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METHYLMERCURY ACCUMULATION IN TISSUES AND ITS EFFECTS ON GROWTH AND APPETITE IN CAPTIVE GREAT EGRETS

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ABSTRACT: To test the hypothesis that fledging wading birds would be more at risk from mercury toxicosis than younger nestlings, captive great egret nestlings were maintained as controls or were dosed from 1- to 14-wk-old with 0.5 or 5 mg methylmercury chloride/kg wet weight in fish. Birds dosed with 5 mg/kg suffered from subacute toxicosis at wk 10–12. Growing feather concentrations were the most closely correlated with cumulative mercury consumed per weight. Blood concentrations of mercury increased more rapidly after 9 wk in all groups when feathers stopped growing. Total mercury accumulated in tissues in concentrations in the following order: growing scapular feathers > powderdown > mature scapular feathers > liver > kidney > blood > muscle > pancreas > brain > bile > fat > eye. The proportion of total mercury that was methylated depended upon tissue type and dose group. Selenium accumulated in liver in direct proportion to liver mercury concentrations. After wk 9, appetite and weight index (weight/bill length) declined significantly in both dosed groups. At current exposure levels in the Everglades (Florida, USA) mercury deposited in rapidly growing feathers may protect nestlings from adverse effects on growth until feathers cease growing.

Key words: Appetite, *Ardea albus*, bioaccumulation, captive, contaminants, feathers, great egret, growth, methylmercury, tissue accumulation.

INTRODUCTION

Methylmercury contamination of wetland food chains has been suspected to cause reduced survival and/or reproduction in top carnivores (Fimreite, 1974; Van der Molen et al., 1982; Barr, 1986; Face-mire et al., 1995; Meyer et al., 1998) and has been suggested as one of the possible causes for reduced reproduction of long-legged wading birds (Ciconiiformes) in the Everglades (Florida, USA) in recent decades (Frederick and Spalding, 1994; Spalding et al., 1994; Sundlof et al., 1994). One mechanism by which methylmercury might affect reproduction would be to directly alter the development of nestlings. A few authors have documented reduced appetite and/or reduced weight gain in captive juvenile raptors and ducks dosed with relatively high concentrations of methylmercury (Borg et al., 1970; Fimreite and Karstad, 1971; Pass et al., 1975; Bhatnagar et al., 1982) and Hoffman et al. (1998) found reduced weights in wild div-

ing ducks with higher liver mercury concentrations. Williams (1997) and Sepulveda et al. (1999b) found effects of mercury on appetite, but not on survival of wild great egret young that were dosed with methylmercury in the Everglades. In their study dosing was confined to the fastest growth period for the chicks. It seemed likely that a large proportion of mercury would be shunted into growing feathers during that time, where it might be unavailable to other tissues (Furness et al., 1986). For this reason, we hypothesized that young chicks with rapidly growing feathers would be protected from the effects of dietary methylmercury and that chicks that continued to receive methylmercury after feather growth ceased would be more likely to exhibit the signs of methylmercury toxicosis.

In the present study, we raised captive great egret nestlings from hatching to 14-wk-old, well after the time that they would normally fledge and be independent in the

wild (9–11 wk, Sepulveda et al., 1999b), on control diets and diets containing 0.5, and 5 mg methylmercury/kg food. The main purpose of this paper is to report the accumulation of mercury in various tissues of these birds and the effects of mercury on growth and appetite. The effects on behavior and foraging skills (Bouton et al., 1999), tissue and plasma biochemistry (D. J. Hoffman et al., unpubl. data), and health and histologic changes (Spalding et al., 2000) are or will be published elsewhere.

METHODS

On 16 March 1996 we collected first-hatched great egret nestlings from broods of 23 different nests in Alley North colony (26°11.25'N, 80°31.05'W) in Water Conservation Area 3 of the central Everglades. This colony is located within an area where high mercury concentrations have been measured in wading birds and fish (Sundlof et al., 1994; Frederick et al., 1999). They were taken from nests of three eggs that had been monitored throughout incubation. Some were collected as pipped eggs, which took several days to hatch, and others were as old as 5 days. The range in ages was 7 days. Seven birds were excluded from the experiment due to early mortality. The birds were transported to the Florida Field Station of the National Wildlife Research Center (United States Department of Agriculture, Gainesville, Florida, USA). Chicks were assigned to group randomly and 5, 5, and 6 birds were in control, low dose, and high dose groups respectively. Initially birds were housed indoors in individual 65 × 40 cm plastic boxes with artificial stick nests. All birds were kept in the same heated room during the first 2 wk of life, and heating pads were applied to the bottoms of the boxes for the first wk of life. Boxes were cleaned daily. At 5 wk, birds were moved to outdoor housing. The plastic boxes were attached to perches within each outdoor cage, and removed after the birds stopped using the boxes for perching and resting. Cages were 3m × 3m × 2m enclosures constructed of chickenwire supported by PVC plastic tubing. The cages had sand floors and each contained a water dish, perches, and, during the latter third of the experiment, one shallowly flooded plastic wading pool. Cages were grouped into blocks of three with common walls between adjacent cages. Each block contained one bird from each dose group, and dose group cage assignment was random with respect to location within a block.

An electrified fence to keep terrestrial predators away surrounded the entire group of pens. 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 four to two times daily, depending on age, on a modified *ad libitum* basis. Uneaten fish were removed and weighed. *Ad libitum* feeding for the first wk allowed us to establish the initial amount to feed each bird. We offered that amount of food to each bird until it either ate all food offered for three consecutive meals, or left any amount of food uneaten for three consecutive meals. We then either increased or decreased food by 10 g, respectively. During the trials on hunting behavior, wk 10–14, the birds were also allowed to forage on live fish (see Bouton et al., 1999). Methylmercury dosing was based upon total daily food offered, including the live fish.

Gelatin dosing capsules were made three times a week from solutions that contained 0, 3, or 30 µg reagent grade methylmercury chloride/µl in acetone. Each gelatin capsule received 0.17 µl solution/g food offered, which was equivalent to 0, 0.5, or 5.0 mg/kg food offered for that day. In addition to the controls, we used a low dose of 0.5 mg/kg in fish because it was similar to what great egret nestlings in the Everglades currently eat (Frederick et al., 1999) and a high dose of 5 mg/kg because that would be expected to produce clinical toxicity. The acetone was evaporated from the capsules and they were stored in sealed containers until time of dosing. Capsules were given to the birds daily just prior to the evening meal by manipulating the capsules within the esophagus to the base of the neck. We never saw birds regurgitate the capsules and never found any capsules in the cages. Assignment to methylmercury dose groups was random and blind to researchers working on the experiment. Only 8 of the original 23 birds were male. Of the birds included in the study, 1 of 5, 2 of 5, and 2 of 6, were male in the control, low, and high dose groups respectively.

Birds were dosed every 3 days starting at about 8 days of age. Dosing then changed to a daily regime beginning on day 20, and continued until the end of the experiment (wk 14). The high dose group received 0.5 mg/kg food until wk 6 due to an error in handling solutions. This error was confirmed by examining blood, feather, and fecal data. Birds were captured weekly for examination, to collect blood and feather samples, and to perform various tests.

Twenty-four hour fecal collections were made during wk 5 and 13, for 1 and 2 birds respectively, from each group. Feces, including embedded fragments of feather sheath, were collected every 8 hr from the individual boxes or from plastic placed on the ground. The sample was mixed thoroughly and an aliquot submitted for total mercury analysis. To determine the conversion ratio for wet to dry weight, feces were dried in an oven to constant mass at 58 C. Birds were killed humanely by an overdose of sodium pentobarbital when they could no longer stand [birds in the high dose group were killed at wk 10 (1 individual), 11 (2), and 12 (3)], and all remaining birds were killed at the end of the experiment (wk 14).

The Department of Environmental Protection Chemistry Laboratory (Tallahassee, Florida) determined total and methylmercury concentrations in tissues of great egrets. Total mercury concentrations were detected by cold vapor atomic absorption spectrophotometry as described by Sepulveda et al. (1999a). Methylmercury concentrations were determined for two birds from each group using aqueous phase ethylation followed by cryogenic gas chromatography with cold vapor atomic fluorescence detection (Bloom, 1989). Unless specified otherwise, reported values are total mercury or methylmercury concentrations on a wet weight basis (ww) for blood and other tissue samples and on a dry weight basis (dw) for feathers and feces. Selenium concentrations in liver were measured using a fluorometric method (Whetter and Ullrey, 1978) and are reported on a dry weight basis.

We used repeated measures analysis of variance (ANOVA) to test for effects of dose group on various responses (SAS, 1988). The responses were all tissue concentrations, weight, weight index (weight divided by bill length), tarsometatarsus length, bill length, tail length, length of the most distal primary feather (emerged portion), primary sheath length (as a proportion of total primary feather length), daily food consumed (food averaged over the 3 days prior to blood collection), and food/weight (daily food divided by body weight). We included age as a covariant in these analyses because there was a 7 day difference in age among individuals. We also included gender as a covariant, since great egrets are somewhat sexually dimorphic in body measurements even during the pre-fledging stage (Palmer, 1962). Measurements were made either weekly or bi-weekly, and the effect of wk was included as a covariant in all models. Significant effect of group \times wk interactions were interpreted as evidence of an effect of methylmercury dose. Probabilities <0.05 were considered significant,

and probabilities of 0.10–0.05 were considered marginally significant. A multiple comparisons test using a probability of <0.01 was used to determine weeks during which effects differed from the control group.

RESULTS

Fish used to feed the birds contained an average (adjusted for proportions of each species fed) of 0.025 mg/kg of total mercury (0.022 mg/kg in silversides, 0.046 mg/kg in capelin). Selenium measured in the fish used to feed the great egrets was 0.87 mg/kg dryweight (dw) for silversides and 1.14 mg/kg dw for capelin. The adjusted average for the diet was 0.90 mg/kg dw selenium. Selenium accumulated in liver in direct proportion to mercury ($n = 20$, $P < 0.001$, $r^2 = 0.93$) (Fig. 1).

The proportion of total mercury that was methylmercury varied depending on the tissue and on the dose group. In some cases the methylmercury measurement exceeded the measurement of total mercury ($>100\%$). In livers the mean proportion of methylated mercury increased with dose group ranging from 56% (range = 51 to 59%) in the control group, to 61% (55 to 67%) in the low dose group to 73% (69 to 80%) in the high dose group. The proportion of mercury that was in the methyl form in the kidneys was similar, mean = 58% (range = 43 to 69%) in the control group, 61% (49 to 70%) in the low dose group, and 90% (74 to 145%) in the high dose group. Virtually all of the mercury in feathers from two birds in each of the dosed groups was methylmercury; mean = 120% (range = 93 to 150%).

Between 11 and 15% of the mercury administered to low dose birds was recovered in feces during a 24 hr period. Although lower concentrations of mercury were recovered in the feces of control birds, the percent excreted was much higher. Large quantities of shed feather sheath fragments contaminated the feces, especially at 5 wk, and probably account for these higher than expected percentages in the control birds. The percent excretion rate for the low dose birds is also

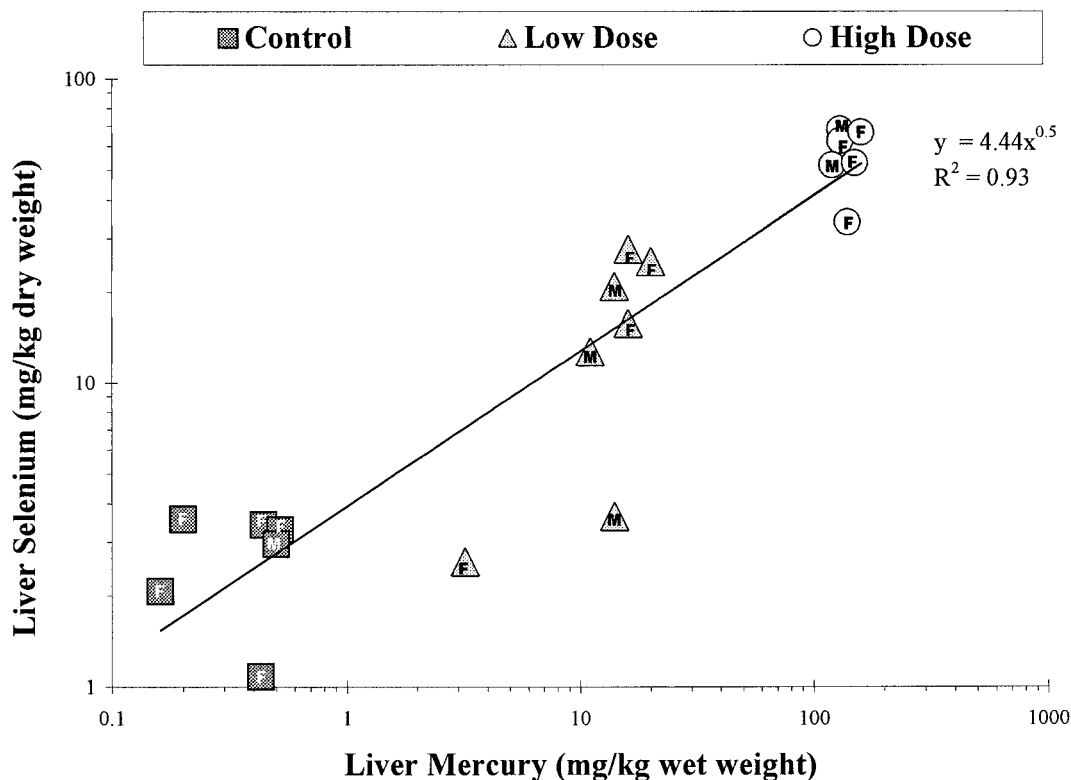


FIGURE 1. Liver selenium (mg/kg dry weight) and total mercury concentrations (mg/kg wet weight) graphed on a log scale for great egrets dosed with methylmercury. M = male, F = female.

undoubtedly artificially increased by this same phenomenon.

We found that mercury concentrations in growing scapular feathers and powderdown were similar. Because growing scapular feathers could not always be found, especially later in the study, the data for these feather categories were combined and are referred to as "growing feather".

Mercury concentrations in blood and growing feathers increased significantly with time over the course of the experiment for all three groups (Fig. 2). In the control group, where background concentrations in the embryo were probably higher than in the food offered, there was an initial decline in blood and feather mercury concentrations.

Concentrations of mercury in various tissues collected at death are listed in Table 1. All tissue concentrations, including those collected repeatedly during the experiment,

increased significantly with the amount of mercury administered (CumHg/Weight, ANOVA, $P < 0.05$). Except for bile and fat, all tissues were also significantly correlated with each other (Pearson correlation coefficient, $P < 0.05$). Generally, mercury concentrated in tissues in the following decreasing order: growing scapular feathers > powderdown > mature scapular feathers > liver > kidney > blood > muscle > pancreas > brain > bile > fat > eye.

We compared mercury concentrations in those tissues that could be sampled repeatedly (blood, mature scapulars, and growing feathers), with several measures of mercury intake to obtain a better understanding of the dynamics of accumulation. These included cumulative methylmercury consumed, cumulative methylmercury consumed divided by weight of the bird (CumHg/Weight), daily methylmercury consumed, and daily methylmer-

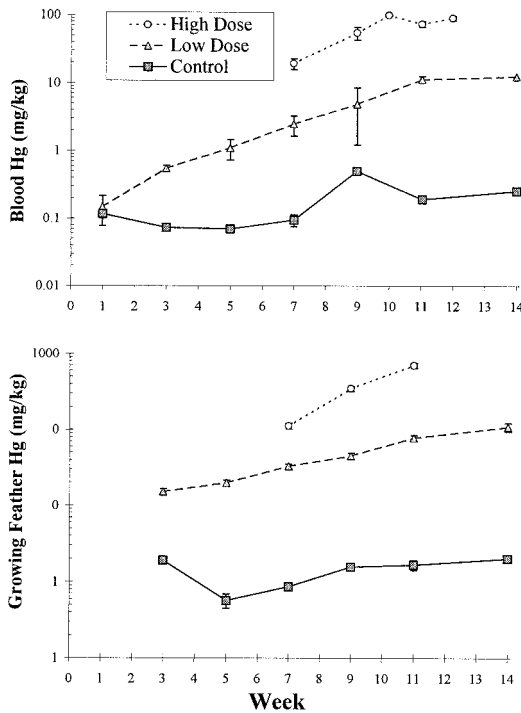


FIGURE 2. Blood and growing feather total mercury concentrations for great egrets dosed with methylmercury at control, 0.5, and 5 mg/kg graphed on a log scale during the course of experiment. Dosing with the high dose began at week 6.

cury consumed divided by weight of the bird. CumHg/Weight was the best predictor of tissue concentration, and growing feather was the best predictor of CumHg/Weight. For all groups, blood concentra-

tions lagged below the regression line early in the experiment, and then rose above it at about 9 wk when feathers ceased to grow (Fig. 3).

The greatest 3-day-average daily food consumed per body weight (Food/Weight) ranged between 6 and 27% of body weight, and peaked during the third wk for all groups (Fig. 4). Methylmercury chloride consumed (mg/kg body weight) varied with amount of food consumed and ranged from a high of 0.135 mg/kg/day during wk 3 to a low of 0.048 mg/kg/day during wk 13. We found a significant effect of dose group on Food/Weight (ANOVA, $P = 0.007$). Food intake differed significantly between the control and low dose birds during wk 11 and between the control and high dose birds during wk 10–11. Note that only two birds remained in the high dose group during wk 12.

We found a significant effect of gender on weight (ANOVA, $P = 0.007$). Weight index (weight/bill length) differed significantly between groups (ANOVA, $P = 0.008$), being lower in the low dose group during wk 11–14, and in the high dose group during wk 10–11 (Fig. 5). Bill length, tarsometatarsus length, primary length, primary sheath, tail length, and tail sheath did not differ significantly between the groups.

TABLE 1. Mean total mercury concentrations in tissues of control, low-dosed, and high-dosed great egrets at death. The mean total methylmercury chloride administered was 0.35, 8.0, and 45 mg/kg for the control, low-dose, and high-dose groups respectively.

Tissue	Mean \pm standard error		
	Control	Low-dose	High-dose
Growing feather	2.0 \pm 0.16	110 \pm 14	810 \pm 46
Mature scapular feather	6.6 \pm 5.6	40 \pm 3.2	150 \pm 15
Liver	0.42 \pm 0.057	15 \pm 1.5	140 \pm 6.0
Kidney	0.33 \pm 0.0080	8.4 \pm 1.3	120 \pm 7.6
Blood	0.25 \pm 0.0093	12 \pm 1.2	93 \pm 3.7
Pancreas	0.20 \pm 0.0071	5.4 \pm 1.3	52 \pm 1.5
Muscle	0.17 \pm 0.012	18 \pm 11	45 \pm 3.3
Brain	0.21 \pm 0.014	3.4 \pm 0.25	35 \pm 1.5
Bile	0.45 \pm 0.39	3.5 \pm 1.7	14 \pm 5.0
Eye	0.031 \pm 0.0063	0.43 \pm 0.096	4.8 \pm 0.20
Fat	0.027 \pm 0.0021	0.25 \pm 0.022	3.6 \pm 1.5

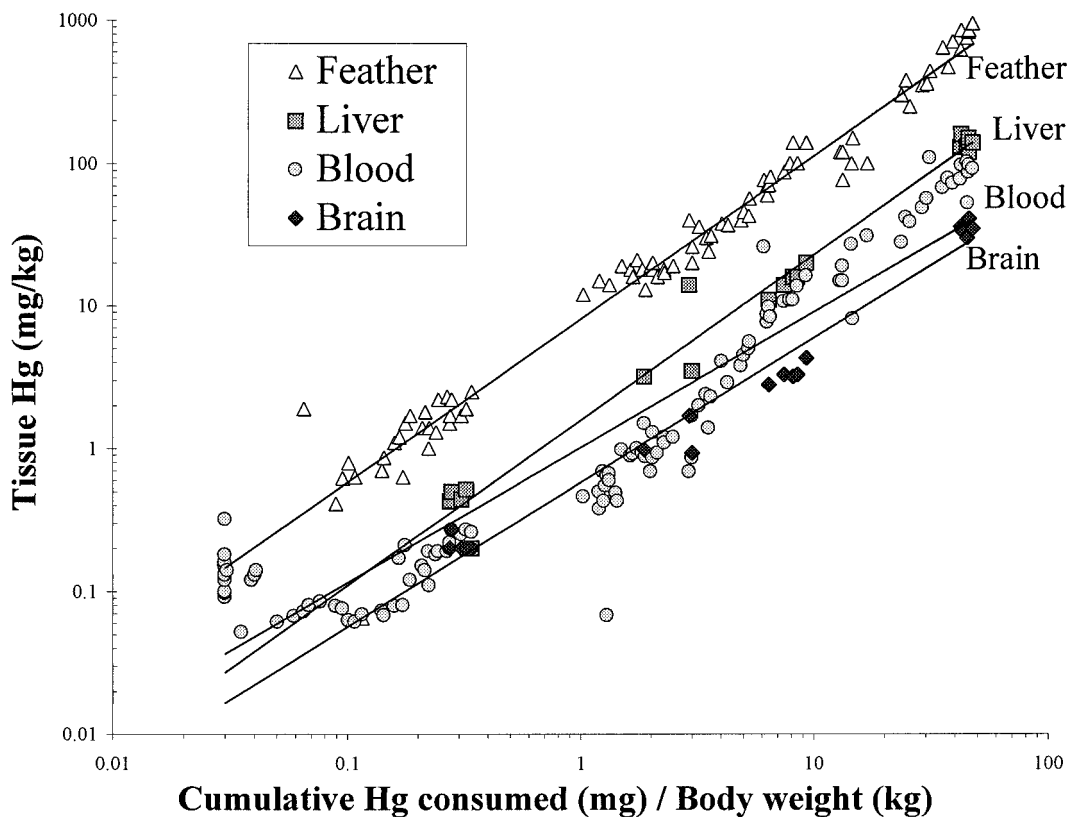


FIGURE 3. Tissue concentrations of total mercury plotted against cumulative mercury ingested/body weight (mg/kg) graphed on a log scale for great egret chicks dosed with methylmercury. Growing feather concentration = $8.0599x^{1.1456}$, blood concentration = $1.0188x^{0.9494}$, liver concentration = $1.5798x^{1.1602}$, and brain concentration = $0.9754x^{1.0136}$, where x = CumHg/Weight in mg/kg.

DISCUSSION

Methylmercury is generally well absorbed by the intestinal tract (Lewis and Furness, 1991), and although we were not able to accurately measure the proportion of methylmercury assimilated in this study due to contamination of feces by feather sheaths, our data generally agree. Based upon estimated food intake for wild nestlings (Frederick et al., 1999) tissue concentrations in our study were similar to those of naturally exposed great egrets in Florida (Sepulveda et al., 1999a).

In our study, although we administered methylmercury chloride, the bulk of the tissue analysis was for total mercury. Several authors have reported that most of the mercury in fish is methylated, and this is true for the Everglades ecosystem (Gard-

ner et al., 1978; Bloom, 1992; Frederick et al., 1999). Essentially all of the mercury in the egret feathers was methylated, concurring with the findings of Thompson and Furness (1989) for seabirds. Only a proportion of mercury that was in liver and kidney was methylated, and that proportion increased with dosing level. This observation supports the hypothesis that birds are capable of demethylating mercury (Thompson and Furness, 1989). It appears that this process is more efficient at lower mercury concentrations than at higher mercury concentrations, or that there are limitations to the demethylation process, such as might occur with cell damage or inadequate materials for detoxification (possibly selenium). Thompson et al. (1991) reported that all mercury in the

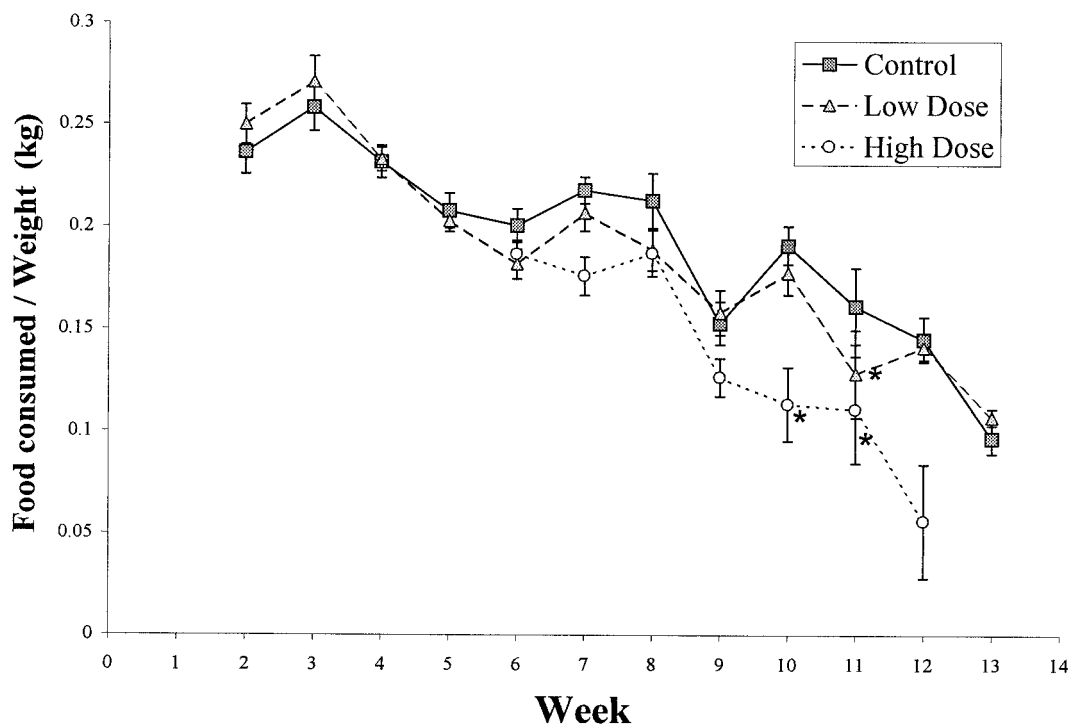


FIGURE 4. Three-day average daily food consumed per weight for great egrets dosed as control, 0.5, and 5 mg/kg methylmercury in their diet. * = values that differ significantly from controls ($P < 0.01$). Only two birds remained in the high dose group by wk 12.

muscles of great skuas (*Catharacta skua*) was methylated, whereas, approximately half that in liver and kidney was. Gardner et al. (1978) found that 40% and 20% of mercury in livers of snowy egrets (*Egretta thula*) and tricolored herons (*Egretta tricolor*) respectively, was methylated. Contrary to our findings, several authors have reported an inverse relationship between the total mercury concentration and the proportion that is methylated in liver and kidney (Norheim and Froslic, 1978 in raptors; Thompson and Furness, 1989 in seabirds; Kim et al., 1996 in seabirds).

Mercury was not distributed uniformly among tissues and our findings generally are similar to other studies of fish-eating birds. (Hesse et al., 1975; Osborn et al., 1979; Nicholson, 1981; Frank et al., 1983; Honda et al., 1985, 1986; Furness et al., 1986; Thompson et al., 1991; Lewis and Furness, 1991; Lock et al., 1992; Elbert and Anderson, 1998; Evers et al., 1998;

Wolfe and Norman, 1998). The tight correlations we observed between tissue concentrations and dose and among tissue types were undoubtedly due to the very controlled nature of this study and would not necessarily apply directly to field situations where dosing would be irregular in duration, magnitude, and chemical form. In a number of experimental dosing studies in captive seabirds, mercury concentrations were higher in kidney than in liver, whereas the opposite was found in wild seabirds (summarized by Lewis and Furness, 1991). We have not observed this in herons and egrets in Florida (M. E. Spalding and M. S. Sepulveda, unpubl. data). In grebes, Elbert and Anderson (1998) found correlations between kidney, muscle and brain concentrations, but not liver, contrary to our findings.

A positive correlation between mercury and selenium in liver has been reported for other piscivorous species (Van der Mo-

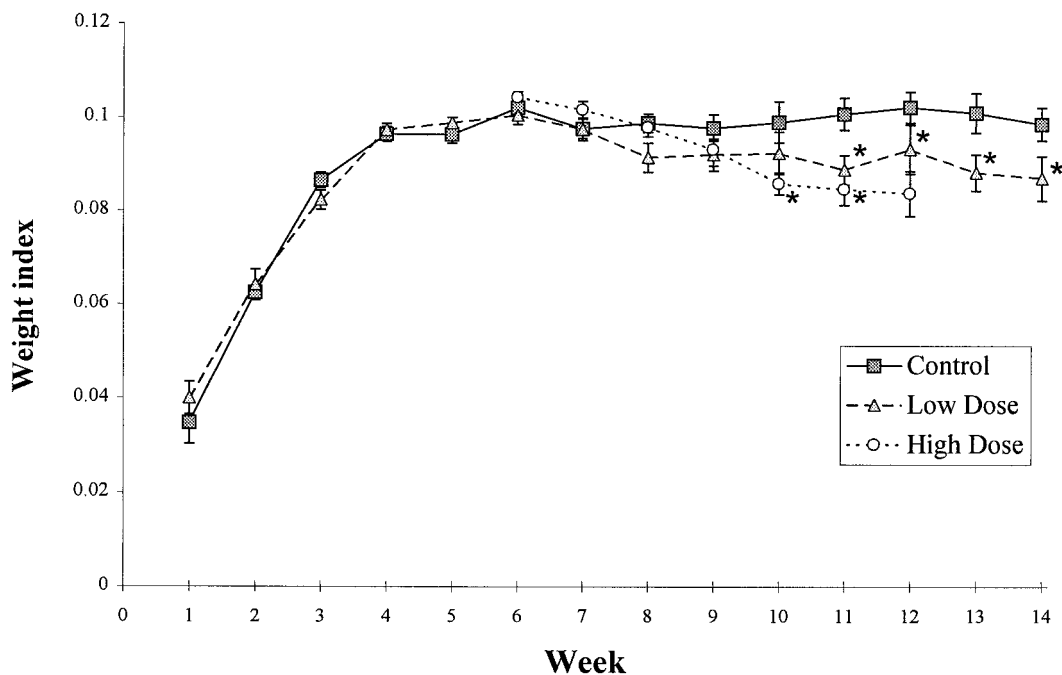


FIGURE 5. Weight index (weight/bill length) for great egrets dosed as control, 0.5, and 5 mg/kg methylmercury in their diet. * = values that differ significantly from controls ($P < 0.01$). Only two birds remained in the high dose group by wk 12.

len, et al., 1982; Sepulveda et al., 1998; Scheuhammer et al., 1998b), and it has been hypothesized that selenium plays an important role in the reduction of the toxic effects of methylmercury (Change et al., 1977; Cuvin-Aralar and Furness, 1991; Hoffman and Heinz, 1998; Heinz and Hoffman, 1998; Scheuhammer et al., 1998b). When methylmercury was fed to adult mallards (*Anas platyrhynchos*), selenium accumulated in liver at a greater rate than in controls, and accumulated at three times the rate in laying females, and 19 times the rate in males (Heinz and Hoffman, 1998). We did not observe any difference in selenium concentrations due to gender in our study of young birds (Fig. 1). Liver selenium concentrations in the high dose group exceeded the 66 ppm dry weight suggested for selenium toxicosis by Heinz (1996) however we did not observe all of the criteria for selenium toxicosis listed by Albers et al. (1996). We could not rule out the possibility that some of our

experimental results were due to the effects of selenium directly, or possibly in concert with the methylmercury.

As reported previously for great egrets and for other fish-eating birds, feather and blood concentrations were highly correlated with each other, and mercury concentrated in feathers at a greater rate than in blood (Gochfield, 1980; Evers et al., 1998; Scheuhammer et al., 1998a; Sepulveda et al., 1999a). Overall, we found concentrations in growing feathers to be roughly eight times those in blood, but this relationship varied with age. We found that growing feathers were excellent predictors of other tissue concentrations and of the cumulative mercury consumed. Blood mercury concentrations, on the other hand, varied relative to the molt status. It seems likely that molt in adult birds would also act to decrease mercury concentrations in blood and other storage organs.

Our finding of increasing feather mercury concentration with age supports the

reports of Furness et al. (1986) and Braune and Gaskin (1987) that feather mercury reflects the cumulative amount of mercury stored in body tissues rather than the dietary intake at the time that the feather grew. Mature feathers collected near the end of the experiment had lower mercury concentrations than growing feathers collected at the same time. This is probably because mature feathers ceased to grow at about 9 wk, whereas growing feathers were exposed to the higher blood and other tissue concentrations near the end of the experiment. Although Lewis and Furness (1991) found higher concentrations in primary feathers grown first in gull chicks, all dosing in their experiment occurred prior to the initiation of feather growth. Therefore, the timing of feather growth in relation to mercury exposure and maturity of feather are important factors when comparing birds or locations, or simply evaluating the exposure history of a single bird of interest. The combination of a mature feather, a growing feather, a blood sample, and knowledge of the molt stage may provide the most comprehensive information about contamination history for a bird.

Even though chicks were ingesting food (and methylmercury, 0.135 mg/kg/day) at the highest rate relative to body size during wk 3, the effects of mercury on appetite and growth were not apparent until much later. In all three groups mercury began to accumulate in blood in higher concentrations relative to dosing after wk 9. This corresponded with the time that feathers stopped growing (wk 9–11 for primary feathers, and wk 9 for tail feathers) and was just before the time that birds also began to show obvious deficits in appetite and growth.

Although appetite declined in both dosed groups, we could find no good explanation for why it occurred after consumption of 6.4 mg/kg in the low dose group, and but not until 44.5 mg/kg had been consumed in the high dose group (Spalding et al., 2000, Table 1). In wild

dosed great egrets (1.8 mg Hg/kg in fish) declines in appetite were detected when feather concentrations were 49 mg/kg (=4.8 CumHg/body weight using regression in Fig. 3, see Williams, 1997). These findings support the hypothesis that wild nestlings might be more sensitive to methylmercury than captive nestlings because of uncontrolled factors that might interact to affect appetite.

The weight loss that occurred in both dose groups was a logical consequence of the methylmercury-induced reduction in appetite. The magnitude of weight loss was small and all birds had abundant body fat at the end of the experiment. The lack of food stress in these captive birds may have masked some of the effects that methylmercury contamination would have caused had the birds been hunting on their own. Williams (1997) could find no evidence of weight declines or changes in skeletal measurements in wild great egret nestlings dosed in the field.

It appears that growing feathers, and possibly other storage organs, provide a sink for mercury during the nestling period that protects chicks from mercury poisoning. When feathers ceased to grow and this sink was no longer available, mercury increased more rapidly in blood. Our findings support the conclusion that there is a period of higher risk of chronic mercury toxicity for young birds when feathers stop growing. This period of elevated risk usually would occur as feathers are finishing their growth which coincides with the time that young birds also encounter the multiple risk factors of having to forage on their own, leave the natal colony, and become exposed to novel predation and disease factors.

We conclude that methylmercury affected appetite and growth of great egrets, even at the 0.5 mg/kg dose rate, a dose similar to current exposure in the Everglades (Frederick et al., 1999). We caution against using these data to designate a lowest observable adverse affect level (LOAEL) for two reasons. Selenium avail-

ability was high in our experiment, and we suspect that in a system where it is less available poisoning might occur earlier. In wild birds with lower nutritional reserves, a reduction of appetite could more quickly result in loss of weight and body condition given the rigors of competition between siblings for limited food resources (Mock et al., 1987), learning to forage, and other stressors. Since the consequences of poor body condition can initiate a downward trend in health due to poorer foraging success, compromised immune system (Grasman and Scanlon, 1995) and increased disease susceptibility, we suggest that the effects of mercury on body condition will lead to higher mortality rates for juveniles in the wild.

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