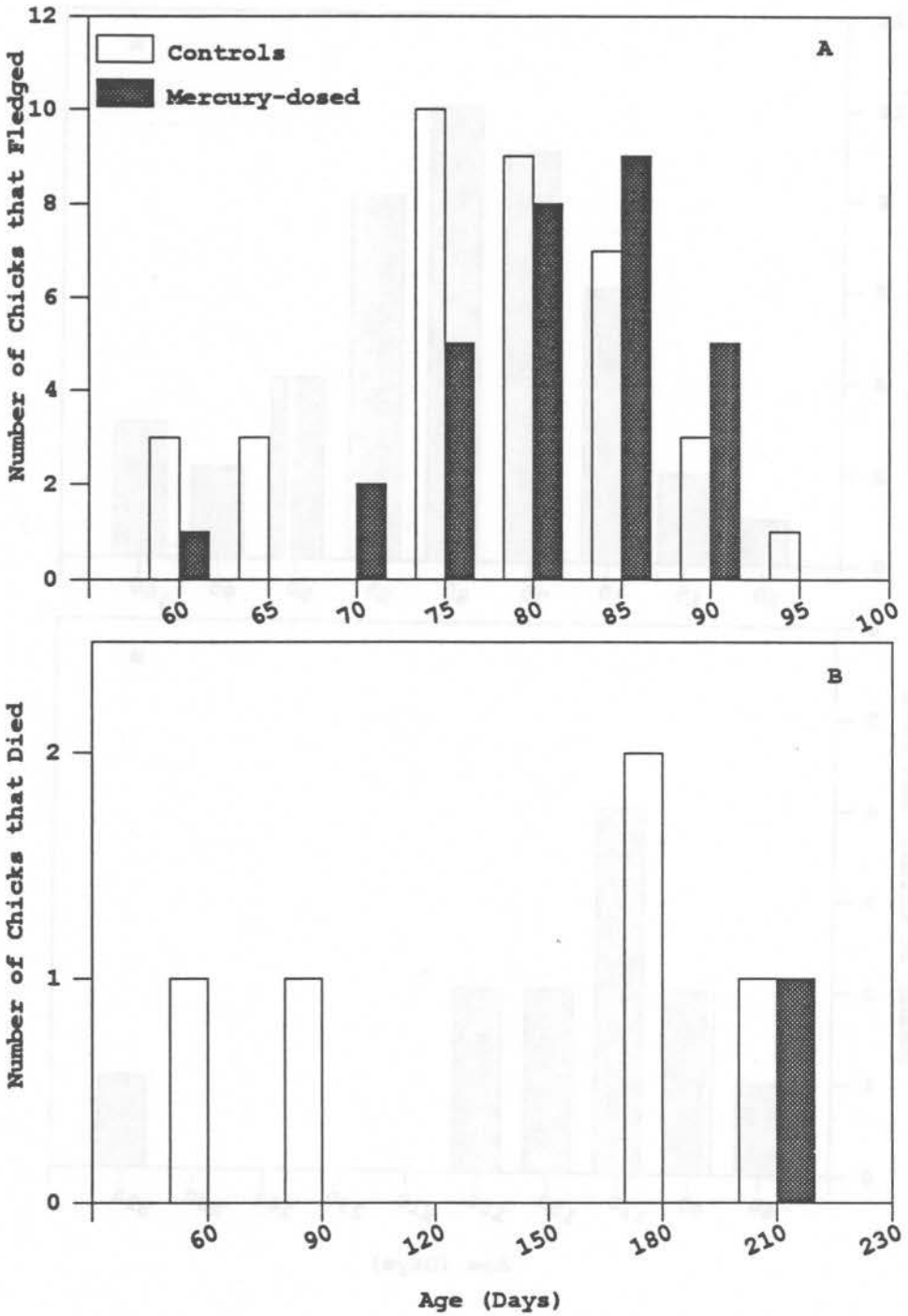


Figure 5.21. Timing of fledging (A) and of post-fledgling mortality (B) of great egret chicks from colonies studied during 1995 in the Everglades. Note that x-axis scales are different.

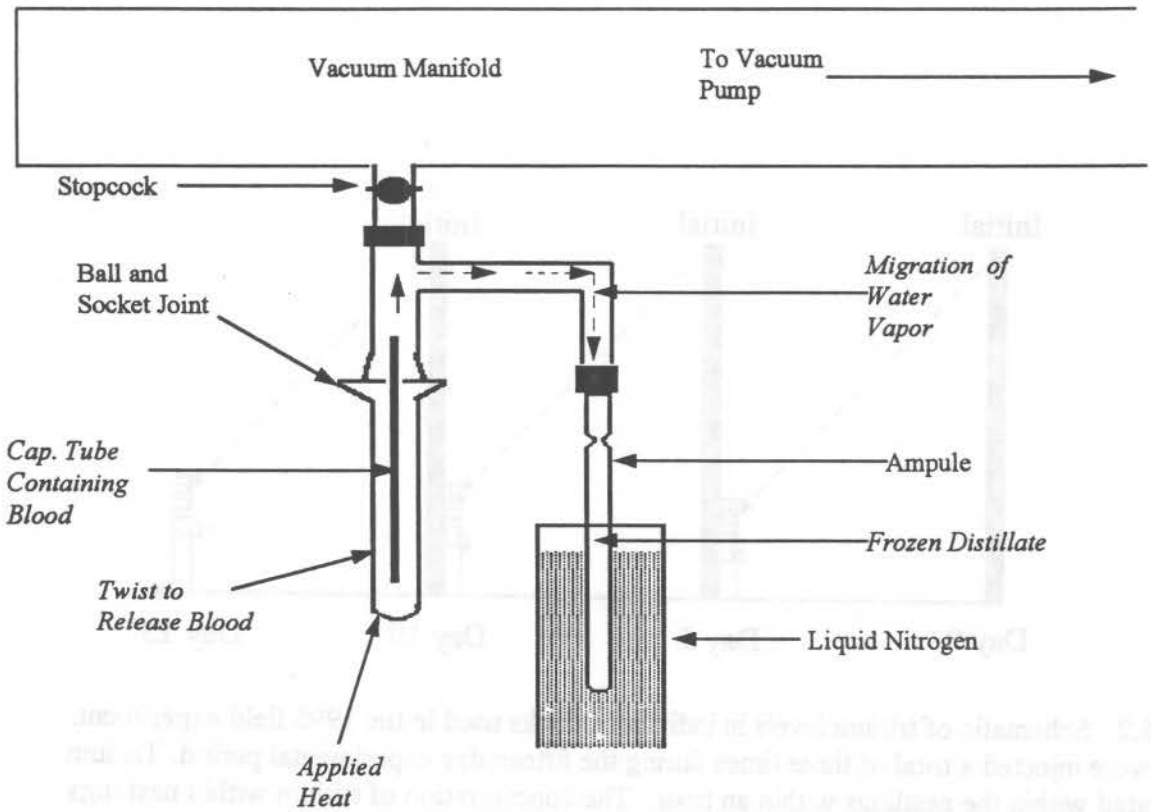


Figure 5.1. Vacuum distillation apparatus and procedure used for distilling labeled water from nestling blood samples.

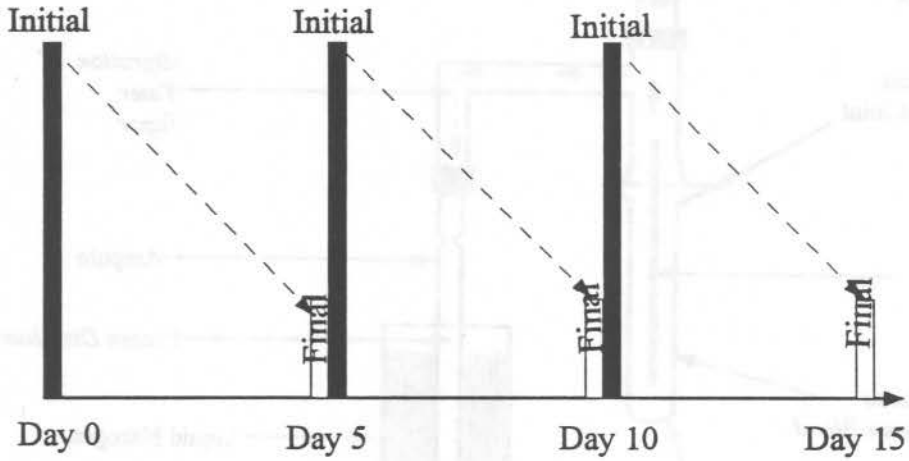


Figure 5.2. Schematic of tritium levels in individual chicks used in the 1995 field experiment. Chicks were injected a total of three times during the fifteen day experimental period. Tritium equilibrated within the nestlings within an hour. The concentration of tritium within nestlings declined over the five day interval between injections. Y-axis denotes relative, not absolute, concentrations of tritium. Note that the pattern of injection-dilution-reinjection also applies to chicks injected with deuterium in 1994, the only difference being that the interval between injections was three days instead of five.

Table 5.13. Summary of 1995 radio-telemetry data.

Colony	Nest N°	Chick Weight(g) at banding	Hatch Order	Weight of Transmitter as a % of body mass	Date of Deployment	Last Information
Hidden/L28	60	630	Largest	1.59%	5/3/95	Last located on 10/28/95(26°39.19'N, 81° 26.57'W). 5m SW of LaBelle.
	61	571	Largest	1.75%	4/30/95	Last located on 9/29/95(26°03.69'N, 81° 37.50'W). 7m SE Naples Airport.
	62	540	Largest	1.85%	5/18/95	Last located on 09/29/95(26°02.49'N, 81° 34.57'W).
	65	730	Largest	1.37%	5/3/95	Found dead on 10/13/95. Collected several mature feathers and bones at Everglades National Park (25°19.02'N, 80°42.60'W).
	66	810	Largest	1.23%	6/7/95	Heard mortality signal on 06/25/95. Collected transmitter at colony but found nothing to indicate death of bird.
	69	740	Largest	1.35%	5/23/95	Heard mortality signal on 08/08/95. Collected transmitter but found nothing to indicate death of bird. South of Loop Road.
	70	650	Largest	1.54%	5/23/95	Found dead on 10/28/95. Collected several mature feathers and bones on small palmetto hammock (26°46.85'N, 81°34.77'W)..
	71	590	Largest	1.69%	5/23/95	Last located on 09/29/95(25°43.41'N, 81° 02.55'W).
	74	600	Largest	1.67%	5/23/95	Last located on 10/28/95(25°43.31'N, 80° 53.90'W).
	75	695	Largest	1.44%	5/18/95	Last located on 10/28/95(25°46.77'N, 81°14.68'W).
	76	610	Largest	1.64%	5/28/95	Last located on 10/28/95(25°48.00'N, 81°17.00'W). In wet prairie.

Table 5.13. Continued.

Colony	Nest N°	Chick Weight(g)	Hatch Order	Weight of Transmitter as a % of body mass	Date of Deployment	Last Information
Hidden/L28	100	760	Largest	1.32%	5/20/95	Last located on 3/5/96(26°00.07'N, 80° 50.76'W).
	101	630	Largest	1.59%	5/20/95	Last located at colony on 07/08/95.
	104	660	Largest	1.52%	5/25/95	Last located at colony on 07/05/95.
	105	630	Largest	1.59%	5/20/95	Last located on 09/29/95(25°53.64'N, 80° 58.05'W).
	109	740	Largest	1.35%	5/20/95	Last located on 12/16/95(25°54.29'N, 80° 56.75'W).
	110	820	Largest	1.22%	5/20/95	Last located at colony on 07/05/95.
	111	745	Largest	1.34%	6/3/95	Heard mortality signal on 06/23/95. Collected transmitter at colony but found nothing to indicate death of bird.
	112	800	Largest	1.25%	5/20/95	Last located on 09/29/95(25°45.12'N, 80°55.49'W). East of Pinecrest.
	113	990	Largest	1.01%	5/20/95	Last located on 12/16/95(25°70.00'N, 80°38.59'W). 4m NE Beard Center.
	114	650	Largest	1.54%	5/20/95	Last located at colony on 06/15/95.
	117	790	Largest	1.27%	5/30/95	Last located on 09/29/95(25°59.47'N, 80°53.17'W).
	126	580	Largest	1.72%	5/20/95	Last located on 12/16/95(25°51.42'N, 81°13.10'W).
	132	600	Largest	1.67%	5/20/95	Last located at colony on 07/01/95.
	441	705	Largest	1.42%	5/28/95	Last located at colony on 07/01/95.
	442	740	Largest	1.35%	5/23/95	Heard mortality signal on 06/05/95 at colony. Found nothing to indicate death of bird.
	443	650	Largest	1.54%	5/18/95	Found dead on 08/08/95. Collected several mature feathers and bones on cypress and cabbage hammock (25°43.96'N, 80°58.34'W).

Table 5.13. Continued.

Colony	Nest N°	Chick Weight(g)	Hatch Order	Weight of Transmitter as a % of body mass	Date of Deployment	Last Information
Tamiami East	780	550	Largest	1.82%	5/26/95	Last located at colony on 07/8/95.
	783	550	Largest	1.82%	5/21/95	Last located on 9/29/95(25°45.73'N, 81° 13.94'W). NW of Harney River.
	784	630	Largest	1.59%	5/6/95	Last located at colony on 07/1/95.
	785	605	Largest	1.65%	5/9/95	Last located at colony on 07/1/95.
	787	485	Largest	2.06%	5/9/95	Found dead on 11/21/95. Collected several mature feathers about 50 feet from Krome Avenue (25°42.73'N, 80°28.83'W).
	789	589	Largest	1.70%	5/11/95	Last located at colony on 06/19/95.
	790	600	Largest	1.67%	5/16/95	Last located on 3/5/96(25°35.59'N, 80° 34.88'W).
	791	660	Largest	1.52%	5/6/95	Last located at colony on 06/26/95.
	792	580	Largest	1.72%	5/6/95	Last located at colony on 06/19/95.
	797	660	Largest	1.52%	5/6/95	Last located on 12/16/95 (25°47.70'N, 81° 07.19'W). 10m SW Monument Lake.

Table 5.14. Summary of previous measurements of nestling food consumption by ciconiiform species.

Species	*Captive (feeding regime) or Free-ranging*	*Method*	*Age Class (d)*	*Food Consumption (g wet/d)*	*Adult Mass (g)*	*Source*
Wood Stork	captive (ad lib)	weighed	23-45	350	2,702[4]	Kahl 1962
Great Blue Heron	captive (ad lib)	weighed	26-42	275[1]	2,390[4,5]	Bennett et al. 1995
White Ibis	captive (ad lib)	weighed	0-40	213	880[6]	Kushlan 1977,78
Great Egret	free ranging	labeled water	8-31	166	875[4]	This study
Great Egret	free ranging	collar	10-28	110-170	875[4]	Min et al. 1984
Great Egret	free ranging	visual est.	0-25	23[2]	875[4]	Mock et al. 1987
Little Egret	free-ranging	nest scales	10-20	118[3]	504[7]	Hafner et al. 1992
Cattle Egret	captive (ad lib)	weighed	7-14	95	383[8]	Siegfried 1973

[1](2027 kj/day)/(25 kj/g dry mass x 0.295 dry matter content). [2]Three-chick broods were provisioned an estimated 70 g food/d. [3]385 g food/day were delivered to 3.25 chicks/brood. [4]Dunning 1993. [5]Mean for adult males. [6]Kushlan 1977 [7]Hafner et al. 1992. [8]Telfair II 1994.

Table 5.13. Continued.

Colony	Nest N°	Chick Weight(g)	Hatch Order	Weight of Transmitter as a % of body mass	Date of Deployment	Last Information
Hidden/L28	447	690	Largest	1.45%	5/28/95	Last located on 09/29/95(25°43.64'N, 80° 53.46'W).
	454	720	Largest	1.39%	5/20/95	Found dead on 11/21/95. Collected several mature feathers and bones at Big Cypress National Park (25°44.66'N, 80°53.27'W)..
	488	930	Largest	1.08%	6/3/95	Last located at colony on 07/14/95.
	494	660	Largest	1.52%	5/30/95	Last located on 12/16/95(25°51.63'N, 81°11.32'W). 1m SW Monument Lake.
	769	820	Largest	1.22%	5/8/95	Last located at colony on 06/15/95.
	770	690	Largest	1.45%	5/23/95	Last located on 10/28/95(26°54.98'N, 81°53.47'W). NW Punta Gorda.
	772	720	Largest	1.39%	6/3/95	Last located at colony on 07/08/95.
	778	590	Largest	1.69%	5/25/95	Last located on 12/16/95(25°57.22'N, 80° 56.47'W). At Raccoon Point.
	798	570	Largest	1.75%	5/18/95	Last located on 10/28/95(25°49.83'N, 80° 53.00'W). South of Dade Collier Airport.
	UM1	1000	Largest	1.00%	6/3/95	Last located on 10/28/95(25°50.48'N, 81°12.12'W).
	UM2	935	Largest	1.07%	6/3/95	Found dead on 07/20/95. Collected several mature feathers in open area, hardwood hammock (26°06.25'N, 81°03.83'W).
	UM3	875	Largest	1.14%	6/3/95	Last located at colony on 07/14/95.
	UM4	880	Largest	1.14%	6/9/95	Last located on 08/09/95(25°45.00'N, 80° 56.22'W). South of Loop Road.
Tamiami East	450	600	Largest	1.67%	5/24/95	Last located at colony on 07/08/95.
	497	440	Largest	2.27%	5/16/95	Last located on 09/29/95, unknown. Homestead Area.

Table 5.13. Continued.

Colony	Nest N°	Chick Weight(g)	Hatch Order	Weight of Transmitter as a % of body mass	Date of Deployment	Last Information
Tamiami East	498	510	Largest	1.96%	5/21/95	Last located on 07/21/95(26°41.83'N, 80°33.47'W). 4m E of Belle Glade.
	709	740	Largest	1.35%	5/19/95	Last located at colony on 07/08/95.
	710	630	Largest	1.59%	5/24/95	Last located at colony on 07/08/95.
	711	620	Largest	1.61%	5/11/95	Last located on 07/5/95(25°34.57'N, 80°34.15'W). 1m SE of Glider Port.
	712	550	Largest	1.82%	5/19/95	Last located at colony on 07/05/95.
	717	500	Largest	2.00%	5/14/95	Last located at colony on 07/05/95.
	718	490	Largest	2.04%	5/9/95	Last located at colony on 07/08/95.
	719	770	Largest	1.30%	5/9/95	Last located at colony on 07/01/95.
	723	620	Largest	1.61%	5/9/95	Last located on 02/11/96(25°25.00'N, 80° 34.78'W).
	725	720	Largest	1.39%	4/29/95	Last located on 10/28/95(25°32.63'N, 80°38.44'W). 2m N of tomato patch.
	727	620	Largest	1.61%	5/4/95	Last located at colony on 06/19/95.
	730	810	Largest	1.23%	5/11/95	Last located at colony on 06/19/95.
	731	610	Largest	1.64%	5/9/95	Last located on 2/11/96(25°14.81'N, 80°42.00'W).
	736	595	Largest	1.68%	5/14/95	Last located on 12/16/95(25°42.49'N, 80° 30.26'W). 5m NW Tamiami Airport.
	739	470	Largest	2.13%	5/4/95	Last located on 7/21/95(26°39.69'N, 80° 45.68'W). Southern edge of Lake Okeechobee.
	740	510	Largest	1.96%	5/29/95	Last located on 7/21/95(26°50.10'N, 80°32.61'W). NW side of Corbett Wildlife Refuge.
	742	620	Largest	1.61%	4/29/95	Last located on 7/12/95(25°27.29'N, 80° 38.22'W). Close to tomato patch.
	779	690	Largest	1.45%	5/21/95	Last located at colony on 07/5/95.

Table 5.11. Continued.

Colony	Nest N°	Chick Weight(g)	Hatch Order	Weight of Transmitter as a % of body mass	Date of Deployment	Last Information
L67	248	580	Largest	2.07%	5/21/94	Last located on 07/13/94 in a pasture agricultural area (approx. 26°35.00'N, 81°03.00'W).
	248	548	Smallest	2.19%	5/21/94	Last located at colony on 07/26/94.
	323	520	Largest	2.31%	5/13/94	Found dead on 09/14/94 (25°37.60'N, 80° 36.48'W), on gravel road close to a campground.
	325	540	Largest	2.22%	5/13/94	Last located on 02/18/95 (26°12.77'N, 81°24.47'W).
	336	500	Only	2.40%	5/13/94	Last located on 02/18/95 (25°35.45'N, 80°35.94'W).
	338	558	Largest	2.15%	5/13/94	Last located at colony on 06/29/94.
	338	414	Smallest	2.90%	5/13/94	Found dead on 10/29/94 in a cypress forest (26°28.24'N, 81°08.62'W).
	341	602	Smallest	1.99%	5/13/94	Last located on 07/22/94 in a wet prairie (25°22.00'N, 80°35.00'W).
Frog City South	312	720	Largest	1.67%	5/14/94	Last located on 02/18/95 (26°32.62'N, 80°43.64'W).
	312	540	Smallest	2.22%	5/14/94	Last located on 10/29/94 in cypress forest with some pastures (26°48.77'N, 81°42.20'W).

Table 5.12. Total mercury concentrations (mg/kg) in tissues from seven radioed great egrets in 1994.

Colony of Origin	Date Last Sampled	Mercury in Blood (mg/kg)	Mercury in Growing Feathers [a] (mg/kg)	Date Found Dead	Age (days)	Mercury in Mature Feathers [b] (mg/kg)	Mercury in Liver (mg/kg)	Mercury in Brain (mg/kg)	Mercury 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/L28	05/06/94	0.83	18	06/15/94	62	19.0	-	-	-
Hidden/L28	05/21/94	2.2	13	08/11/94	110	14.0	-	-	-
L67	05/13/94	- [c]	9.7	10/29/94	195	11.0	-	-	-
L67	05/10/94	1.2	15	09/14/94	136	26.0	-	-	-
L67	05/13/94	1.5	13	09/14/94	149	22.0	-	-	-
L67	05/21/94	-	18	07/22/94	97	26.0	-	-	-

[a] Growing scapular feathers.
[b] Mature primary feathers.
[c] Not determined.

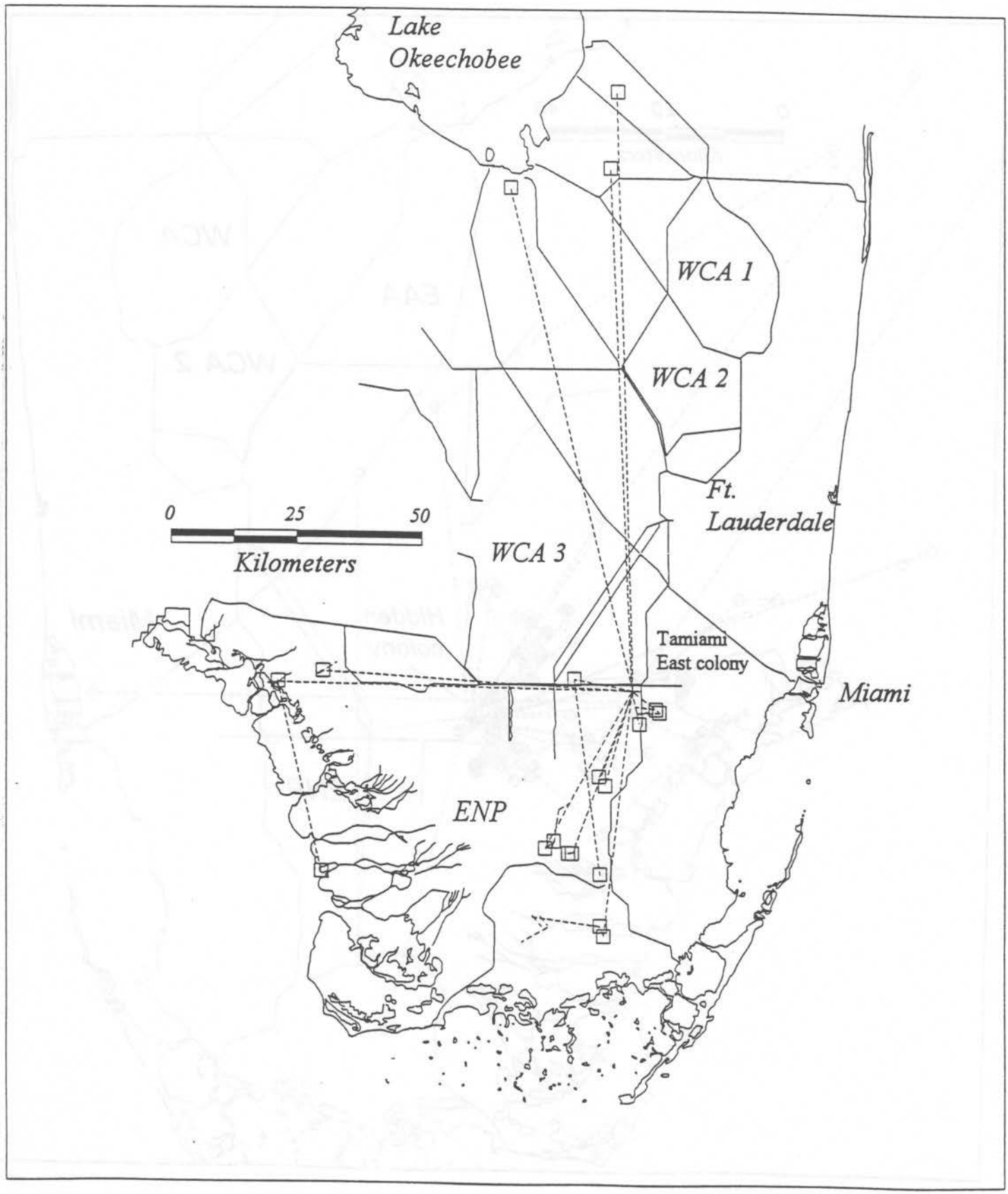


Figure 5.22a. Map of movements of radio-tagged great egret juveniles, originating from Tamiami West colony during 1995, during the first 9 months of life.

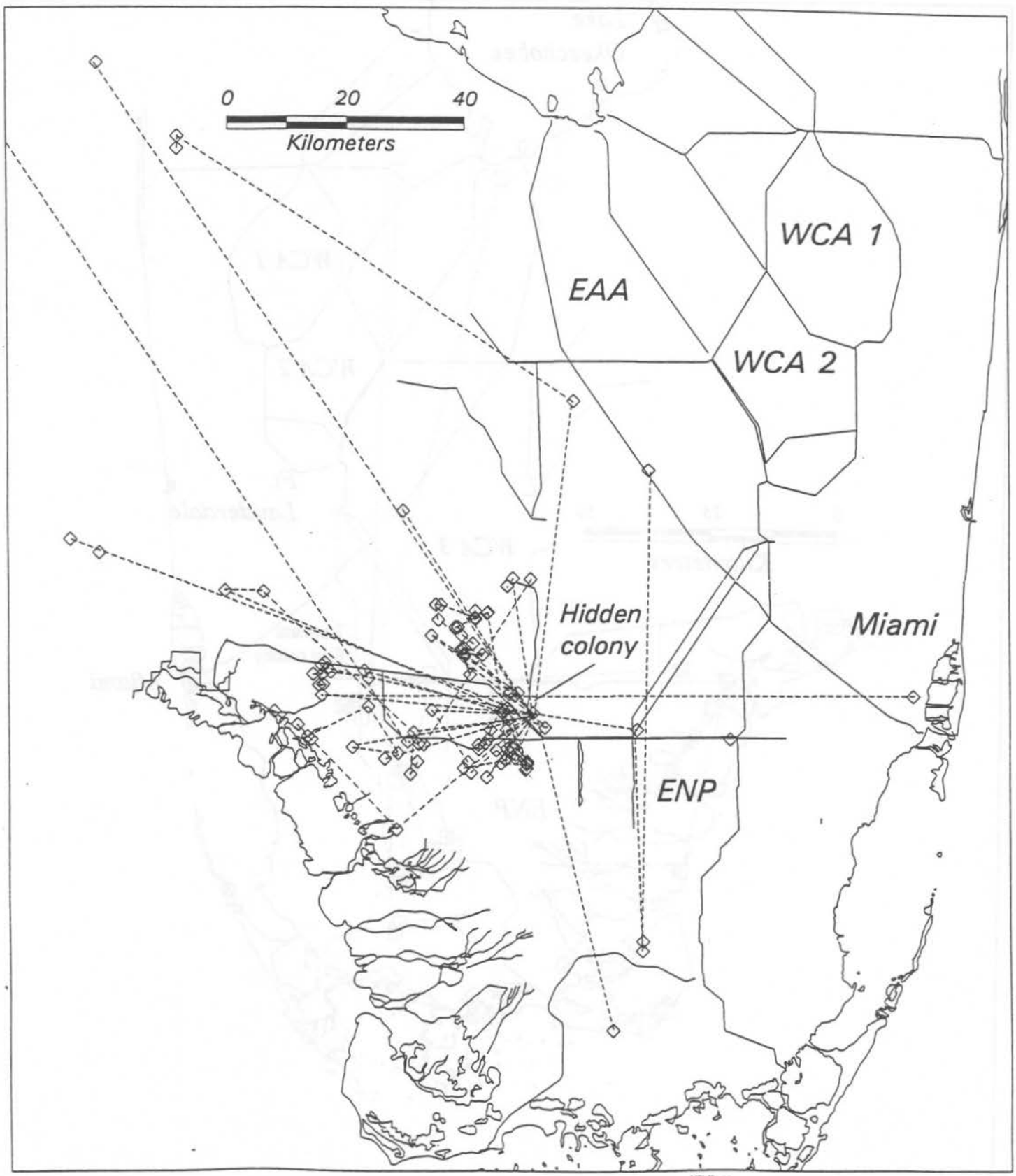


Figure 5.22b. Map of movements of radio-tagged great egret juveniles, originating from Hidden colony during 1995, during the first 9 months of life.

CHAPTER VI. EFFECTS OF METHYLMERCURY ON CAPTIVE-REARED GREAT EGRET NESTLINGS

INTRODUCTION

During the spring of 1996, we raised captive great egret nestlings from hatching to 3.5 months of age, well after the time that they would normally be independent in the wild, on diets containing several different doses of methylmercury. We then compared growth, appetite, health, immune function, survival, behavior, and hunting ability in relation to mercury dose. Several paths led us to this experiment. First, the field experiment (Chapter V) had implied that there were few or no effects of methylmercury on growing chicks prior to 28 days of age. This seemed quite counter to findings reported in the literature, and we suspected that our results were strongly influenced by the short duration of dosing (15 days). Because dosing had been confined to a very short part of what turned out to be the very fastest growth period for the chicks, it seemed likely that the chicks were able to shunt mercury quickly into feathers and so render it largely unavailable to the body. Since dosing stopped well before feathers were grown out, and at least a month prior to independence from parental feedings, it seemed probable that the chicks in the field had been able to depurate the dose we had given them before they were exposed to the rigors of surviving without parental care.

In addition to allowing dosing throughout the late nestling and fledgling period, the captive work would also allow much closer control of mercury intake. In the field, we estimated the total mercury intake that occurred naturally, and we had no real checks on those estimates. Captive work would also allow evaluation of a dose-response relationship, something we had not been able to achieve in the field.

A captive situation would also give us the chance to document mercury effects through many measures that were unobtainable in the field, particularly on health parameters, and behavior, and especially during the latter half of the period of parental dependence amd early independence, when we had been unable to study wild birds.

The 1996 experiment was designed to look at any and all effects we could measure, on chicks that were given very few environmental stresses. The chicks were all housed individually; although they could see and hear one another, there was no opportunity for the sibling aggression and competition for food that typifies great egret broods. Although we attempted to limit food to some degree, the birds were never food stressed. The effects we wished to assay for were those that would occur in the wild as a result of feeding on much the same food source each day, which implied chronic rather than periodic exposure to mercury.

The philosophy behind the choice of dosing concentrations was to establish effects at three levels: 1) a control, or placebo group, 2) a group receiving high enough mercury to be assured of some effects, and 3) a group that was at or slightly below the levels that we estimate are currently being ingested by great egret nestlings in the Everglades. Although many more groups would have been required to establish a true dose-response relationship, we were limited by space requirements, and by a desire to keep the number of experimental animals to a bare minimum. The three groups were thought to be adequate for answering a number of questions about the dynamics of mercury deposition in body tissues, the mode of action, the types of effects manifested, and their potential for affecting birds in the wild.

In addition to measuring food intake, blood parameters behavior, and mercury accumulation in different tissues we also used blood from our collections, and tissues from organs saved following necropsy to investigate the effects of mercury on biochemical parameters and enzymes in blood plasma, liver, kidney, and brain. The biochemical parameters measured include ones known to be indicative of mercury exposure in birds and mammals. Mercury exposure has been shown to result in decreased glutathione (GSH) peroxidase activity in plasma and liver of at least four species of birds including mallards (Hoffman and Heinz 1997), surf scoters (*Melanitta perspicellata*) and ruddy ducks (*Oxyura jamaicensis*) (Hoffman et al. 1997). Similarly in mammals, methylmercury is known to decrease hepatic GSH peroxidase activity by as much as 60% in rats relative to untreated controls (Hirota 1986). In fact, a unique profile of hepatic response following mercury exposure in birds has been reported, and includes decreased glutathione peroxidase activity, with sometimes decreased glucose-6-phosphate dehydrogenase activity, and reduced thiol concentrations with an increased ratio of oxidized to reduced glutathione. This profile has also been reported in mallards (Hoffman and Heinz 1997), surf scoters and ruddy ducks (Hoffman et al. 1997). Thiol depletion has also been reported in great blue heron nestlings (Custer et al. 1997). Other environmental contaminants including selenium, lead and PCBs do not result in the same profile. In mammals, and also apparently in birds, the liver is the major site of reduced (GSH) synthesis. Since methylmercury is a sulfhydryl-binding toxicant, GSH and other thiols are important factors in hepatobiliary excretion of methylmercury in mammals, accounting for diminished hepatic GSH concentrations with increasing concentrations of mercury (Klassen et al. 1985). This process appears to involve the formation of a mercury-glutathione complex in liver cells, followed by the secretion of the complex through a process closely linked to GSH secretion (Ballatori and Clarkson 1985, Ballatori 1994). This experiment seemed an excellent opportunity to look at the profile of mercury-induced changes in enzyme activity, since mercury was the only contaminant administered to the birds.

METHODS

Captive rearing and dosing

We collected 23 first-hatched great egrets from Alley North colony in Water Conservation Area 3 on 16 March 1996, as recently hatched chicks or pipped eggs. The eggs were taken from nests of three eggs that had been monitored throughout incubation. Ages were estimated from having observed hatching in the colony (or in the lab in the case of birds collected as pipped eggs), or from having found evidence of hatching (wet chicks, younger chicks having hatched, etc.). The estimated range in ages was 7 days. After being placed in captivity, 7 chicks (2, 3, and 2 chicks in groups 1, 2, and 3, respectively) died or were humanely killed after having developed bacterial infections or malformed legs. These animals were not included in the experiment.

The birds were transported on 16 March 1996 to the Florida Filed Station of the National Wildlife Research Center, U.S.D.A., where they were housed for the rest of the experiment. Birds were initially housed indoors in 65 × 40 cm plastic boxes with sticks of 0.5-1 cm diameter in them. All birds were kept in the same heated room during the first two

weeks of life, and heating pads were applied to the bottoms of the boxes for the first week of life. Boxes were cleaned daily. At 5 weeksof age, birds were moved to outdoor housing. The plastic boxes were attached to perches within each outdoor cage, and removed only after the birds had stopped using the boxes for perching and resting (ca 2 weeks following the move to outdoor cages).

Cages were 3 m × 3 m enclosures constructed of chickenwire supported by PVC plastic tubing. The cages were semicircular in cross-section (maximum height of 2 m) and had sand floors. Each cage contained a water dish, two parallel east-west running perches made of dried bamboo, and one set of ladder-like perches running from the northern of the two bamboo perches to the ground. During the latter third of the experiment, we placed one shallowly flooded plastic wading pool in each cage (see under Behavior).

Cages were grouped into blocks. Each elongate block contained three cages, with one cage on each end and one in the middle, with common walls between adjacent cages. Access to each block of cages was through a single door, at the east end of half the blocks and the west end of the other half. Each block contained one bird from each dose group, and dose group cage assignment was random with respect to location within a block. The entire group of blocks were surrounded by an electrified fence to keep terrestrial predators away. The outdoor housing units were surrounded by pine flatwoods forest, and were not subject to any routine disturbance other than our visits.

All birds received the same diet of thawed Atlantic silversides (*Menidia menidia*), with small but regular (ca 10% by weight) additions of capelin (Mallotus villosus). Food was provided in dishes for 0.5 hr three times daily. Fish remaining in dishes (or picked up after having been moved from the dish) after 0.5 hr was weighed after each meal. Food quantity was provided on a modified ad lib basis. Ad lib feeding for the first week allowed us to establish the initial food amount for each bird. We offered that amount of food to the individual until it either ate all food offered for 3 consecutive meals, or left any amount of food uneaten for three consecutive meals. We then either increased (finished meals) or decreased food (food uneaten) by 10 g (wet weight). During the trials on hunting behavior during the latter quarter of the experimental period, the birds were allowed to forage on live fish. The amount they ate was included in the calculation of total food consumed, and both mercury dose and total food offered was adjusted accordingly. As below, mercury dosing was based upon daily food offered. Prior to the morning foraging trials, food was withheld the previous evening.

Gelatin dosing capsules were made from mercury solutions prepared by DEP that contained 0, 3, or 30 μg reagent grade methylmercury chloride/μl in acetone. Each gelatin capsule received 0.17μl solution/g food offered, which equaled 0, 0.5, or 5.0 mg/kg food offered for that day, depending on the solution used. These correspond to groups labeled I, II, and III, or placebos, "low" dose, and "high" dose groups, respectively. The acetone was evaporated from the capsules in a fume hood, the capsules were closed, and stored in sealed containers until time of dosing. Capsules were given to the birds just prior to the evening meal. Capsules were quickly dipped in oil or water, dropped into the open mouth, and manipulated within the esophagus to the base of the neck. As with the field dosing experiment, we never saw birds regurgitate the capsules, and never found any capsules in the cages. Assignment to mercury dose groups was blind to all but one (MGS) of the researchers working on the experiment.

Birds were dosed every 3 days starting at 8 days of age (week 2). Dosing then changed to a daily regime beginning on day 20, and continued until the end of the experiment (week 14). The highest daily dose was 15 mg/kg body weight, during the time that birds were being dosed every three days; the high daily dose was a function of the time between doses. Once the daily dosing was begun, the highest dose was 1.6 mg/kg body weight for group 3, and 0.16 mg/kg for group 2. Both of these were achieved on day 21 (the time period during which they ate the greatest proportion of body weight daily). Birds were humanely killed by lethal injection of sodium pentobarbital when they could no longer stand [birds in group 3 were killed at week 10(1 individual), 11(2 individuals), and 12(3 individuals)], and all remaining birds were humanely killed at the end of the experiment (week 14).

Effects of mercury on health parameters

Blood was collected weekly beginning at week 1. Blood collection volumes were 0.5 ml for week 1, 1.6 ml for weeks 3, 5, and 7; 0.5 ml for weeks 4, and 6; 1 ml for weeks 8, 10, 12, and 13; 2.6 ml for weeks 9, and 11; and 10 ml just prior to euthanasia for all birds. On each collection day, blood was collected prior to dosing with mercury. Blood for white cell counts and differentials was collected on weeks 3, 5, 7, 9, 11, and 13. Packed cell volume and refractive index were determined during the alternate weeks. Plasma (EDTA) for EEE and BSA titers was collected weekly from week 8 to week 14, and for enzymes during weeks 5, 7, 9, and 14.

The immune system responses of all birds were tested during the latter half of the experimental period. All birds were vaccinated with 0.3 ml of a killed eastern equine encephalitis (EEE, Fort Detrick) in the right pectoral muscle on weeks 8, 9, 11, and in the left pectoral muscle with 200 μg bovine serum albumin (BSA) in 0.1 ml sterile saline (Fraction V Heat shock tx Fischer BP 1600-100) on weeks 8 and 10. Phytohemagglutinin (PHA, 0.1 ml (1 mg/ml)) in phosphate buffered saline was injected intradermally into the left wing web during week 11. Twenty-four hours later the response area was measured with calipers and a biopsy collected and preserved in 10% NBF.

Plasma and organ biochemistry

Blood was separated within two hours of collection and preserved frozen at -70°C. Liver, kidney, and brain tissue collected at necropsy were stored in cryovials at -70°C. Samples were shipped to Patuxent Wildlife Research Center in Laurel, Maryland on dry ice. We measured the following variables from these tissues:

1) Blood: Plasma enzyme activities measured included: glutathione peroxidase (GSH-peroxidase), and glutathione reductase (GSSG-reductase). The following plasma enzyme activities were measured as indicators of possible organ damage: alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), creatine kinase (CK), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LDH-L). Three of these enzymes have been linked to hepatotoxicity in birds (ALT, AST, and LDH-L), whereas GGT is specific for kidney, CK for neural and muscle tissue, and AP has been related to bone growth in nestlings.

2) Liver: A series of tissue enzymes related to glutathione metabolism and oxidative stress were recorded: GSH peroxidase, GSSG reductase, glutathione-S-transferase (GSH-S-

transferase), and glucose-6-phosphate dehydrogenase (G-6-PDH). As part of this, reduced glutathione (GSH), oxidized glutathione (GSSG) and total hepatic sulfhydryl concentration (total SH) were also measured. Thiobarbituric acid reactive substances (TBARS) was determined as an estimate of lipid peroxidation.
3) Kidney: All of the above indicators of oxidative stress, and additionally, GGT, and uric acid were determined.
4) Brain: All of the above indicators of oxidative stress, and additionally acetylcholinesterase (AChE) was recorded.

Effects of mercury dose on maintenance and hunting behavior

Cleanliness and motor control

Birds were evaluated for cleanliness at weeks 7, 9, and 10 by placing them in one of 4 categories. They were evaluated for weakness by pushing them at the shoulder when standing. They were placed in one of 3 categories of response. They were also tested for the time to return to a standing position after being placed on their backs at weeks 6, 8, 10, and 12.

Differences in behavior due to mercury contamination were measured in two ways. We measured the stances, positions used, and other maintenance behaviors of birds during normal activity periods in their cages, and compared behaviors among the three treatment groups. We also allowed the birds to forage on live fish in wading pools in their cages, with varying levels of difficulty imposed by background coloration of the pool. We compared the numbers of strikes necessary to capture each fish, the time necessary to capture each fish, and the numbers of fish presented that were actually pursued and captured. The comparisons of hunting behavior occurred during the latter part of the experiment, and since all of the high-dose birds had died by this point, we compared only low dose and control birds.

Activity and Position

The aim of this part of the study was to detect any differences in general activity and position of the birds in the cage in relation to mercury dose group. We used scan samples to document behavior. A scan sample is a repeated "snap-shot" of what each bird is doing, in this case sampled for all birds once every five minutes during an observation bout. These observations can then be compiled to give an indication of percentage of total time spent in various positions or activities. All observation sessions took place between nine and eleven in the morning, or one and five in the afternoon. A total of approximately 600 observations were collected for each bird.

Observations were made from a viewing post attached to a tree, approximately 7 m above the ground, which gave a clear view into every cage. The birds were well habituated to human activity by the time that this work was conducted, and at this distance, we saw no evidence that the observer's presence disturbed the birds. In addition, since members of each experimental group were evenly distributed with reference to distance from the tower, it is unlikely that disturbance effects could explain any group differences in behavior.

Our observation bouts were conducted between 18 May and 15 June 1996 (weeks 10-

14). During observation bouts, we collected scan samples every five minutes for two to four hour periods. After an initial 5 minute period at the beginning of an observation bout, the observer noted the Position (pool edge, perch, or ground), Activity (pecking, preening, gullar fluttering, head under wing, delta wing, head down, vocalizations, swaying, stretching, rouse) and Stance (walking, standing still, flying and sitting/laying down) of each bird. Whether the bird was in the sun or not was also noted. The sequence of sampling the individual birds was the same for all observations.

Hunting behavior

We compared foraging behavior among dose groups when the great egrets were between the ages of 60 and 100 days, by which time the birds would have fledged in the wild. The timing of these tests therefore has biological relevance, since this is the time during which wild birds would be learning to forage on their own. Since we found that the majority of first-year deaths occur during the early post-fledging period (Chapter V), we felt that any evidence of impairment in hunting behavior in the experimental birds could reasonably be linked with decreased survival rate in the field.

The tests were designed to explore differences in foraging ability between the low dose and control groups. We placed light blue circular plastic children's wading pools (1.2 m diameter) in each bird's cage. Water depth was maintained at 15 cm by a system of hoses that fed the pools with fresh water, and by holes in the sides of the pools that drained excess water above the target depth. The pools were scrubbed and re-filled with fresh water every 3 to 4 days, and generally remained clear.

Tests were performed between 0700 and 1000 hours, and always prior to the first feeding of the day. The live fish were therefore the first food available to birds following a night of fasting. During tests, we placed a single fish in each pool, and the birds were individually monitored for time to capture each fish, number of strikes to capture and whether each fish was caught during the 10 minutes following fish presentation. Five fish were presented sequentially in each bout. Birds were observed in groups of three, with one researcher observing and recording, and the other presenting the fish. Latency to capture was measured from the time at which the fish was placed in the water. If the fish was not caught after ten minutes it was considered "uncaught" and replaced with a fresh fish during the next presentation. The fish used were juvenile largemouth bass (*Micropterus salmoides*) between 2 and 5 cm in total length (ave. = 61.75 mm, s.d. = 8.69 mm, n = 56) . These fish were relatively uniform in size, having been raised in the same hatchery (Welaka, Fl.).

Live fish presentations were divided into two treatment groups: pools with a light blue background against which the bass showed up clearly (contrasting pools), and pools that were painted flat black with patterned dull yellow-gold spotting to camouflage the fish (camouflage pools). The different backgrounds were designed to detect any differences between the two groups in the ability to see prey.

During the initial two week habituation period when we regularly offered the birds live fish, we discovered that the birds were completing the task quickly and uniformly. In order to induce greater complexity to the task, we placed a wire grid into each pool, that completely covered the area of the pool at just below the water surface; the grid was removed when the fish presentations were not in progress so that some of the novelty of the

Table 6.5. Results of repeated measure ANOVAs for significant differences between groups of great egrets dosed with methylmercury. Group 1 = placebo, group 2 = low dose, group 3 = high dose.

Parameter	Groups compared	Tests of Fixed Effects		Direction	Weeks with Significant Effects[1]												
		Group	Group*Week		Week												
					1	2	3	4	5	6	7	8	9	10	11	12	13	14
Feather Hg	1-2	0.0001	0.0001	^					**		**		**		**			**
	1-2-3	0.0001	0.0001	^							**		**		**			
Blood Hg	1-2	0.0008	0.0001	^			**		**		**		**		**			**
	1-2-3	0.0001	0.0001	^							**		**		**			
Weight Index	1-2	0.1045	0.0300	v	**										*		*	*
	1-2-3	0.0550	0.0084	v	**									*	**			
Weight	1-2	0.2653	0.3439	v										*	+	+	*	*
	1-2-3	0.5297	0.1206	v											*			
Food/Mass	1-2	0.3512	0.3337	v											*			
	1-2-3	0.0651	0.1430	v							+			**	*			
Food	1-2	0.0663	0.3030	v								*			**			
	1-2-3	0.0613	0.0073	v										**	**			
PCV	1-2	0.0036	0.1638	v				+	**	*	**	**	**	**	*	+		
	1-2-3	0.0012	0.0002	v					**		**	*	**	**	**			
RI	1-2	0.2702	0.7357	v														*
	1-2-3	0.1440	0.0295	v							**	*		*	*			
Heterophils	1-2	0.9787	0.9847	^														
	1-2-3	0.0027	0.0095	^											**			
Monocytes	1-2	0.4859	0.8316	^														
	1-2-3	0.0667	0.0185	^											**			
Basophils	1-2	0.1943	0.0282	^			**											
	1-2-3	0.2050	0.0963	^			*											
Eosinophils	1-2	0.1752	0.1355	^			*											
	1-2-3	0.3176	0.1107	^			**											
Lymphocytes	1-2	0.4048	0.9632	v														
	1-2-3	0.2212	0.6327	v							*							
WBC	1-2	0.3274	0.5637															
	1-2-3	0.3050	0.9293															
Polychromasia	1-2	0.1595	0.9420	^														
	1-2-3	0.0947	0.7629	^							**							

+ = P<0.1; * = P<0.05; ** = P<0.001

^ =indicates the parameter was higher in dosed than placebo group

v = indicates the parameter was lower in dosed than placebo group

[1]Significant differences detected in specific weeks using comparson of least square means

Table 6.6. Summary of statistical comparisons of organ and plasma biochemistries among the great egret dose groups. Arrows indicate significant differences between high or low dose groups, and placebos. Note that samples form liver, kidney and brain were available only after necropsy at about week 11 for high dose and week 14 for placebo and low dose birds.

		Sample collection times (weeks) / Methyl-Hg dosing groups									
Sample	Biochemical	5		7		9		11		14[a]	
	Parameter*	Hi	Lo	Hi	Lo	Hi	Lo	Hi	Lo	Lo	
Plasma	GSH-peroxidase			↑[b]		↓[c]		↓↓[d]		↓	
	GSSG-reductase			↓	↓						
	ALT									↑	
	AST							↑	↑		
	CK									↑	
	LDH-L							↓			
	Glucose					↑				↑	
	Albumin					↓		↓			
	TPP					↓		↓			
	Cholesterol							↑	↑		
	Calcium									↓	
	Triglycerides									↓	
	Uric acid					↓		↓		↓	
	Phosphorus							↓			
Liver	GSH-peroxidase							↓			
	GSSG-reductase							↑			
	GSH-S-transferase							↑			
	TBARS							↑		↑	
	Total thiol							↓			
	Protein-bound SH							↓			
Kidney	GSH-peroxidase							↓			
	GSH-S-transferase							↑			
	G-6-PDH							↑			
	Glutathione							↑			
	Protein-bound SH							↓			
Brain	GSH-peroxidase							↓↓			
	TBARS							↑			
	Glutathione							↑			

* GSH-peroxidase: glutathione peroxidase; GSSG-reductase: glutathione reductase; ALT: alanine aminotransferase; AP: alkaline phosphatase; AST: aspartate aminotransferase; CK: creatine kinase; LDH-L: lactate dehydrogenase; TPP: total plasma protein; GSH-S-transferase: glutathione-S-transferase; G-6-PDH: glucose-6-phosphate dehydrogenase; TBARS: thiobarbituric acid reactive substances.

[a] Plasma chemistries reflect only controls and low dose survivors

[b] ↑ Refers to an increase when compared to the control group

[c] ↓ Refers to a decrease when compared to the control group

[d] ↓↓ Severely decreased

Table 6.6a. Summary of timing of significant differences in various measurement parameters relative to mercury concentrations in blood, growing feathers and cumulative mercury consumed/mass (Cum/Mas). Rows arranged approximately in order of increasing blood mercury concentrations.

Week	Dose group	Blood Hg mg/kg	Growing feather Hg mg/kg	Cum/Mass Hg mg/kg	Week of first significant change
1	3	0.1		0.0	
1	2	0.1		0.0	
3	2	0.5	17.0	1.3	(basophils and eosinophils this week only)
3	3	0.7	13.0	9.8	(basophils and eosinophils this week only)
6	3	0.69	40.0	29.57	
5	3	1.0	17.3	20.7	PCV
5	2	1.1	19.4	2.3	PCV
7	2	2.4	32.6	3.6	
9	2	4.8	44.8	5.0	Cleanliness
11	2	11.9	77.0	6.4	Weight index, Food/Mass weakness
14	2	12.3	107.6	8.0	RI, lymphocytes, polychromasia, GSH peroxidase glucose, uric acid
7	3	19.2	111.2	34.6	RI, lymphocytes,
9	3	54.2	346.7	48.2	Cleanliness, GSH peroxidase, glucose, uric acid
10	3	98	850.0	67.294	Food/Mass, ataxia, time to right
11	3	73	692.5	63.292	Weight index, monocytes, heterophils, weakness

A. Percentage of scan samples spent in each stance.

Exp Grp	Stance Standing	Walking	Flying	Sitting/Laying Dow	n
Placebo	62.85	11.8	2.64	22.71	2950
Low Dose	64.64	7.65	2.1	25.62	2955
High Dose	29.83	2.13	0.33	67.71	1502

B. Percentage Time Spent in Each Position

Exp Grp	Position Ground	Pool	Perch	Pool Edge	n
Placebo	58.33	3.55	26.34	11.78	2954
Low Dose	58.48	6.57	27.31	7.65	2955
High Dose	94.55	1.33	3.32	0.80	1505

Table 6.7. A: Percentages of scan samples in which great egrets were noted in different stances, by dose group. B: Percentages of scan samples in which great egrets were noted in different positions, by dose group.

Table 6.3. Mean concentrations of mercury in tissues of great egrets at time of death (14 weeks) that received placebo (group 1), low (group 2), or high (group 3) doses of methylmercury.

Tissue	Dose group	Mean Cum mg Hg/mass	N	Mean Hg (mg/kg)	Range Min	Range Max
Growing feather	1	0	5	2	1.7	2.5
	2	8	5	110.00	71.00	140.00
	3	68	6	770.00	620.00	950.00
Liver	1	0	5	0.42	0.20	0.52
	2	8	5	15.00	11.00	20.00
	3	68	6	140.00	120.00	160.00
Mature scapular	1	0	5	6.60	0.70	29.00
	2	8	5	40.00	34.00	52.00
	3	68	6	150.00	100.00	200.00
Kidney	1	0	5	0.33	0.31	0.35
	2	8	5	8.40	5.50	13.00
	3	68	6	120.00	99.00	140.00
Blood	1	0	5	0.25	0.22	0.27
	2	8	5	12.00	9.80	16.00
	3	68	6	82.00	38.00	100.00
Pancreas	1	0	5	0.20	0.18	0.22
	2	8	5	5.40	0.39	8.00
	3	68	6	52.00	47.00	55.00
Muscle	1	0	5	0.17	0.14	0.20
	2	8	5	18.00	6.10	61.00
	3	68	6	45.00	36.00	59.00
Brain	1	0	5	0.21	0.20	0.27
	2	8	5	3.40	2.80	4.30
	3	68	6	35.00	30.00	41.00
Bile	1	0	5	0.45	0.02	2.00
	2	8	5	3.50	0.76	10.00
	3	68	6	14.00	4.10	37.00
Eye	1	0	5	0.03	0.02	0.06
	2	8	5	0.43	0.06	0.60
	3	68	6	4.80	4.20	5.60
Fat	1	0	5	0.03	0.02	0.03
	2	8	5	0.25	0.19	0.30
	3	68	3	3.60	0.87	5.90

Table 6.4. Relationship between tissue concentrations of mercury and cumulative mercury consumed per mass (mg Hg/Kg mass) at the time of death for experimentally dosed great egrets, n = 16. Probabilities are associated with significance of regression relationship.

Tissue	Slope	P	Pearson Correlation Coefficient
Growing feather	6.989	<0.0001	0.96
Liver	2.015	<0.0001	0.99
Mature scapular	1.898	<0.0001	0.92
Kidney	1.795	<0.0001	0.97
Blood	1.183	<0.0001	0.94
Pancreas	0.759	<0.0001	0.99
Muscle	0.563	0.0004	0.77
Brain	0.518	<0.0001	0.99
Bile	0.205	0.003	0.69
Eye	0.07	<0.0001	0.98
Fat	0.055	0.0004	0.83

A. Percentage of scan samples in each activity

Exp Grp	Activity Nothing Noted	Head U/ Wing	Gular Flutter	Preening	Delta Wing	Head Down
Placebo	56.77	0.64	4.2	19.7	1.46	4.91
Low Dose	60.17	0.41	2.54	20.3	2.57	3.65
High Dose	53.49	4.19	4.98	5.51	17.54	9.83

Exp Grp	Activity Vocalizations	Pecking	Swaying	Stretching	Rouse	n
Placebo	0.64	9.99	0.07	0.88	0.74	2954
Low Dose	0.85	7.55	0.1	1.29	0.58	2955
High Dose	0.13	1.06	2.52	0.53	0.2	1505

B. Percentage Time Spent in Sun vs. Shade

Experimental Group	Shade	Sun	n
Placebo	57.3	42.7	1623
Low Dose	69.43	30.57	1570
High Dose	71.45	28.55	704

Table 6.8. A: Percentage of scan samples in which great egrets in the three dose groups were noted in various activities; B: Percentage of scan samples in which great egrets were noted in sun or shade.

Table 6.9. Summary of statistical tests comparing the effects of mercury on hunting behavior, tendency to finish fish presented, and several types of maintenance activity.

Behavior Analyzed	Treatment/ Groups Compared	Statistical Test	P - value	Slope
Foraging Focal Samples				
# Stikes to capture:				
vs. Experimental Group	Contrasting	ANCOVA	0.290	n/a
	Camouflage	ANCOVA	0.169	n/a
vs. Blood Hg	Contrasting	ANCOVA	0.097	negative
	Camouflage	ANCOVA	0.077	negative
vs. Powderdown Hg	Contrasting	ANCOVA	0.083	negative
	Camouflage	ANCOVA	0.169	negative
Time to Capture:				
vs. Experimental Group	Contrasting	ANCOVA	0.041	negative
	Camouflage	ANCOVA	0.980	n/a
vs. Blood Hg	Contrasting	ANCOVA	0.042	positive
	Camouflage	ANCOVA	0.135	positive
vs. Powderdown Hg	Contrasting	ANCOVA	0.006	positive
	Camouflage	ANCOVA	0.281	negative
Fish Finished:				
	Contrasting	Fisher's Exact	0.003	
	Camouflage	Fisher's Exact	0.0003	
Scan Samples				
Activity	Placebo/ Low Dose	2 by K Chi -Sq	0.001	
e.g. preening, head down,	Placebo/ High Dose	2 by K Chi -Sq	0.001	
gullar flutter, vocalizing, etc	Low Dose/ High Dose	2 by K Chi -Sq	0.001	
Position	Placebo/ Low Dose	2 by K Chi -Sq	0.001	
i.e. ground, perch, pool,	Placebo/ High Dose	2 by K Chi -Sq	0.001	
or pool edge	Low Dose/ High Dose	2 by K Chi -Sq	0.001	
Stance	Placebo/ Low Dose	2 by K Chi -Sq	0.001	
i.e. standing, walking,	Placebo/ High Dose	2 by K Chi -Sq	0.001	
flying or sitting/laying down	Low Dose/ High Dose	2 by K Chi -Sq	0.001	
Sun	Placebo/ Low Dose	2 by 2 Chi -Sq	0.001	
Time spend sitting in sun	Placebo/ High Dose	2 by 2 Chi -Sq	0.001	
	Low Dose/ High Dose	2 by 2 Chi -Sq	0.33	

Table 6.10. Table of P-values generated by repeated measures ANCOVAs, examining the effects of sex, age, experimental group, and day of presentation on striking efficiency and capture time, in the two types of pool treatments.

Number of Strikes	**Contrasting Pools**	**Camouflage Pools**
sex	0.927	0.375
age	0.627	0.498
experimental group	0.288	0.170
day of presentation	0.052	0.405
exp. group x time	0.785	0.612
Time to Capture	**Contrasting Pools**	**Camouflage Pools**
sex	0.004	0.091
age	0.004	0.040
experimental group	0.041	0.975
day of presentation	0.224	0.037
exp. group x day	0.717	0.291

Table 6.11. Mean time in seconds that great egrets took to capture fish, grouped by treatment group, and presentation date within the experimental period, for unpainted (contrasting) pools. Note that mean capture times decreased for both groups over the course of the samplings.

Presentation session	Placebos	Low Dose Group
1	47.78	55.83
2	28.33	61.93
3	18.45	65.05
4	18.33	45.69
5	15.37	57.65
6	20.05	25.81
7	12.29	30.29
8	7.41	48.14

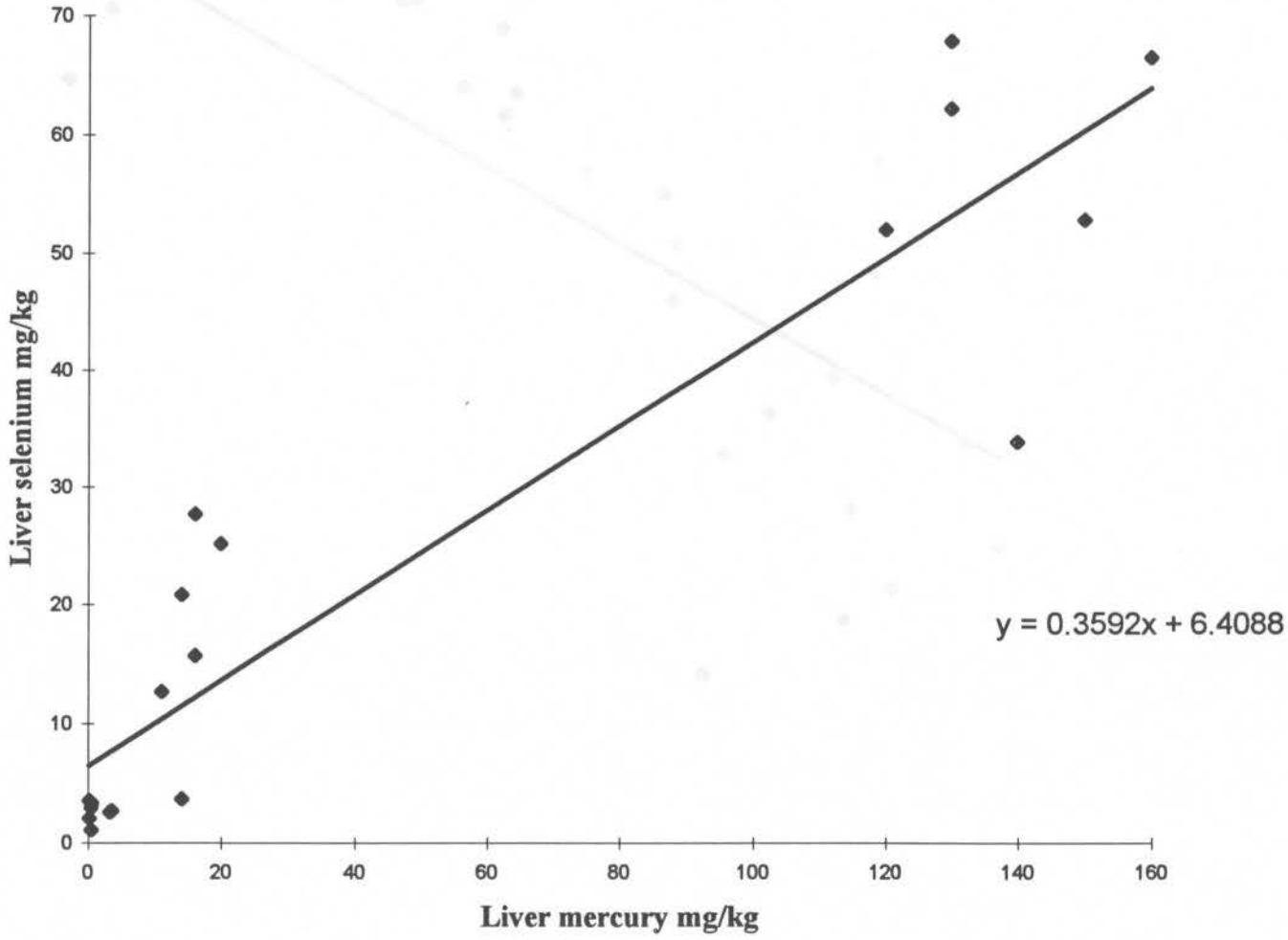


Figure 6.1. Regression analysis of mercury and selenium (mg/kg wet weight) in liver tissue collected from methylmercury dosed great egrets.

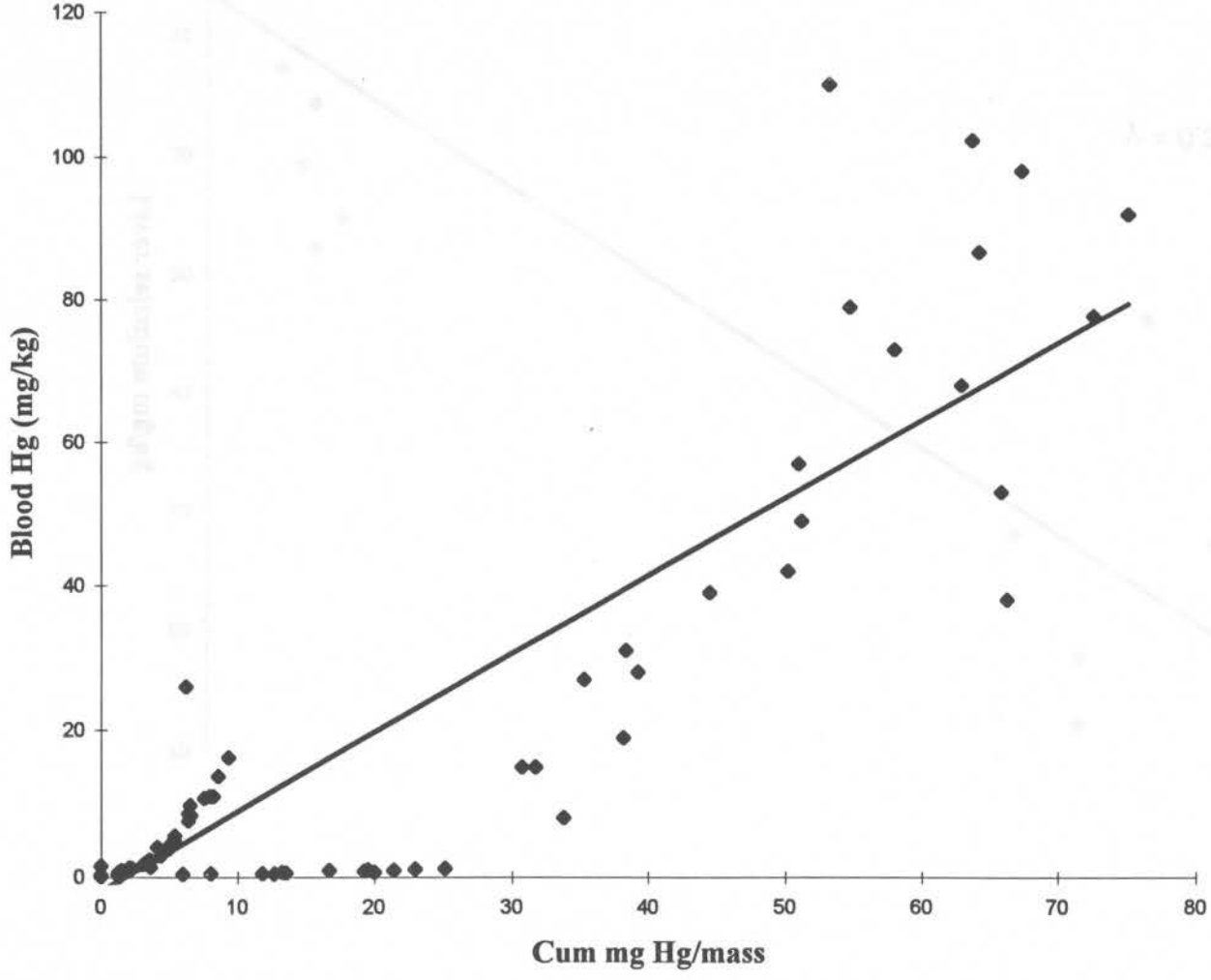


Figure 6.2. Blood mercury concentrations relative to the cumulative amount of methylmercury fed to great egrets.

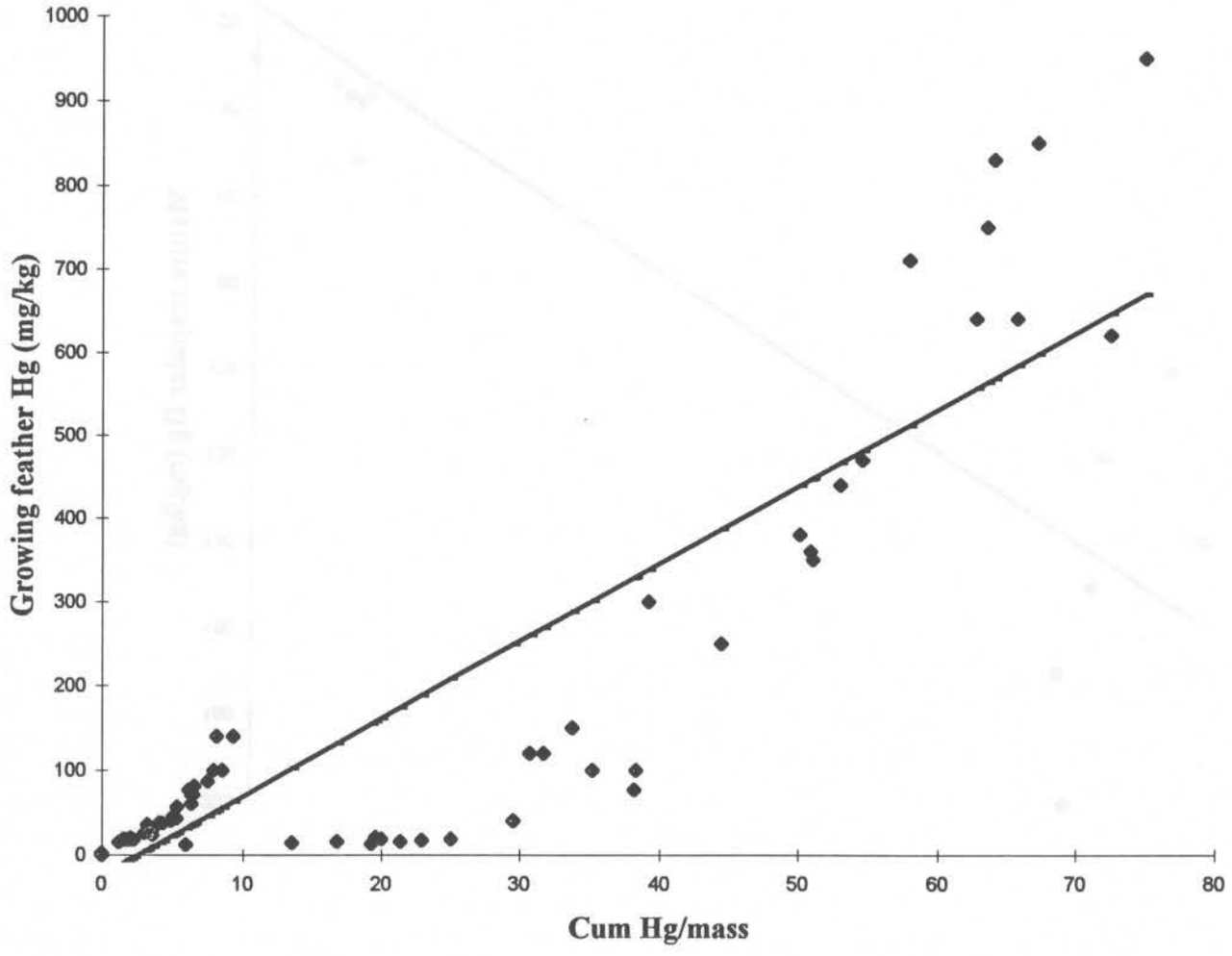


Figure 6.3. Growing feather mercury concentrations relative to the cumulative amount of methylmercury fed to the great egrets.

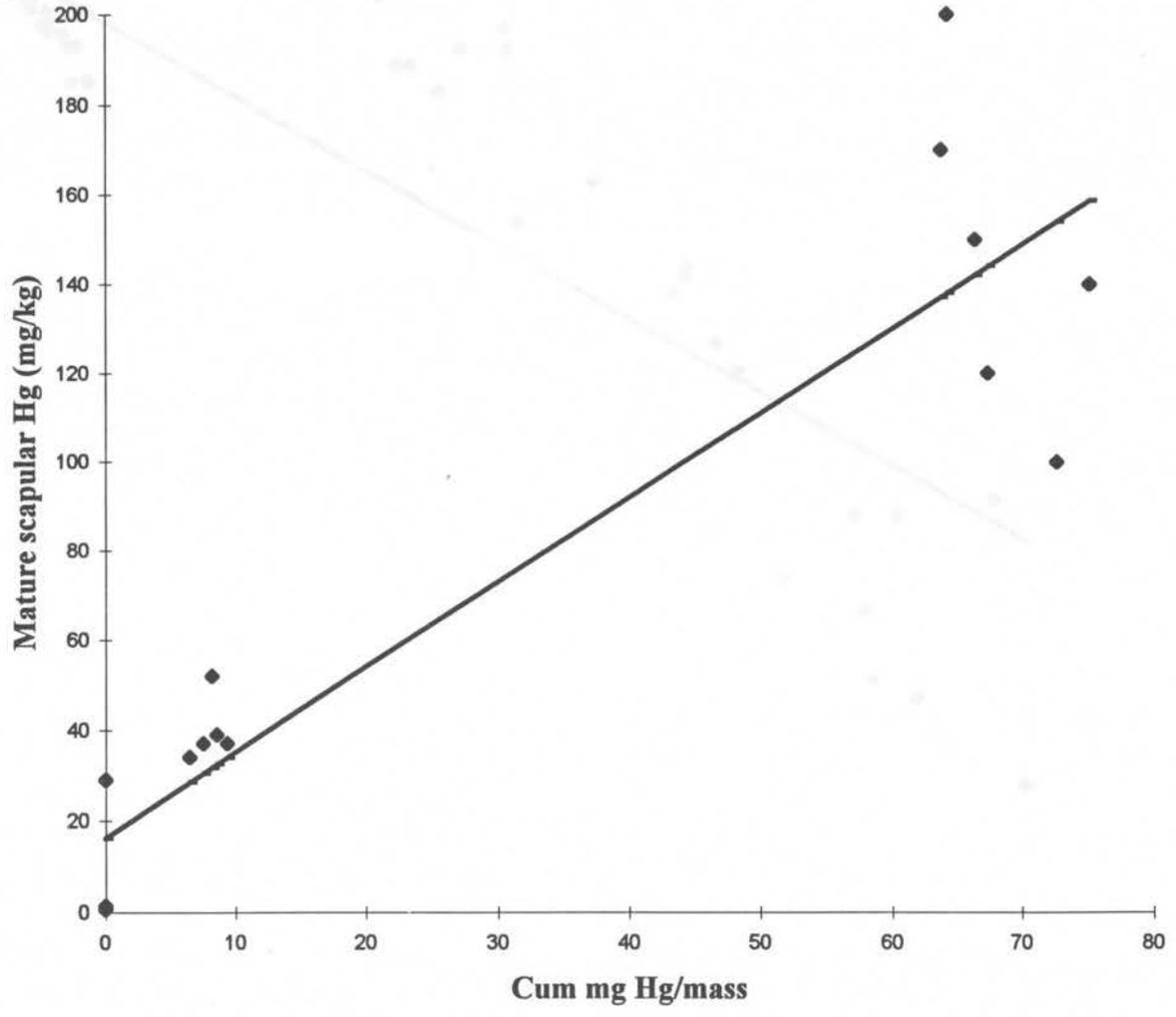


Figure 6.4 Mature scapular feather mercury concentrations relative to the cumulative amount of methyl mercury fed to great egrets.

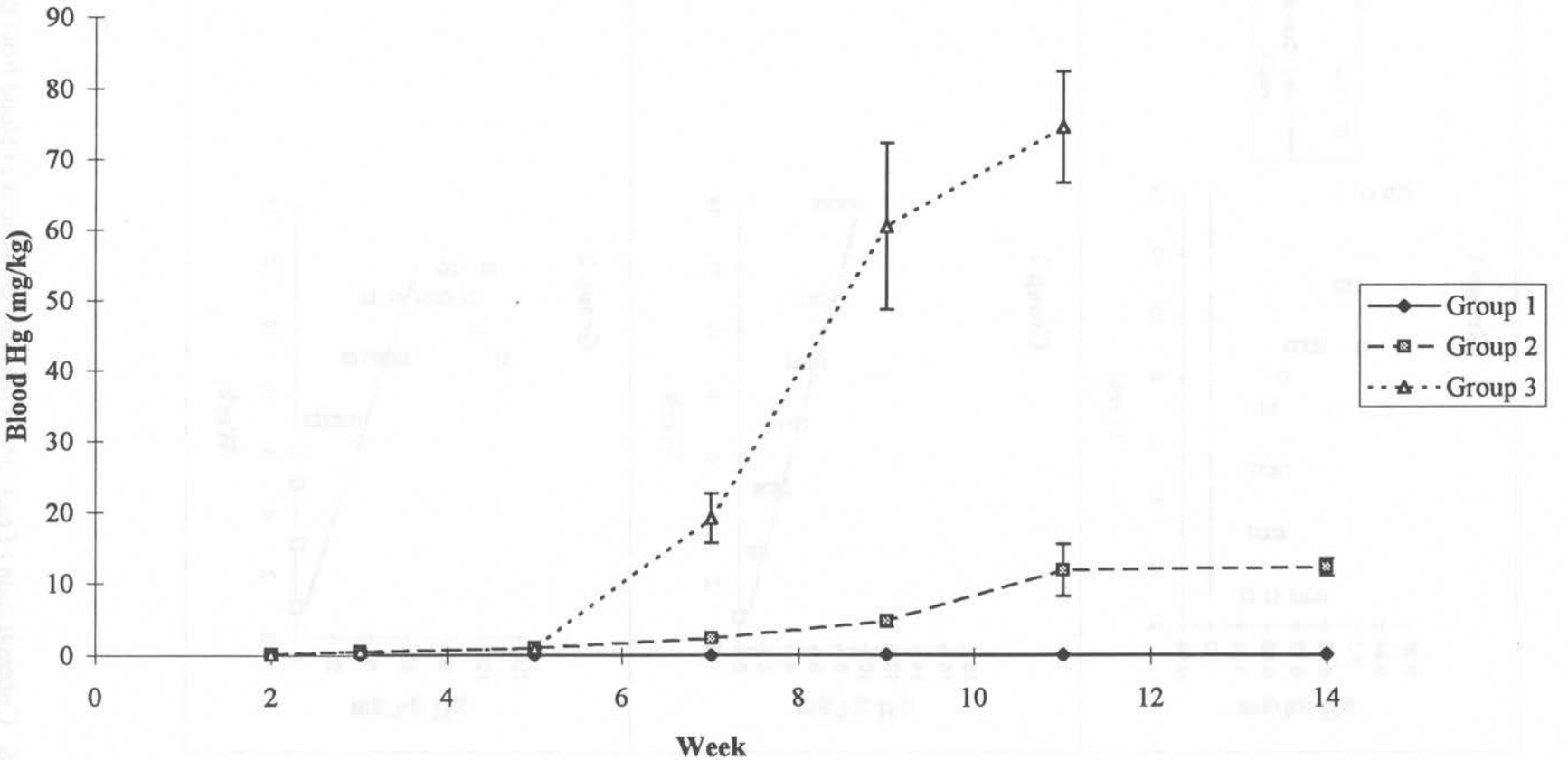


Figure 6.5. Concentration of mercury in repeated collections of blood from three groups of great egrets dosed with different amounts of methylmercury.

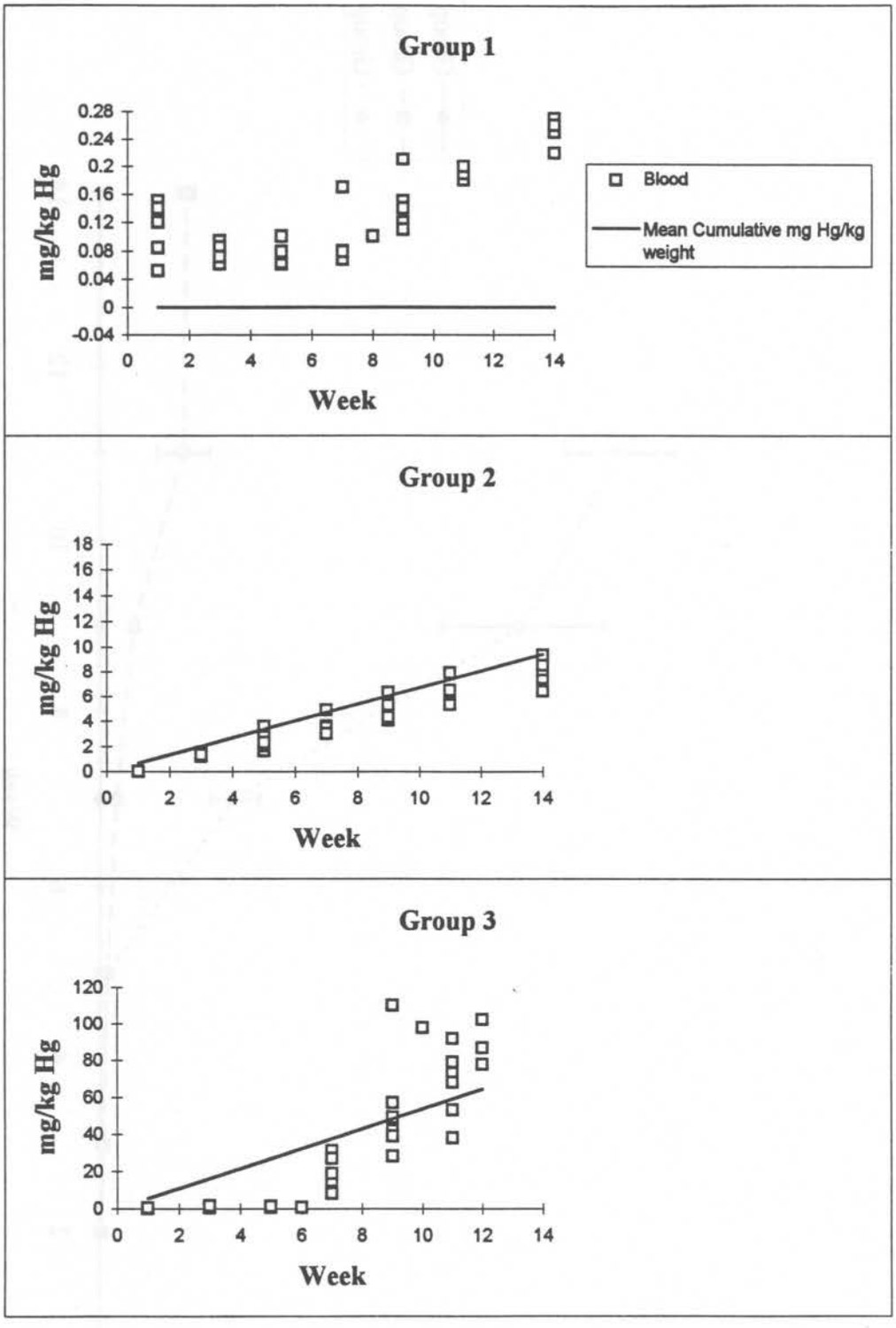


Figure 6.6. Concentration of mercury in repeated collections of blood from three groups of great egrets dosed with different amounts of methylmercury. Group 1=controls, Group 2= low dose, Group 3= high dose.

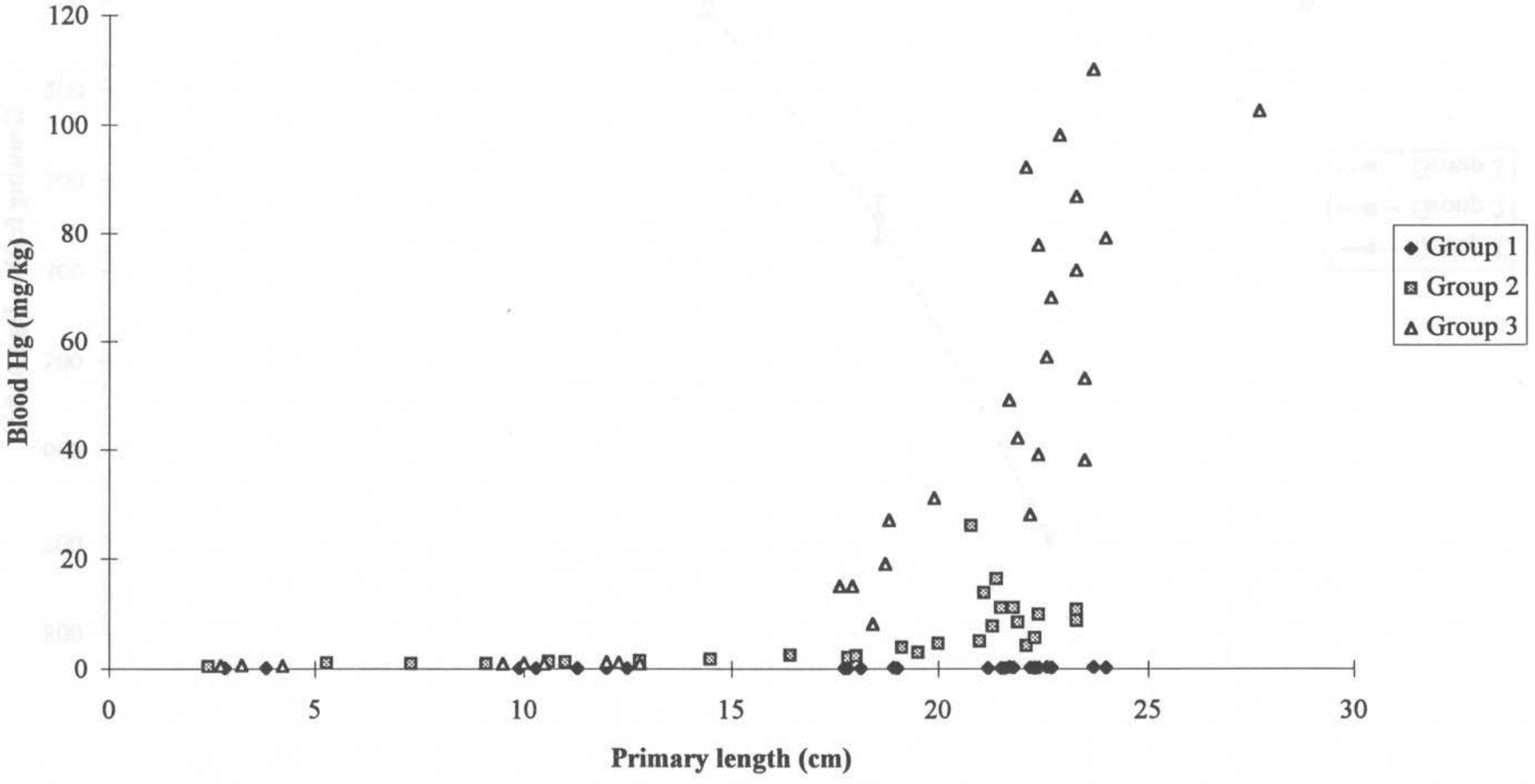


Figure 6.7. Concentration of mercury in blood as a function of the primary feather length for the three groups of experimentally dosed great egrets.

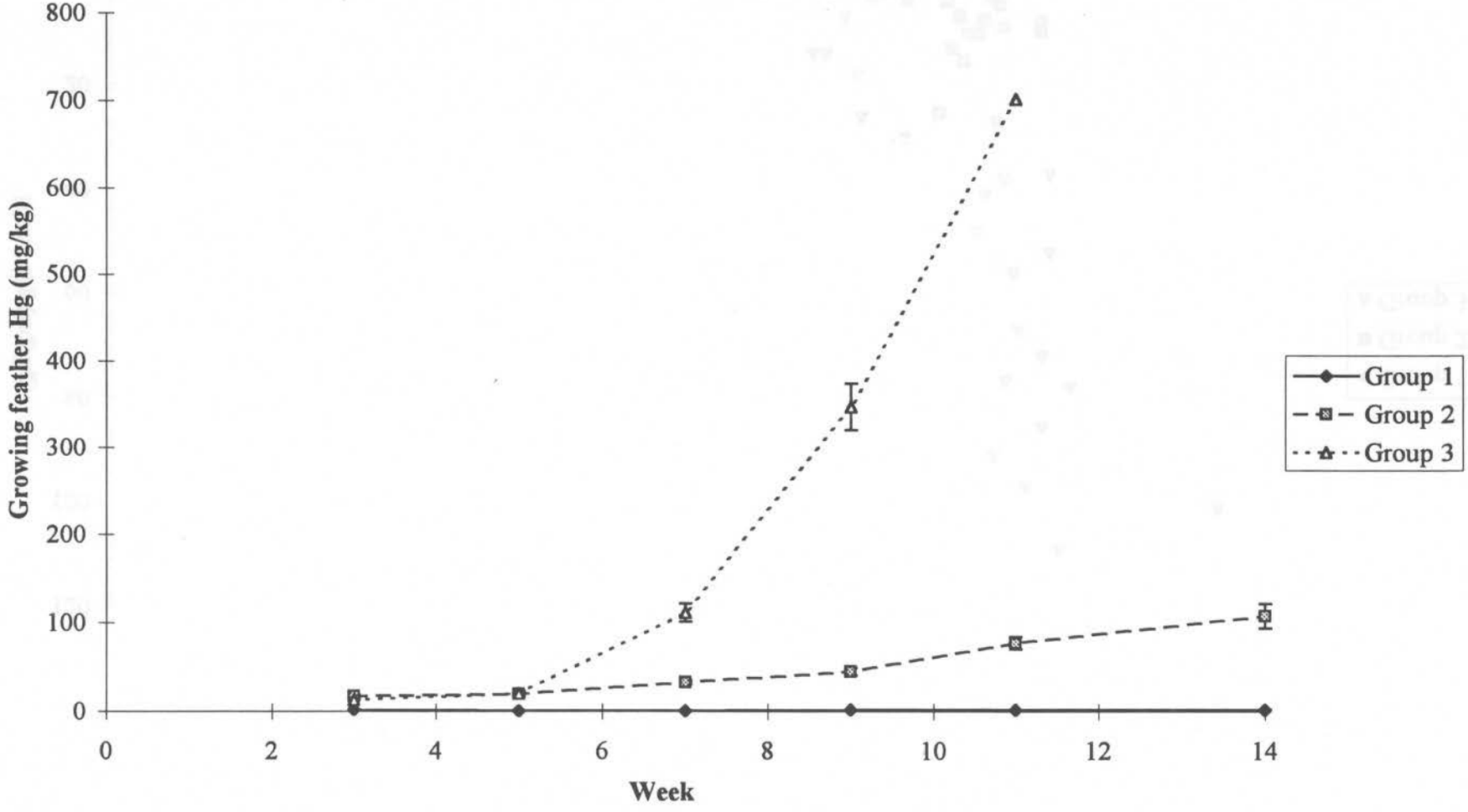


Figure 6.8. Concentration of mercury in growing feathers (body feathers and powderdown from three groups of experimentally dosed great egrets.

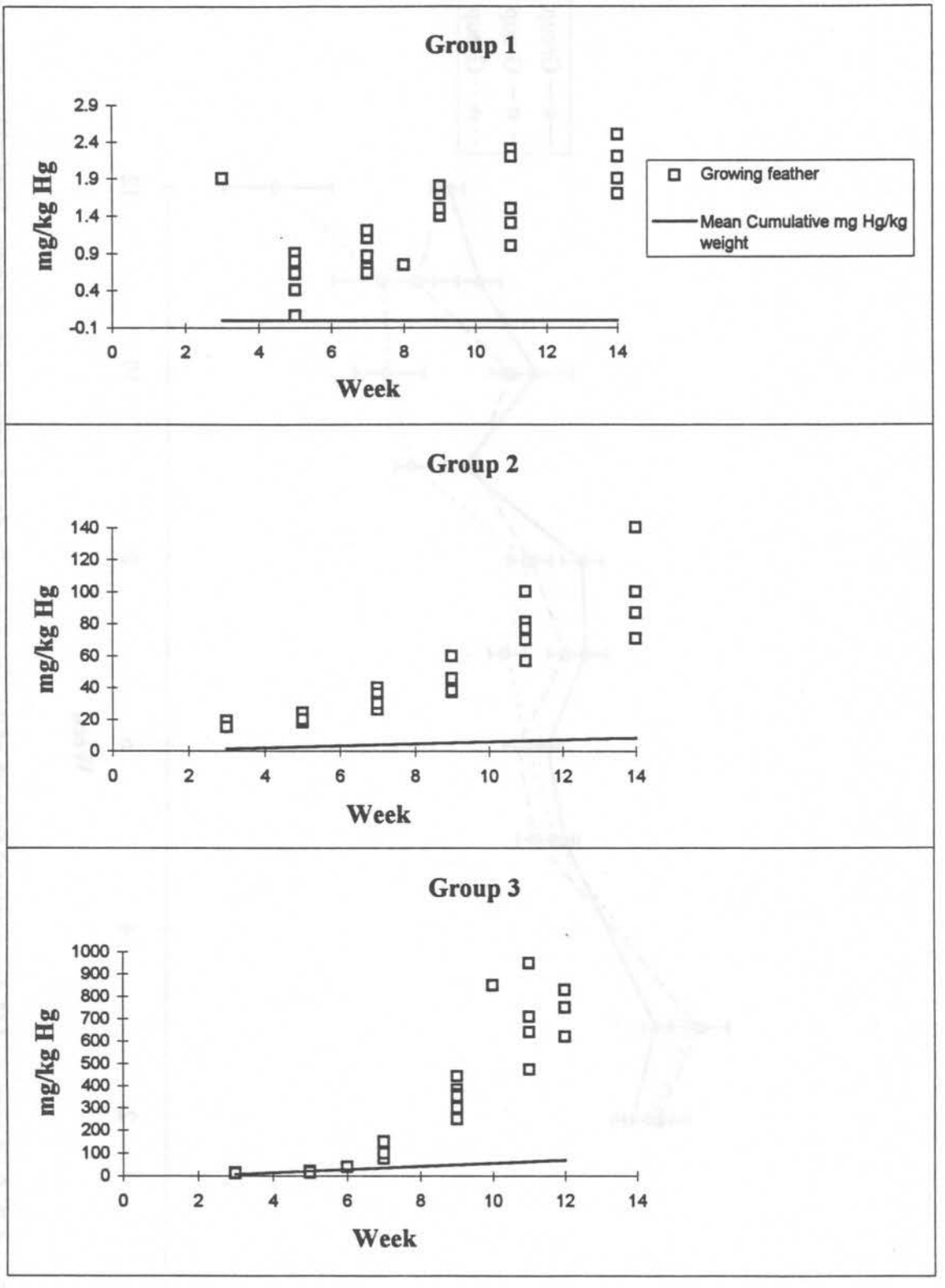


Figure 6.9. Concentration of mercury in growing feathers over the course of the experiment for each of the three groups of experimentally dosed great egrets.

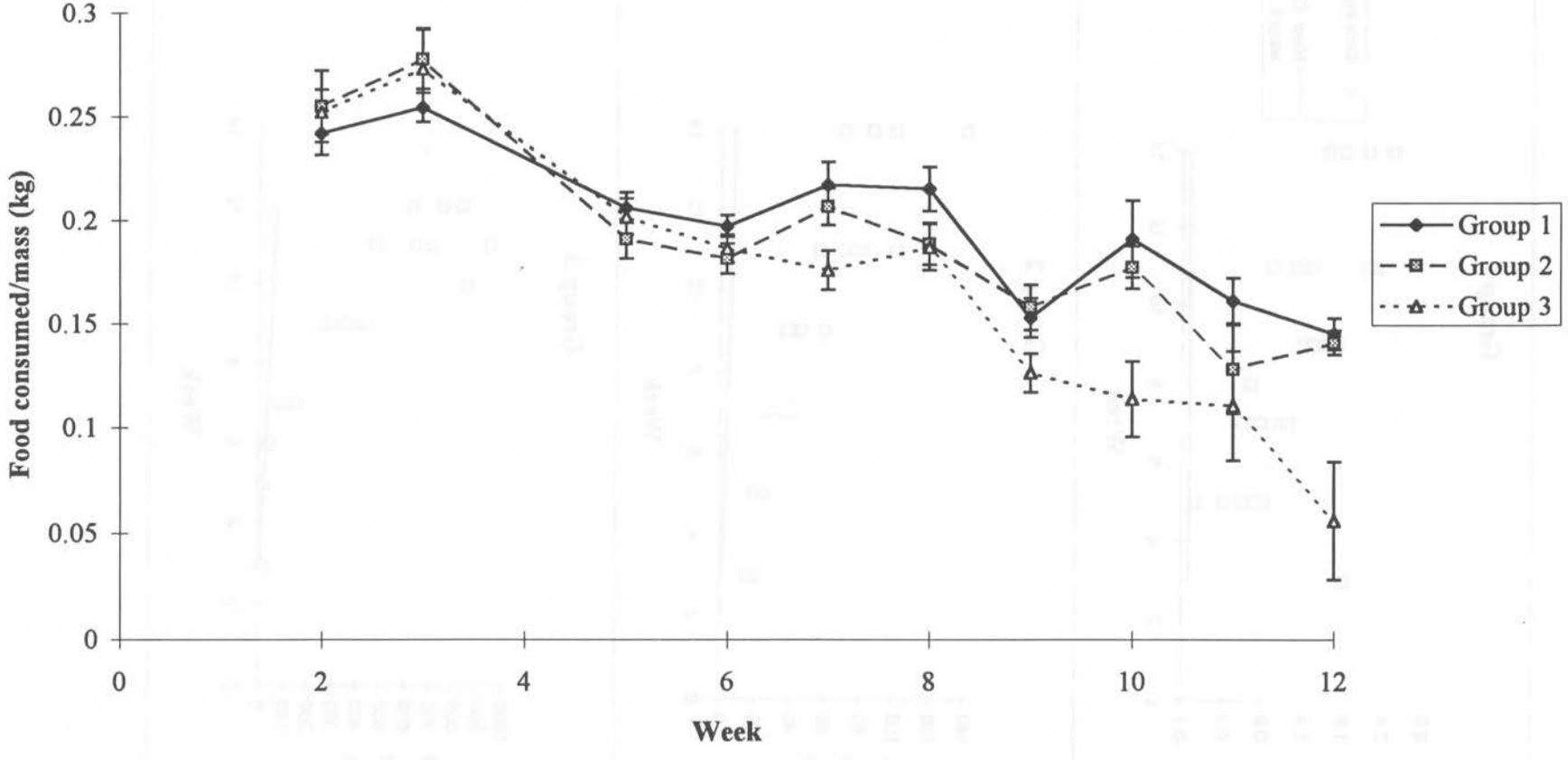


Figure 6.10. Mean daily food consumed (3 day averages) for each of the experimentally dosed groups of great egrets. Vertical bars are one standard error.

structure could be preserved. The mesh of the grid was made of rectangles that measured 5.0 × 10.1 cm. All feeding trials reported here included the wire grid, requiring the egrets to stab through the mesh in order to catch the bass. A total of 40 observations were made with each bird using the unpainted contrasting pools, and 25 observations using the painted camouflage pools.

Statistical analysis

Effects of group on growth, development, and health

We used repeated measures ANOVAs to test for effects of group on various responses by the chicks to the three mercury doses. The potential responses were mass, mass index (mass divided by bill length), tarsometatarsus length, bill length, tail length, length of last primary feather (emerged portion), primary sheath length (as a proportion of total primary feather length), Food consumed (food averaged over the three days prior to blood collection and Food/Mass (food divided by mass), packed cell volume (PCV), refractive index of plasma (RI), white blood cells (WBC), and counts of heterophils, basophils, eosinophils, lymphocytes and monocytes. We included age as a covariate in these analyses, simply because there was a possible 7-d difference in age between individuals due to the collection of chicks from nests that were not entirely synchronous. We also included sex as a covariate, since adult great egrets are somewhat sexually dimorphic in body measurements, apparently even during the pre-fledging stage (see Results). Measurements were made either weekly or biweekly, and the effect of week was included as a covariate in all models. Significant effects of group, and significant group × week interactions were both interpreted as evidence of an effect of mercury dose. Probabilities of 0.05 or less were considered significant, and probabilities of 0.10 - 0.05 were considered marginally significant.

Effects of group and mercury on maintenance and hunting behavior

Activity and position: The effect of mercury group on position, stance and activity was analyzed using two-by-k Chi-squared tests. In these tests, we compared the proportions of time in each activity, stance, or position category, by group. Time spent in the sun vs. shade by members of each group was compared using a two-by-two Chi-squared test.

Live Fish Presentations: The two treatment groups, contrasting and camouflage pools, were analyzed separately in all cases. The effect of experimental group was tested for association with average time to capture and average number of strikes using a repeated measures analysis of covariance (ANCOVA) adjusted for age and sex (PROC GLM, SAS Institute, 1988). In addition, the relationships between blood and feather mercury concentrations, and both time to capture or number of strikes were tested using an ANCOVA, with responses adjusted for age and sex by using these latter parameters as covariates. All values for time to capture and number of strikes to capture were averages of bouts of five sequentially presented fish. Lastly, using a 2-tailed Fisher's Exact Test we assayed for group related differences in the proportion of all fish presented that were actually eaten. Results were considered significant when p-values were less than 0.05, and marginally significant when between 0.05 and 0.10.

RESULTS

Distribution of mercury in tissues

Fish used to feed the birds contained an average (adjusted for proportions of each species fed) of 0.025 mg/kg of mercury (0.022 mg/kg in silversides, 0.046 mg/kg in capelin) of mercury. This amount of mercury is insignificant when compared to the 0.5 mg/kg fed to the low dose birds, and 5 mg/kg fed to the high dose birds.

Selenium measured in the fish used to feed the great egrets was 0.87 mg/kg for silversides and 1.14 mg/kg for caplin. The adjusted average for the diet was 0.90 mg/kg selenium. Selenium accumulated in liver in proportion to mercury ($n = 20$, $P < .001$, slope -0.359, Pearson correlation coefficient = 0.93) (Fig. 6.1). The Hg:Se ratio in liver was highest for high dose birds (ave. = 2.4, range = 1.9 - 4.1), intermediate for low dose birds (ave. = 0.8, range = 0.6 - 1.0) and lowest for control birds (ave. = 0.2, range = 0.05-0.4).

Growing scapular feathers and powderdown mercury concentrations were similar (Table 6.1). Because growing scapular feathers could not always be found, especially later in the study, the data for these feather categories were combined (e.g. powderdown used if growing scapular data not available) and are referred to as "growing feather."

Mercury concentrations in blood, mature scapulars, and growing feathers were compared with several measures of the doses given better understand the dynamics of accumulation. These included cumulative mercury consumed in mg (Cum), cumulative mercury consumed divided by mass of the bird (Cum/Mass), daily mercury consumed (Daily) and daily mercury consumed divided by mass of the bird (Daily/Mass). Both blood and feather mercury concentrations were most closely correlated with Cum/Mass (Table 6.2, Figures 6.2-6.4). Correlations were also significant for cumulative mercury consumed (CumHg) and daily mercury dose (DailyHg), but were not for daily mercury dose divided by body weight (DailyHgBW).

Mercury concentrations in blood increased over the course of the experiment for all three groups (Fig. 6.5). Low concentrations of mercury were undoubtedly present in the embryos and in the fish used to feed the birds. Mercury concentrations in blood closely mirrored the Cum/Mass values for low dose birds but not for high dose birds (Fig. 6.6). Toward the end of the experiment, it appeared that the blood mercury began to decline relative to the dose in the low dose birds. In the high dose birds blood mercury remained below 2 mg/kg until week 7, when it very rapidly increased. When blood mercury is plotted as a function of primary feather length (Fig. 6.7), it becomes apparent that blood mercury remained low in all dose groups (<10 mg/kg) until feathers were nearly grown (~ 20 cm).

Mercury concentrations increased in growing feathers in all groups (Fig. 6.8) in much greater proportion than differences in dose rates would suggest (Fig. 6.9). Growing feathers accumulated mercury at 7.6 times the rate of blood. Similar rates occurred when both high and low dose groups were examined separately.

Concentrations of mercury in various tissues collected at death are listed in Table 6.3. They were significantly correlated with CumMass (Table 6.4). Growing scapular feathers were not found at death and were replaced with powderdown as "growing feathers." Five tissues acted as storage organs, accumulating mercury in greater concentrations than dosed.

These were (in declining order): powderdown, liver, mature scapular feathers, kidney, and blood. Bile, eye, and fat accumulated little mercury relative to dose. Of tissues that can be sampled from live birds, powderdown had a slightly higher correlation coefficient than blood when correlated with CumMass (Table 6.4).

Table 6.1 compares the correlation analyses for various tissues in the experimentally dosed birds. Correlation coefficients were consistently high except for muscle, bile, and fat. Generally concentrations decreased in tissues in the follow order: growing scapular feather > powderdown > mature scapular feathers > liver > kidney > blood > muscle > pancreas > brain > bile > fat > eye.

Effects of mercury on growth and health

Appetite

The greatest 3-day-average percent of body weight consumed (Food/Mass) ranged between 22 and 36%, and peaked during the second and third weeks for all groups (Figure 6.10). When effects of age, week, and sex were controlled for, we found a significant effect of dose group on food eaten/body mas (Food/Mass) when data from all three groups were lumped (repeated measures ANOVA, group × week, $P = 0.007$) (Table 6.5). When the same variables were controlled for using data from only low dose and placebo groups, we found a marginally significant effect of group on Food/Mass (repeated measures ANOVA, $P = 0.066$). Least squares means were significantly different between the placebo and low dose birds during week 11 and between the placebo and high dose birds during weeks 10-11.

Growth

When the effects of week, age and sex were controlled for, we found a marginally significant effect of dose group on weight index (weight/bill length) when the data from all three dose groups were combined (repeated measures ANOVA, group × week, $P = 0.088$). When using data from only placebo and low dose groups, we found a significant effect of group on weight index (repeated measures ANOVA, group × week, $P = 0.03$). Weight index was significantly lower in the low dose group during weeks 1, and 11-12, and in the high dose group during weeks 1, and 10-11 (Fig. 6.11). Weight itself was not as clearly different, but least square means were significantly lower for low dose birds in weeks 10, and 13-14, and lower for high dose birds in week 11 (Fig. 6.12). Sex was a significant factor in the repeated measures ANOVA of the effect of dose group on weight for all three groups ($P = 0.007$) and probably explains the greater separation found when weight index was used. Both dose groups began to diverge in weight index from the placebo group at about 8-9 weeks of age (Fig. 6.11). Bill length, tarsometatarsus length, primary length, primary sheath, tail length, and tail sheath did not differ significantly between the groups (Figures 6.13-6.18).

Effects of mercury on blood parameters

Dose group had a significant effect on packed cell volume (PCV) when all dose groups were combined, and effects of age, sex, and week were controlled for (repeated measures ANOVA, $P = 0.0012$ for group, and $P = 0.0002$ for group × week). When data for placebo and low dose birds were used, we also found a significant effect of group on PCV

(repeated measures ANOVA, group effect $P = 0.004$). PCV gradually increased with age for all birds (Figure 6.19). PCV was significantly lower in the low dose group than in placebos for weeks 5-11 and lower in the high dose group than placebos during weeks 5, and 7-11. A decrease in PCV was observed on alternate weeks in all groups, suggesting an additional effect of blood collection on PCV. The blood collected ranged from 0.1 to 0.3 % of body weight.

We found a significant effect of dose group on refractive index of plasma (RI) when data from all three groups were used, and when effects of age, sex and week were controlled for (repeated measures ANOVA, group x week, $P = 0.030$), but no significant effects of group when data from placebo and low dose groups were compared. RI was lower in the low dose birds by week 14, and in the high dose birds in weeks 7-8, and 10-11 (Fig. 6.20).

We noted several significant differences in the number of certain cell types, but not in the overall white blood cell count (Fig. 6.21, Table 6.5). When data from all three groups were combined, we found a significant effect of dose group on numbers of heterophils (repeated measures ANOVA, $P = 0.003$ for group, $P = 0.01$ for group × week, effects of age, sex and week controlled for), and monocytes (repeated measures ANOVA, group effect, $P = 0.0185$, effects of age, sex, and week controlled for). Heterophils (Fig. 6.22) and monocytes (Fig. 6.23) increased significantly in the high dose group by week 11. The number of basophils (Fig. 6.24) and eosinophils (Fig. 6.25) increased significantly in both the high and low dose groups as compared to placebos on week 3 and then returned to normal. When data from placebos and low dose birds were combined, there was a significant effect of dose group on numbers of basophils (repeated measures ANOVA, group x week effect, $P = 0.028$). The numbers of lymphocytes were significantly lower in the high dose group than in placebos on week 7. Although significant differences were not observed at other times, the shape of the line for the high dose group was very different from the other groups (Figure 6.26). Lymphocyte numbers were initially low and remained low throughout the experiment.

Immune function tests

There were no significant differences between the groups for peak or duration of titers or for titers at week 11, for BSA titer or EEE titer or the sum of BSA and EEE titers.

Effects of mercury dose on plasma and organ biochemistry

Plasma biochemistry

By week 5, we found no significant differences in any blood chemistry parameters among dose groups (Table 6.6). We found significantly lower GSSG-reductase in both dosed groups, and marginally higher GSH-peroxidase in the high dose group by week 7. By the 9th week, we found no differences between the low dose and placebo groups. When compared with placebos, we found high dose birds had significant depression of plasma GSH-peroxidase activity, lower albumin and total plasma protein concentrations, lower uric acid concentrations, and higher plasma glucose.

By the 11th week low dose birds had significantly elevated AST and cholesterol when compared with placebos. High dose birds had severe depression of GSH-peroxidase activity, elevated AST activity, lower LDH-L activity, lower albumin and total plasma protein

concentrations, lower uric acid concentrations, lower inorganic phosphorus concentrations, and higher cholesterol than did controls.

At the end of the experiment, week 14, low dose birds showed significant depression of plasma GSH-peroxidase activity, elevated ALT activity, elevated CK, lower uric acid concentrations, lower plasma calcium concentrations, lower plasma triglyceride concentrations, and higher plasma glucose concentrations than did placebo birds. No high dose birds were alive by this time for comparison.

Effects on liver biochemistry

Compared with placebos, livers of high dose birds showed significantly increased hepatic lipid peroxidation measured as TBARS (thiobarbituric reactive substances), lower hepatic total thiol concentration, lower hepatic protein-bound sulfhydryl concentration, and lower hepatic GSH-peroxidase activity. Compared with placebos, the livers of birds from the low dose group showed significantly increased hepatic lipid peroxidation. We found significant overall effects of dose on increased glutathione transferase and reductase activities.

Effects on kidney biochemistry

Compared with placebos, we found kidneys of high dose birds had significantly lower protein-bound sulfhydryl concentrations, lower GSH-peroxidase activity, increased glutathione concentrations, increased glucose-6-phosphate dehydrogenase activity, and increased glutathione transferase activity. We found no significant differences between low dose birds and placebos in kidney biochemistry.

Effects on brain biochemistry

We found brains of birds from the high dose group had, by comparison with placebos, increased lipid peroxidation measured as TBARS (thiobarbituric reactive substances) increased glutathione concentrations, and decreased GSH-peroxidase activity. We found no significant differences in brain biochemistry when comparing low dose and placebos.

Summary of temporal and dose related changes

The first change to occur was a short term increase in basophils and eosinophils at week 3, shortly after dosing began (Table 6.6a). These numbers returned to the level of the placebo group by week 5. Mean blood (0.5-0.7 mg/kg) and growing feather concentrations were relatively low (13-17 mg/kg) at this time. PCV was significantly lower in both dosed groups by week 5 when blood mercury concentrations were near 1 mg/kg and growing feather near 18 mg/kg. Changes in RI, lymphocyte numbers, and polychromasia appeared to be very closely linked with blood mercury concentrations near 15 mg/kg and feather concentrations near 110 mg/kg. Decreases in food intake (Food/Mass), weight index, and weakness appeared to be more related to week than to dose or to mercury concentrations in

blood or feathers. The timing of first changes in weakness is somewhat artifactual because weakness was only measured on week 11. Changes in numbers of heterophils and monocytes, and terminal ataxia developed only in the high dose group when blood concentrations exceeded 70 mg/kg and growing feathers exceeded 600 mg/kg. Although correlations between Cum/Mass and blood and growing feather are significant (see above), few of these changes occurred at the same Cum/Mass. In fact, Cum/Mass was always 10 times higher in the high dose group than the low dose group when the same effect occurred in both.

Changes in plasma enzymes and biochemical parameters also appeared to be somewhat dose related (Table 6.6a). Three parameters that were observed in high dose birds at week 9 were first observed in low dose birds at week 14. These included a decrease in GSH-peroxidase, increase in glucose, and a decrease in uric acid. Mercury concentrations in blood at these times were 54, and 12 mg/kg respectively, and for growing feathers 350 and 110 mg/kg respectively. Changes in GSH-peroxidase began at week 7 for the high dose birds and by week 11 for the low dose birds (except for a transient decrease in glutathione reductase at week 7). This corresponds to blood mercury concentrations of 19 and 12 mg/kg respectively, and growing feather concentrations of 111 and 77 mg/kg respectively.

Effects of mercury on behavior

Cleanliness and motor control

There was a significant difference in weakness between the high dose group and placebos at week 10, (Figure 6.27). Both the low and high dose groups were significantly less clean at week 9 than placebos, and the high dose birds continued to be so at week 10 (Fig. 6.29). It took high dose birds significantly longer to return to a standing position when placed on their backs by week 10 than placebo birds (Fig 6.28). No effect was observed for low dose birds compared to placebos.

Activity and position samples

We found highly significant differences between all three experimental groups for position, stance and activity (all p-values < 0.001, Tables 6.7-6.9). The percentage of time spent in each position, stance and activity by the high dose group differed more in comparison to the other two groups than the low dose and placebo groups differed from each other. High dose birds spent proportionally more time with head down, head under-wing, delta wing and swaying, and less time preening or pecking. This suggests a general decline in activity level, and a shift towards activities that were less energetically demanding, or those that used fewer motor skills.

Differences between low dose and placebo groups were less easily interpretable. Low dose birds spent less time pecking but more time preening than placebos (Table 6.8). We considered these two activities to be among the more active ones. In addition, the placebo and low dose groups spent similar amounts of time standing and sitting while the high dose group spent approximately 30% less time standing and 40% more time sitting than the other two groups (Table 6.7). Again, the differences between the low dose and control groups are less obvious, but are still statistically significant. The differences in stance

between the placebo and low dose groups lie in the fact that the low dose group spent relatively less time walking and/or flying than the placebo group (Table 6.7). Finally, the high dose group spent the majority of their time on the ground while the low dose group spent more time on the perch or in the pool than the placebos but less time on the pool edge. Generally, there seemed to be a negative relationship between mercury dose and amount of time spent in active, energetic behaviors.

We found that mercury dosed birds spent significantly more time in the shade than did controls. This difference was significant between the placebo group and the low dose group, and the placebo and high dose group (both $P < 0.001$) but not between the two mercury dosed groups ($P = 0.33$, Table 6.7 and 6.9). This analysis used only observations in which the birds had the option of sun or shade, and overcast days or times when a specific cage was completely shaded by a tree, were not included in our analysis. Shade is not necessarily linked with any one position in the cage since both sun and shade options existed for ground, perch and pool locations, and changed with time of day. Thus mercury dosed birds at both dose levels appeared to avoid sunny locations within their cages.

Hunting behavior

By the time we began live fish presentations, all of the high dose birds had been euthanized. Thus the results of live fish presentations refer exclusively to low dose and control birds.

No statistically significant association was found between average numbers of strikes, and sex, age, experimental group, test date (= time) or experimental group × test date, for either pool treatment (Table 6.10). The contrasting pools treatment showed a significant decline in average numbers of strikes necessary to capture fish over the duration of the experiment ($P = 0.052$).

After accounting for variation due to age and sex we measured for an association between average number of strikes and mercury concentrations in blood and powder down. Again, the effects were not statistically significant at a p-value of 0.05 but three out of the four analyses were marginally significant (Table 6.9). The slope of the regression was negative in all cases, indicating a slight tendency for fewer number of strikes necessary to capture fish as mercury levels increased, which is contrary to an interpretation of impairment of hunting ability or coordination as a result of mercury contamination.

Analyses of time necessary to capture fish in relation to mercury dose group gave contradictory results. For the contrasting pools there were statistically significant effects of sex, age and experimental group on average time to capture ($P = 0.004$, 0.004, 0.041 respectively, Table 6.10). A graph of the least squares means by experimental group shows that placebo birds were consistently faster at capturing fish than low dose birds throughout the duration of the contrasting pools treatment (Figure 6.30). For the camouflage pools treatment there was a marginally significant effect of sex on time to capture ($P = 0.091$), with males being slower than females. We also found significant effects of age and time on time to capture ($P = 0.04$ and 0.037, respectively). As the experiment progressed, capture time in the camouflage pools decreased significantly (Table 6.11).

After accounting for variation due to age and sex, we tested for an association between average time to capture and mercury concentrations in blood and powder down. These relationships were statistically significant for the contrasting pool treatment (blood

mercury, ANCOVA, $P = 0.042$; powder down mercury, $P = 0.006$, Table 6.9), but not for the camouflage pool treatment. In addition, both slopes for the relationship with blood mercury and the slope for the significant powder down result were positive. In other words, there was an increase in time to capture with increasing mercury contamination, which may be interpreted as an impairment of hunting ability. This result is statistically significant in the predicted direction for the contrasting pools treatment and significant in the opposite direction or not significant for the camouflage pools (Table 6.9).

Mercury dosed birds in both dose groups were significantly less likely to eat fish presented to them in the camouflage ($P = 0.0003$) and contrasting ($P = 0.003$) pool treatments (Table 6.9).

DISCUSSION

Dynamics of tissue mercury deposition

The repeated sampling of feathers and blood throughout the course of the experiment provided a large series of tissue mercury concentrations with which we could evaluate the dynamics of tissue mercury in response to an essentially static dose rate. As expected, mercury concentrated in feathers at a much greater rate than in blood. Initially (weeks 3 to 5) mercury blood concentrations in the low dose group actually exceeded those of the high dose group. We can find no obvious explanation for this pattern. In the low dose group, blood mercury initially mirrored the cumulative mercury dose/mass, but gradually began to fall below that level, probably because mercury was taken up preferentially by other tissues such as feathers. In the high dose birds, however, mercury initially remained low and began to concentrate in blood at a higher rate than the dose rate at about week 9. This occurred at about the time that the birds began to show obvious health problems and had to be euthanized (weeks 10-12). The time at which blood mercury began to exceed dose corresponded to the time that feathers stopped growing (weeks 9-11 for primary feathers, and week 9 for tail feathers).

Thus, it appears that growing feathers provide a sink for mercury during the nestling period. When this sink is no longer available, mercury apparently increases in other tissues. This explains the results obtained from Chapter V, in which we hypothesized that no effects were observed because mercury was being "dumped" into relatively inert feather tissue throughout the dose and monitoring period. It also helps to explain why the only significant effects of mercury on health index (Chapter V) occurred in older chicks. An obvious conclusion to be drawn from these results is that the time of greatest risk of mercury toxicity for young birds is the period when feathers stop growing. It is probably that this process usually occurs at or near the time that young birds also encounter the multiple risk factors of having to forage on their own, having to leave the natal colony, and being exposed to novel predation and disease factors.

Mercury accumulated in several tissues in greater concentrations than dose rate (growing feather > powderdown > mature scapular feathers > liver > kidney > blood). This was not expected for blood mercury. Closer examination of the blood tissue correlations may provide an explanation for this. As above, blood mercury remained at relatively low concentrations until feathers (primaries) were no longer in sheath and ceased to grow at about

9 weeks.

We were initially puzzled by the fact that mature feathers collected from the chicks were lower in mercury concentration than growing feathers. However, this can be explained by the fact that mercury concentrations in most tissues increased over time. Feathers that are plucked regrow. Growing feathers collected from birds near the end of the experiment, when blood mercury values were highest, were probably these regrown feathers. Thus growing feathers collected sequentially during the experiment will have higher average concentrations than mature feathers. A comparison between growing feathers and mature feathers collected *at the same time* should therefore give a good indication of recent versus previous exposure.

We found differences in the distribution of mercury among tissues of the captive chicks, and of birds collected from the wild (Table 2.5). There are two potential sources for these differences. The first is the method of exposure. The nearly pure methylmercury presented in the capsules to the captives might be different that the possible mix of mercury types present in naturally contaminated fish, and this might affect the relative uptake by various tissues. The second explanation is that wild birds had a different (and unfortunately unknown) history of mercury exposure, whereas the captive birds had constant and consistent exposure. It is particularly interesting that the feathers in captives had higher concentrations than other tissues in the captive birds, and did not in the wild birds. Mature feathers in wild birds were generally higher in mercury than were liver, but growing feathers, powderdown and plumes were not. If we assume from the captive results that mercury preferentially accumulates at a higher rate in feathers than it does in liver, then it would appear that current exposure in wild great blue herons (as reflected in growing feather mercury), was lower than past exposure, as reflected by liver concentration.

Efficiency of various tissues for sampling mercury

One of the objectives for establishing correlations between tissue types was to define a tissue that would be representative of mercury contamination and that could be sampled without killing the bird. Growing feathers, whether scapular feathers or powderdown, appear to serve this purpose best. Although the correlation coefficient between mature scapulars and Cum/Mass was slightly higher than for growing feathers, it is difficult to tell in anything but a nestling bird when mature feathers were grown. It should be possible, especially in nestlings, to estimate the actual quantity of mercury consumed to date by the nestling by sampling growing feathers.

Blood mercury also had a similarly high correlation coefficient with cumulative dose. However, blood mercury may be quite dynamic over time (see above), and so may be misleading, especially if the timing of molt is not known. In addition, blood mercury collection and storage in the field is considerably more difficult than feather collection. Growing feathers therefore seem to be the best general sampling medium in herons and egrets.

Effects of mercury on growth and development

Appetite

The results of this experiment supported the hypothesis that methylmercury exposure

results in reduced appetite in nestling and post-fledging birds. In the high dose group, it resulted in a cessation of eating. The effect was found both in the amounts of thawed fish eaten from feeding dishes, and in the numbers of live fish captured and eaten during feeding trials. This finding agrees with the results of the field dosing experiment, and in that sense is a very robust finding. The fact that significant reduction in appetite occurred in the low dose group illustrates that appetite can be affected at 0.5 mg/kg in the diet, a level that is likely to be regularly exceeded by wild nestlings in the Everglades (see Chapters V and VII).

Growth

We found reductions in body mass in response to mercury dose, when body mass was indexed to bill length. This occurred in both low and high dose groups when compared to placebos, and is a logical consequence of the mercury-induced reduction in appetite (above). The differences between the groups were not striking, and even the high dose birds had abundant body fat when euthanized. The lack of food stress in these birds probably masked some of the effect that mercury contamination might have caused. Mercury dosing did not result in any decreases in skeletal measurements. This implies that, even at the rather high dose that we gave (5 mg/kg), mercury does not typically have an effect on skeletal growth of egrets. In wild birds exposed to mercury, a reduction of appetite could quickly result in weight loss and body condition given the rigors of competition between siblings for limited food resources. The consequences of low body condition depend entirely on severity, but given the typically high juvenile mortality rate for wading birds, it can be presumed that poor body condition would lead to increased mortality rates for juveniles in the wild.

Blood parameters

A pattern of alternate peaking and declining was apparent in the PCV of all three groups for most of the experiment. This was probably due to the larger volume of blood that we took on alternate weeks to meet the requirements of various tests, resulting in a depression of PCV that was evident at the next sampling. However, this is a surprising finding given that the volume of blood collected never exceeded 0.3% of body weight. Generally, it is recommended that 1 to 3% of body weight can be collected with no ill effects (Cambell 1994). We were surprised to see such a pronounced effect on PCV. These results warrant further investigation into the effects of blood collection, especially in very young birds.

The effects of mercury on PCV were obvious even in the low dose group. These effects occurred early in the dosing and at relatively low concentrations. In the low dose group the depression of PCV averaged about 20% between weeks 5 and 11, and the differences became insignificant after that. Because of the timing of the experiment relative to the age of these birds (during the period of PCV increase) it is impossible to tell if this depression of PCV has to do with damage to red cells themselves, or to the suppression of production of new cells. Polychromasia, evidence of red cell regeneration, was only increased in dosed birds at much higher mercury concentrations in blood.

A 20% reduction in PCV, although not life threatening, is likely to have an effect on stamina, especially in birds that are stressed. Little to no quantitative information is available about the effects of this degree of anemia on behavior. It is possible that anemia can cause a

decrease in maintenance activities such as preening. It is possible but unlikely that it can cause the weakness (measured by pushing) and increase in righting time. The threshold concentration of 1 mg/kg for depression of PCV is equivalent to the mean concentration of mercury in blood of nestlings in the Everglades. Thus, it is possible that great egret nestlings in the Everglades are presently experiencing the chronic, sublethal effects of mild anemia. This would be difficult to prove in a field setting. The very marked depression of PCV that occurred terminally in the high dose birds was clearly life-threatening.

Other effects on blood parameters that occurred at about 15 mg/kg of mercury in blood, (decreased RI and lymphocyte numbers), occurred at blood mercury concentrations above those found in Everglades great egret nestlings, but at within the high range of adult and juvenile great blue herons. We cannot, with the information presently available, extrapolate these results to older birds. A decrease in refractive index can be caused by a wide range of factors, from over hydration, to a decrease in plasma proteins. Lymphopenia, if severe, can result in immunosuppression. In vitro studies have demonstrated death of lymphocytes in other species with mercury exposure (Koller and Roan 1980, Lawrence 1981, Steffensen et al. 1994).

The increases in heterophils and monocytes that occurred terminally in the high dose birds were extreme. Heterophils and monocytes usually increase in number in response to infectious agents. No cause for such an increase could be found at necropsy. The reason for these increases remains unknown at this time.

Immune function

Our failure to demonstrate an effect of mercury on the serologic responses to antigens presented is confounded by several factors. The great egrets in this experiment were only 3 to 3.5 months of age. We know nothing about the age at which these birds become immunocompetent. The fact that all birds did develop titers to EEE and BSA suggests that it occurs before or at this age. Extreme individual variability in responses, may reflect the individual differences in maturation of the immune system. Second, the high dose birds had to be euthanized in the middle of the test period, and thus could not be compared with other groups. And finally, the length of the experiment limited our ability to separate responses to initial vaccination from repeat vaccination. Thus, these results are inconclusive.

Blood and organ biochemistry and enzyme production

Many significant changes in plasma chemistries and tissue biochemistry were apparent due to mercury exposure in great egret nestlings. A number of the mercury-associated effects in this study were similar to those found in a pen study with mallards as well as in several field studies with aquatic birds, and therefore may serve as good field bioindicators of mercury exposure at toxic levels. These included depression of the enzyme GSH-peroxidase accompanied by decreases in one or more categories of hepatic reduced thiols. In the present study with great egret nestlings, GSH-peroxidase activity (plasma, liver, kidney and brain) were significantly decreased in the high dosed group. Hepatic total thiol and protein-bound sulfhydryl concentrations decreased in the high dosed group, kidney protein-bound sulfhydryl concentration decreased in this group, and kidney GSH increased. Effects of methylmercury in adult mallards at 10 mg/kg in a dry diet included decreased

activity of the enzyme GSH-peroxidase (plasma and liver) with oxidative stress (increased oxidized glutathione relative to reduced glutathione and reduced thiols; Hoffman and Heinz 1997).

The following significant relationships also occurred in one or more of three species of diving ducks in the San Francisco Bay area with increasing hepatic Hg concentration: hepatic enzyme activity for GSH-peroxidase and G-6-PDH decreased; and reduced hepatic thiol concentrations decreased but the ratio of oxidized (GSSG) to GSH increased (Hoffman et al. 1997). Similarly, reduced hepatic thiol concentrations including GSH, PBSH, and total thiols in livers of pipping great blue heron embryos were found to decrease with increasing concentrations of mercury in eggs from the same colony (Custer et al. 1997).

In the mallard study, ducks became ataxic and unable to walk associated with lipid peroxidation of the CNS as detected by increased thiobarbituric reactive substances (TBARS) in the brain. In the present study similar effects occurred in high-dosed great egrets where brain TBARS increased. In egret liver, TBARS increased in both dose groups. In japanese quail, methylmercury at 8 mg/kg in the diet resulted in approximately 10% mortality, with some loss of muscular control by 8 weeks (Hill and Soares 1984).

A number of other indicators of tissue damage were apparent as judged on the basis of increased plasma enzyme activities for AST, ALT, and CK. The increases in AST and ALT are indicative to of liver alterations whereas increased CK is related to neural or muscular injury. Hill and Soares (1984) reported on subchronic maturational and physiological effects of methylmercury up to 8 mg/kg in the diet of japanese quail during the first 9 weeks post-hatching. Notable elevations in plasma LDH (over 4 fold) and the isozyme HBD (2-3 fold) occurred indicative of liver and heart biochemical lesions. Brain AChE in quail was depressed by 38%. In the present study brain AChE was not significantly affected.

Temporal and dose related effects

Some of the changes observed in the experimentally dosed great egrets were closely tied to specific blood concentrations, whereas others occurred at similar times after dosing began, irrespective of degree of tissue contamination. Number of lymphocytes, polychromasia and RI were all significantly different when blood concentrations reached about 15 mg/kg (growing feather about 110 mg/kg). This is well above the average concentration for wild great blue herons collected in this study. Only a single adult, non-breeding great blue heron that was found in the Everglades, weak and unable to fly, exceeded this concentration (18 mg/kg in blood). Weight index, Food/Mass, and weakness changes were observed in both the dosed groups at week 11 of the experiment at widely divergent concentrations in blood and feather. This suggests that, within some bounds, the duration of exposure may be as important, or more important than magnitude of exposure. A longer chronic dosing experiment would be necessary to tease these apart from the effects of age. The tissue concentrations at which these changes occurred in the low dose group (mean blood mercury = 12 mg/kg, growing feather = 77 mg/kg) are at the high end of the range for wild great blue herons in the Everglades. Other changes became important early in the experiment, such as PCV, and number of eosinophils and basophils, and differed significantly from placebos during the same week and at the same blood and growing feather concentrations. The number of eosinophils and basophils returned to levels comparable with

control birds within a week, so it appears that this was only a temporary change. The blood concentration at which PCV declined was 1 mg/kg, well below the mean for wild nestling great egrets, and juvenile/adult great blue herons in the Everglades. Thus, it is likely that wild great egrets and great blue herons in the Everglades are presently experiencing a reduction in PCV compared with an uncontaminated condition. What this might mean in terms of health, reproduction, and survival cannot be surmised from information available at this time.

The above measured thresholds are expected to be conservative for a number of reasons. These birds all had abundant food, abundant body fat, some protection from environmental extremes, and no competition from siblings. All of these factors should contribute to the conservative nature of the above estimated thresholds. Young birds, however, are likely to be more sensitive to toxicosis, and so caution should be taken when using these same thresholds with adult birds and with birds of other species.

Effects of mercury on behavior

Activity and position

Our results indicate that there were considerable changes in the maintenance behavior (feather cleanliness, weakness, and ability to return to a standing position) of post-fledging great egrets, that were directly attributable to even low doses of mercury (0.50 mg/kg). The most obvious differences were found in high dose birds but the variation between placebo and low dose groups was also statistically significant. As levels of mercury intoxication increased, the birds spent proportionally more time in behaviors or positions that we interpreted to be physically less demanding, such as more time sitting, using perches in shade rather than sun, and less time flying. This suggests that mercury makes birds more lethargic, possibly by interrupting metabolic pathways that make energy available, or birds may be less able to perform active behaviors that require motor skills.

The fact that mercury can sensitize birds to environmental stress has been shown for extreme cold weather conditions (Van der Molen et al. 1982). Our study showed that mercury birds in both high and low dose groups spent significantly more time in the shade than did placebo birds. This may indicate a lower tolerance of heat. In comparison to the placebo group, both classes of dosed groups spent more time in the delta wing position which is thought to be associated with cooling. In contrast, gular fluttering, which is also thought to help with heat regulation, was observed less often in the low dose group than in the placebo birds, but more often in the high dose group than in placebos. Thus although mercury apparently results in an increased threshold for sitting in the sun, the results are only partially supportive of the hypothesis that mercury dosed birds are having trouble thermoregulating.

The analysis of scan samples from this study show that mercury intoxication causes behavioral differences in juvenile great egrets, even at very low dose rates (0.5 mg/kg in diet). The effects are not obviously linear with increasing mercury contamination, and they become less interpretable as mercury dose decreases. Whether these mercury effects are the result of lower energy levels, or impaired ability to perform motor skills, is not known.

Hunting behavior

Striking efficiency: The relationship between number of strikes to capture fish and mercury contamination was not statistically significant for any group. In addition, the slopes of the regression analyses were contrary to our prediction that increasing mercury would lead to increasing number of strikes necessary to capture fish, presumably reflecting deteriorating perceptive abilities or motor coordination.

The interpretation of these results, however, depends heavily on our assumptions that more strikes and more time to capture fishes is indicative of impaired hunting abilities. This assumption may not be valid. During the course of the experiment we noted that the hunting tactics consistently fell into one of two groups: the patient watcher, which generally caught the fish on the first strike but waited, watched, and stalked before making any attempt to strike, and very active foragers that jumped into the pools immediately and struck repeatedly, with little apparent concentration, until they caught the fish. These two tactics were not related to mercury group and individual birds seemed to adopt one or the other method consistently. As a result, it may be that our results for striking efficiency merely reflect the difference between two quite naturally variable foraging strategies.

Time to capture fish: The analysis of time necessary to capture fish showed inconsistent results. When variation due to age and sex were taken into account, our tests showed that Placebo birds took significantly longer to capture fish than did low dose birds. These results do not support our prediction that mercury dosing would impair the ability of young great egrets to capture fish.

However, two of the other tests produced significant results in the opposite direction. In contrasting pools, blood and powderdown mercury concentrations both had significant positive effects on capture times. Thus the evidence is contradictory-- we have shown significant results in both positive and negative directions.

In addition, there were two tests that were not significant in either direction. Neither of the tests of association between blood/powderdown mercury and time to capture were significant in the camouflage pools.

This combination of contradictory, and null evidence suggests that there is only weak evidence of an increase in time to capture with methylmercury exposure, at least at the 0.5 mg/kg diet.

It is interesting to note that the analysis of time to capture revealed a significant effect of both week and sex, with older birds, and females, having shorter capture times. Since great egrets are sexually dimorphic one might expect some differences in hunting strategies, and consequently capture time, due to sex. An effect of age on capture time has been noted in great blue herons (Butler 1995) and may simply reflect a learning effect with juveniles reaching adult capacity by two years of age.

Willingness to hunt fish: Under both mercury dose levels, mercury dosed birds were consistently less likely to hunt and eat fish than were undosed birds. This may reflect a lack of motivation or appetite. It did not, however, reflect a lack of motor ability since we found no differences in striking efficiencies, and only weak and inconsistent differences in capture time.

In conclusion, the live fish presentation studies showed that, when motivated to hunt,

post-fledging great egrets dosed at 0.5 mg/kg dietary methylmercury do nearly as well as undosed birds at catching fish, but they are consistently less likely to eat fish that are available. This is most likely due to a decrease in activity level or motivation to eat, rather than a deterioration of motor skills. The result is consistent with the other evidence of reduction of appetite in this experiment, and in the field dosing experiment (Chapter V) that showed that increased mercury intoxication levels result in lowered appetite in wild great egret chicks.

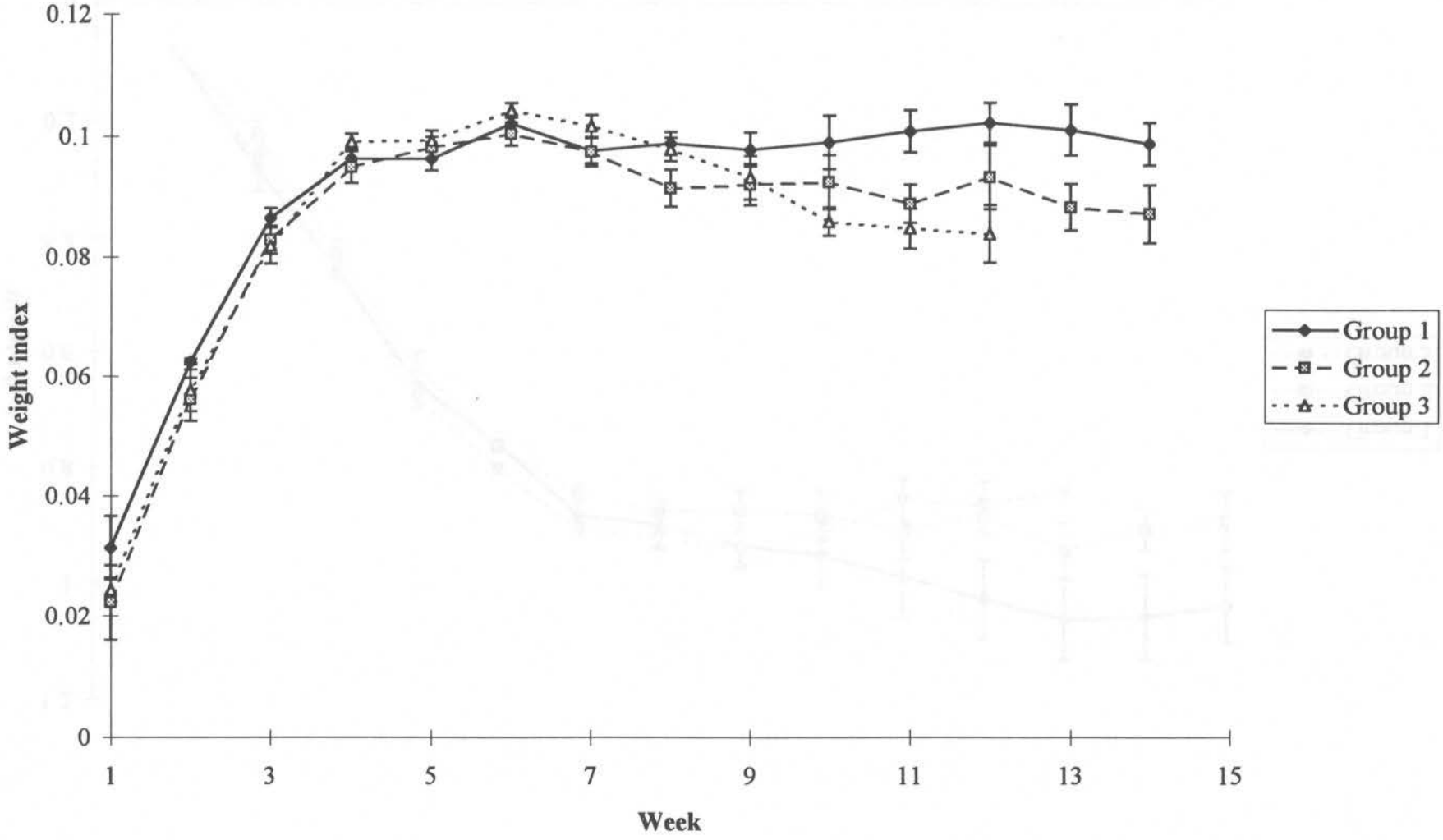


Figure 6.11. Mean weight index (weight/bill length) for each of the groups of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

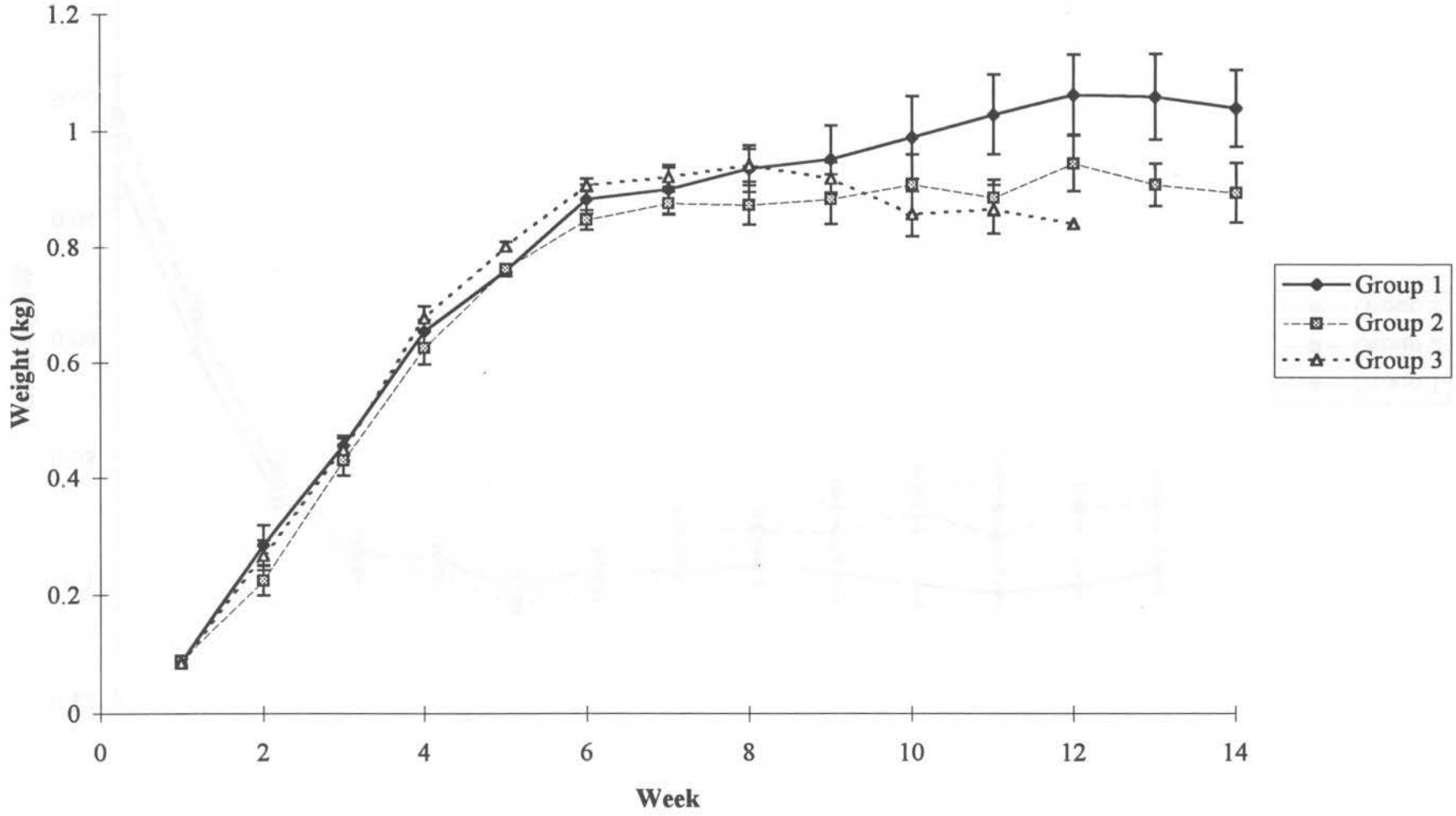


Figure 6.12. Mean weight for each of the groups of experimentally dosed birds. Vertical bars represent standard error.

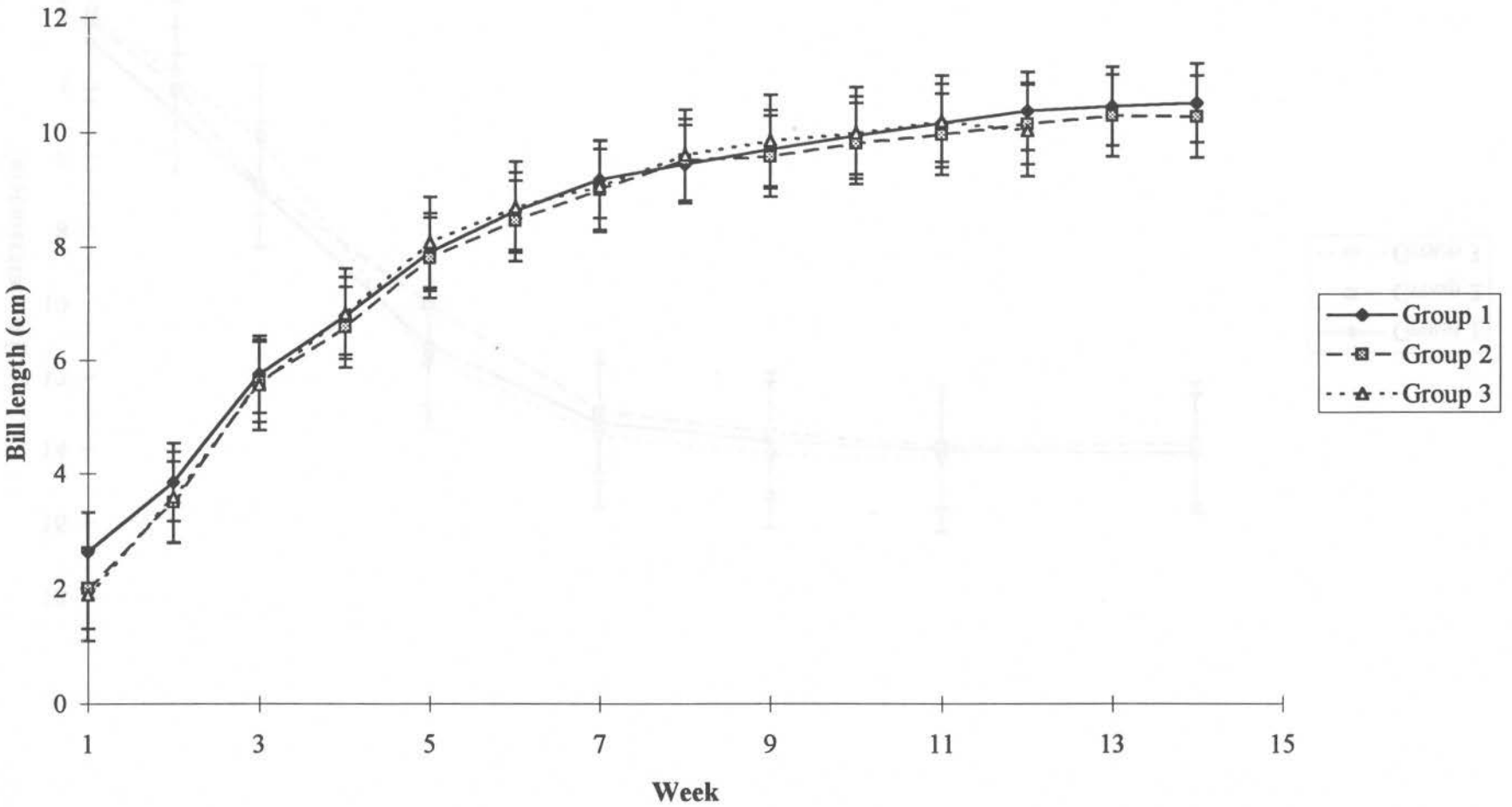


Figure 6.13. Mean bill length (culmen) for each of the groups of experimentally dosed birds. Vertical bars represent standard error.

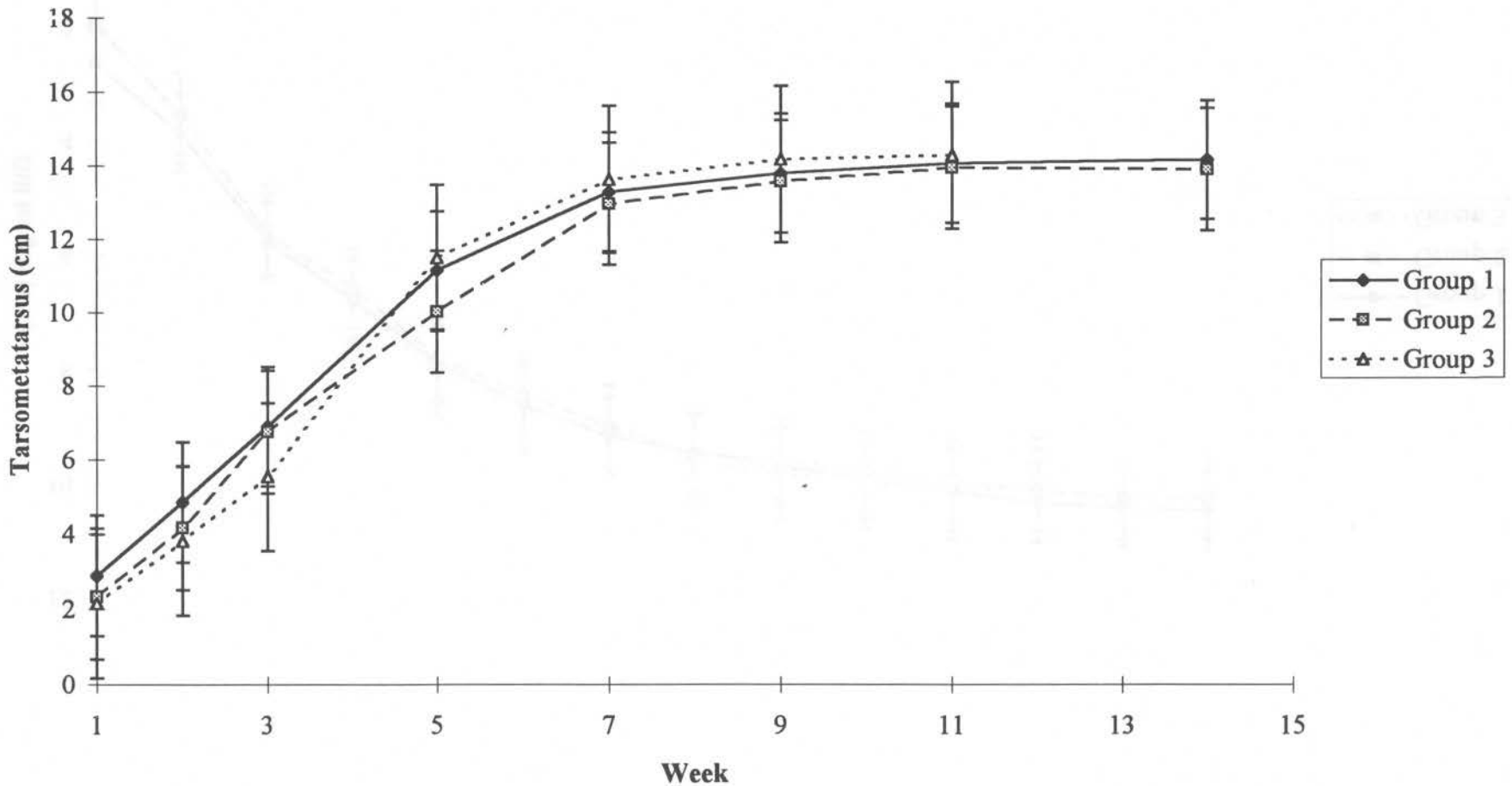


Figure 6.14. Mean tarsometatarsus length for each of the groups of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

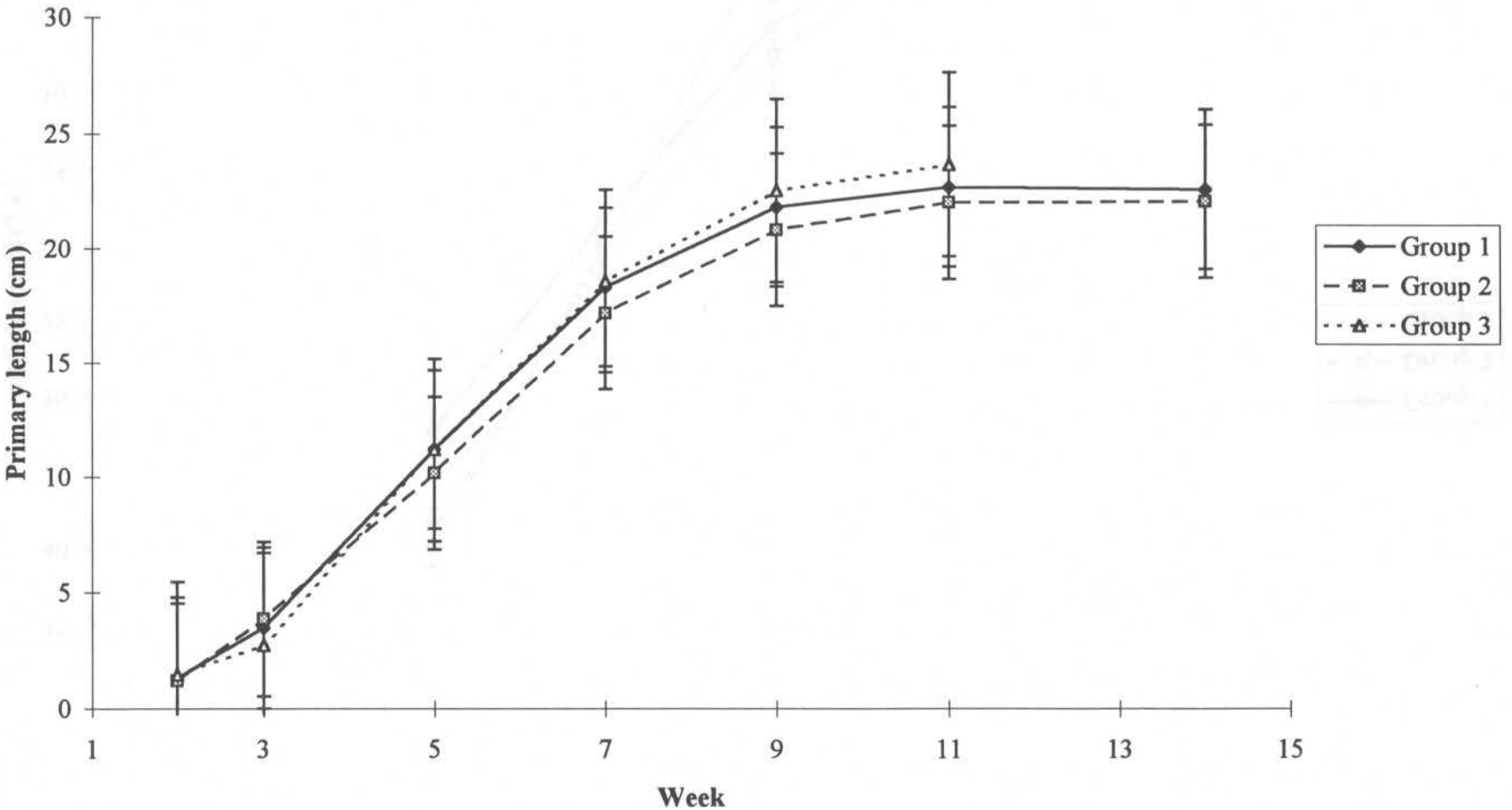


Figure 6.15. Mean primary feather (last) length for each of the groups of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

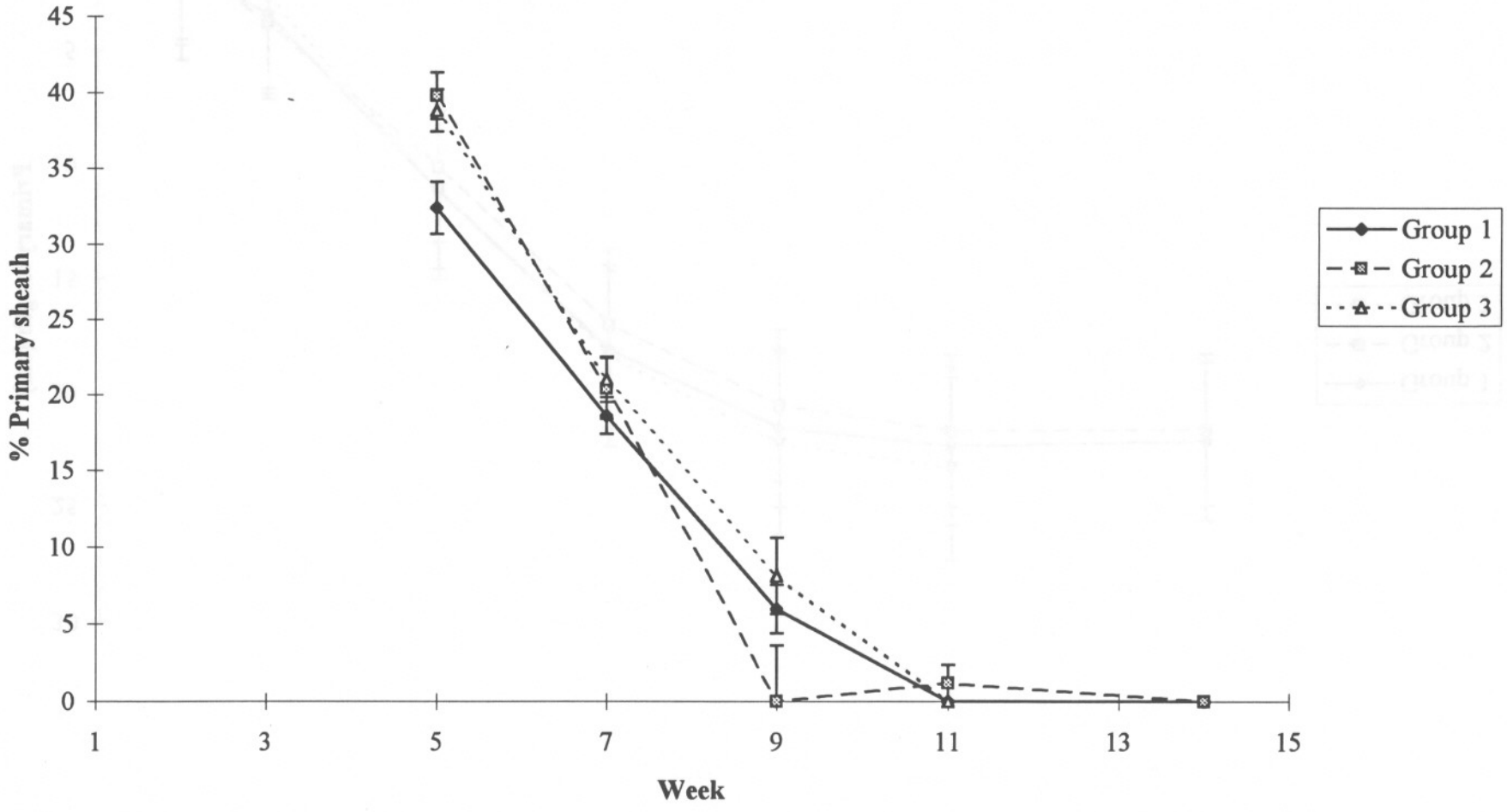


Figure 6.16. Mean primary feather sheath length (expressed as a percent of primary length) for each of the groups of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

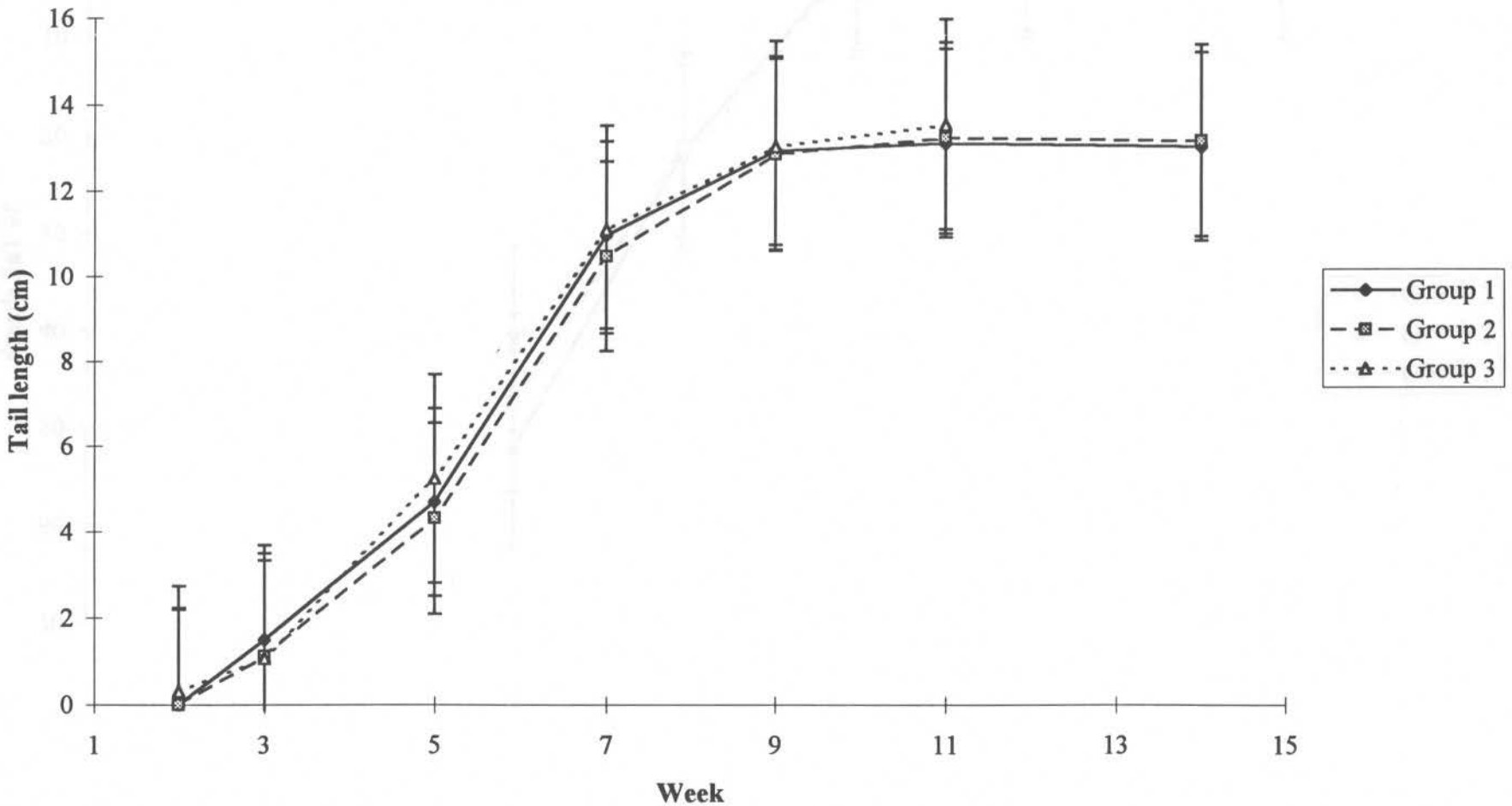


Figure 6.17. Mean tail feather (middle) length for each one of the groups of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

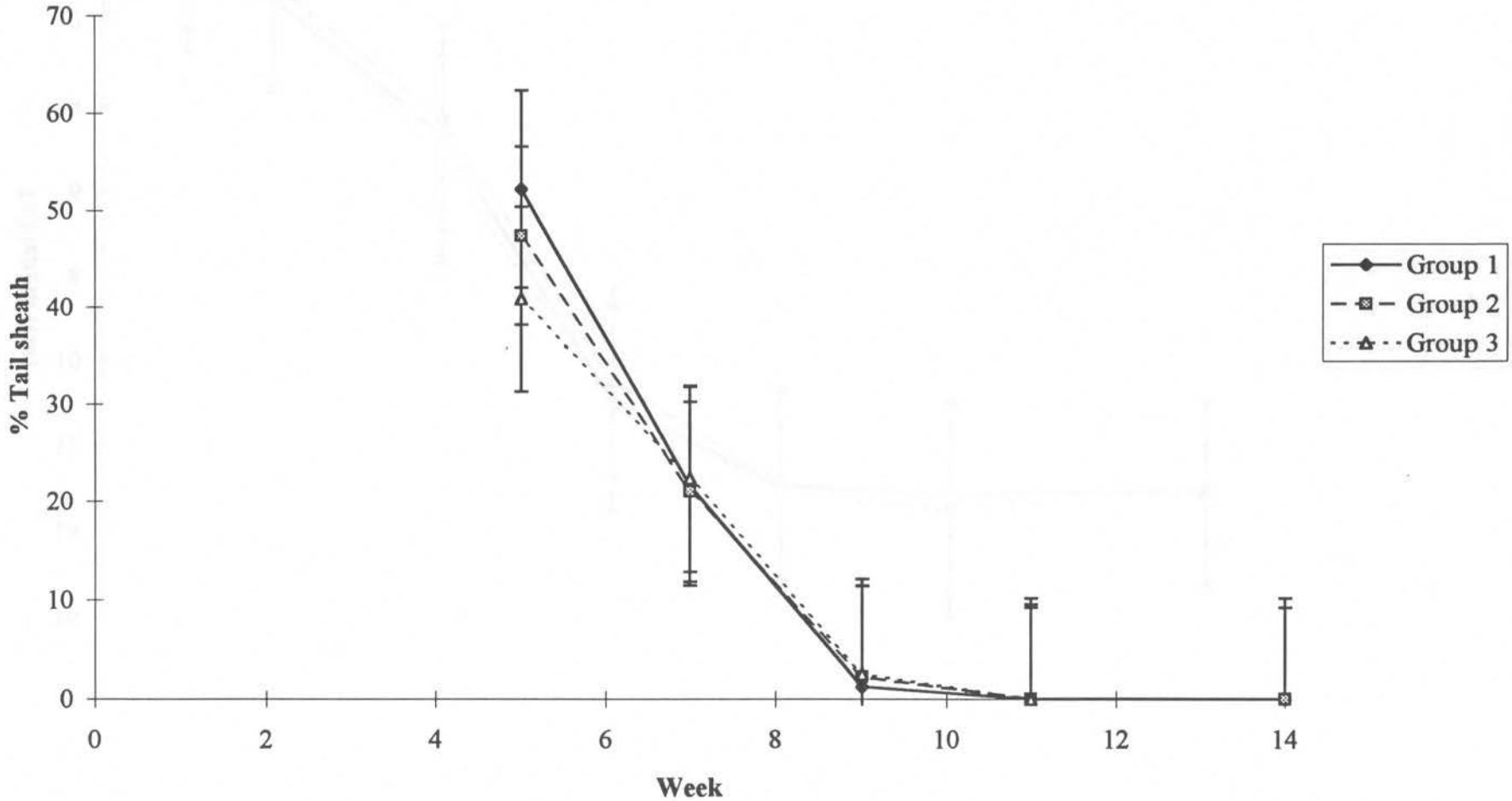


Figure 6.18. Mean tail sheath length (expressed as a percent of tail length) for each of the groups of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

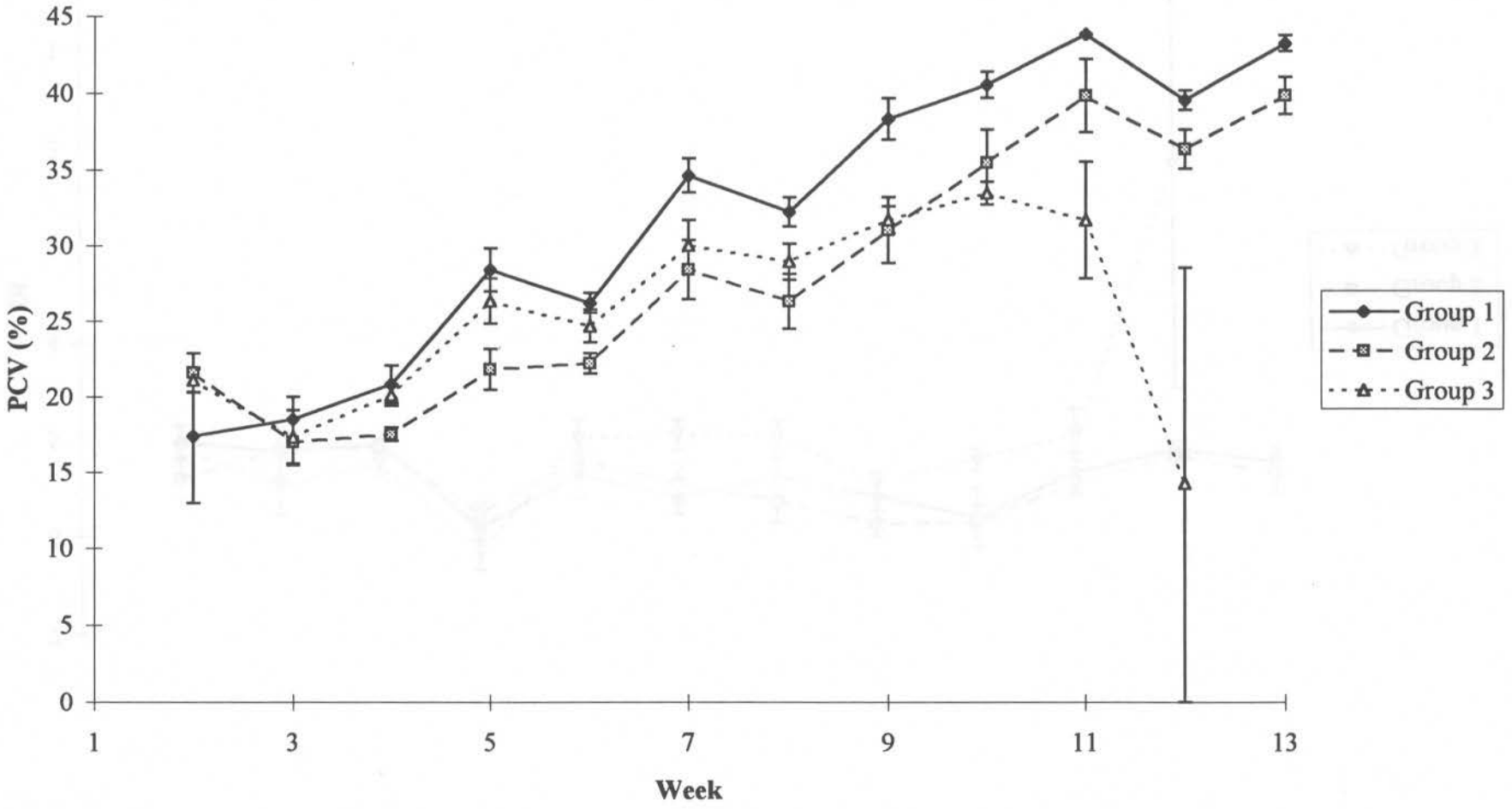


Figure 6.19. Mean packed cell volume (PCV) for each of the groups of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

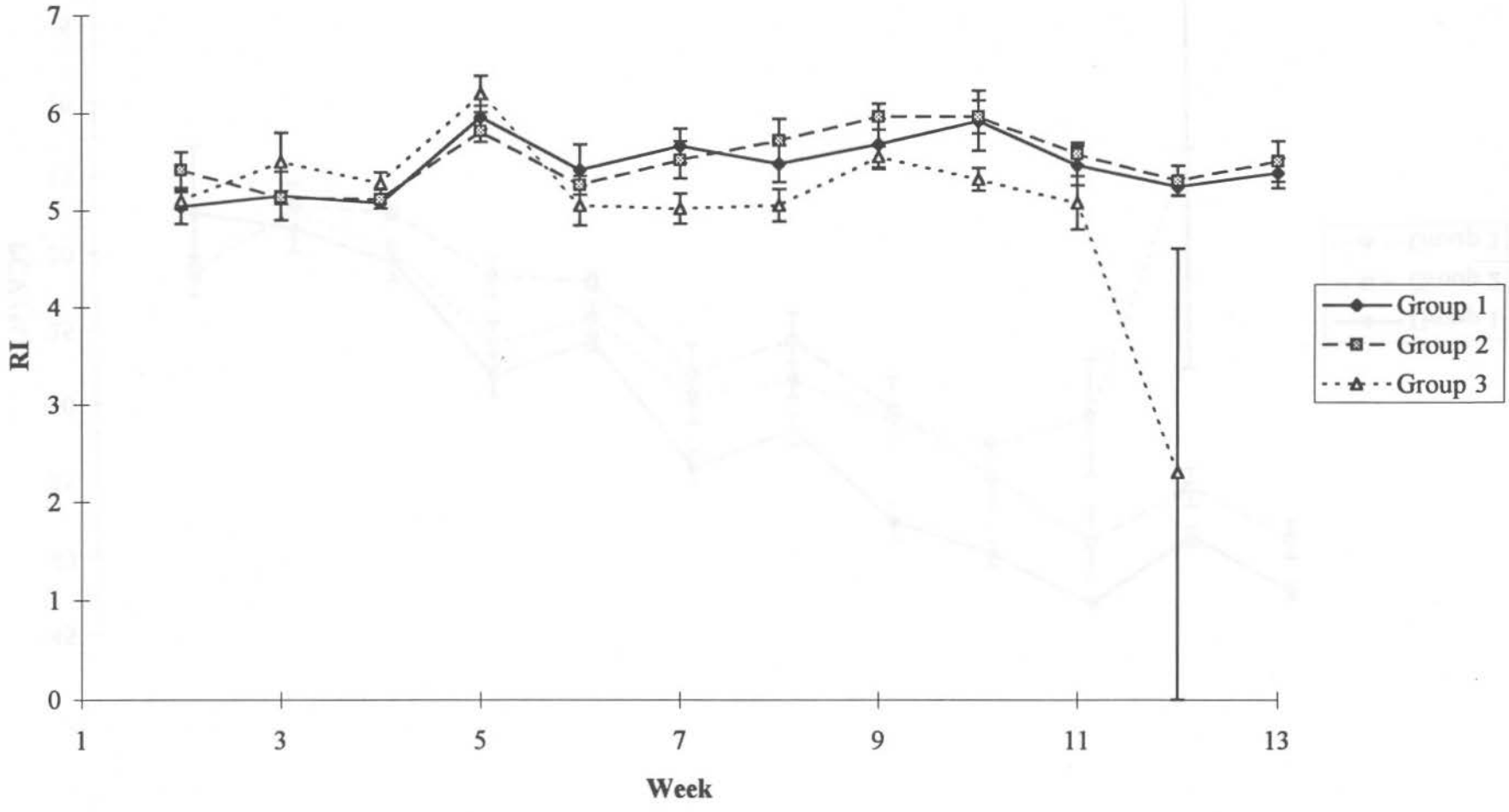


Figure 6.20. Mean refractive index (RI) for the three groups of great egrets experimentally dosed with methyl mercury. Vertical bars represent standard error.

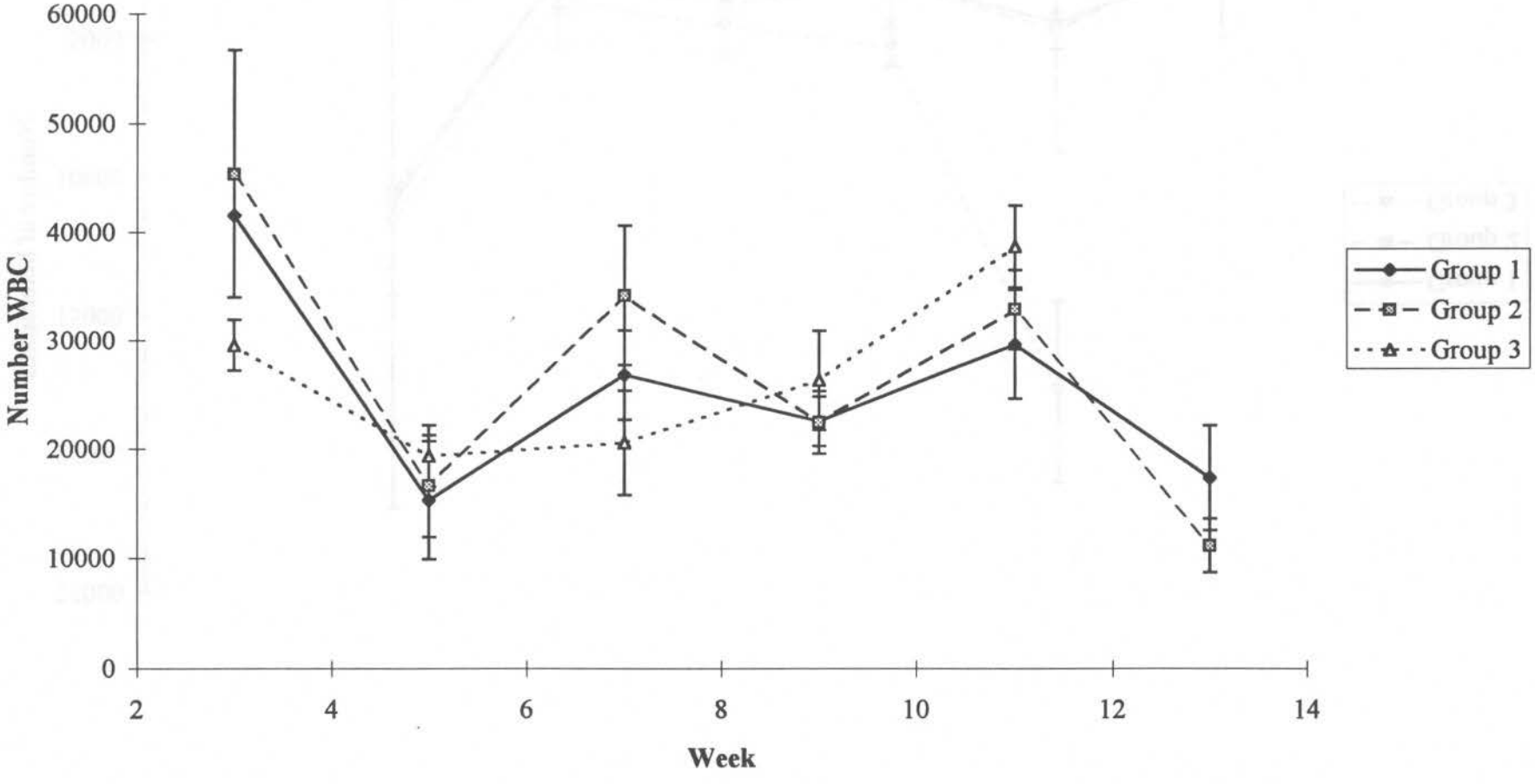


Figure 6.21. Mean number of white blood cells/mm^3 for each of the groups of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

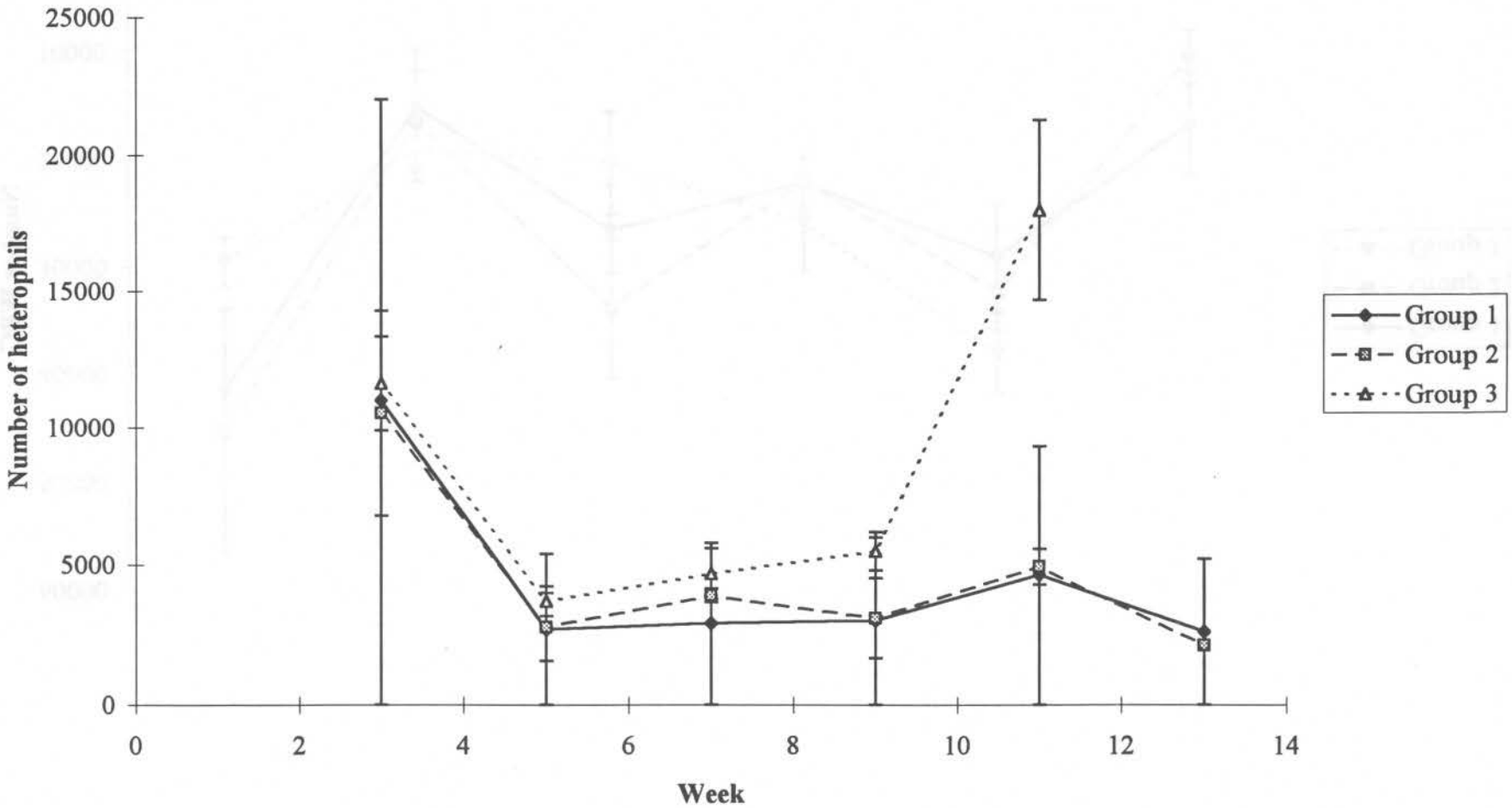


Figure 6.22. Mean number of heterophils/mm^3 for each of the groups of great egrets experimentally dosed with methylmecury. Vertical bars represent standard error.

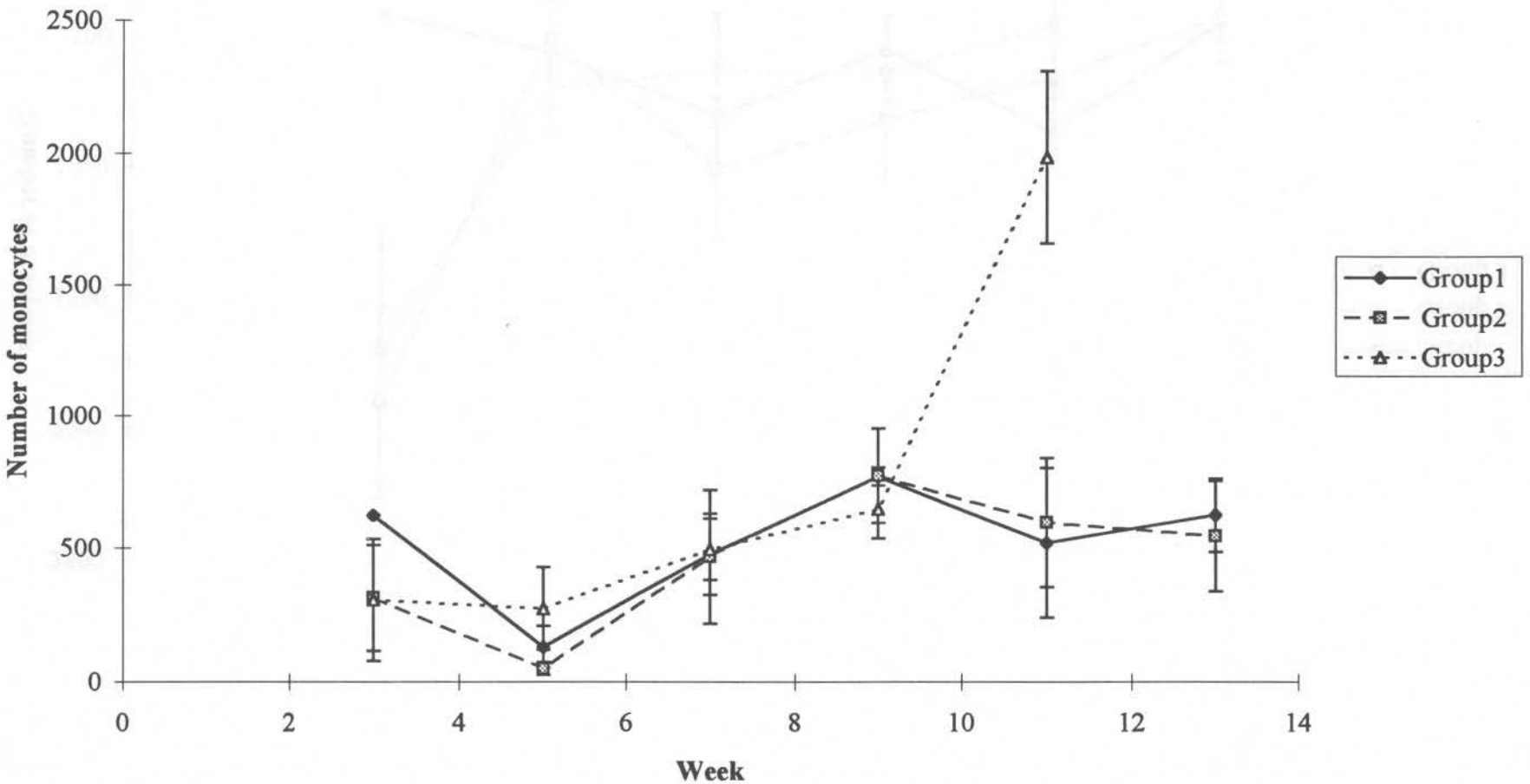


Figure 6.23. Mean number of monocytes/mm^3 for each of the groups of great egrets experimentally dosed with methyl mercury. Vertical bars represent standard error.

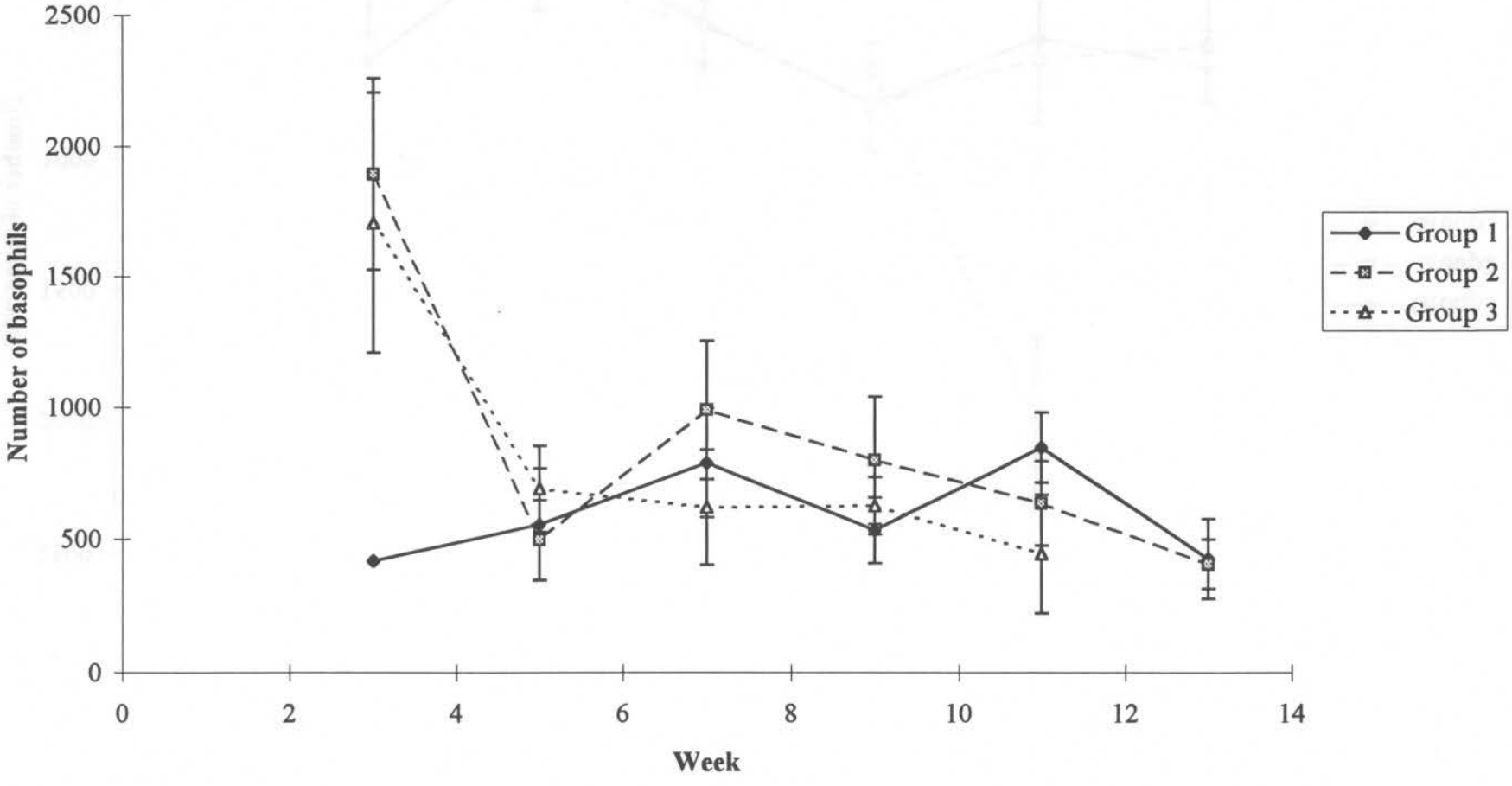


Figure 6.24. Mean number of basophils/mm^3 for each of the groups of great egrets experimentally dosed with methyl mercury. Vertical bars represent standard error.

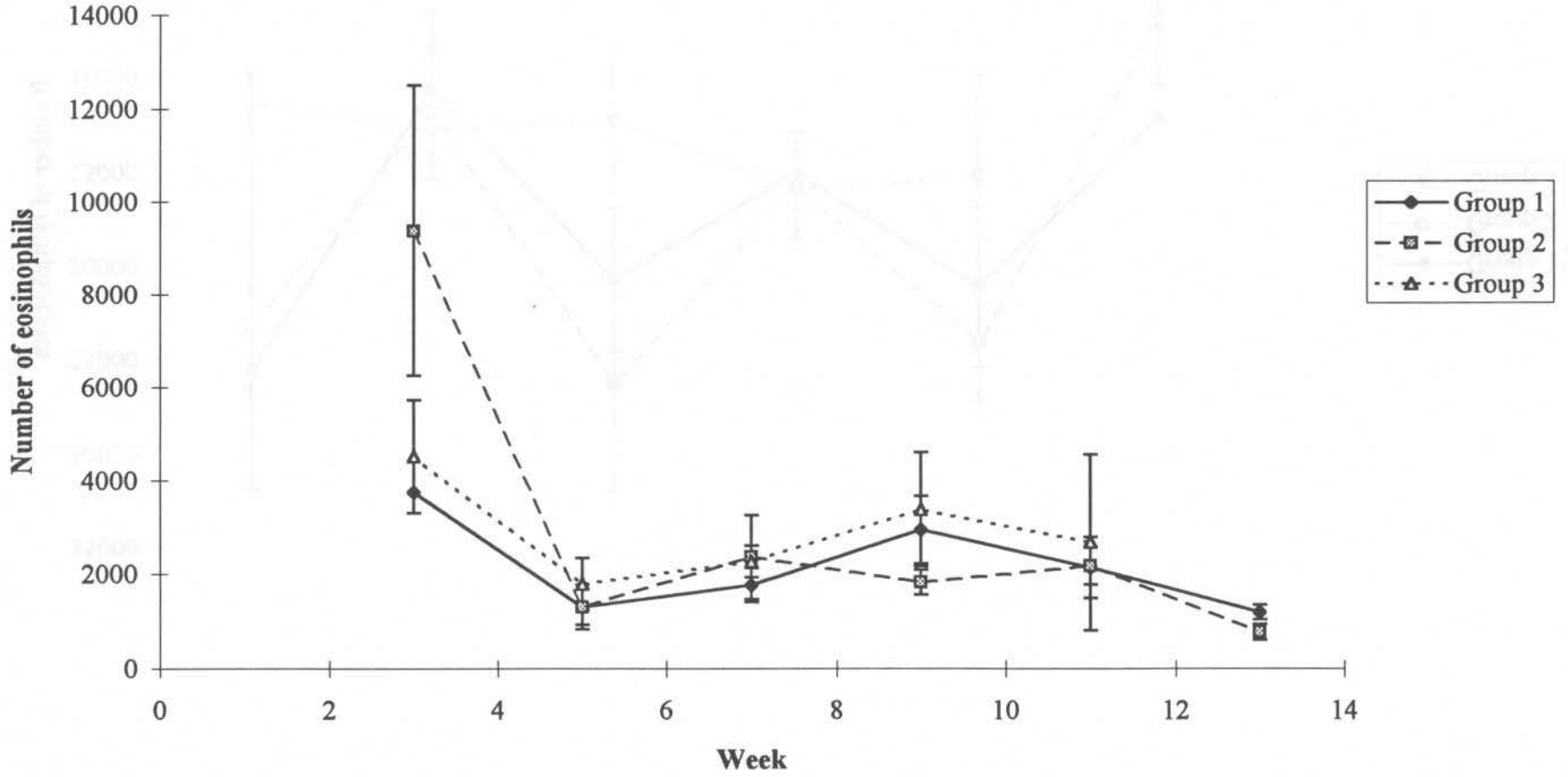


Figure 6.25. Mean number of eosinophils/mm^3 for each of the groups of great egrets experimnetally dosed with methylmercury. Vertical bars represent standard error.

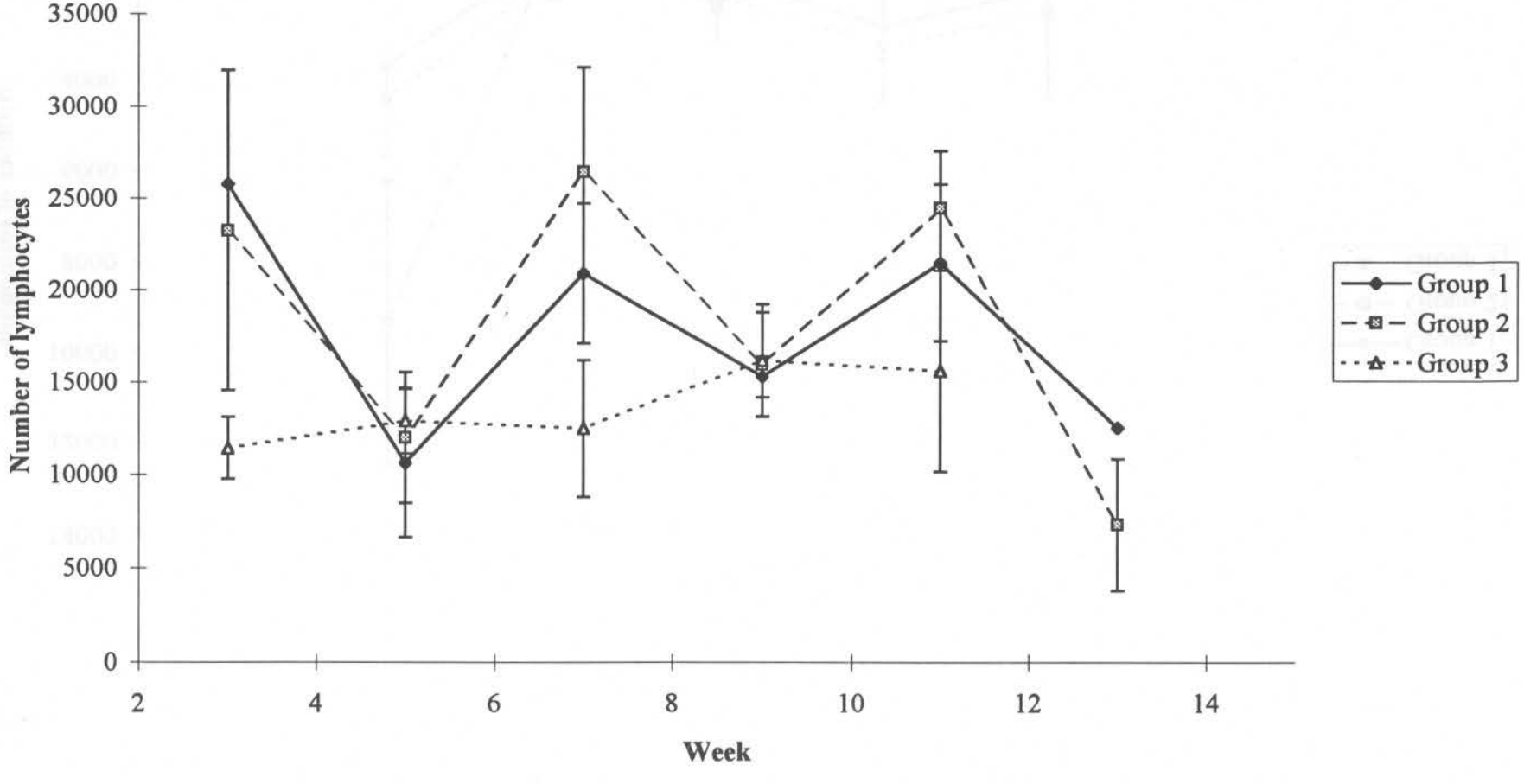


Figure 6.26. Mean number of lymphocytes/mm^3 for each of the groups of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

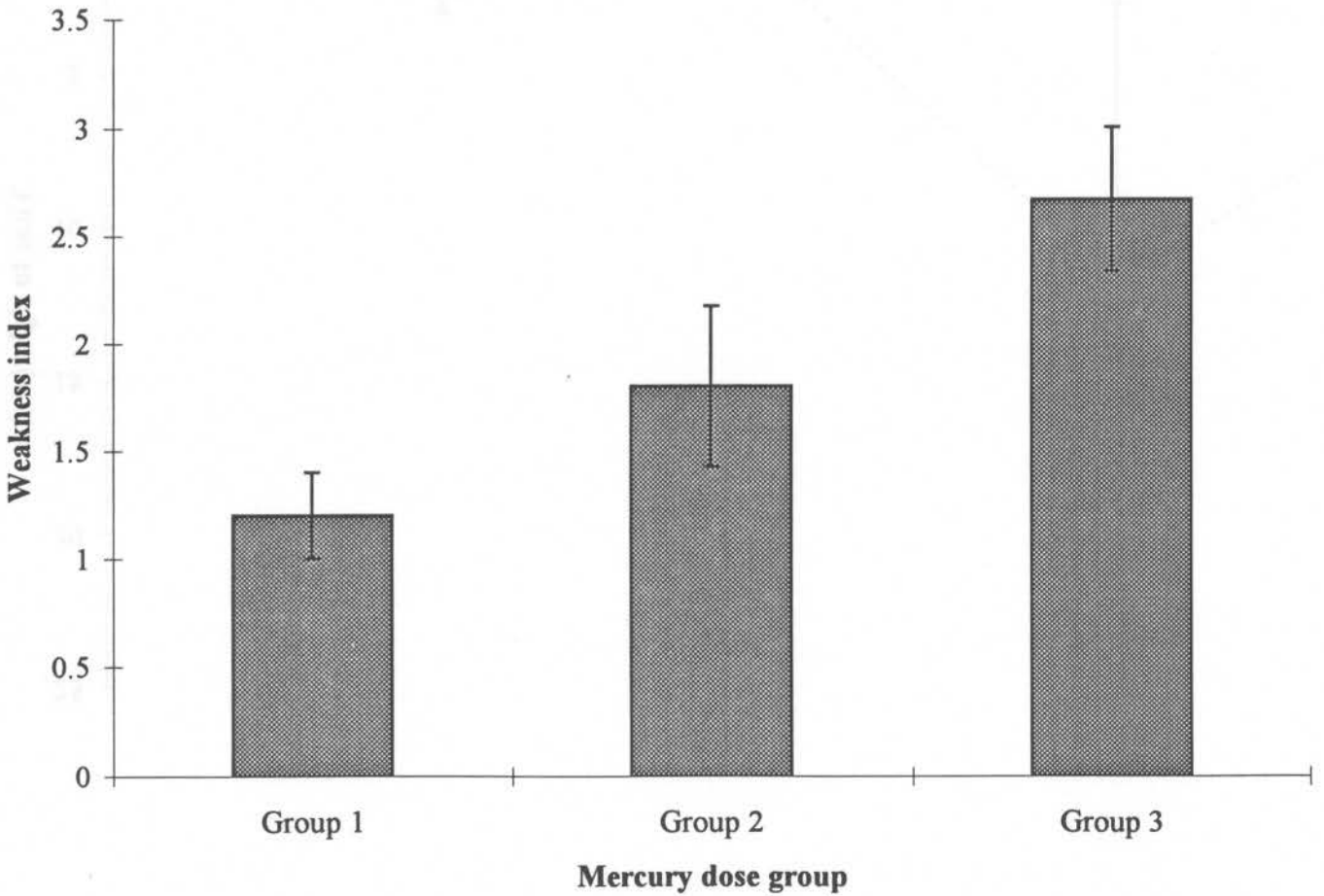


Figure 6.27. Mean weakness index (increasing number=increasing weakness) for each group of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

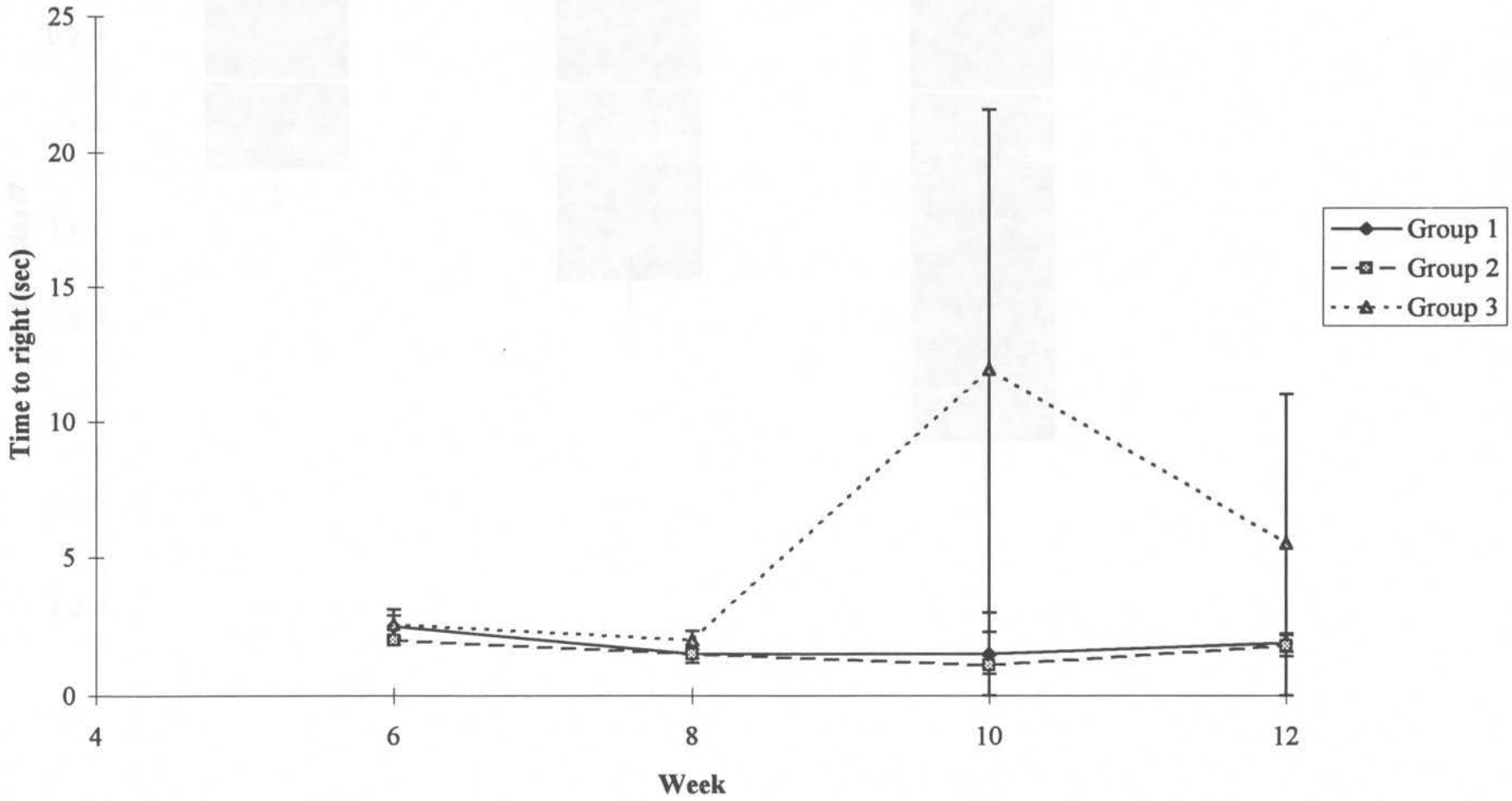


Figure 6.28. Mean time to return to standing position when placed on back for each group of great egrets experimentally dosed with methyl mercury. Vertical bars represent standard error.

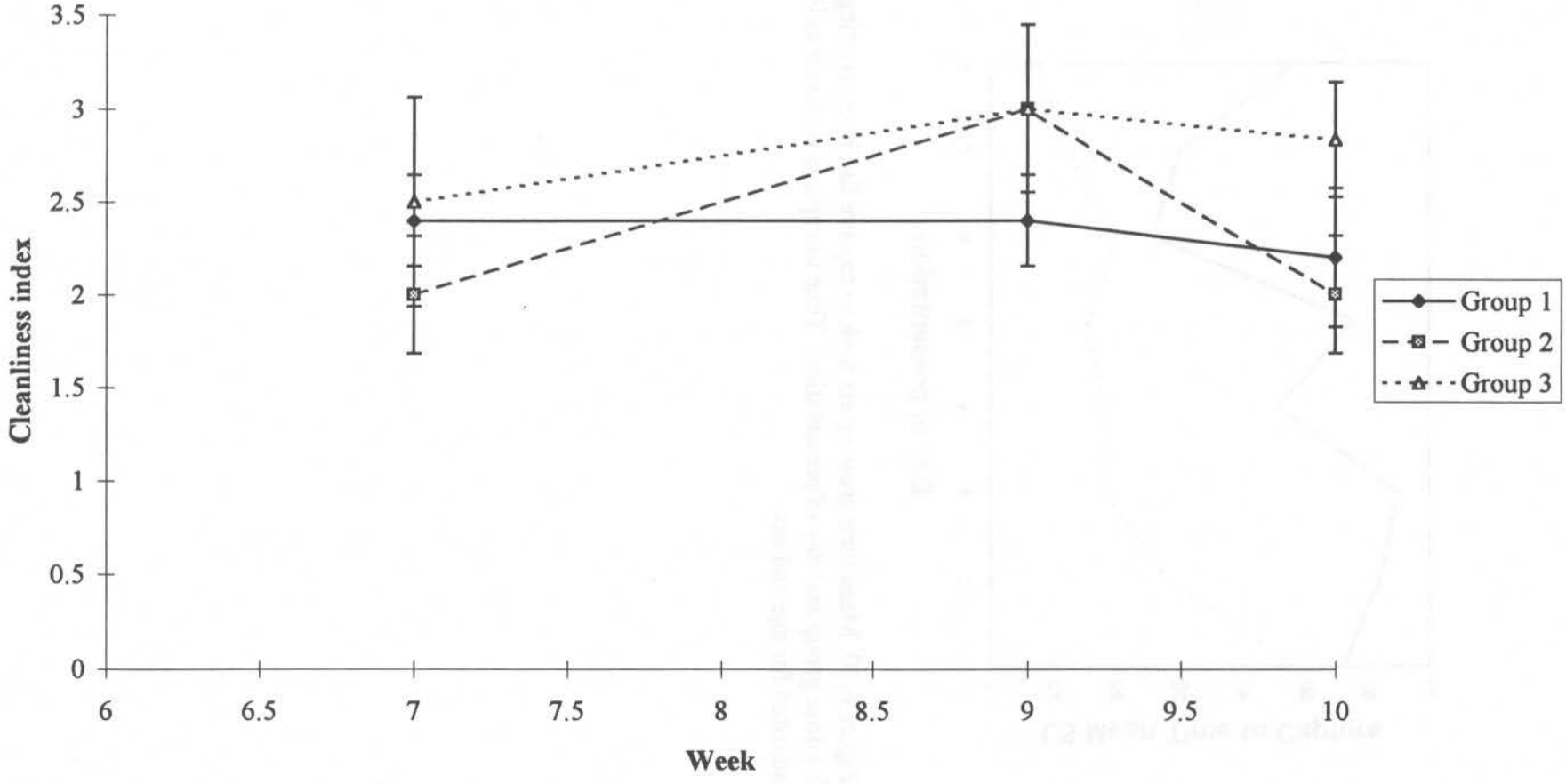


Figure 6.29. Mean cleanliness index (increasing number=increased staining of feathers) for each group of great egrets experimentally dosed with methylmercury. Vertical bars represent standard error.

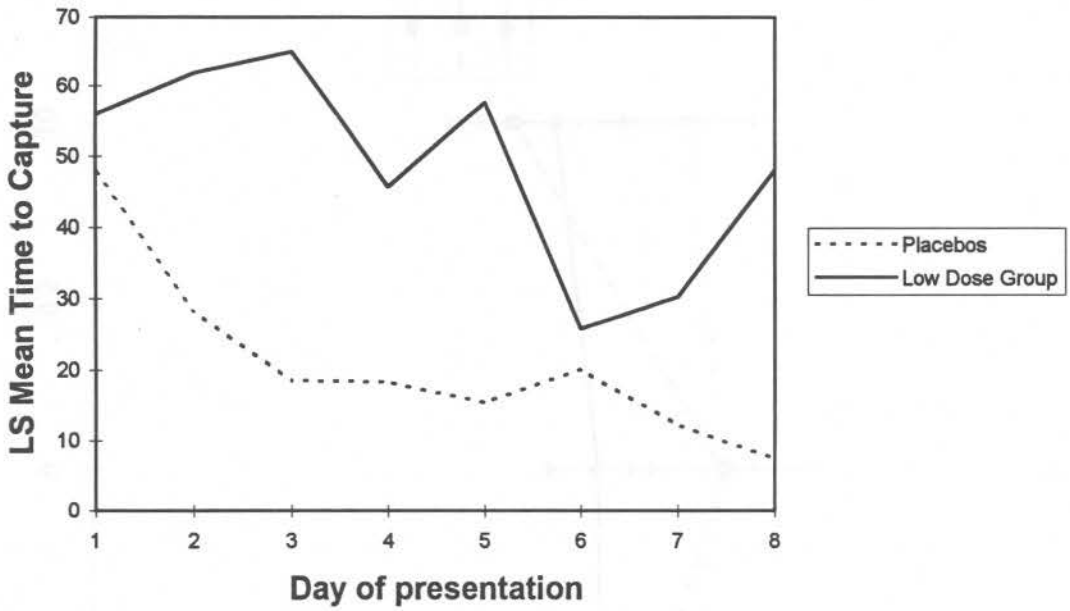


Figure 6.30. Mean time great egrets took to capture fish in contrasting pools, shown by dose group and day of presentation. Time to capture is shown as least-square means, adjusted for age and sex.

CHAPTER VII. EXPOSURE OF GREAT EGRETS TO MERCURY THROUGH DIET IN THE EVERGLADES ECOSYSTEM

INTRODUCTION

Numerous studies have demonstrated contamination and bioaccumulation of mercury in tissues of wild birds, particularly carnivores and piscivores (see Chapter 1). In contrast, relatively little study has been devoted to how those levels are produced - that is, the functional response of mercury in tissues to mercury concentration in diet. This is particularly true of piscivorous birds. Hoffman and Curnow (1979) reported mercury concentrations in both adult birds and prey of black-crowned night herons (Nycticorax nycticorax). Similarly, Gariboldi et al. (in prep.) reported mercury content in the diet of nestling wood storks. Goutner and Furness (1997) measured mercury in feathers and prey of little egrets (Egretta garzetta) in Greece. All three studies indicated considerably higher mercury content in freshwater than in saltwater prey animals, and concluded that large, predatory fish from freshwater areas constituted a significant source of mercury exposure to young birds. Although each study indicated that significant exposure of mercury occurred at the diets measured, there was little attempt to link different dietary levels with specific tissue levels.

The work presented so far has illustrated that great egret chicks in the Everglades have significant mercury exposure, as expressed in feather and liver mercury values, and that these concentrations may have effects on health and survival of young birds. The purpose of this chapter is to document the diet of great egret nestlings in the Everglades, to report mercury concentrations in prey animals taken as regurgitant from nestlings, and to estimate mercury exposure to nestlings by using mercury concentrations in the diet, and food consumption measurements (from Chapter V).

METHODS

Food habits of great egrets: Many ciconiiform birds will regurgitate entire boluses of food material upon close approach or handling by humans. We collected regurgitated food samples opportunistically from nestling and branchling great egrets during our visits to colonies. Some samples were collected during regular visits for the collection of feathers, blood, and other information, and some were from visits that were specifically designed for collecting regurgitant at colonies we did not visit regularly. These samples were collected only from chicks that regurgitated spontaneously as we approached, or which regurgitated while we were handling them for other reasons. Marked regurgitant samples were stored individually in sealed plastic bags and frozen for later analysis. Any regurgitation or series of regurgitations from the same bird on the same date were defined as a single "bolus".

Boluses were analyzed individually at the end of the nesting season. All intact fishes were dissected and examined for presence of the nematode parasite *Eustrongylides ignotus* and other parasites. For all samples, individual prey items were identified, weighed to the nearest 0.1 gm and measured to the nearest mm (total length). Fishes that were not intact (broken or partly digested) were identified to species, and their mass included as the total for

that species. Unidentified fishes were almost always parts, and their masses were combined for an "unidentified fish" category. For crayfish, shrimps and insects for which body parts usually break off rapidly after ingestion, we weighed the total of all parts from all individuals together. We measured carapace length of crayfishes and shrimps where possible.

Mercury concentrations in fish

We selected a total of 52 fish from regurgitated boluses of great egret chicks during 1995 for later analysis of whole-body fish mercury concentrations. The species were selected based on relative importance in the diet (e.g. we chose to analyze relatively large numbers of centrarchid fishes than any other group, and relatively few smaller fishes such as mollies (*Poecilia latipinna*), or killifishes (*Fundulus* spp.). In addition, we did not analyze any mosquitofish (*Gambusia holbrooki*), since this species has been the subject of extremely intensive and extensive sampling throughout the Everglades by researchers from the U.S. EPA and Florida International University. Specimens were chosen for freshness and completeness, and to represent the size range of individuals most frequently taken by the birds. The fish were sealed individually in plastic bags, and sent frozen to the FDEP analytical chemistry lab for mercury analysis. When expressing average mercury values, we averaged all sunfishes (*Lepomis* sp.) to get an unidentified "sunfish mean", and averaged the mean concentrations for all species to estimate a value for "unidentified fishes."

RESULTS AND DISCUSSION

Food habits of great egret nestlings

In 1993, we collected 21 regurgitated boluses from great egret nestlings at Tamiami East, Frog City South, Frog City North, and Hidden colonies during April and May. The contents of these boluses are summarized in Figure 7.1, and Appendix 1.

Great egret boluses in 1993 were composed primarily of sunfishes and largemouth bass (Centrarchidae, 58% of biomass, occurring in over 82% of samples). Second and third in importance, both in biomass and frequency, were cichlids (Cichlidae), and pike killifish (*Bellenesox belizanus*) respectively. These exotic species together constituted 32% of the biomass, and occurred in over 57% of boluses. Although smaller native fishes such as flagfish (*Jordanella floridae*) and several species of killifishes (primarily *Fundulus chrysotus*) were found in approximately 20% of the boluses, they represented a very small proportion of the biomass consumed.

In 1994, we collected 24 regurgitations from great egret nestlings Alley North (n = 2), Deer Island (n = 3), Frog City South (n = 2), Hidden/L-28 (n = 7), JW1 (n = 6), L-67 (n = 2) and Mud Canal (n = 2) colonies (Figure 7.1, Appendix 1). All of the regurgitations examined had some species of fish, 7 (29%) also contained insect parts (primarily dragonfly larvae or adults), 7 (29%) contained crustacean parts (crayfish or grass shrimp), 4 (17%) contained plant material and one sample included a single frog.

Ten different species of fresh water fishes were identified (Appendix 1). Warmouth (*Lepomis gulosus*) was the most common prey item in 1994, and was found in 42% of the samples. Golden topminnows (*Fundulus chrysotus*) were found in 17% of samples, and mosquitofish (*Gambusia holbrooki*) and least killifish (*Heterandria formosa*) were found in

12.5 and 8.3% of the samples, respectively. Only one sample contained an exotic fish species, the pike killifish. This prevalence of exotic species (4.2%) is quite low by comparison with the 57% found in 1993. Since sample size, water conditions and foraging locations of great egrets were similar in both years, it is unclear why the prevalence of exotic species was so different. As the winter of 1993/4 was quite warm by comparison with the long-term record, and showed no severe or extended freezes, it is unlikely that the exotic fishes became less abundant between the two breeding seasons as a result of their reduced cold tolerance relative to native species (Goodkin 1993).

In 1995, we collected a total of 51 regurgitated boluses from great egret nestlings, predominantly at Hidden (n = 29) and Tamiami West (n = 13) colonies, with the remainder split about evenly among Alley North, JW1, Mud Canal, and L-67 colonies (Appendix 1, Figure 7.1). The prevalence of unknown fish is high in this series because a large number of the fish specimens could not be identified to species. As in 1993 and 1994, centrarchids were the most abundant prey item, occurring in over 67% of samples. Other prey items recorded frequently were mollies (24% of samples), killifishes (29% of samples), and pike killifish (14% of samples). Exotic fishes (cichlids and pike killifish) were found in 22% of samples.

We collected a total of 29 boluses from great egret chicks at Hidden and JW1 colonies during 1996 (Appendix 1, Figure 7.1). Great egrets ate large fishes, and particularly sunfishes. A large proportion of the boluses (50% of biomass) were not identifiable to species, but consisted of large-bodied fishes. Sunfishes dominated the identifiable portion of the diet, and all of those identifiable to species were spotted sunfishes (*Lepomis punctatus*). The diet was entirely fish at both colonies. By comparison with previous years, great egrets in 1996 ate a greater proportion of spotted sunfishes, and ate far fewer exotic fishes.

Thus, although the biomass of great egret nestling diets fluctuates with location and year within the Everglades, the composition can be typified as being almost completely fish. The larger species and individuals are certainly favored, with heavy concentration on the sunfishes, and similarly-shaped and sized cichlids. Over the four years, we found large fluctuations in the proportion of the diet occupied by exotic fishes, ranging from 32% of the diet in 1993 to 0 in 1996. During 1996, the diet also became much less diverse, and was almost completely composed of a single species, the spotted sunfish. Since this species is usually found only in open marsh, this may indicate that great egrets were hunting much more in the marsh than in other years. The 1996 season was at least the third and perhaps the fourth year in a row of what could be classified as high water in the Everglades, meaning both deep depths and very long hydroperiod, compared to previous years (Frederick et al. 1996). How this dramatic hydroperiod factors into the change in diet during 1996 is unknown.

Mercury concentrations in regurgitated fish

We found mercury concentrations in whole fish recovered from regurgitated great egret boluses to range between 0.035 and 1.4 mg/kg ww (Table 7.1, Figure 7.2). Among the fish species most frequently consumed by great egrets, we found average concentrations ranging from 0.05 ppm ww (sailfin mollies) to 0.79 ppm ww (largemouth bass). Using species-specific wet mass - dry mass conversions (Kushlan et al. 1986), we estimated 0.22 - 3.55 ppm dw among species. These values are generally within the ranges reported from

other studies in the Everglades (Ware et al. 1990, W. Loftus pers. comm.), and are high in the context of mercury measurements of fish in similar sizes and trophic niches at other locations (Hoffman and Curnow 1979). Combining all species, we found a significant relationship between mass of fish and whole-body mercury concentration (ANOVA, $p = 0.0001$, $df = 43$, Figure 7.3). We also found a significant relationship between mass and mercury concentration when looking only at the sunfishes (ANOVA, $p = 0.00019$, $df = 25$, Figure 7.4). Although there was no suggestion of a relationship between whole body mercury concentration and mass in the cichlid fishes (Figure 7.5), it should be recognized that there were only 8 individual fishes analyzed for mercury. These findings demonstrate that within the diet of great egret nestlings, larger fish tend to have higher mercury concentrations.

Mercury exposure through diet of great egret nestlings

We combined the information on fish species composition in diet, with the species-specific concentrations of mercury, to estimate concentrations of mercury in the diet of nestling great egrets during the different years of study. For each fish species, the proportion in the diet was multiplied by either the average, or median mercury concentrations (both are expressed in Table 7.1). The mean pro-rated concentrations were then summed over all fish species to give an average mercury concentration in the diet, during each of the years of study. For this estimation, we used all fish species listed in Table 7.1, which accounted for between 85 and 95% of the diet between 1993 and 1996 (Appendix 1). We estimate that annual mean mercury concentrations varied between 0.33 and 0.43 mg/kg ww (mean across years = 0.412 mg/kg) in the diet of great egret nestlings over the course of the four year study, and that median mercury concentrations varied between 0.21 and 0.30 mg/kg (Table 7.2).

We then estimated daily and cumulative doses of mercury to great egret nestlings over the course of the 80-day nestling and pre-independence period. This was done using age-specific food intake data taken from 1) field measurements of control birds (Chapter V), 2) measurements from captive-reared birds (Chapter VI), and, for the very early nestling period, 3) measurements of age-dependent chick mass from Black et al. (1986). We assumed that chicks received rapidly decreasing amounts of food during the last ten days prior to fledging, that chicks weighed approximately 1,100 gm immediately prior to fledging, and that they were receiving the average annual value of 0.412 mg mercury/kg dietary fish for these calculations. The estimation of total mercury consumed includes the fact that great egret chicks in the Everglades begin life with an average of 0.40 mg/kg mercury ww as embryos (whole egg measurements, n = 76 eggs from the Water Conservation Areas of the Everglades, Dan Day pers. comm.).

Under these modeled conditions, daily mercury doses as a proportion of body mass are highest immediately following hatching, and then decline slowly over the nestling period, remaining largely in the range of 0.06 - 0.15 mg mercury/kg body mass (Figure 7.6). Over the course of the nestling period, we estimate that great egret chicks ingest a total of 5.02 mg hg, which, if completely retained in body tissues, would result in a concentration of 4.57 mg hg/kg body mass.

It should be noted that these estimates depend strongly on mercury measurements taken from a relatively small sample of fishes. The virtue of these estimates is that the fish were taken directly from great egret food samples, and no assumptions about availability to

the birds have to be made. Since the samples were taken from small and medium-sized young (to about 28 d), and since larger young might well take larger fish, we suggest that our estimates may be biased somewhat low for estimating actual mercury intake of prefledging great egrets in the Everglades. Similarly, these data were collected during three years when large fish were hypothesized to be relatively unavailable due to high water conditions (Chapter V), which would have biased our measurements towards smaller fishes of relatively low mercury concentration.

Using the modeling conditions described above, we have also estimated mercury intake at dietary mercury concentrations higher than what we measured directly in the diet, but which are, under the right conditions, quite possible in the Everglades (Table 7.3). The first is the 0.63 mg/kg level, suggested as the best estimate for actual dietary levels in Chapter V, which comes largely from fish concentrations provided by W. Loftus (pers. comm). The second is roughly three times that concentration (1.76 mg/kg) that we used in the field dosing experiment, and which could be achieved if great egrets fed only on larger, predatory fish, which is possible in a year in which much of the marsh surface is exposed through drying. Although this latter estimate is considered a current worst-case scenario, it should be noted that, in light of imperfect understanding of mercury bioaccumulation and methylation processes in the Everglades, it is possible that mercury levels could increase to the point that the worst case becomes the mean. We estimate that mercury ingested over the 80-day prefledging period would be 7.65 mg per bird at the 0.63 mg/kg intake rate, and 21.38 mg at the 1.76 mg/kg dose rate. Mercury ingested by the captive dosed birds by 80 days at the 0.1 mg/kg level averaged 6.6 mg/kg (dosing began at about 2 weeks of age).

Table 7.1 Mercury concentrations (mg/kg) in whole body samples of fish taken from regurgitated boluses of great egret nestlings during 1995, with average mass and length of each species.

Species	n	Mean mass (gm)	s.e. mass	Mean total length (cm)	s.e. length	Mean Mercury (wet weight)	s.e. Mercury	Mean Mercury (dry weight)	Median Mercury (wet weight)
Bellenesox belizanus	1	9.10	N/A	8.00	N/A	0.42	0.000	1.89	0.42
Cichlasoma bimaculatu	5	7.26	3.085	4.86	0.663	0.10	0.039	0.45	0.11
C. urophthalmus	4	27.95	11.880	8.43	1.285	0.14	0.053	0.65	0.10
Lepomis gulosus	9	26.96	8.879	8.38	1.292	0.49	0.146	2.21	0.18
Lepomis marginatum	3	N/A	N/A	N/A	N/A	0.09	0.003	0.41	0.09
Lepomis punctatus	19	12.27	3.078	6.31	0.488	0.33	0.048	1.47	0.33
Micropterus salmoides	4	43.90	35.748	11.80	3.787	0.79	0.341	3.55	0.53
Poecilia latipinna	2	1.05	0.050	3.60	0.100	0.05	0.001	0.22	0.05

Table 7.2. Estimated concentrations of mercury (mg/kg) in the diet of great egret nestlings, by year and prey animal species, as computed from species-specific prey mercury concentrations, and representation of each species in the diet, by biomass. Results are shown using both mean mercury and median mercury values.

Prey Species	1993		1994		1995		1996	
	mean	median	mean	median	mean	median	mean	median
Belenesox belizanus	0.063	0.063	0.013	0.013	0.008	0.008	0.000	0.000
Cichlasoma bimaculatum	0.001	0.001	0.000	0.000	0.005	0.005	0.000	0.000
Cichlasoma urophthalmus	0.018	0.013	0.000	0.000	0.018	0.012	0.000	0.000
Lepomis gulosus	0.114	0.042	0.388	0.142	0.047	0.017	0.000	0.000
Lepomis marginatum	0.005	0.005	0.000	0.000	0.002	0.002	0.000	0.000
Lepomis punctatus	0.004	0.004	0.001	0.001	0.108	0.108	0.103	0.103
Micropterus salmoides	0.018	0.012	0.000	0.000	0.103	0.069	0.000	0.000
Poecilia latipinna	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.000
unknown *Lepomis*	0.094	0.061	0.002	0.001	0.004	0.003	0.050	0.032
unknown fish	0.097	0.076	0.075	0.059	0.093	0.073	0.220	0.172
Total	**0.415**	**0.276**	**0.479**	**0.216**	**0.389**	**0.299**	**0.373**	**0.307**

Table 7.3. Estimation of total accumulation of mercury aquired through diet by great egret nestlings during the nestling and prefledging period in the Everglades.

Mercury content of food (mg/kg prey)	Total mercury ingested (mg)	Total mercury per kg body weight at independence (mg/kg)	Average daily dose (mg)
0.412	5.023	4.566	0.063
0.630	7.653	6.957	0.096
1.760	21.380	19.436	0.267

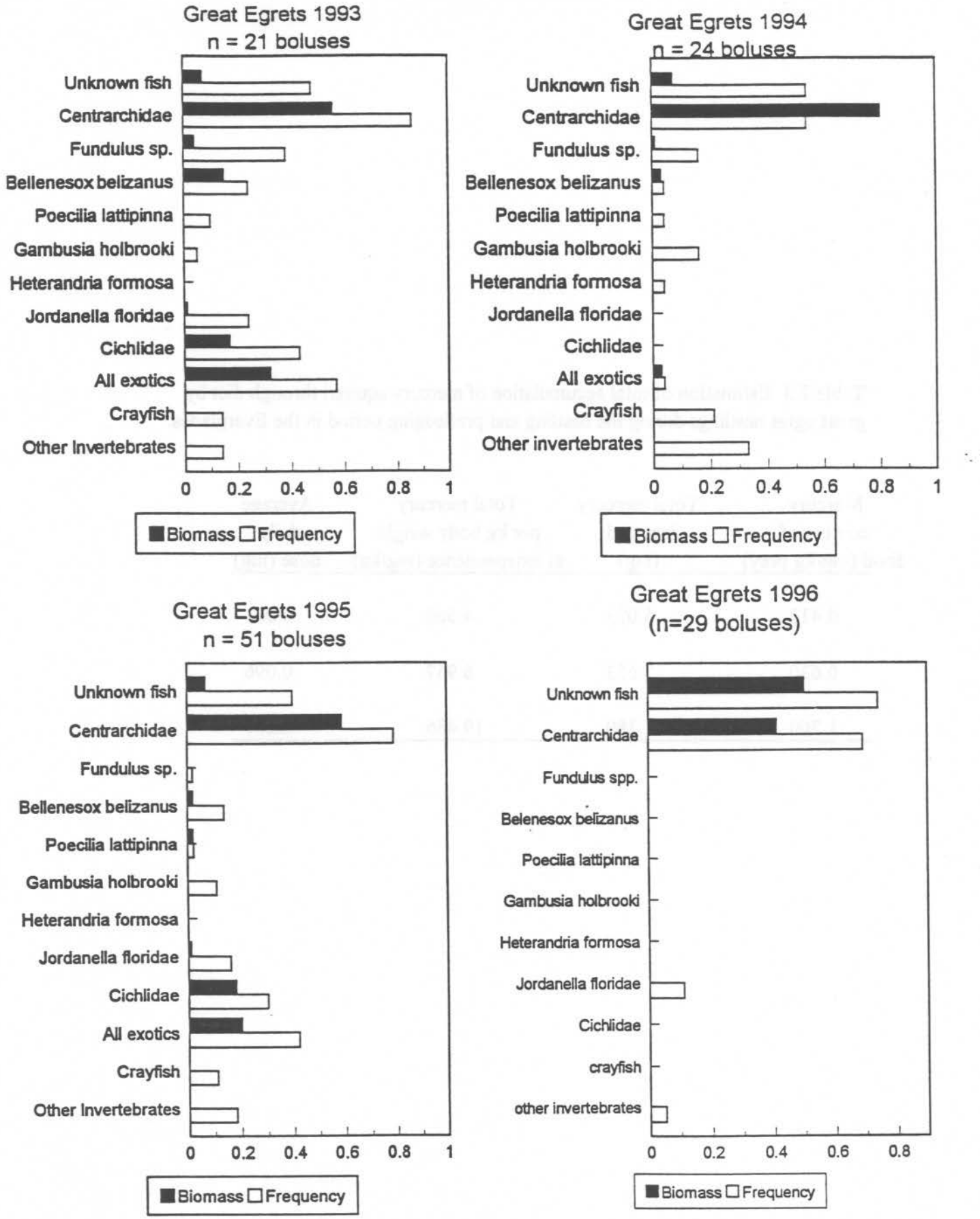


Figure 7.1. Proportional representation of dominant prey items in boluses regurgitated by great egret nestlings during four years in the freshwater Everglades, shown both as a proportion of the total biomass and frequency of occurrence.

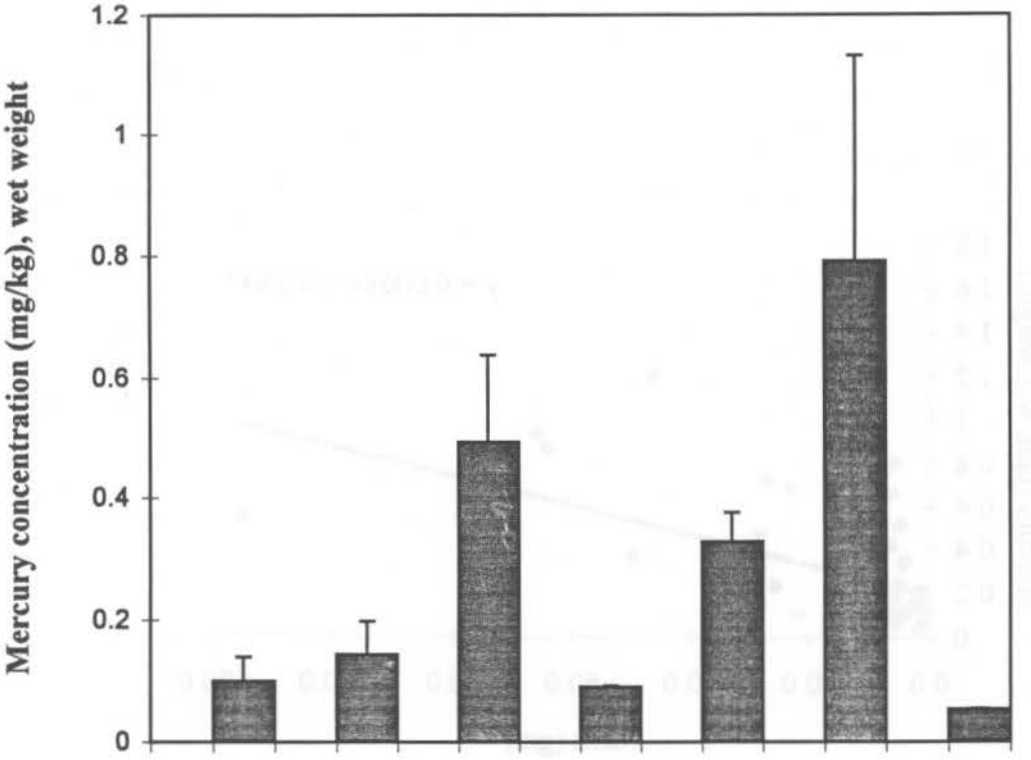


Figure 7.2. Mean whole-body mercury concentrations (ww) in fishes collected as regurgitant from great egret nestlings during 1995 in the freshwater Everglades, during 1995.

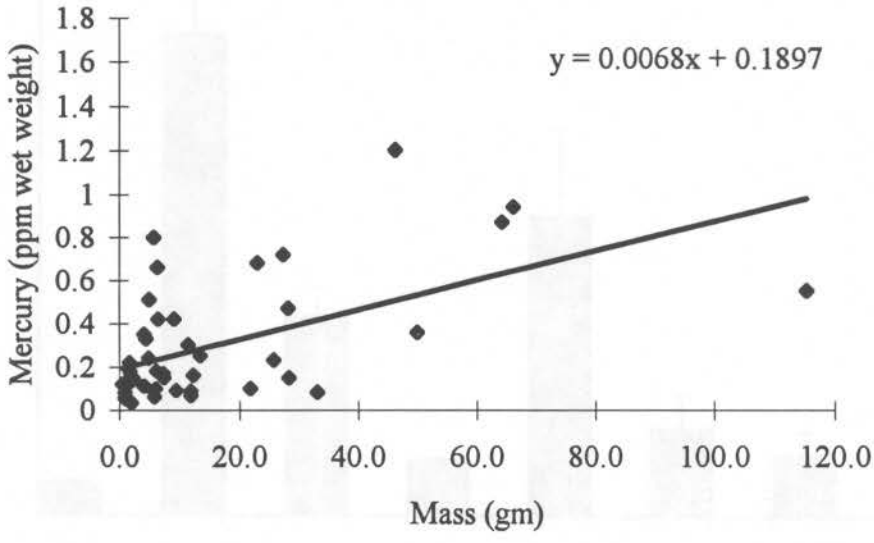


Figure 7.3. Graph of fish mass vs. whole body mercury concentrations, from all species of fishes collected in regurgitated boluses from great egret chicks in the Everglades, 1995.

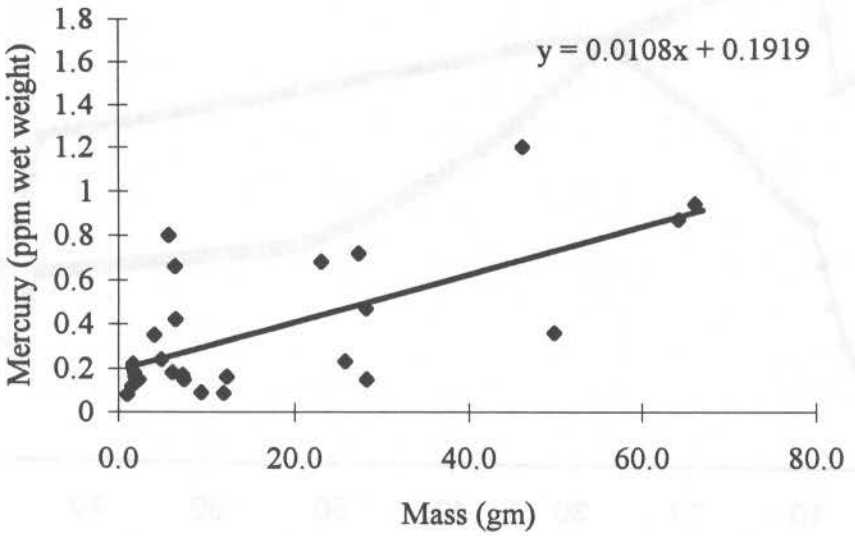


Figure 7.4. Graph of fish mass vs. whole body mercury concentrations, from sunfishes collected in regurgitated boluses from great egret chicks in the Everglades, 1995.

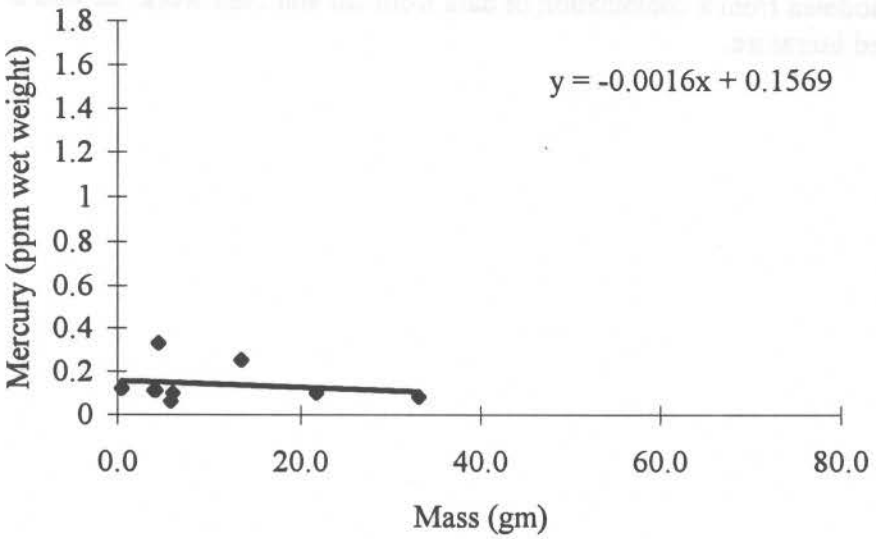


Figure 7.5. Graph of fish mass vs. whole body mercury concentrations, from cichlid fishes collected in regurgitated boluses from great egret chicks in the Everglades, 1995.

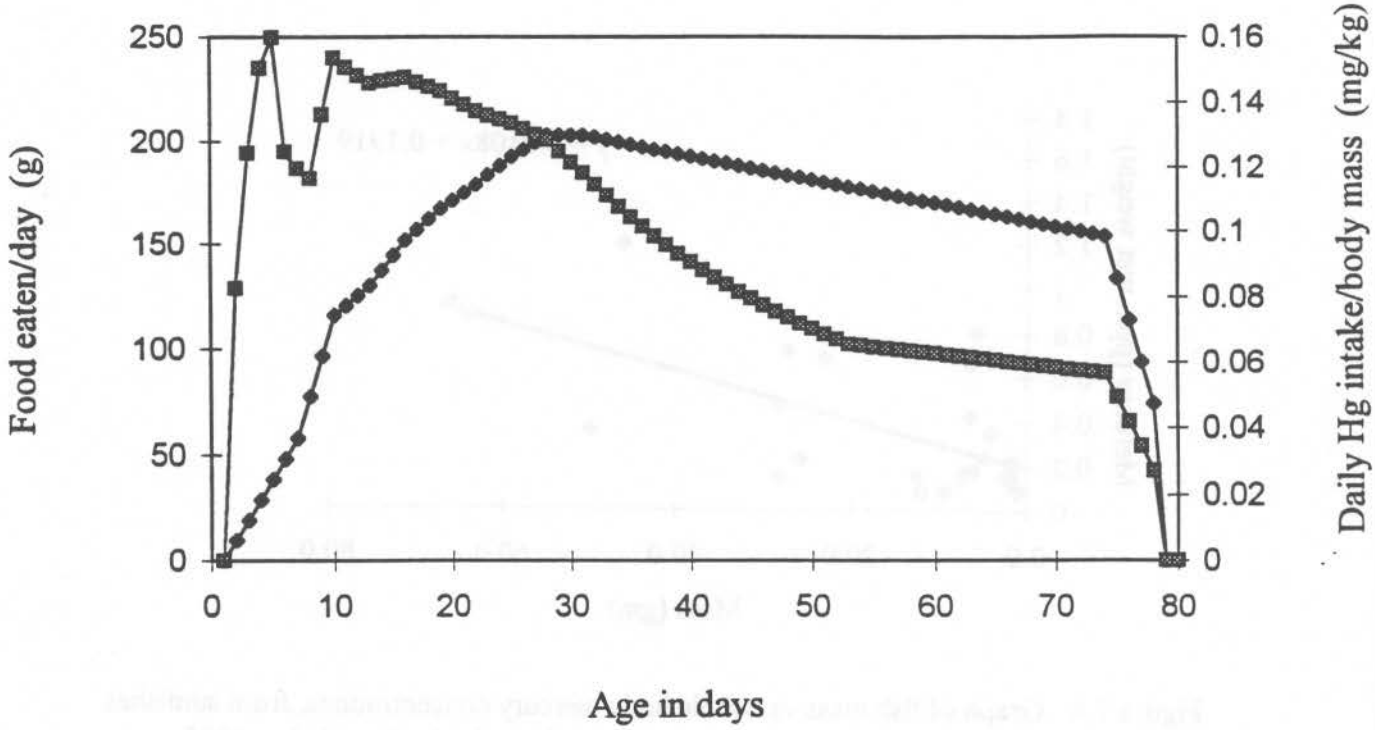


Figure 7.6. Graph of food consumed (diamonds, left hand axis), and daily mercury intake (squares, right hand axis) in great egrets during the nestling and prefledging period. Both parameters are modeled from a combination of data from lab and field work, as well as from the published literature.

CHAPTER VIII. SYNTHESIS OF RESULTS AND CONCLUSIONS

The purpose of this chapter is to bring together the diverse threads of the preceding chapters. This will be done by summarizing the results, and synthesizing them into as comprehensive a picture as possible of the current and future role of mercury in the ecology of wading birds in the Everglades.

We have shown first, that total mercury can be accurately and nondestructively sampled in great egrets through measurement of growing feather tissue (growing body feathers or powderdown). Mercury occurs in these tissues at concentrations that are high enough that commonly accepted mercury determination practices are accurate. The extremely high correlations (0.96 - 0.99) between mercury concentrations in blood or growing feathers, and levels in liver, kidney and muscle, demonstrate that feather mercury measurements are, in growing chicks, highly predictive of mercury concentrations in other tissues. We have also shown, through captive dosing, that growing feather mercury is a very good predictor of doses given. So feathers seem to be very useful as media for noninvasive sampling of mercury contamination.

Our work has also shown very strong relationships between mercury in growing feather and powderdown. Since the latter is available on adult, nonmolting, as well as molting and juvenile egrets and soome other birds, powderdown and growing feather provides an excellent alternative to blood sampling in almost any situation. Powderdown has the distinct advantage of being a constantly growing tissue, and is therefore likely to serve as a barometer of current, rather than past circulating mercury levels in birds. These results indicate that sampling of mercury in wading birds in the future may be done nondestructively by sampling growing feather tissue, and that the mercury concentrations from feathers are predictive of concentrations in other tissues, especially in chicks. We do not feel, however, that we fully understand the relative proportions of methylmercury and inorganic mercury in tissue samples, since we have no measurements of methyl mercury.

Our sampling of tissues in field and lab have also shown that the distribution of mercury in the body follows a characteristic pattern. The highest concentrations appear in tissues that are highly keratinized, such as feathers. Concentrations are intermediate in liver, blood, and kidney, and lowest in brain, fat and eye tissue. The high concentrations in feather have also been found in numerous other bird species. Whether the low values we found in brain and eye are similar to or different from the pattern in other birds is not known, simply because very few studies have reported concentrations from these tissues.

We have also shown that rates of mercury deposition in various tissues may be interdependent. Mercury concentration in blood is, for instance, highly dependent upon whether or not growing feathers are available as deposition sites, and similar dependencies are quite possible in other tissues. The concentrations among tissues therefore covary to some extent, and are far from independent of one another. This fact has important ramifications for the interpretation of results. For instance, concentrations in a single sampling of blood from individuals that are growing new feathers might well give an impression of very low exposure, when actual exposure is high, but the burden is being absorbed largely by feather tissue. We have also found that mature feathers must

be sampled with the understanding that they represent a history of mercury accumulation that is strongly dependent on exposure, and some function of body burden, at the time of growth.

We have also found that the strong affinity of mercury for feather tissue determines the period of maximum mercury risk for growing wading birds. Our field and lab studies have collectively shown that even large amounts of mercury (5.0 mg/kg) can be tolerated in the diet during the period of rapid feather growth without obvious effects on mass gain or behavior, because the mercury is sequestered relatively harmlessly in the growing feather tissue. Once feathers cease growing, however, circulating mercury concentrations increase dramatically, and effects of mercury on appetite, mass, and blood parameters become suddenly apparent. The timing could not be worse for the birds, since the reduced feather growth is followed closely by the various stresses associated with independence - learning to fly, uncertain food supply, the need for rapid development of hunting skills, and exposure to diseases and predators. Thus the period of maximum susceptibility to the effects of mercury is almost undoubtedly during the fledging period, rather than during the early growth phase. This pattern probably has relevance for many other bird species. It should be noted that we do not discount the possibility of effects of mercury contamination early in the nestling period, as some effects such as PCV were noted prior to feather maturation. Certainly at high dietary levels, or at high concentrations in the egg, this is probable. Our work also implies that the effects of mercury contaminationare also closely linked with molt cycles in adult birds - adults should also be least susceptible to mercury at the time that feathers are growing.

Our field sampling has shown that hatch order has no significant effects on mercury concentrations in young great egrets. This is not surprising, since they eat food from the same source once hatched. This result implies, however, that there are not lasting differences in mercury concentrations among eggs of different laying order. The effect of age of young birds on tissue mercury concentration, however, is very strong, and any routine measurements of mercury contamination in nestlings need to be standardized for age in order to be comparable. We also have found strong differences that are due to geographic location within the Everglades. Surprisingly, our highest values by far have consistently come from a single colony in north-central Water Conservation Area 3A. The relative values obtained from the different colonies match in large part with the relative magnitude of mercury concentrations in mosquitofish in the foraging range of each of the colonies (Stober et al. 1996). We take these results collectively as evidence that tissue samples from wading bird nestlings may serve as local indicators of ecosystem contamination in the Everglades.

Our standardized measurements of feather and blood mercury in great egrets also showed annual differences. Since diet composition, and mercury content of the diet, varied considerably among years, this may be indicative only of the changing availability of fish, rather than some underlying change in the capacity of the wetland environment to produce mercury. This question may only be addressed by measuring mercury concentrations at various points in the aquatic food web over a period of years.

We have estimated mercury exposure in the diet of great egrets as 0.33-0.43 mg/kg wet weight using regurgitated samples (Chapter VII), and as 0.65 mg/kg ww in diet by using fish mercury concentrations in the Everglades that are reported elsewhere

(Chapter V). While this probably reflects the realistic range for wild birds in the Everglades, the differences in estimation are of interest, and may have several possible explanations. First, the differences could be due to size differences in fish. The fish taken from regurgitation samples were collected from relatively young chicks, (<28 d of age), and may represent size classes of fishes that are small relative to the sizes of fishes collected in other studies for mercury assay. The other possibility is that the discrepancies in fish concentrations are due to geographic differences, which can be large (Stober et al. 1996). We suspect that our estimates of dietary mercury exposure are biased low simply because we have studied great egrets during a period of prolonged high water. This is likely to lead to relatively small fish being consumed. In contrast, a breeding season with a strong, continuous drying event would be likely to make larger fish available that would presumably have higher mercury content.

Dietary mercury has a number of measurable effects on great egrets, at concentrations that are likely to be encountered in the Everglades. The first and most widely supported of these effects is a reduction in food consumed. This effect was shown clearly in both years of field studies, and confirmed experimentally during the captive dosing work. The effect occurred at existing levels of mercury in wild birds, it occurred with chicks dosed with 1.7 mg/kg mercury in their diet, and it occurred in captive animals at both 0.5 and 5.0 mg/kg dietary levels. The mechanism by which this effect is manifested is unclear, but it does not appear to be a result of poor motor control, since experimental birds that had reduced appetite showed no impairment of hunting skills (striking efficiency and time to capture). This implies that the effect is instead induced by a reduced motivation to eat, or perhaps is a result of lethargy that was also manifested in decreased activity levels (see below). The decrease in appetite may have survival value, since reduced appetite would presumably lead to reduced mercury exposure. There is a possibility that birds can taste methylmercury- our initial attempts to dose birds with powdered methylmercury placed directly in fish resulted in immediate regurgitations and avoidance of fish offered. There is no evidence that birds can taste methylmercury as it occurs in live contaminated fish.

Our field experiments indicated that the reduction in bird appetites due to mercury resulted in no decrease in the rate of weight gain. However, the weakness of the effect in the field study may be explained most parsimoniously by the fact that (as above) we were measuring effects during the part of the nestling period when rapid feather growth of chicks conferred the greatest ability to sequester the contaminant. In the captive experiment, however, we found significant though small effects of mercury on appetite, as well as effects on body condition at both 0.5 and 5.0 mg/kg dose rates. It is important to note that the effects on mass did not take place until after feathers had stopped growing. This suggests that if mercury contamination continues through the post-fledging period, the effects on appetite would become more obvious than they were in the field work. Exactly what these effects would mean for wild birds is not entirely predictable. The birds in our captive experiments were well fed, and they were buffered from any environmental stressors. Birds from all three dose groups had abundant fat at death, unlike wild fledglings. Thus even high doses did not affect the ability of birds to put on fat, when food was extremely easy to come by. The effects in the field, however, might be considerably more severe than can be extrapolated from the lab study. Generally, birds

fledging in reduced body condition are less likely to survive the first year of life. How much less likely, and what minimum rate of exposure causes decreases in survival, are questions that we are currently unable to answer.

Mercury also seems to make birds weaker and more lethargic. We found a significant change in frequencies of behaviors among captive mercury dose groups relative to placebos, with the mercury dosed birds tending to use behaviors that were less active. In addition, mercury-dosed birds spent less time in the sun than placebos. These changes may be interpreted as a mercury-induced tendency towards lethargy. The dose-related anemia that was observed did not appear to be severe enough to cause the weakness observed, but might have caused the decrease in the length of the foraging bout and might cause a general decrease in maintenance activities such as preening. The avoidance of the sun in mercury dosed birds may be interpreted as a decreased ability to thermoregulate, though other explanations are also possible. The lethargic state may be related to decreased appetite, and the decrease in cleanliness noted in both classes of dosed birds.

It is unlikely that the lethargy was a result of impaired motor skills, except in the most advanced cases of toxicosis. This is because lethargic birds usually had no obvious impairment of hunting abilities, which we felt should be demanding tests of motor skills. While this was truly testable only for the low dose birds in captivity, we had several opportunities to observe hunting behavior in captive high dose birds that were exhibiting classic symptoms of mercury toxicosis (difficulty standing, unable to right themselves, little or no preening or maintenance behavior, and poor appetite). Although barely able to stand, these birds were able to accurately strike and capture fish, usually in less time than placebos. This suggested to us that striking accuracy was not a parameter that was sensitive to the effects of mercury. At low doses, we also found no evidence of poor striking efficiency, and only very weak evidence of slower capture times. It therefore appears that mercury acts to decrease body condition by inducing lethargy and lowering motivation to hunt, rather than by reducing the hunting efficiency of birds. We also have noted that there may be a variety of foraging strategies involved in capturing fishes and that our simple measures of time to capture and striking ability in highly artificial circumstances may not be very relevant to natural hunting situations.

Mercury may have effects on vision in wading birds. In a single bird, we have shown that mercury can affect properties of the transmission of signals from eye to brain, as well as the histology of the eye itself. These effects were manifested only at very high doses of mercury, we had relatively poor controls, and minimal sample size. The importance of the result to wild birds is not clear-- this may be an effect of severe toxicosis, or it may be the result of acute, rather than chronic, dosing. As above, in tests of striking accuracy, we saw no effects of mercury, even in our highly dosed and obviously sick birds (as above), suggesting that the measured neurological and histological effects on vision may not have been manifested as impairment of behavior.

We have also demonstrated an obvious decrease in packed-cell volume in blood in proportion to mercury consumed. In the lab, this effect was significant at both 5.0 and 0.5 mg/kg dose rates as compared to the placebos, and appears to be a robust result. A tendency toward lowered PCV was also noted in the field dosing experiment. In the captive dosing, PCV changes were noted at 5 weeks of age, whereas the field experiment

terminated before 4 weeks of age. The possible effects of anemia on stamina, weakness, and maintenance activities have already been discussed. The reduction in PCV was not severe enough to be life threatening but was likely to result in lethargy or reduced stamina.

Other blood parameters may also be altered, such as significant increases in heterophils, basophils, eosinophils and decreases in lymphocytes and monocytes. Trends toward these same changes were also observed in the field dosing experiment. Reduced sizes of bursa and spleen that we noted incidentally in high dosed captive great egrets at time of death also contributes to the suggestion that mercury affects the immune system. Whether this translates to a functional change in responses to challenges can only be speculated upon at this time.

Although we did not see any significant effects of mercury on immunocompetence, our test was probably confounded by considerable variability among the placebos, the immaturity of the birds, the early death of the high dose birds, as well as a small sample size. At this point, we feel we have no direct evidence to prove or disprove the hypothesis that mercury affects the immune system.

As in other studies, mercury caused a number of changes in blood chemistry in the high dose birds, some of which seem result in a profile unique to mercury, including reduced hepatic thiol concentrations and depression of GSH peroxidase. In addition we also found significant differences in blood chemistry between low dose and placebo birds by the end of the experiment. These differences are suggestive of increased oxidative stress, and may also imply metabolic imbalances (increased cholesterol, increased plasma glucose, decreased uric acid, and decreased plasma calcium).

We found no evidence to indicate that mercury is affecting the ability of birds to come into reproductive condition, or to reproduce successfully. Feather concentrations of mercury in successfully breeding great blue herons were actually consistently higher than those of nonbreeding birds during the same season and in the same ecosystem. While this evidence is suggestive that mercury does not affect reproductive abilities, we would like to emphasize that this result may be confounded in several ways, and so may not be an accurate test of the hypothesis. First, we compared mercury in breeding plumes (breeding birds), with feathers of a different type in nonbreeding birds. Considerable differences in mercury concentration have been shown in feathers of different types in the same bird (Furness et al. 1986). Second, although we are confident that the feathers from breeding birds were grown during the late winter and early spring, and are good indicators of mercury contamination at that time, we cannot say the same about other feather types were collected from nonbreeding birds. Growing feathers and mature feathers from the same bird represent exposure at different times. Thus it is difficult to confidently measure the mercury contamination in these nonbreeding birds at the time that they could *potentially* have come into reproductive condition. Third, there is an overall larger pattern of higher mercury concentrations in feathers collected during the breeding season. This suggests that during the dry season, when larger fishes are exposed to predation, mercury risk may increase for birds of all types. Further, if breeding birds eat more than nonbreeding birds in order to establish body reserves, breeding birds may show increased mercury simply as a function of food intake. Thus the differences in mercury concentration among breeding and nonbreeding birds may be a function of metabolism,

and may be unrelated to breeding ability. Therefore, while it is clear that successfully breeding great blue herons may have relatively high mercury concentrations in their feathers at the time of breeding initiation, it is not obvious that nonbreeding birds have less mercury contamination at the same point in the reproductive cycle.

The relative importance of mercury as a stressor for wading birds in the Everglades

We have shown that dietary mercury has several effects on wading birds at dietary concentrations that are realistically encountered by piscivorous birds in the current Everglades ecosystem. We feel it is important to comment to the extent possible on the potential effects of mercury relative to other stressors in the environment, and to speculate on the possible effects of mercury should concentrations increase above current levels in the Everglades.

Our assessment of mercury concentrations in food of great egrets in the Everglades suggests that a diet of 0.33 - 0.65 mg/kg wet weight is probably a realistic and if anything, conservative range of average values for nestling great egrets in the Everglades. The effects of mercury that we have demonstrated with the birds dosed at 0.50 mg/kg in captivity are therefore most applicable for divining the effects that mercury might have on birds in the current Everglades ecosystem. Thus if we compare a nestling in the Everglades with a bird in an uncontaminated environment, we might expect to find, by time of fledging, decreased appetite, increased weakness, increased lethargy, decreased stamina, poorer body condition, altered blood chemistry and composition, and the possibility of increased susceptibility to disease. Our results indicate that these effects would all be manifested at the time of fledging, when the risks and stresses of becoming independent of parental feedings are most severe. Our findings allow us to predict that the young Everglades birds would be likely to have reduced survival over the next several months as a result. The *degree* to which this is true is impossible to predict from our results. It seems clear from our captive dosing work that the field-dosed birds had ample time to depurate mercury from their bodies prior to fledging. Our estimation of survival in the field-dosed birds therefore cannot be used to compare dosed and undosed birds. However, it is clear that reduced survival of juvenile birds can have a large effect on the demographics of the great egret population.

There is also the possibility of intergenerational effects of mercury contamination. Heinz (1979) found that mallard ducklings produced by parents that had been exposed to mercury, showed marked behavioral changes without having been exposed to dietary mercury themselves. This is certainly a possibility with Everglades wading birds as well, and we have almost no information on whether chicks produced in the Everglades ever return to breed, or even breed successfully elsewhere. Therefore, the possibility exists that, in addition to producing chicks with decreased survival and increased susceptibility to disease, the Everglades is also producing substandard birds that have decreased potential as future breeders.

The extent to which the effects of mercury that we noted in young are also found in adults was not studied directly in this research project. The effects of mercury on vision were studied in an adult bird, indicating that vision can be affected in adults. A brief and uncontrolled captive dosing study in spring of 1994 (Sepúlveda et al. 1995)

suggested that the appetites of two adult great egrets and one great blue heron were dramatically reduced when dosed at very high levels. We see no obvious reasons why the effects on behavior, blood parameters and organ function would be fundamentally different in adults, but we caution that the dose-response relationships may well be different in adults. Generally, tissue concentrations are higher in adults than in fledglings.

Our work suggests that great blue herons with high mercury concentrations in feathers manage to breed successfully. In addition, great egret breeding populations have increased dramatically in the Everglades during the past ten years (Frederick 1995), despite the high mercury exposure we have demonstrated. This also may be taken as evidence that current levels of mercury contamination do not have a strongly depressive effect on reproduction of great egrets and great blue herons. These patterns do not exclude the possibility that mercury in the Everglades results in less frequent breeding, since we have no control situation for comparison. We also must consider the possibility that the Everglades could be acting as a reproductive sink, with contaminated birds passing out of the population after breeding once or twice, and/or by producing offspring with decreased reproductive potential.

In the Everglades, mercury is certainly not alone as a potential stressor of free-ranging populations of wading birds. The availability of food, drought, pesticides, inappropriate water management, a salinized estuary, predation, and parasitic diseases have all been identified as strongly affecting wading bird reproductive success (Frederick and Spalding 1994). Though the effects of mercury are less easily measured than these effects, and its mode of action more subtle, mercury contamination may also have strong effects, including reduced survival of offspring, reduced appetite, and increased susceptibility to diseases. Each of these impairments could strongly affect the reproduction and demography of the population, especially if acting in concert with the other ecological problems faced by wading birds in the current Everglades ecosystem.

Applicability of our results to other species

The literature suggests large differences in the effects of mercury among species, and it is not clear to what extent the effects we have shown in great egrets and great blue herons can be translated to other species of wading birds. We picked two species that are at greatest risk of exposure in the Everglades. However, they are also the only two species which have not shown declines in reproduction in the ecosystem. As in Chapter I of this report, sensitivity to the effects of mercury seems lower in fish-eating and raptorial birds than herbivorous or omnivorous birds. Because great egrets and great blue herons have been at the top of the aquatic food chain during much of their evolutionary history, they are perhaps more likely than other avian species to have developed adaptations that reduce the toxicity of mercury, or that ameliorate the effects. Until other species of wading birds are examined for susceptibility to mercury toxicity, we are therefore hesitant to apply our results to species other than great egrets and great blue herons.

CONCLUSIONS

1) Measurement of mercury in growing feathers is an accurate, nondestructive way to sample mercury in long-legged wading birds. Growing feathers give an accurate prediction of the relative contamination in other tissues and are easily sampled and accurately measured for mercury content.

2) Mercury contamination in nestling wading birds reflects local mercury contamination in prey animals.

3) Selenium accumulates in liver of great egrets and great blue herons in to mercury accumulation. The significance of this finding is presently unknown.

4) Deposition of mercury in tissues is nonrandom, with highest affinities for mercury in highly keratinized tissues such as feathers, and lowest in brain, fat and eye. Tissue concentrations are also interdependent in the sense that the relative availability of high affinity tissues determines the concentrations in tissues of lower affinity.

5) Concentrations of mercury in nestling wading birds are strongly dependent upon geographic location, age, tissue sampled, prey species and year. These factors must be taken into account when comparisons are made.

6) At dietary mercury concentrations encountered by young great egrets in the current Everglades ecosystem (conservatively estimated at 0.33-0.65 mg/kg wet weight), mercury (in a laboratory setting) significantly decreases appetite, decreases body mass, reduces blood packed cell volume, reduces or increases counts of several types of white blood cells, and affects several aspects of blood chemistry, and circulating and tissue enzymes. The measured changes in immune system parameters could not be directly related to functional disorders.

7) At dietary mercury concentrations typically encountered in the Everglades, young great egrets are likely to behave less energetically than at background dietary mercury levels, and are less likely to hunt and consume fish. They do not show any impairment of hunting skills, and once engaged in hunting behavior they strike and capture prey efficiently.

8) Young wading birds are most susceptible to the effects of mercury contamination when their feathers cease growing. Prior to this time, feathers serve as a sink for mercury absorbed from food, due to the high affinity of mercury for keratinized tissue. At about the same time feathers stop growing, the birds are also forced to become independent. They must simultaneously encounter the multiple problems of independence at the same time that mercury levels in tissues increase, and that the effects of mercury manifest themselves.

9) While many of the effects of mercury that were shown in fledglings may also affect adults, we found no evidence that mercury affects the ability of adults to come into reproductive condition, or to produce chicks successfully. However, our tests for these effects may have been confounded by sampling artifacts.

10) It may be concluded that the cumulative effects of current dietary mercury concentrations in the Everglades (decreased appetite, decreased body mass, increased susceptibility to disease) lead to reduced survival of post-fledging birds as compared with birds on a low mercury diet. We are unable to estimate the magnitude of this effect on survival with the results available.

11) The effects of mercury on great egret and great blue heron reproduction in the Everglades can probably be felt at the population level. Reduced survival of young birds is a likely consequence at 0.5 mg/kg mercury in the diet, and population size is likely to be sensitive to changes in juvenile survival.

12) The effects demonstrated in this report have been focused on the only two bird species in the Everglades that have undergone little or no reproductive decline. Given the wide variation in responses among avian species to mercury exposure, and the higher tolerances to mercury shown by carnivorous birds, it is possible that these two species are among the least susceptible to mercury. We believe it is unwise to extrapolate our results for great egrets and great blue heron to other wading bird species.

13) The results presented in this study are relevant only to total mercury concentrations. The extent to which demethylation occurs in body tissues, as is suggested by a few measurements in this study, and in other studies, is very relevant to the conclusions of this study and needs to be further investigated.

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Appendix 1. Relative importance of prey items in boluses regurgitated by nestling great egrets during four years in the central Everglades.

		1993	1994	1995	1996
n boluses		21.00	24.00	57.00	29.00
animals per	mean	6.64	4.33	4.98	2.41
bolus	s.d.	4.83	6.04	5.54	n.a.
mass of	mean	47.96	7.58	25.52	27.07
boluses	s.d.	32.13	15.60	10.88	n.a.
Relative importance of prey items					
Osteichthys:					
Unknown	biomass	71.09	44.70	188.85	390.00
fish	proportion of total biomass	0.07	0.07	0.07	0.50
	sample occurrence	10.00	13.00	23.00	21.00
	proportion of samples	0.48	0.54	0.40	0.74
Erimyzon	biomass	0.00	34.40	30.60	0.00
sucetta	proportion of total biomass	0.00	0.05	0.02	0.00
	sample occurrence	0.00	1.00	1.00	0.00
	proportion of samples	0.00	0.04	0.01	0.00
All	biomass	0.00	34.40	30.60	0.00
Catostomidae	proportion of total biomass	0.00	0.05	0.02	0.00
	sample occurrence	0.00	1.00	1.00	0.00
	proportion of samples	0.00	0.04	0.01	0.00
Unknown	biomass	184.80	1.80	12.30	72.80
Centrarchids	proportion of total biomass	0.18	0.00	0.01	0.09
	sample occurrence	12.00	1.00	5.00	11.00
	proportion of samples	0.57	0.04	0.09	0.37
Lepomis	biomass	12.80	1.40	0.00	0.00
macrochirus	proportion of total biomass	0.01	0.00	0.00	0.00
	sample occurrence	3.00	1.00	0.00	0.00
	proportion of samples	0.14	0.04	0.00	0.00

Appendix 1. (cont.)

Lepomis microlophus	biomass	49.10	0.00	0.00	0.00
	proportion of total biomass	0.05	0.00	0.00	0.00
	sample occurrence	2.00	0.00	0.00	0.00
	proportion of samples	0.10	0.00	0.00	0.00
Lepomis marginatus	biomass	54.90	0.00	26.60	0.00
	proportion of total biomass	0.05	0.00	0.02	0.00
	sample occurrence	2.00	0.00	5.00	0.00
	proportion of samples	0.10	0.00	0.09	0.00
Lepomis punctatus	biomass	12.80	2.80	482.60	246.20
	proportion of total biomass	0.01	0.00	0.33	0.31
	sample occurrence	1.00	1.00	30.00	17.00
	proportion of samples	0.05	0.40	0.53	0.58
Lepomis gulosus	biomass	244.10	490.90	140.40	0.00
	proportion of total biomass	0.23	0.79	0.10	0.00
	sample occurrence	4.00	10.00	5.00	0.00
	proportion of samples	0.19	0.41	0.09	0.00
Micropterus salmoides	biomass	24.50	0.00	190.00	0.00
	proportion of total biomass	0.02	0.00	0.13	0.00
	sample occurrence	2.00	0.00	7.00	0.00
	proportion of samples	0.10	0.00	0.12	0.00
Elassoma evergladei	biomass	3.20	0.00	0.00	0.00
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	1.00	0.00	0.00	0.00
	proportion of samples	0.05	0.00	0.00	0.00
All Centrarchidae	biomass	586.20	496.90	851.90	319.00
	proportion of total biomass	0.56	0.80	0.59	0.41
	sample occurrence	18.00	13.00	45.00	20.00
	proportion of samples	0.86	0.54	0.79	0.69
Fundulus sp.	biomass	11.70	0.00	2.40	0.00
	proportion of total biomass	0.01	0.00	0.00	0.00
	sample occurrence	3.00	0.00	1.00	0.00
	proportion of samples	0.14	0.00	0.02	0.00

Appendix 1. (cont.)

Fundulus seminolis	biomass	0.00	0.00	2.30	0.00
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	0.00	0.00	1.00	0.00
	proportion of samples	0.00	0.00	0.02	0.00
Fundulus chrysotus	biomass	8.10	9.30	28.90	0.00
	proportion of total biomass	0.01	0.01	0.02	0.00
	sample occurrence	1.00	4.00	11.00	0.00
	proportion of samples	0.05	0.16	0.19	0.00
Fundulus confluentis	biomass	23.50	0.00	0.00	0.80
	proportion of total biomass	0.02	0.00	0.00	0.00
	sample occurrence	4.00	0.00	0.00	2.00
	proportion of samples	0.19	0.00	0.00	0.05
Fundulus lineolatus	biomass	0.00	0.00	16.40	0.00
	proportion of total biomass	0.00	0.00	0.01	0.00
	sample occurrence	0.00	0.00	6.00	0.00
	proportion of samples	0.00	0.00	0.11	0.00
Lucania parva	biomass	0.00	1.10	0.30	0.00
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	0.00	1.00	1.00	0.00
	proportion of samples	0.00	0.04	0.02	0.00
Bellenesox belizanus	biomass	157.70	19.90	27.50	0.00
	proportion of total biomass	0.15	0.03	0.02	0.00
	sample occurrence	5.00	6.00	7.00	0.00
	proportion of samples	0.24	0.04	0.12	0.00
Poecilia latipinna	biomass	3.80	0.40	24.40	0.00
	proportion of total biomass	0.00	0.00	0.02	0.00
	sample occurrence	2.00	1.00	13.00	0.00
	proportion of samples	0.10	0.04	0.23	0.00
Gambusia holbrooki	biomass	0.50	1.60	2.70	0.00
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	1.00	4.00	6.00	0.00
	proportion of samples	0.05	0.16	0.11	0.00
Heterandria	biomass	0.00	0.20	0.00	0.00

formosa	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	0.00	1.00	0.00	0.00
	proportion of samples	0.00	0.04	0.00	0.00
Jordanella floridae	biomass	13.05	0.00	8.60	0.60
	proportion of total biomass	0.01	0.00	0.01	0.00
	sample occurrence	5.00	0.00	9.00	3.00
	proportion of samples	0.24	0.00	0.16	0.11
Unidentified catfish	biomass	9.00	0.00	0.00	0.00
	proportion of total biomass	0.01	0.00	0.00	0.00
	sample occurrence	1.00	0.00	0.00	0.00
	proportion of samples	0.05	0.00	0.00	0.00
Unknown cichlid	biomass	59.10	0.00	2.00	0.00
	proportion of total biomass	0.06	0.00	0.00	0.00
	sample occurrence	2.04	0.00	1.00	0.00
	proportion of samples	0.10	0.00	0.02	0.00
Cichlasoma urophthalmus	biomass	133.00	0.00	179.10	0.00
	proportion of total biomass	0.13	0.00	0.12	0.00
	sample occurrence	3.00	0.00	9.00	0.00
	proportion of samples	0.14	0.00	0.16	0.00
Cichlasoma bimacculatum	biomass	10.00	0.00	72.00	0.00
	proportion of total biomass	0.01	0.00	0.05	0.00
	sample occurrence	2.00	0.00	6.00	0.00
	proportion of samples	0.10	0.00	0.11	0.00
Hemichromis letourneaux	biomass	0.00	0.00	3.80	0.00
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	0.00	0.00	1.00	0.00
	proportion of samples	0.00	0.00	0.02	0.00
All Cichlidae	biomass	175.90	0.00	256.90	0.00
	proportion of total biomass	0.17	0.00	0.18	0.00
	sample occurrence	9.00	0.00	17.00	0.00
	proportion of samples	0.43	0.00	0.30	0.00

Appendix 1. (cont.)

All exotic fishes	biomass	333.60	19.90	284.40	0.00
	proportion of total biomass	0.32	0.03	0.20	0.00
	sample occurrence	12.00	6.00	24.00	0.00
	proportion of samples	0.57	0.04	0.42	0.00
Odonata adults	biomass	0.10	0.00	3.50	0.20
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	1.00	5.00	8.00	2.00
	proportion of samples	0.05	0.21	0.14	0.05
Crayfish (Procambarus spp.)	biomass	2.00	0.00	3.80	0.00
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	3.00	5.00	6.00	0.00
	proportion of samples	0.14	0.21	0.11	0.00
Paleomenetes paludosus	biomass	0.10	0.00	0.13	0.10
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	1.00	2.00	2.00	2.00
	proportion of samples	0.05	0.08	0.04	0.05
Unidentified insect	biomass	1.00	0.00	0.00	0.00
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	1.00	3.00	1.00	0.00
	proportion of samples	0.05	0.13	0.02	0.00
Rana utricularia	biomass	1.30	0.00	0.00	0.00
	proportion of total biomass	0.00	0.00	0.00	0.00
	sample occurrence	1.00	0.00	0.00	0.00
	proportion of samples	0.05	0.00	0.00	0.00
Rana sp.	biomass	0.00	3.80	0.00	0.00
	proportion of total biomass	0.00	0.62	0.00	0.00
	sample occurrence	0.00	1.00	0.00	0.00
	proportion of samples	0.00	0.04	0.00	0.00

George Noguchi
08/02/2002 11:09 AM

To: Daniel Welsh/SAC/R1/FWS/DOI@FWS
cc: Elaine Snyder-Conn/ARL/R9/FWS/DOI@FWS, Roxanna Hinzman/ARL/R9/FWS/DOI@FWS, Tony Hawkes/KFFWO/R1/FWS/DOI@FWS
Subject: Re: draft interim -Please email to George Noguchi today!!

Dan,

I spoke with Elaine about this interim and it is apparently complete except that the period in the last sentence was accidentally left off (I'll make the edit). Given our past conversations regarding this project I think this interim will satisfy the FY03 performance requirement. Looking forward to seeing the final report by next FY.

George

>--)> >--)> >--)> >--)> >--)> >--)>
George Noguchi, Ph.D.
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Daniel Welsh

Daniel Welsh
08/01/02 07:00 PM

To: Elaine Snyder-Conn/ARL/R9/FWS/DOI@FWS
cc: George Noguchi/ARL/R9/FWS/DOI@FWS, Tony Hawkes/KFFWO/R1/FWS/DOI@FWS
Subject: Re: draft interim -Please email to George Noguchi today!!

Elaine,

It looks like part of the file got cut off. Please send it again.

Thanks, Dan

Elaine Snyder-Conn

Elaine Snyder-Conn
08/01/2002 02:34 PM

To: Daniel Welsh/SAC/R1/FWS/DOI@FWS
cc:
Subject: draft interim -Please email to George Noguchi today!!

DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION 1

1W40
updated status to Interim 8/19/02

Environmental Contaminants Program
On-Refuge Investigations Sub-Activity

Interim Final Report

PESTICIDE IMPACT ASSESSMENT IN TULE LAKE AND LOWER KLAMATH NATIONAL WILDLIFE REFUGES, 1998 - 2000 Growing Seasons

WO Project ID: 199810004.3
(filename: PIMreport1998-2000.*doc*)

by

Elaine Snyder-Conn

and

Tony Hawkes
Environmental Contaminants Specialist

For

Phil Norton, Refuge Manager
Klamath Basin Refuges
Route 1, Box 74
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July 31, 2002

This report will summarize results of a comprehensive, refuge-wide pesticide monitoring program conducted for three years, from 1998-2000, to assess potential impacts of pesticides to refuge wildlife, including fish, mammals, birds, amphibians, other wildlife, and habitat on Tule Lake and Lower Klamath NWRs. The objectives of this study were (1) to survey both refuges for dead or impaired wildlife, with an emphasis on mortality events in/near agricultural areas (2) to determine whether pesticide exposure was implicated in any death or impairment discovered, and (3) to investigate the source of any pesticide exposure detected. Results are collated from mortality incidents identified during five general types of surveys conducted: (1) agricultural field surveys, (2) aquatic surveys, (3) driving surveys, (4) response surveys, and (5) in-route surveys.

Interim reports for individual years have already been submitted for this monitoring study. The final summary report, anticipated to be completed by December 1, 2002, is in preparation and is approximately 75% complete. The report will summarize and synthesize information on major sources of mortality to refuge wildlife in and near agricultural fields on the two refuges and also add results and interpretations of additional analytical and water quality analyses not received prior to completion of the interim reports.

In addition, the report profiles pesticide use patterns on the refuges each year and provides tables summarizing the mortality records, including the species, location of death, laboratory analysis results, and a diagnosis of probable cause of death (when possible) as determined by field observations, gross external necropsies, gross internal necropsies, histopathology, specific tests for pathogens, acetylcholinesterase inhibition tests, and/or pesticide residue analysis for carcasses. Causes of mortalities could not be established in all cases and depended on carcass condition and age. However, probable cause of death was established in the majority of cases when fresh, relatively intact carcasses were located.

During the study surveys, a number of pesticide, fertilizer, and petroleum spills were located in and adjacent to the refuge lands. No dead wildlife was found at any of the sites, despite intensive monitoring of several of the pesticide-treated spills. However, diesel was implicated in the case of several fish kills at LKNWR, as were poor water quality conditions. Spills observed as a result of farm equipment failures and/or accidents led to definitive management actions and an apparent reduction in spill incidents, as well as more rapid spill response, over the study period. In comparison to other causes of mortality identified (fish kills related to poor water quality and avian and mammalian mortalities as a result of avian botulism, vehicle strikes, and predation), pesticide-related mortalities appear to be infrequent on the refuges. Pesticide residues were not detected in most analyzed wildlife, and AChE depression, when observed, was usually not definitive. Additional integration of observational, necropsy, and analytical data, especially QA/QC data on AChE inhibition tests, will be needed prior to report completion for a more accurate interpretation of several mortality records before this report is finalized. While pesticide- related mortalities appear rare on the refuge, acrolein-related fish kills in waters adjacent to TLNWR will remain a problem until completion of additional fish screens on the irrigation system, such as the A Canal screen now being installed